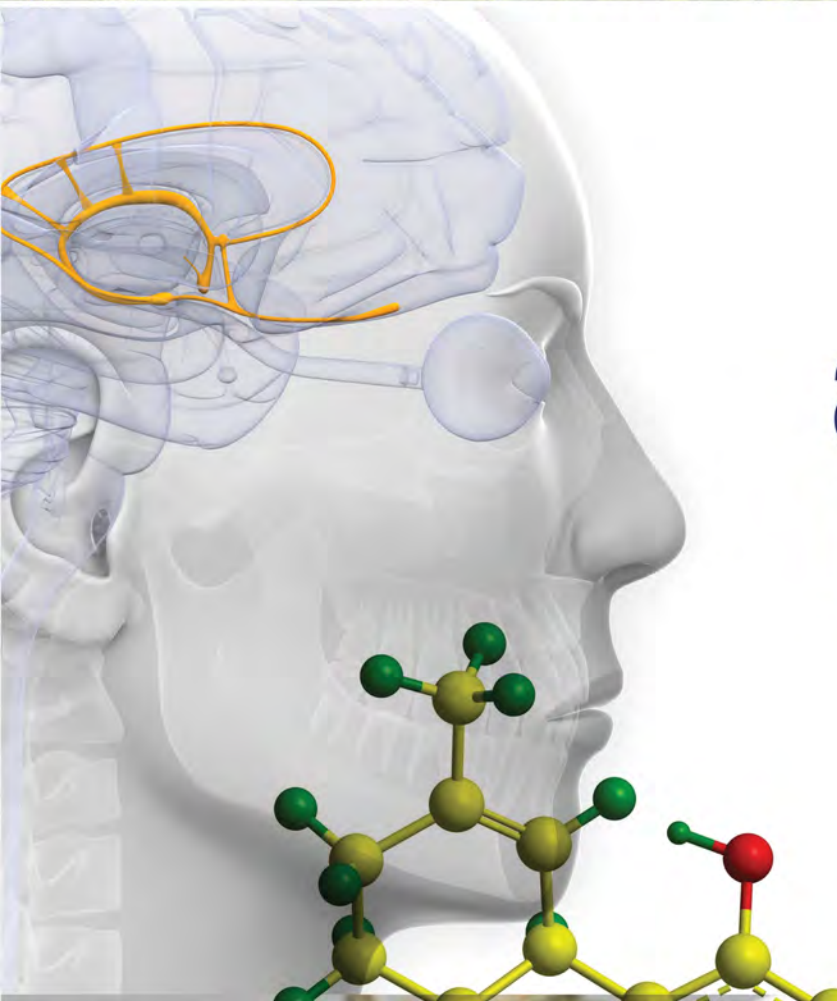
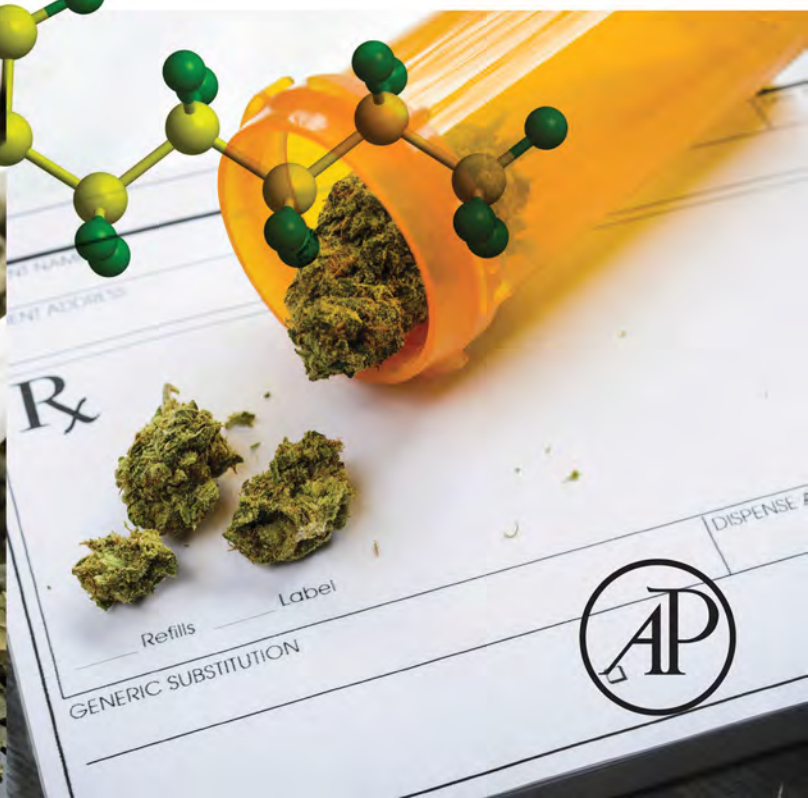
Handbook of Cannabis and Related Pathologies

Biology, Pharmacology, Diagnosis, and Treatment

EDITED BY
VICTOR R. PREEDY





HANDBOOK OF CANNABIS AND RELATED PATHOLOGIES

ELSEVIER science & technology books

Companion Web Site:

http://store.elsevier.com/product.jsp?&isbn=9780128007563

Handbook of Cannabis and Related Pathologies
V.R. Preedy, Editor

Available Resources:

20 additional chapters are available online - see contents list.

TOOLS FOR ALL YOUR TEACHING NEEDS
textbooks.elsevier.com

ACADEMIC PRESS

To adopt this book for course use, visit http://textbooks.elsevier.com.

HANDBOOK OF CANNABIS AND RELATED PATHOLOGIES

BIOLOGY, PHARMACOLOGY, DIAGNOSIS, AND TREATMENT

Edited by

V.R. PREEDY

BSC, PHD, DSC, FRSB, FRSH, FRIPHH, FRSPH, FRCPATH, FRSC

Faculty of Life Sciences and Medicine,
King's College London, London, United Kingdom

ACADEMIC PRESS

An imprint of Elsevier
elsevier.com

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1800, San Diego, CA 92101-4495, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

Copyright © 2017 Elsevier Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: www.elsevier.com/permissions.

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-0-12-800756-3

For information on all Academic Press publications visit our website at https://www.elsevier.com/

Publisher: Mara Conner
Acquisitions Editor: April Farr
Editorial Project Manager: Timothy Bennett
Production Project Manager: Chris Wortley
Designer: Mark Rogers

Typeset by Thomson Digital

Dedicated to my friend Chris Bird

Page left intentionally blank

Contents

List of Contributors xv
Preface xxv

I
SETTING THE SCENE, BOTANICAL, GENERAL AND INTERNATIONAL ASPECTS

1. The *Cannabis* Plant: Botanical Aspects 3
S. FARAG, O. KAYSER

2. The Biosynthesis of Cannabinoids 13
F. DEGENHARDT, F. STEHLE, O. KAYSER

3. Increasing Plant Concentrations of THC and Implications on Health Related Disorders 24
V. VINDENES, J. MØRLAND

4. Age as a Predictor of Cannabis Use 33
D. BERGEN-CICO, R.D. CICO

5. Lifetime Cannabis Use and Cognition in Psychosis Spectrum Disorders 44
M.J. CUESTA, A.M. SÁNCHEZ-TORRES, R. LORENTE-OMEÑACA, L. MORENO-IZCO

6. A Profile of Synthetic Cannabinoid Users 53
A.N. SANDERS, J.M. STOGNER

7. Dual Disorders in Cannabis Misuse 61
F. ARIAS-HORCAJADAS, N. SZERMAN, P. VEGA, I. BASURTE, B. MESÍAS

8. Cannabis Use and Cognitive Function 70
C. EVREN

9. Cannabis, Migration, and Psychosis Onset 79
A. KOKONA, I. TARRICONE, M. DI FORTI, E. CARRA

10. The Global Epidemiology and Disease Burden of Cannabis Use and Dependence 89
L. DEGENHARDT, A.J. FERRARI, W.D. HALL

11. International Aspects of Cannabis Use and Misuse: the Australian Perspective 101
D.J. ALLSOP, W.D. HALL

12. International Aspects of Cannabis Use and Misuse: Egypt 110
O.M.E. ABDEL-SALAM, A.F. GALAL, S.A. ELSHEBINEY, A.E.D.M. GAAFAR

13. Cannabis Body Packing: A Caribbean Perspective 122
S.O. CAWICH, D. DAN, V. NARAYNSINGH

II
PERSONAL, SOCIAL AND COMMUNITY ASPECTS OF CANNABIS USE

14. Gender Differences in Cannabis Use Disorders 131
R. SECADES-VILLA, S. FERNÁNDEZ-ARTAMENDI

15. The Role of Age in the Onset and Further Development of Cannabis Use Disorders 138
S. BEHRENDT

16. Effects of Cannabis Use on Neurocognition in Adolescents and Emerging Adults 151
N.E. WRIGHT, K.E. MAPLE, K.M. LISDAHL

17. Correlates and Consequences of Prenatal Cannabis Exposure (PCE): Identifying and Characterizing Vulnerable Maternal Populations and Determining Outcomes for Exposed Offspring 160
L.K. BRENTS

18. Cannabis and Clubbing: Relevance of Cannabis and Polydrug Use in the Clubbing Culture Today 171
D.A. HERZIG, S. BACHMANN

19. Cannabis and Sexual Behavior 180
G. SCIMECA, C. CHISARI, M.R.A. MUSCATELLO, C. CEDRO, G. PANDOLFO, R. ZOCCALI, A. BRUNO

20. Friendships and Cannabis Use 188
J.H. BOMAN IV, C. HECK

21. Students' Knowledge of Cannabis 198
M. DROZD, J. SOBCZYŃSKI

22. Childhood Trauma and Cannabis: Risk Factors in Severe Mental Disorders? 208
M. AAS, I. MELLE

23. Parent's Influence on Children's Cannabis Use 215
S. MILLER, J.T. SIEGEL, W.D. CRANO

24. Cannabis Users and Premorbid Intellectual Quotient 223
L. FERRARO, L. SIDELI, D. LA BARBERA

25. Cannabis and Traffic Accidents 234
R.B. DE BONI, R.P. LIMBERGER, T.R.V. SOUSA

III
CANNABIS, BEHAVIOR, PSYCHOPATHOLOGY AND NEUROPATHOLOGY

26. Drug-Related Pictures, Attentional Bias, and Cannabis Use 247
D. ASMARO

27. Cannabis Use and First-Episode Psychosis Patients (FEP) 257
I. GONZÁLEZ-ORTEGA, M. MARTÍNEZ-CENGOTITABENGOA, A. GONZÁLEZ-PINTO

28. Cannabis, Associative Memory, fMRI, and the Implicit Association Test 267
S.L. AMES, A.W. STACY

29. Stress Response in Cannabis Users and Psychosis 278
M. BIOQUE, H.-H. TSENG, R. MIZRAHI

30. Motivation in Chronic Cannabis Use 288
R. HIRST, L. SODOS, S. GADE, L. RATHKE

31. Cannabis Use and Its Association to Mental Illness: A Focus on Mood and Anxiety Disorders 298
S. LEV-RAN, D. FEINGOLD

32. Cannabis Use and Well-Being 308
J. ALLEN, M.D. HOLDER, Z. WALSH

33. Craving and Cannabis: A Potential Paradox 317
M.J. LOFLIN, M. EARLEYWINE

34. Delta-9-Tetrahydrocannabinol and Catalepsy-Like Immobilization 326
N. EGASHIRA

35. The Interactive Nature of Cannabis and Schizophrenia Risk Genes 335
T. KARL, J.C. ARNOLD

36. Neuroimaging Findings in Adolescent Cannabis Use and Early Phase Psychosis 345
C.E. CROCKER, J. COOKEY, P.G. TIBBO

37. Cannabis Smoking in Adult Schizophrenia: A Cognitive and Functional Magnetic Resonance Imaging Perspective 357
K. PAQUIN, T. LECOMTE, S. POTVIN

38. The Long-Lasting Effects of Cannabis Use on Movement and Brain Regions that Control Movement 372
G. TODD, J.M. WHITE

39. Assessment of Cannabis Acute Effects on Driving Skills: Laboratory, Simulator, and On-Road Studies 379
P. BONDALLAZ, H. CHTIOUI, B. FAVRAT, E. FORNARI, C. GIROUD, P. MAEDER

40. Chronic Cannabis Use and Axonal Fiber Connectivity 391
N. SOLOWIJ, A. ZALESKY, V. LORENZETTI, M. YÜCEL

41. Microglial Activation and Cannabis Exposure 401
L. CUTANDO, R. MALDONADO, A. OZAITA

42. Cannabis and Psychosis: Correlation, Causality, and Consequences 413
D. BASU, P. PARAKH

43. Cannabis Use in Bipolar Disorder 422
T.V. LAGERBERG

44. Cannabis Use in Epilepsy—Risks and Benefits 431
M. HOLTKAMP, M. HAMERLE

45. Cannabis, Cannabinoids, and Visceral Pain 439
R. ABALO, M. ISABEL MARTÍN-FONTELLES

46. Cannabis and Postoperative Analgesia 450
S.O CAWICH, U. DEONARINE, H.E. HARDING, D. DAN, V. NARAYNSINGH

IV

CANNABIS, ORGANS, TISSUES AND NON-CNS ASPECTS

47. Chronic Cannabis Abuse and Thyroid Function 461
U. BONNET

48. Cannabis Hyperemesis Syndrome 466
U. BONNET

49. Cannabis and Cannabinoids and the Effects on Gastrointestinal Function: An Overview 471
M. SAŁAGA, R. ABALO, J. FICHNA

50. Cardiovascular Effects of Cannabis Usage 481
S. MENAHEM

51. Cannabis and Stroke 486
P.A. BARBER

52. Cannabis Smoking and the Lung 494
D.P. TASHKIN

53. Cannabis and Hepatic Injury 505
S.A. NADA, O.M.E. ABDEL-SALAM, A.A. SLEEM

54. Cannabis Allergy: More Than a Bad Trip 517
A.L. VAN GASSE, V. SABATO, M.M. FABER, C.H. BRIDTS, D.G. EBO

55. Marijuana and Breastfeeding 527
M.G. HILL, K.L. REED

56. Hypocretins/Orexins and Addiction: Role in *Cannabis* Dependence 533
Á. FLORES, R. MALDONADO, F. BERRENDERO

57. Regulatory Role of Cannabinoids for Skin Barrier Functions and Cutaneous Inflammation 543
T. TÜTING, E. GAFFAL

V

PHARMACOLOGY AND CELLULAR ACTIVITIES OF CANNABINOIDS AND ENDOCANNABINOIDS

58. Cannabinoids and the Cannabinoid Receptors: An Overview 553
D. LU, D.E. POTTER

59. Signaling and Regulation of the Cannabinoid CB_1 Receptor 564
M.R. HUNTER, D.B. FINLAY, M. GLASS

60. Allosteric Modulation of the Cannabinoid CB1 Receptor 573
E.E. CAWSTON, M.R. HUNTER, M. GLASS

61. Polymorphisms of the CB2 Cannabinoid Receptor 584
P. KUMAR, Z.-H. SONG

62. Chemistry of Cannabinoid Receptor Agonists 592
M. AGHAZADEH TABRIZI, P.G. BARALDI

63. The Endocannabinoid System as a Target for New Antiseizure Drugs 606
L.R. VILELA, A.C.P. DE OLIVEIRA, M.F. MORAES, F.A. MOREIRA, R.N. TAKAHASHI

64. Pharmacological Aspects of Anandamide and 2-Arachidonoyglycerol as Bioactive Lipids 616
M. ALHOUAYEK, G.G. MUCCIOLI

65. Pharmacological Aspects of NMDA Receptors, mGluR5, and Endocannabinoids 630
Y. IZUMI, C.F. ZORUMSKI

66. Peripheral CB1 Receptors and Ghrelin in Feeding Regulation: Pharmacological Implications 639
L. ORIO, R. GÓMEZ DE HERAS, F. RODRÍGUEZ DE FONSECA

67. Pharmacological Aspects of Novel Antiobesity Agents Related to Cannabinoids 649
L. HERNANDEZ-FOLGADO

68. Cannabinoid Reward and Dependence: Focus on the Main Psychoactive Ingredients of Marijuana in Preclinical Studies 659
G. PANAGIS

69. Peroxisome Proliferator Activated Receptors and Cannabinoids 671
E. MUÑOZ, F. POLLASTRO, O. TAGLIALATELA-SCAFATI, G. APPENDINO

70. The Protein–Protein Interactions of Cannabinoid Receptor Interacting Protein 1a (CRIP1a) and Cannabinoid 1 Receptor: The Molecular Mechanism Study Through an Integrated Molecular Modeling Approach 680
M.H. AHMED, Y. ZHANG

71. Synthetic Cannabinoids: a Summary of Selected Phenomena with Respect to Behavioral Pharmacology and Abuse Liability 691
B.T. BURROWS, L.R. WATTERSON, J. EGNATIOS, M.F. OLIVE

VI

EFFECTS OF SPECIFIC NATURAL AND SYNTHETIC CANNABINOIDS

72. The Role of 5-HT_{1A} Receptor, and Nausea and Vomiting Relief by Cannabidiol (CBD), Cannabidiolic Acid (CBDA), and Cannabigerol (CBG) 703
E.M. ROCK, L.A. PARKER

73. Genetic and Molecular Aspects of Addiction with Tetrahydrocannabinol 713
T. JANUS, A. MACHOY-MOKRZYŃSKA, K. BOROWIAK

74. Effects of Δ^9-Tetrahydrocannabinol in Human Breast Cancer 722
S. TAKEDA, E. IKEDA, H. OKAZAKI, K. WATANABE, H. ARAMAKI

75. Δ^9-THC and COX-2 Signaling 729
J. ZHANG, C. CHEN

76. Cannabinoids and the Addictive Effects of Nicotine 739
L.V. PANLILIO, S.R. GOLDBERG

77. Cannabinoid Regulation of Intraocular Pressure: Human and Animal Studies, Cellular and Molecular Targets 748
A. ALOWAY, A. KUMAR, A.S. LAUN, Z.H. SONG

78. Ocular Delivery of Tetrahydrocannabinol 760
G.R. ADELLI, P. BHAGAV, M.A. REPKA, W. GUL, M.A. ELSOHLY, S. MAJUMDAR

79. The Role of γ-Aminobutyric Acid in the Interoceptive Effects of Oral Δ^9-Tetrahydrocannabinol in Humans 770
J.A. LILE, J.S. FOGEL, T.H. KELLY

80. The Role of Δ^9-Tetrahydrocannabinol in Diabetes Mellitus 779
Z.M. COSKUN, S. BOLKENT

81. Cannabidiol: An Overview of its Antipsychotic Properties 787
F.F. PERES, V. ALMEIDA, V.C. ABILIO

82. Cannabidiol for the Treatment of Epilepsy: An Overview of Possible Mechanisms of Action and Preclinical and Human Studies 795
R. GUIMARÃES DOS SANTOS, J.E.C. HALLAK, A.W. ZUARDI, A.C. DE SOUZA CRIPPA, J.A. DE SOUZA CRIPPA

83. Cannabidiol and Neuroprotection: Evidence from Preclinical Studies 802
N. SCHRÖDER, V.K. DA SILVA, J.E.C. HALLAK, A.W. ZUARDI, J.A. DE SOUZA CRIPPA

84. Cannabinoids as Potent Inhibitors of Human CYP1 Enzymes 813
K. WATANABE, S. YAMAORI, K. MASUDA, T. KATSU, S. NARIMATSU, I. YAMAMOTO

85. The Synthetic Analog of Δ^9-Tetrahydrocannabinol (THC): Nabilone. Pharmacology and Clinical Application 821
R.E BALTER, M. HANEY

86. Synthetic Cannabinoids in Dementia 828
S. AMANULLAH, K. SHIVAKUMAR, S. HASSAN, A. CANFIELD, J. COLE

87. Synthetic Cannabinoid Receptor Agonists (Spice) as New Recreational Psychoactive Substances 839
A. HELANDER

88. Accidents and Synthetic Cannabinoids in Blood of Drivers 848
S.S. TUV, V. AUWÄRTER, V. VINDENES

VII
MEDICINAL CANNABIS USE

89. Cannabis and Synthetic Cannabinoids for Cancer Patients: Multiple Palliative Indications Together With Promising Laboratory Antineoplastic Effects 859
D. ZALMAN, G. BAR-SELA

90. The Use of Medical Marijuana in the Treatment of Psychiatric Disorders 869
T. TELLIOĞLU, Z. TELLIOĞLU

91. Beneficial Effects of Cannabis and Related Compounds on Sleep 877
I.M.P. LINARES, J.A.S. CRIPPA, M.H.N. CHAGAS

92. Cannabinoid-Based Medicines for the Treatment of Gilles de la Tourette Syndrome 883
A.S. KANAAN, K.R. MÜLLER-VAHL

93. Cannabidiol and Multiple Sclerosis 893
M. MECHA, A. FELIÚ, F.-J. CARRILLO-SALINAS, C. GUAZA

94. Cannabinoids and Their Effects on Painful Neuropathy 905
D. SELVARAJAH, R. GANDHI, S. TESFAYE

95. Cannabis for Basal Ganglia Disorders (Parkinson Disease and Huntington Disease) 917
O.M.E. ABDEL-SALAM

96. Medical Cannabis for the Treatment of Inflammatory Bowel Disease 931
A. LAHAT

97. Cannabidiol for the Treatment of Drug Use Disorders 939
R.G. DOS SANTOS, J.E.C. HALLAK, A.W. ZUARDI, J.A. DE SOUZA CRIPPA

98. Cannabinoids and Effects on the Gastrointestinal Tract: A Focus on Motility 947
G. VERA, J. FICHNA, R. ABALO

99. Potential Medical Uses of Cannabigerol: A Brief Overview 958
S. DEIANA

VIII
SCREENING, DIAGNOSIS, AND TREATMENT

100. The Cannabis Abuse Screening Test (CAST) and Its Applications 971
L. BASTIANI, R. POTENTE, M. SCALESE, V. SICILIANO, L. FORTUNATO, S. MOLINARO

101. Screening of Synthetic Cannabinoids 981
E.L. ØIESTAD, R. KARINEN, K. HAUGLAND, Å.M.L. ØIESTAD

102. On-Site Drug Testing for Cannabis 998
A.A. FERNÁNDEZ

103. Cannabinoids in Oral Fluid: Identification and Interpretation of Analytical Results 1007
C. MOORE

104. Cannabinoids in Exhaled Breath 1018
O. BECK

105. Barriers to Treatment Seeking for Cannabis Dependence 1025
P. GATES, J. COPELAND

106. Pharmacotherapies for Cannabis Use Disorders 1030
A.L. McRAE-CLARK

107. Self-Initiated Cannabis Use Cessation in Adolescents and Emerging Adults 1036
J. TSAI, M. LITTLE, S. SUSSMAN

108. Treating Cannabis-Dependent Adolescents with Family Therapy: The Case of Multidimensional Family Therapy 1047
H. RIGTER

109. Cognitive Behavioral Therapy in Cannabis Use Disorder 1056
F.M. GUVEN, U.M. CAMSARI, O. SENORMANCI, G. OGUZ

110. The Cannabis Withdrawal Syndrome—Symptoms and Time Course 1066
M. HESSE, B. THYLSTRUP

111. School-Based Cannabis Prevention Programs 1074
C. ARIZA, F. SÁNCHEZ-MARTÍNEZ, A. PÉREZ

112. The CapOpus Trial for Cannabis Use Disorders 1086
C.R. HJORTHØJ, M. NORDENTOFT

113. Treating Cannabis Use Disorders Through Technology-Assisted Interventions: The Telephone and Internet 1093
P. GATES, J. COPELAND

114. Reducing Cannabis Use With a Real-Time Intervention Using Mobile Technology 1101
M. KELLS, L.A. SHRIER

Cannabis Neuropathology Resources and Recommended Reading 1111
R. RAJENDRAM, V.B. PATEL, V.R. PREEDY

Index 1115

Online Contents

I

SETTING THE SCENE, BOTANICAL, GENERAL AND INTERNATIONAL ASPECTS

e1. Tetrahydrocannabinol Concentration and Genetic Characterization of Cannabis e1
F. CASCINI, I. BOSCHI

II

PERSONAL, SOCIAL AND COMMUNITY ASPECTS OF CANNABIS USE

e2. Cannabis Use in Youth Subcultures e11
M. PAWSON, B.C. KELLY

III

CANNABIS, BEHAVIOR, PSYCHOPATHOLOGY AND NEUROPATHOLOGY

e3. Aggressive Behavior and Cannabis Use e19
W. LIU, H. PETRAS

e4. COMT Genotypes, Cannabis Use, and Psychosis: Gene-Environment Interaction Evidence from Human Populations, and Its Methodological Concerns e29
M. FATJÓ-VILAS, C. PRATS, L. FAÑANÁS

e5. Neuroimaging and Genetics of the Acute and Chronic Effects of Cannabis e42
R. MARTÍN-SANTOS, J.A. DE SOUZA CRIPPA, S. BHATTACHARYYA

e6. Gray Matter, Lateral Ventricle Volumes, and Executive Functioning in Cannabis Users with First-Episode Psychosis e53
P.J. CUNHA, P.G.P. ROSA, F.L.S. DURAN, L.C. SANTOS, J.A.S. CRIPPA, G.F. BUSATTO, M.S. SCHAUFELBERGER

e7. Cannabis Use and Attention-Deficit/Hyperactivity Disorder: Potential Moderators e64
K.E. MAPLE, N.E. WRIGHT, K.M. LISDAHL

IV

CANNABIS, ORGANS, TISSUES AND NON-CNS ASPECTS

e8. Cannabis and Oral Health: Deleterious Effects on Periodontitis and Dental Implants e72
G. NOGUEIRA-FILHO

e9. Does Cannabis Use Increase the Risk of Developing Cancer in Humans? e80
R.C. CALLAGHAN, M. VERDICHEVSKI, T.M. FYFE, J.M. GATLEY

e10. Cannabis and the Use of Amphetamine-Like Substances e101
A. PORCU, M.P. CASTELLI

V

PHARMACOLOGY AND CELLULAR ACTIVITIES OF CANNABINOIDS AND ENDOCANNABINOIDS

e11. Cannabinoid Signaling in Glioma Cells and Therapeutic implications e111
A. ELLERT-MIKLASZEWSKA, I. A. CIECHOMSKA, B. KAMINSKA

VI

EFFECTS OF SPECIFIC NATURAL AND SYNTHETIC CANNABINOIDS

e12. Cannabidiol as an Antioxidant e122
R.S. BORGES, A.B.F. DA SILVA

e13. The Anxiolytic Effects of Cannabidiol (CBD) e131
A.W. ZUARDI, J.A. DE SOUZA CRIPPA, J.E.C. HALLAK, A.C. CAMPOS, F.S. GUIMARÃES

e14. New Ethological and Morphological Perspectives for the Investigation of Panicolytic-Like Effects of Cannabidiol e140
N.C. COIMBRA, J. MENDES-GOMES, J.A. DA SILVA, T. DOS ANJOS-GARCIA, F. ULLAH, R.C. ALMADA

e15. Spice Use Among United States Military Personnel e150
H.A. MORRIS, J.M. STOGNER

VII

MEDICINAL CANNABIS USE

e16. Cannabis Use in Fibromyalgia e158
M. FARRÉ, A. FARRÉ, J. FIZ, M. TORRENS

VIII

SCREENING, DIAGNOSIS, AND TREATMENT

e17. Short Instruments to Screen for "Problematic" Cannabis Use in General Population Surveys e168
B. ANNAHEIM, S. LEGLEYE

e18. Self-report of Cannabis Use e185
T. VAN DER LINDEN

e19. CANDIS Program: Modular Treatment of Cannabis Use Disorders e193
E. HOCH, H. ROHRBACHER

e20. Engaging Cannabis Users in Treatment e202
F. KAY-LAMBKIN, A. HEALEY, A. BAKER, W. SWIFT, L. THORNTON, A. TURNER

List of Contributors

M. Aas NORMENT, KG Jebsen Centre for Psychosis Research, Division of Mental Health and Addiction, Oslo University Hospital & Institute of Clinical Medicine, University of Oslo, Oslo, Norway

R. Abalo Area of Pharmacology and Nutrition, Faculty of Health Sciences, University Rey Juan Carlos, Alcorcón; Associated Unit I+D+i of the Institute of Medicinal Chemistry (IQM) and of the Institute of Research in Food Sciences (CIAL), Spanish National Research Council (CSIC), Madrid, Spain

O.M.E. Abdel-Salam Department of Toxicology and Narcotics, National Research Centre, Dokki, Greater Cairo, Egypt

V.C. Abilio Department of Pharmacology, Federal University of Sao Paulo; Integrated Laboratory of Clinical Neurosciences (LiNC), Federal University of Sao Paulo, Sao Paulo, Brazil

G.R. Adelli Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, Oxford, MS, United States

M.H. Ahmed Department of Medicinal Chemistry, School of Pharmacy and Institute for Structural Biology and Drug Discovery, Virginia Commonwealth University, Richmond, VA, United States

M. Alhouayek Bioanalysis and Pharmacology of Bioactive Lipids Research Group, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium

J. Allen Psychology Department, IKBSAS, University of British Columbia, Kelowna, BC, Canada

D.J. Allsop Psychopharmacology Laboratory, School of Psychology, University of Sydney, Sydney, NSW, Australia

R.C. Almada Laboratory of Neuroanatomy & Neuropsychobiology, Department of Pharmacology, Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP), Ribeirão Preto, São Paulo, Brazil

V. Almeida Department of Pharmacology, Federal University of Sao Paulo; Integrated Laboratory of Clinical Neurosciences (LiNC), Federal University of Sao Paulo, Sao Paulo, Brazil

A. Aloway Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY, United States

S. Amanullah Woodstock General Hospital, Woodstock, ON, Canada; School of Medicine, University of Western Ontario, London, ON, Canada; and Faculty of Medicine, Dalhousie University, NS, Canada

S.L. Ames School of Community and Global Health, Claremont Graduate University, Claremont, CA, United States

B. Annaheim Institute for Biomedical Ethics (IBMB), University of Basel, Basel, Switzerland

G. Appendino Department of Pharmaceutical Science, University of Piemonte Orientale, Novara, Italy

H. Aramaki Department of Molecular Biology, Daiichi University of Pharmacy; Drug Innovation Research Center, Daiichi University of Pharmacy, Fukuoka, Japan

F. Arias-Horcajadas Psychiatric Department, Doce de Octubre Hospital, Madrid, Spain

C. Ariza Evaluation and Intervention Methods Service, Public Health Agency, Barcelona, Spain

J.C. Arnold School of Medicine, Western Sydney University, Campbelltown; University of Sydney, Department of Pharmacology, Bosch Institute, Sydney; Brain and Mind Research Institute, Camperdown, NSW, Australia

D. Asmaro Department of Psychology, Simon Fraser University, Burnaby, BC, Canada

V. Auwärter Department of Drug Abuse Research, Division of Forensic Sciences, Norwegian Institute of Public Health, Oslo, Norway; Forensic Toxicology Department, Medical Center, University of Freiburg, Institute of Forensic Medicine, Freiburg, Germany

S. Bachmann Clienia AG, Littenheid, Switzerland

A. Baker Priority Research Centre for Translational Neuroscience and Mental Health, University of Newcastle, Callaghan, NSW, Australia

R.E Balter Division on Substance Abuse, New York State Psychiatric Institute and Department of Psychiatry, Columbia University Medical Center, New York, NY, United States

P.G. Baraldi Department of Chemistry and Pharmaceutical Science, University of Ferrara, Ferrara, Italy

P.A. Barber Department of Medicine, Centre for Brain Research, University of Auckland, Auckland, New Zealand

G. Bar-Sela Division of Oncology, Rambam Health Care Campus and Faculty of Medicine, Technion—Israel Institute of Technology, Haifa, Israel

L. Bastiani Institute of Clinical Physiology, The Italian National Research Council (IFC-CNR), Pisa, Italy

D. Basu Drug De-addiction and Treatment Centre, Department of Psychiatry, Postgraduate Institute of Medical Education and Research, Chandigarh, India

I. Basurte Gregorio Marañon Hospital, Madrid, Spain

O. Beck Department of Laboratory Medicine, Section of Clinical Pharmacology, Karolinska Institutet, Stockholm, Sweden

S. Behrendt Institute of Clinical Psychology and Psychotherapy, Technische Universitaet Dresden, Dresden, Germany

D. Bergen-Cico Department of Public Health, Addiction Studies, Syracuse University, Syracuse, NY, United States

F. Berrendero Department of Experimental and Health Sciences, Laboratory of Neuropharmacology, School of Health and Life Sciences, Pompeu Fabra University, Barcelona, Spain

P. Bhagav Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, Oxford, MS, United States

S. Bhattacharyya Department of Psychosis Studies, King's College London, Institute of Psychiatry, Psychology & Neuroscience, London, United Kingdom

M. Bioque Barcelona Clínic Schizophrenia Unit, Hospital Clínic de Barcelona, Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Barcelona, Spain

S. Bolkent Department of Medical Biology, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey

J.H. Boman IV Department of Criminal Justice, University of Wyoming, Laramie, WY, United States

P. Bondallaz Traffic Medicine and Psychology Unit, University Center of Legal Medicine, University Hospital of Geneva, Geneva, Switzerland

U. Bonnet Department of Psychiatry, Psychotherapy and Psychosomatics, Evangelisches Krankenhaus Castrop-Rauxel, Academic Teaching Hospital of the University of Duisburg-Essen, Castrop-Rauxel, Germany

R.S. Borges Faculty of Pharmacy, Institute of Health Sciences, Federal University of Pará, Belém, Para, Brazil

K. Borowiak Department of Clinical and Forensic Toxicology, Pomeranian Medical University, Szczecin, Poland

I. Boschi Public Health Institute, Department of Forensic Medicine, Università Cattolica del Sacro Cuore, Rome, Italy

L.K. Brents Brain Imaging Research Center, Psychiatric Research Institute, University of Arkansas for Medical Sciences, Little Rock, AR, United States

C.H. Bridts Faculty of Medicine and Health Science, Department of Immunology-Allergology-Rheumatology, University of Antwerp, Antwerp, Belgium

A. Bruno Department of Biomedical, Dental Sciences and Morpho-functional Imaging, University of Messina, Messina, Italy

B.T. Burrows Department of Psychology, Arizona State University, Tempe, AZ, United States

G.F. Busatto Department of Psychiatry, Faculty of Medicine, Laboratory of Psychiatric Neuroimaging (LIM-21), University of São Paulo; Center for Interdisciplinary Research on Applied Neurosciences (NAPNA), University of São Paulo, São Paulo, Brazil

R.C. Callaghan Northern Medical Program, University of Northern British Columbia, Prince George, BC; Dalla Lana School of Public Health, University of Toronto, Toronto, ON; Human Brain Lab, Centre for Addiction and Mental Health, Toronto, ON, Canada

A.C. Campos Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

U.M. Camsari Department of Psychiatry and Psychology, Mayo Clinic Health System, and Mayo Clinic College of Medicine, Rochester, MN, United States

A. Canfield University of Toronto, Toronto, ON, Canada

E. Carra Department of Psychosis Studies, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom

F.-J. Carrillo-Salinas Neurobiology and Functional Systems Department, Cajal Institute, CSIC, Madrid, Spain

F. Cascini Public Health Institute, Department of Forensic Medicine, Università Cattolica del Sacro Cuore, Rome, Italy

M.P. Castelli Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, Cittadella Universitaria, Monserrato, CA, Italy

S.O. Cawich Department of Clinical Surgical Sciences, University of the West Indies, St. Augustine Campus, St Augustine, Trinidad and Tobago

E.E. Cawston Department of Pharmacology and Clinical Pharmacology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

C. Cedro Department of Biomedical, Dental Sciences and Morpho-functional Imaging, University of Messina, Messina, Italy

M.H.N. Chagas Department of Neurosciences and Behavior, Faculty of Medicine, Ribeirão Preto, University of São Paulo, Ribeirão Preto; Barretos School of Health Sciences, Dr. Paulo Prata, Barretos, São Paulo, Brazil

C. Chen Neuroscience Center of Excellence, School of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA, United States

C. Chisari University of York, York, United Kingdom

H. Chtioui Department of Clinical Pharmacology and Toxicology, University Hospital of Lausanne, Lausanne, Switzerland

R.D. Cico Columbia University, New York, NY, United States

I.A. Ciechomska Laboratory of Molecular Neurobiology, Neurobiology Center, Nencki Institute of Experimental Biology, Warsaw, Poland

N.C. Coimbra Laboratory of Neuroanatomy & Neuropsychobiology, Department of Pharmacology, Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP); Neurobiology of Emotions Research Centre (NAP-USP-NuPNE), Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP), Ribeirão Preto, São Paulo, Brazil

J. Cole Queen Elizabeth Hospital, Charlottetown, PE, Canada

J. Cookey Department of Psychiatry, Dalhousie University, Halifax, NS, Canada

J. Copeland National Cannabis Prevention and Information Centre, University of New South Wales, Randwick, Sydney, NSW, Australia

Z.M. Coskun Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Istanbul Bilim University, Istanbul, Turkey

W.D. Crano Division of Behavioral and Organizational Sciences, School of Social Science, Policy and Evaluation, Claremont Graduate University, Claremont, CA, United States

J.A.S. Crippa Department of Neuroscience and Behavior, Faculty of Medicine, Ribeirão Preto, University of São Paulo, Ribeirao Preto, Brazil

C.E. Crocker Department of Psychiatry, Dalhousie University; Division of Neurology, Department of Medicine, Dalhousie University, Halifax, NS, Canada

M.J. Cuesta Department of Psychiatry, IdiSNA, Navarra Institute for Health Research, Pamplona, Spain

P.J. Cunha Department of Psychiatry, Faculty of Medicine, Laboratory of Psychiatric Neuroimaging (LIM-21), University of São Paulo; Center for Interdisciplinary Research on Applied Neurosciences (NAPNA), University of São Paulo, São Paulo, Brazil

L. Cutando Department of Experimental and Health Sciences, Laboratory of Neuropharmacology, School of Health and Life Sciences, Pompeu Fabra University, Barcelona, Spain

A.B.F. da Silva Institute of Chemistry of São Carlos, University of São Paulo, São Carlos, Sao Paulo, Brazil

J.A. da Silva Laboratory of Neuroanatomy & Neuropsychobiology, Department of Pharmacology, Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP), Ribeirão Preto, São Paulo, Brazil

V.K. da Silva Neurobiology and Developmental Biology Laboratory, Faculty of Biosciences, Pontifical Catholic University, Porto Alegre, Rio Grande do Sul, Brazil

D. Dan Department of Clinical Surgical Sciences, University of the West Indies, St. Augustine Campus, St Augustine, Trinidad and Tobago

R.B. De Boni INI Evandro Chagas, FIOCRUZ, Rio de Janeiro, Rio de Janeiro, Brazil

F. Rodríguez de Fonseca Department of Psychobiology, Faculty of Psychology, Complutense University of Madrid, Pozuelo de Alarcón, Madrid, Spain

R. Gómez de Heras Department of Psychobiology, Faculty of Psychology, Complutense University of Madrid, Pozuelo de Alarcón, Madrid, Spain

A.C.P. de Oliveira Department of Pharmacology, ICB, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

A.C. de Souza Crippa Health Sciences Sector, Federal University of Paraná, Curitiba, Parana, Brazil

J.A. de Souza Crippa Department of Neuroscience and Behavior, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Sao Paulo, Brazil

F. Degenhardt Laboratory of Technical Biochemistry, Department of Biochemical and Chemical Engineering, TU Dortmund University, Dortmund, Germany

L. Degenhardt National Drug and Alcohol Research Centre, University of New South Wales, Sydney, NSW, Australia

S. Deiana CNS Diseases Research Department, Boehringer Ingelheim Pharma GmbH & Co. KG, Birkendorfer straße, Biberach an der Riss, Germany

U. Deonarine Department of Clinical Surgical Sciences, University of the West Indies, St. Augustine Campus, St Augustine, Trinidad and Tobago

M. Di Forti Department of Psychosis Studies, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom

T. dos Anjos-Garcia Laboratory of Neuroanatomy & Neuropsychobiology, Department of Pharmacology, Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP), Ribeirão Preto, São Paulo, Brazil

R. Guimarães dos Santos Department of Neuroscience and Behavior, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Sao Paulo, Brazil

M. Drozd Department of Pharmaceutics, Medical University of Lublin, Lublin, Poland

F.L.S. Duran Department of Psychiatry, Faculty of Medicine, Laboratory of Psychiatric Neuroimaging (LIM-21), University of São Paulo; Center for Interdisciplinary Research on Applied Neurosciences (NAPNA), University of São Paulo, São Paulo, Brazil

M. Earleywine Department of Psychology, School of Arts and Sciences, University at Albany, State University of New York, Albany, NY, United States

D.G. Ebo Faculty of Medicine and Health Science, Department of Immunology-Allergology-Rheumatology, University of Antwerp, Antwerp, Belgium

N. Egashira Department of Pharmacy, Kyushu University Hospital; Department of Neuropharmacology, Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka, Japan

J. Egnatios School of Medicine, University of California San Diego, La Jolla, CA, United States

A. Ellert-Miklaszewska Laboratory of Molecular Neurobiology, Neurobiology Center, Nencki Institute of Experimental Biology, Warsaw, Poland

S.A. ElShebiney Department of Toxicology and Narcotics, National Research Centre, Cairo, Egypt

M.A. ElSohly ElSohly Laboratories Inc., Oxford, MS, United States

C. Evren Research, Treatment and Training Center for Alcohol and Substance Dependence (AMATEM), Bakirkoy Training and Research Hospital for Psychiatry, Neurology and Neurosurgery, Istanbul, Turkey

L. Fañanás Faculty of Biology, Anthropology Unit, Department of Animal Biology, University of Barcelona, Biomedicine Institute of the University of Barcelona (IBUB), Barcelona; CIBER of Mental Health (CIBERSAM), Madrid, Spain

M.M. Faber Faculty of Medicine and Health Science, Department of Immunology-Allergology-Rheumatology, University of Antwerp, Antwerp, Belgium

S. Farag Technical University Dortmund, Technical Biochemistry Dortmund, Dortmund, Germany

A. Farré Dual Disorder Unit, Addiction Program, Institute of Neuropsychiatry and Addiction—INAD, and Hospital del Mar Medical Research Institute—IMIM, Barcelona, Spain

M. Farré Clinical Pharmacology Unit, Germans Trias i Pujol University Hospital—IGTP, and Human Pharmacology Unit, Hospital del Mar Medical Research Institute—IMIM, and Autonomous University of Barcelona, Barcelona, Spain

M. Fatjó-Vilas Faculty of Biology, Anthropology Unit, Department of Animal Biology, University of Barcelona, Biomedicine Institute of the University of Barcelona (IBUB), Barcelona; CIBER of Mental Health (CIBERSAM), Madrid, Spain

B. Favrat Traffic Medicine and Psychology Unit, University Center of Legal Medicine, University Hospital of Geneva, Geneva; Department of Ambulatory Care and Community Medicine, University Hospital of Lausanne, Lausanne, Switzerland

D. Feingold Department of Psychiatry, Sheba Medical Center, Tel Hashomer; Ariel University, Ariel, Israel

A. Feliú Neurobiology and Functional Systems Department, Cajal Institute, CSIC, Madrid, Spain

A.A. Fernández Forensic Laboratory Institute of Legal Medicine of Catalonia, Barcelona, Spain

S. Fernández-Artamendi Addictive Behaviors Research Group, Department of Psychology, University of Oviedo, Oviedo, Spain

A.J. Ferrari School of Public Health, University of Queensland, Herston; Institute for Health Metrics and Evaluation, University of Washington, Seattle, WA, United States; Queensland Centre for Mental Health Research, Wacol, QLD, Australia

L. Ferraro Biomedical Department of Internal and Specialist Medicine; Department of Experimental Biomedicine and Clinical Neuroscience, School of Medicine, University of Palermo, Palermo, Italy

J. Fichna Department of Biochemistry, Faculty of Medicine, Medical University of Lodz, Lodz, Poland

D.B. Finlay Department of Pharmacology and Clinical Pharmacology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

J. Fiz EDIR, Center for Diagnosis and Rehabilitation, San Martín Santa Fe, Argentina

Á. Flores Department of Experimental and Health Sciences, Laboratory of Neuropharmacology, School of Health and Life Sciences, Pompeu Fabra University, Barcelona, Spain

J.S. Fogel Department of Psychology, University of Kentucky College of Arts and Sciences, Lexington, KY, United States

E. Fornari CIBM, University Hospital of Lausanne, Lausanne, Switzerland

L. Fortunato Institute of Clinical Physiology, The Italian National Research Council (IFC-CNR), Pisa, Italy

T. Fyfe Northern Medical Program, University of Northern British Columbia, Prince George, BC, Canada

A.E.D.M. Gaafar Department of Photochemistry, Chemical Industries Division, National Research Centre, Cairo, Egypt

S. Gade Palo Alto University, Palo Alto, CA, United States

E. Gaffal Laboratory of Experimental Dermatology, Department of Dermatology and Allergy, University Hospital of the Friedrich-Wilhelm-University Bonn, Bonn, Germany

A.F. Galal Department of Toxicology and Narcotics, National Research Centre, Cairo, Egypt

R. Gandhi Academic Department of Diabetes and Endocrinology, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom

P. Gates National Cannabis Prevention and Information Centre, University of New South Wales, Randwick, Sydney, NSW, Australia

J.M. Gatley Northern Medical Program, University of Northern British Columbia, Prince George, BC; Dalla Lana School of Public Health, University of Toronto, Toronto, ON; Human Brain Lab, Centre for Addiction and Mental Health, Toronto, ON, Canada

C. Giroud Forensic Toxicology and Chemistry Unit, University Center of Legal Medicine, University Hospital of Lausanne, Lausanne, Switzerland

M. Glass Department of Pharmacology and Clinical Pharmacology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

S.R. Goldberg Preclinical Pharmacology Section, Behavioral Neuroscience Branch, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD, United States

I. González-Ortega Department of Psychiatry, University Hospital of Alava-Santiago, CIBERSAM; University of the Basque Country; National Distance Education University (UNED)-Centro Asociado de Vitoria, Vitoria, Spain

A. González-Pinto Department of Psychiatry, University Hospital of Alava-Santiago, CIBERSAM; University of the Basque Country, Vitoria, Spain

C. Guaza Neurobiology and Functional Systems Department, Cajal Institute, CSIC, Madrid, Spain

V. Guillon Service des enquêtes et des sondages, Paris, France

F.S. Guimarães Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

W. Gul ElSohly Laboratories Inc., Oxford, MS, United States

F.M. Guven Turkish Association for Cognitive and Behavioural Therapies, Istanbul; Department of Psychiatry, Alcohol & Substance Use Disorders Treatment Center, Lara Anatolia Hospital, Antalya, Turkey

W.D. Hall Centre for Youth Substance Abuse Research, University of Queensland, Herston, QLD, Australia

J.E.C. Hallak Department of Neuroscience and Behavior, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Sao Paulo, Brazil

M. Hamerle Department of Psychiatry and Psychotherapy, Ludwig-Maximilian-University Hospital, Munich, Germany

M. Haney Division on Substance Abuse, New York State Psychiatric Institute and Department of Psychiatry, College of Physicians and Surgeons of Columbia University, New York, NY, United States

H.E. Harding Department of Surgery, University of the West Indies, Mona Campus, Kingston, Jamaica

S. Hassan Dalhousie University, Dartmouth, NS, Canada

K. Haugland National Criminal Investigation Service (NCIS) Norway, Forensic Science Department, Oslo, Norway

A. Healey School of Psychology, The University of Newcastle, University Drive, Callaghan, NSW, Australia

C. Heck City and County of Denver, Crime Prevention and Control Commission, Denver, CO, United States

A. Helander Department of Laboratory Medicine, Karolinska Institutet, and Karolinska University Laboratory, Stockholm, Sweden

L. Hernandez-Folgado Institute of Medical Chemistry, CSIC, Madrid, Spain

D.A. Herzig Clienia AG, Littenheid, Switzerland

M. Hesse Center for Alcohol and Drug Research, Aarhus University, Copenhagen Department, Copenhagen, Denmark

M.G. Hill The University of Arizona, Tucson, AZ, United States

R. Hirst Palo Alto University, Palo Alto, CA, United States

C.R. Hjorthøj Mental Health Center Copenhagen, Copenhagen University Hospital, Copenhagen, Denmark

E. Hoch Department of Psychiatry, Ludwig Maximilian University, Munich, Germany

M.D. Holder Psychology Department, IKBSAS, University of British Columbia, Kelowna, BC, Canada

M. Holtkamp Epilepsy-Center Berlin-Brandenburg, Department of Neurology, Charité – Universitätsmedizin Berlin, Berlin, Germany

M.R. Hunter Department of Pharmacology and Clinical Pharmacology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

E. Ikeda Department of Molecular Biology, Daiichi University of Pharmacy, Fukuoka, Japan

Y. Izumi Department of Psychiatry; The Taylor Family Institute for Innovative Psychiatric Research, Washington University School of Medicine, St. Louis, MO, United States

T. Janus Department of Clinical and Forensic Toxicology, Pomeranian Medical University, Szczecin, Poland

B. Kaminska Laboratory of Molecular Neurobiology, Neurobiology Center, Nencki Institute of Experimental Biology, Warsaw, Poland

A.S. Kanaan Clinic of Psychiatry, Social-Psychiatry and Psychotherapy, Hannover Medical School, Hannover; Nuclear Magnetic Resonance Unit, Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany

R. Karinen Norwegian Institute of Public Health, Division of Forensic Sciences, Oslo, Norway

T. Karl Neuroscience Research Australia, Randwick; School of Medicine, Western Sydney University, Campbelltown, NSW, Australia

T. Katsu Department of Pharmacy, Faculty of Pharmacy, Yasuda Women's University, Hiroshima, Japan

F. Kay-Lambkin NHMRC Centre for Research Excellence in Mental Health and Substance Use, National Drug and Alcohol Research Centre, University of New South Wales, Randwick, NSW, Australia

O. Kayser Technical University Dortmund, Technical Biochemistry Dortmund, Dortmund, Germany

M. Kells Division of Adolescent/Young Adult Medicine, Boston Children's Hospital, Boston, MA, United States

B.C. Kelly Department of Sociology, Purdue University, West Lafayette, IN, United States

T.H. Kelly Departments of Behavioral Science and Psychiatry, University of Kentucky College of Medicine, and Department of Psychology, University of Kentucky College of Arts and Sciences, Lexington, KY, United States

A. Kokona Department of Medical and Surgical Sciences, Bologna University, Bologna, Italy

A. Kumar Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY, United States

P. Kumar Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY, United States

D. La Barbera Department of Experimental Biomedicine and Clinical Neuroscience, School of Medicine, University of Palermo, Palermo, Italy

T.V. Lagerberg NORMENT KG Jebsen Centre for Psychosis Research, Institute of Clinical Medicine, University of Oslo; Division of Mental Health and Addiction, Oslo University Hospital, Oslo, Norway

A. Lahat Department of Gastroenterology, Chaim Sheba Medical Center, Tel-Hashomer, Israel

H.J. Larsen Mental Health Center Copenhagen, Copenhagen University Hospital, Copenhagen, Denmark

A.S. Laun Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY, United States

T. Lecomte Research Centre of the University of Montreal Institute for Mental Health; Department of Psychology, University of Montreal, Montreal, QC, Canada

S. Legleye Institut national d'études démographiques (INED), Paris; University of Paris-Saclay, Univ. Paris-Sud, UVSQ, CESP, Inserm, Versailles, France

S. Lev-Ran Department of Psychiatry, Sheba Medical Center, Tel Hashomer; Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

J.A. Lile Departments of Behavioral Science and Psychiatry, University of Kentucky College of Medicine, and Department of Psychology, University of Kentucky College of Arts and Sciences, Lexington, KY, United States

R.P. Limberger LABTOXICO: Laboratory of Toxicology, Faculty of Pharmacy, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

I.M.P. Linares Department of Neuroscience and Behavior, Faculty of Medicine, Ribeirão Preto, University of São Paulo, Ribeirao Preto, Brazil

K.M. Lisdahl Department of Psychology, University of Wisconsin-Milwaukee, Milwaukee, WI, United States

M. Little Center for Population Sciences, Department of Preventive Medicine, University of Tennessee Health Science Center, Memphis, TN, United States

W. Liu NORC at the University of Chicago, Bethesda, MD, United States

M.J. Loflin Department of Psychology, School of Arts and Sciences, University at Albany, State University of New York, Albany, NY, United States

R. Lorente-Omeñaca Department of Psychiatry, IdiSNA, Navarra Institute for Health Research, Pamplona, Spain

V. Lorenzetti Melbourne Neuropsychiatry Centre, The University of Melbourne and Melbourne Health, Melbourne; Brain & Mental Health Laboratory, Monash Institute of Cognitive and Clinical Neurosciences, School of Psychological Sciences, Monash University, Clayton, VIC, Australia

D. Lu Department of Pharmaceutical Sciences, Rangel College of Pharmacy, Texas A&M University, Kingsville, TX, United States

J. Mørland Department of Drug Abuse Research, Division of Forensic Sciences, Norwegian Institute of Public Health, Oslo, Norway

K.R. Müller-Vahl Clinic of Psychiatry, Social-Psychiatry and Psychotherapy, Hannover Medical School, Hannover, Germany

A. Machoy-Mokrzyńska Department of Pharmacology, Pomeranian Medical University, Szczecin, Poland

P. Maeder Department of Radiology, University Hospital of Lausanne, Lausanne, Switzerland

S. Majumdar Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, Oxford, MS, United States

R. Maldonado Department of Experimental and Health Sciences, Laboratory of Neuropharmacology, School of Health and Life Sciences, Pompeu Fabra University, Barcelona, Spain

K.E. Maple Department of Psychology, University of Wisconsin-Milwaukee, Milwaukee, WI, United States

M. Martínez-Cengotitabengoa Department of Psychiatry, University Hospital of Alava-Santiago, CIBERSAM; National Distance Education University (UNED)-Centro Asociado de Vitoria, Vitoria, Spain

M. Isabel Martín-Fontelles Area of Pharmacology and Nutrition, Faculty of Health Sciences, University Rey Juan Carlos, Alcorcón; Associated Unit I+D+i of the Institute of Medicinal Chemistry (IQM) and of the Institute of Research in Food Sciences (CIAL), Spanish National Research Council (CSIC), Madrid, Spain

R. Martín-Santos Department of Psychiatry and Psychology, Hospital Clinic, IDIBAPS, University of Barcelona, CIBERSAM, Barcelona, Spain

K. Masuda Department of Physical Chemistry, Graduate School of Clinical Pharmacy, Shujitsu University, Okayama, Japan

A.L. McRae-Clark Medical University of South Carolina, Charleston, SC, United States

M. Mecha Neurobiology and Functional Systems Department, Cajal Institute, CSIC, Madrid, Spain

I. Melle NORMENT, KG Jebsen Centre for Psychosis Research, Division of Mental Health and Addiction, Oslo University Hospital & Institute of Clinical Medicine, University of Oslo, Oslo, Norway

S. Menahem Paediatric Cardiology Unit, Monash Health, Melbourne, Melbourne; Faculty of Medicine, Nursing and Health Sciences, Monash University, Clayton, VIC, Australia

J. Mendes-Gomes Laboratory of Neuroanatomy & Neuropsychobiology, Department of Pharmacology, Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP), Ribeirão Preto, São Paulo, Brazil

B. Mesías Instituto de Adicciones, Madrid, Spain

S. Miller Division of Behavioral and Organizational Sciences, School of Social Science, Policy and Evaluation, Claremont Graduate University, Claremont, CA, United States

R. Mizrahi Centre for Addiction and Mental Health (CAMH), Research Imaging Centre, Toronto, ON, Canada

S. Molinaro Institute of Clinical Physiology, The Italian National Research Council (IFC-CNR), Pisa, Italy

C. Moore Toxicology Research and Development, Immunalysis Corporation, Pomona, CA, United States

M.F. Moraes Department of Physiology and Biochemistry, ICB, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

F.A. Moreira Department of Pharmacology, ICB, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

L. Moreno-Izco Department of Psychiatry, IdiSNA, Navarra Institute for Health Research, Pamplona, Spain

H.A. Morris Department of Criminal Justice and Criminology, University of North Carolina at Charlotte, Charlotte, NC, United States

E. Muñoz Department of Cell Biology, Physiology and Immunology, University of Córdoba, Córdoba, Spain

G.G. Muccioli Bioanalysis and Pharmacology of Bioactive Lipids Research Group, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium

M.R.A. Muscatello Department of Biomedical, Dental Sciences and Morpho-functional Imaging, University of Messina, Messina, Italy

S.A. Nada Department of Pharmacology, National Research Centre, Dokki, Greater Cairo, Egypt

V. Naraynsingh Department of Clinical Surgical Sciences, University of the West Indies, St. Augustine Campus, St Augustine, Trinidad and Tobago

S. Narimatsu Department of Health Chemistry, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan

G. Nogueira-Filho School of Health Sciences, University Salvador, Laureate International Universities, Salvador, Bahia, Brazil

M. Nordentoft Mental Health Center Copenhagen, Copenhagen University Hospital, Copenhagen, Denmark

G. Oguz Turkish Association for Cognitive and Behavioural Therapies, Istanbul; Department of Psychology, Canik Basari University, Samsun, Turkey

Å.M.L. Øiestad Norwegian Institute of Public Health, Division of Forensic Sciences, Oslo, Norway

E.L. Øiestad Norwegian Institute of Public Health, Division of Forensic Sciences, Oslo, Norway

H. Okazaki Drug Innovation Research Center, Daiichi University of Pharmacy, Fukuoka, Japan

M.F. Olive Department of Psychology, Arizona State University, Tempe, AZ, United States

L. Orio Department of Psychobiology, Faculty of Psychology, Complutense University of Madrid, Pozuelo de Alarcón, Madrid, Spain

A. Ozaita Department of Experimental and Health Sciences, Laboratory of Neuropharmacology, School of Health and Life Sciences, Pompeu Fabra University, Barcelona, Spain

A. Pérez Evaluation and Intervention Methods Service, Public Health Agency, Barcelona, Spain

G. Panagis University of Crete, Department of Psychology, Laboratory of Behavioral Neuroscience, Rethymnon, Crete, Greece

G. Pandolfo Department of Biomedical, Dental Sciences and Morpho-functional Imaging, University of Messina, Messina, Italy

L.V. Panlilio Preclinical Pharmacology Section, Behavioral Neuroscience Branch, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD, United States

K. Paquin Research Centre of the University of Montreal Institute for Mental Health; Department of Psychology, University of Montreal, Montreal, QC, Canada

P. Parakh Department of Psychiatry, Ruby General Hospital, Kolkata, India

L.A. Parker Department of Psychology and Collaborative Neuroscience Program, University of Guelph, Guelph, ON, Canada

V.B. Patel University of Westminster, School of Life Sciences, Department of Biomedical Science, London, United Kingdom

M. Pawson Department of Sociology, The Graduate Center, City University of New York, New York, NY, United States

F.F. Peres Department of Pharmacology, Federal University of Sao Paulo; Integrated Laboratory of Clinical Neurosciences (LiNC), Federal University of Sao Paulo, Sao Paulo, Brazil

H. Petras American Institutes for Research, Washington, DC, United States

F. Pollastro Department of Pharmaceutical Science, University of Piemonte Orientale, Novara, Italy

A. Porcu Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, Cittadella Universitaria, Monserrato, CA, Italy

R. Potente Institute of Clinical Physiology, The Italian National Research Council (IFC-CNR), Pisa, Italy

D.E. Potter Department of Pharmaceutical Sciences, Rangel College of Pharmacy, Texas A&M University, Kingsville, TX, United States

S. Potvin Research Centre of the University of Montreal Institute for Mental Health; Department of Psychiatry, Faculty of Medicine, University of Montreal, Montreal, QC, Canada

C. Prats Faculty of Biology, Anthropology Unit, Department of Animal Biology, University of Barcelona, Biomedicine Institute of the University of Barcelona (IBUB), Barcelona; CIBER of Mental Health (CIBERSAM), Madrid, Spain

V.R. Preedy Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom

R. Rajendram Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom

L. Rathke Palo Alto University, Palo Alto, CA, United States

K.L. Reed The University of Arizona, Tucson, AZ, United States

M.A. Repka Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, Oxford, MS, United States

H. Rigter Youth Interventions Foundation, Curium, Department of Child and Adolescent Psychiatry, Leiden University Medical Center, Leiden, The Netherlands

E.M. Rock Department of Psychology and Collaborative Neuroscience Program, University of Guelph, Guelph, ON, Canada

H. Rohrbacher Practice for Cognitive Behaviour Therapy, Munich, Germany

P.G.P. Rosa Department of Psychiatry, Faculty of Medicine, Laboratory of Psychiatric Neuroimaging (LIM-21), University of São Paulo; Center for Interdisciplinary Research on Applied Neurosciences (NAPNA), University of São Paulo, São Paulo, Brazil

F. Sánchez-Martínez Evaluation and Intervention Methods Service, Public Health Agency, Barcelona, Spain

A.M. Sánchez-Torres Department of Psychiatry, IdiSNA, Navarra Institute for Health Research, Pamplona, Spain

M. Sałaga Department of Biochemistry, Faculty of Medicine, Medical University of Lodz, Lodz, Poland

V. Sabato Faculty of Medicine and Health Science, Department of Immunology-Allergology-Rheumatology, University of Antwerp, Antwerp, Belgium

A.N. Sanders Department of Criminal Justice and Criminology, University of North Carolina at Charlotte, Charlotte, NC, United States

L.C. Santos Department of Psychiatry, Faculty of Medicine, Laboratory of Psychiatric Neuroimaging (LIM-21), University of São Paulo; Center for Interdisciplinary Research on Applied Neurosciences (NAPNA), University of São Paulo, São Paulo, Brazil

M. Scalese Institute of Clinical Physiology, The Italian National Research Council (IFC-CNR), Pisa, Italy

M.S. Schaufelberger Department of Psychiatry, Faculty of Medicine, Laboratory of Psychiatric Neuroimaging (LIM-21), University of São Paulo; Center for Interdisciplinary Research on Applied Neurosciences (NAPNA), University of São Paulo, São Paulo; Department of Neuroscience and Behavior, Faculty of Medicine, Ribeirão Preto, University of São Paulo, Ribeirao Preto, Brazil

N. Schröder Neurobiology and Developmental Biology Laboratory, Faculty of Biosciences, Pontifical Catholic University, Porto Alegre, Rio Grande do Sul, Brazil

G. Scimeca Department of Biomedical, Dental Sciences and Morpho-functional Imaging, University of Messina, Messina, Italy

R. Secades-Villa Addictive Behaviors Research Group, Department of Psychology, University of Oviedo, Oviedo, Spain

D. Selvarajah Department of Human Metabolism, Medical School, University of Sheffield, Sheffield, United Kingdom

O. Senormanci Department of Psychiatry, Bülent Ecevit University School of Medicine, Zonguldak, Turkey

K. Shivakumar Health Sciences North, Department of Psychiatry, and Northern Ontario School of Medicine, Sudbury, ON, Canada

L.A. Shrier Division of Adolescent/Young Adult Medicine, Boston Children's Hospital, and Department of Pediatrics, Harvard Medical School, Boston, MA, United States

V. Siciliano Institute of Clinical Physiology, The Italian National Research Council (IFC-CNR), Pisa, Italy

L. Sideli Department of Experimental Biomedicine and Clinical Neuroscience, School of Medicine, University of Palermo, Palermo, Italy

J.T. Siegel Division of Behavioral and Organizational Sciences, School of Social Science, Policy and Evaluation, Claremont Graduate University, Claremont, CA, United States

A.A. Sleem Department of Pharmacology, National Research Centre, Dokki, Greater Cairo, Egypt

J. Sobczyński Department of Pharmaceutics, Medical University of Lublin, Lublin, Poland

L. Sodos Palo Alto University, Palo Alto, CA, United States

N. Solowij School of Psychology, University of Wollongong, Wollongong, NSW, Australia

Z.-H. Song Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY, United States

A.W. Stacy School of Community and Global Health, Claremont Graduate University, Claremont, CA, United States

F. Stehle Laboratory of Technical Biochemistry, Department of Biochemical and Chemical Engineering, TU Dortmund University, Dortmund, Germany

J.M. Stogner Department of Criminal Justice and Criminology, University of North Carolina at Charlotte, Charlotte, NC, United States

S. Sussman Departments of Preventive Medicine and Psychology, and School of Social Work, Institute for Health Promotion and Disease Prevention Research, University of Southern California, Los Angeles, CA, United States

W. Swift NHMRC Centre for Research Excellence in Mental Health and Substance Use, National Drug and Alcohol Research Centre, University of New South Wales, Randwick, NSW, Australia

N. Szerman Gregorio Marañon Hospital, Madrid, Spain

T. Tüting Laboratory of Experimental Dermatology, Department of Dermatology and Allergy, University Hospital of the Friedrich-Wilhelm-University Bonn, Bonn, Germany

M. Aghazadeh Tabrizi Department of Chemistry and Pharmaceutical Science, University of Ferrara, Ferrara, Italy

O. Taglialatela-Scafati Department of Pharmacy, University of Napoli Federico II, Napoli, Italy

R.N. Takahashi Department of Pharmacology, CCB, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil

S. Takeda Laboratory of Xenobiotic Metabolism and Environmental Toxicology, Faculty of Pharmaceutical Sciences, Hiroshima International University (HIU), Kure, Hiroshima, Japan

I. Tarricone Department of Medical and Surgical Sciences, Bologna University; Department of Mental Health, Bologna, Italy

D.P. Tashkin Division of Pulmonary and Critical Care, Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, United States

T. Tellioğlu Brown University, Alpert Medical School, Substance Abuse Division, Rhode Island Hospital, Providence, RI, United States

Z. Tellioğlu Brown University, Alpert Medical School, Rhode Island Hospital, Providence, RI, United States

S. Tesfaye Academic Department of Diabetes and Endocrinology, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom

L. Thornton NHMRC Centre for Research Excellence in Mental Health and Substance Use, National Drug and Alcohol Research Centre, University of New South Wales, Randwick, NSW, Australia

B. Thylstrup Center for Alcohol and Drug Research, Aarhus University, Copenhagen Department, Copenhagen, Denmark

P.G. Tibbo Department of Psychiatry, Dalhousie University, Halifax, NS, Canada

G. Todd School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA, Australia

M. Torrens Addiction Program, Institute of Neuropsychiatry and Addiction—INAD, and Hospital del Mar Medical Research Institute—IMIM, Barcelona, Spain

J. Tsai Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, United States

H.-H. Tseng Centre for Addiction and Mental Health (CAMH), Research Imaging Centre, Toronto, ON, Canada; Department of Psychosis Studies, Institute of Psychiatry, King's College London, London, United Kingdom

A. Turner Priority Research Centre for Translational Neuroscience and Mental Health, University of Newcastle, Callaghan, NSW, Australia

S.S. Tuv Department of Drug Abuse Research, Division of Forensic Sciences, Norwegian Institute of Public Health, Oslo, Norway

F. Ullah Laboratory of Neuroanatomy & Neuropsychobiology, Department of Pharmacology, Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP), Ribeirão Preto, São Paulo, Brazil

T. Van der Linden Department Drugs and Toxicology, National Institute of Criminalistics, Brussels, Belgium

A.L. Van Gasse Faculty of Medicine and Health Science, Department of Immunology-Allergology-Rheumatology, University of Antwerp, Antwerp, Belgium

P. Vega Instituto de Adicciones, Madrid, Spain

G. Vera Area of Pharmacology and Nutrition, Faculty of Health Sciences, University Rey Juan Carlos, Alcorcón; Associated Unit I+D+i of the Institute of Medicinal Chemistry (IQM) and of the Institute of Research in Food Sciences (CIAL), Spanish National Research Council (CSIC), Madrid, Spain

M. Verdichevski Northern Medical Program, University of Northern British Columbia, Prince George, BC, Canada

T.R. Vieira Sousa Center for Drug and Alcohol Research, Hospital de Clinicas de Porto Alegre, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

L.R. Vilela Department of Pharmacology, ICB, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

V. Vindenes Department of Drug Abuse Research, Division of Forensic Sciences, Norwegian Institute of Public Health, Oslo, Norway

Z. Walsh Psychology Department, IKBSAS, University of British Columbia, Kelowna, BC, Canada

K. Watanabe Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa; Pharmaceutical Education Support Center, Daiichi University of Pharmacy, Fukuoka, Japan

L.R. Watterson Department of Psychology, Arizona State University, Tempe, AZ, United States

J.M. White School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA, Australia

N.E. Wright Department of Psychology, University of Wisconsin-Milwaukee, Milwaukee, WI, United States

M. Yücel Melbourne Neuropsychiatry Centre, The University of Melbourne and Melbourne Health, Melbourne; Brain & Mental Health Laboratory, Monash Institute of Cognitive and Clinical Neurosciences, School of Psychological Sciences, Monash University, Clayton, VIC, Australia

I. Yamamoto Hokuriku University, Kanazawa, Japan

S. Yamaori Department of Pharmacy, Shinshu University Hospital, Matsumoto, Japan

A. Zalesky Melbourne Neuropsychiatry Centre, The University of Melbourne and Melbourne Health, Melbourne, VIC, Australia

D. Zalman Division of Oncology, Rambam Health Care Campus and Faculty of Medicine, Technion—Israel Institute of Technology, Haifa, Israel

J. Zhang Neuroscience Center of Excellence, School of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA, United States

Y. Zhang Department of Medicinal Chemistry, School of Pharmacy and Institute for Structural Biology and Drug Discovery, Virginia Commonwealth University, Richmond, VA, United States

R. Zoccali Department of Biomedical, Dental Sciences and Morpho-functional Imaging, University of Messina, Messina, Italy

C.F. Zorumski Department of Psychiatry; The Taylor Family Institute for Innovative Psychiatric Research, Washington University School of Medicine, St. Louis, MO, United States

A.W. Zuardi Department of Neuroscience and Behavior, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Sao Paulo, Brazil

Page left intentionally blank

Preface

Cannabis is probably one of the most commonly used drugs of misuse. It has a wide range of adverse effects including impairing learning and memory. At the same time, the medical use of cannabis has been advocated due to its ability to relieve pain as an example. Understanding the nature of the pleasure-seeking, disinhibition, and other effects have also paved the way for the specialized field of cannabis pharmacology. This includes elucidating the nature of cannabinoid receptors, which has led to the development of synthetic cannabinoid agonists. However, the aforementioned is a rather simplistic synopsis. The long-term use of cannabis may also increase the risk of schizophrenia, paranoia, and other psychoses. Its use can affect cells, organs, individuals, families, subcultures, groups, and communities. This is because the interrelationships between cannabis and individual components, diagnosis, screening, social and community effects, psychopathology, neuropathology, non-CNS effects, polydrug use, medicinal applications, treatments, and pharmacology are complex. Furthermore, although the active agents in cannabis are known, the individual steps between exposure by ingestion or inhalation and effects on cells and the body are multifactorial, and cut across many scientific disciplines. It is thus important to learn from these interrelationships to embrace a multidisciplinary approach to understand all the threads and ramifications of cannabis use, misuse, and applications. For example, some cellular mechanisms elucidated by studying one anatomical CNS component may also be relevant to other areas of the CNS, or other fields of cannabis toxicity and pharmacology. Another example relates to the impact of cannabis on social dysfunction, which may also be relevant to other psychosocial scenarios, or useful in devising new treatment strategies. An additional example relates to preclinical studies which may be relevant to understanding clinical pathologies, psychomorbidities, or therapeutic drugs. Unraveling these complex relationships is difficult, as there is a wide myriad of material related to cannabis. In simple terms, the material on cannabis use and misuse has hitherto been either scattered, diffused, or crosses different disciplines. These limitations are, however, addressed in *The Handbook of Cannabis and Related Pathologies: Biology, Pharmacology, Diagnosis, and Treatment* which embraces all aspects of cannabis in a one-stop-shop approach. Where appropriate, positive aspects of cannabis and related metabolites are described.

The book is divided into eight major parts as follows:

1. Setting the Scene, Botanical, General, and International Aspects
2. Personal, Social, and Community Aspects of Cannabis Use
3. Cannabis, Behavior, Psychopathology, and Neuropathology
4. Cannabis, Organs, Tissues, and non-CNS Aspects
5. Pharmacology and Cellular Activities of Cannabinoids and Endocannabinoids
6. Effects of Specific Natural and Synthetic Cannabinoids
7. Medicinal Cannabis Use
8. Screening, Diagnosis, and Treatments

The Editor recognizes the difficulties in ascribing chapters to particular sections, and even their location within separate sections. This is because some chapters can be categorized in many ways. However, this issue is resolved with the excellent indexing carried out by Elsevier.

The Handbook of Cannabis and Related Pathologies: Biology, Pharmacology, Diagnosis, and Treatment transcends both multiple disciplinary and intellectual divides, as each chapter has:

- *Key Facts*
- *Mini-Dictionary*
- *Summary Points*

Finally, there is a chapter on Resources and Recommended Reading, suggested by some of the book's contributors.

The Handbook of Cannabis and Related Pathologies: Biology, Pharmacology, Diagnosis, and Treatment has been designed for those working in the field of cannabis and cannabinoids, drug abuse workers, neurologists, specialists in addictive behaviors, health scientists, public health and community workers, doctors,

pharmacologists, research scientists, and other specialists. The book is valuable as a personal reference book, and also for academic libraries that cover the domains of health sciences or addictions. Contributions are from leading national and international experts, including those from world renowned institutions. It is suitable for undergraduates, postgraduates, lecturers, and academic professors.

Professor V.R. Preedy, BSc, PhD, DSc, FRSB, FRSH, FRIPHH, FRSPH, FRCPath, FRSC
King's College London, London, United Kingdom

PART I

SETTING THE SCENE, BOTANICAL, GENERAL AND INTERNATIONAL ASPECTS

1	*The cannabis plant: Botanical aspects*	*3*
2	*The biosynthesis of cannabinoids*	*13*
3	*Increasing plant concentrations of THC and implications on health related disorders*	*24*
4	*Age as a predictor of cannabis use*	*33*
5	*Lifetime cannabis use and cognition in psychosis spectrum disorders*	*44*
6	*A profile of synthetic cannabinoid users*	*53*
7	*Dual disorders in cannabis misuse*	*61*
8	*Cannabis use and cognitive function*	*70*
9	*Cannabis, migration, and psychosis onset*	*79*
10	*The global epidemiology and disease burden of cannabis use and dependence*	*89*
11	*International aspects of cannabis use and misuse: the australian perspective*	*101*
12	*International aspects of cannabis use and misuse: Egypt*	*110*
13	*Cannabis body packing: A caribbean perspective*	*122*

Page left intentionally blank

CHAPTER

1

The *Cannabis* Plant: Botanical Aspects

S. Farag, O. Kayser

Technical University Dortmund, Technical Biochemistry Dortmund, Dortmund, Germany

SUMMARY POINTS

- This chapter focuses on the botanical aspects of *Cannabis*.
- *Cannabis* trichomes can come in glandular and nonglandular shapes, including oil resin.
- Resin glands are the main producer of cannabinoids.
- Recently, hybrid cannabis strains have been developed.
- Modern hydroponic techniques, coupled with selective artificial lighting, are used in order to solve the issue of low-potency cannabis.
- However, we argue that it is necessary to apply transgenic *Cannabis* plants to facilitate the metabolic pathway for cannabinoid production or agronomic traits.

KEY FACTS

- Most popular varieties of *Cannabis* are a combination of two or three of *C. sativa*, *C. indica* or *C. ruderalis.*
- *Cannabis* cultivated for fiber or oil, or narcotics production.
- Cannabinoids are the main active ingredient.
- Cultivation and breeding of narcotic strains is not permitted in most countries.
- Only female plants are economically important for producing resin in narcotic strains.
- Indoor horticultural lighting is a new system to mimic sunlight.
- Indoor hydroponic technology is used for maximizing cannabinoids.

LIST OF ABBREVIATIONS

CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBN	Cannabinol
GPP	Geranylpyrophosphate
GRIN	Germplasm Resources Information Network
ISSR	Inter simple sequence repeat
NPGS	National Plant Germplasm System
RAPD	Random amplified polymorphic
RFLP	Restriction fragment length polymorphism
RH	Relative humidity
RFLP	Restriction fragment polymorphisms
THCA	Tetrahydrocannabinolic acid
THC	Δ^9-Tetrahydrocannabinol
USDA	United States Department of Agriculture

INTRODUCTION

Cannabis sativa L. (marijuana; Cannabaceae) is an annual dioeciously flowering plant. The first appearance of *Cannabis* was believed to be central Asia about 5000 BC. For millennia, the plant has also been used for fiber, oil production, and traditional uses. It contains a number of medicinally important compounds, such as, cannabinoids (Appendino, Chianese, & Taglialatela-Scafati, 2011), terpenoids (Ross & ElSohly, 1996), flavonoids (Vanhoenacker, Van Rompaey, De Keukeleire, & Sandra, 2002), alkaloids (Turner & Elsohly, 1976), and others (Brenneisen, 2007). Cannabinoids are a unique class of terpenophenolic compounds to *Cannabis* plants, accumulated mainly in the cavity of trichomes (Kim & Mahlberg, 1997). More than 80 cannabinoids have been isolated from *C. sativa* (Elsohly & Slade, 2005). The main psychoactive compound is Δ^9-tetrahydrocannabinol (THC), with well-known medicinal effects (Elbatsh,

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00001-6
Copyright © 2017 Elsevier Inc. All rights reserved.

Moklas, Marsden, & Kendall, 2012). At present, cultivation and breeding of *Cannabis* is prohibited in most countries, except by permission for purposes of research and pharmaceutical uses (ElSohly, 2002). *Cannabis* plants are usually propagated through the seed (sexual reproduction; outdoor cultivation) or by vegetative propagation, using stem cuttings (asexual reproduction; indoor cultivation) (Potter, 2004). However, both techniques have advantages and disadvantages. This chapter is dedicated to botanical aspects, including morphology, taxonomy, genetics, conservation, geographical distribution, and cultivation forms.

BOTANY OF *CANNABIS*

Macroscopical Features

Information was published elsewhere, giving detailed technical descriptions of *Cannabis* morphology (Clarke, 1981; UNODC, 2009) Fig. 1.1. However, this information has been simplified in the present text. *C. sativa* is an annual, dioeciously (ie, male and female flowers are found on separate plants), pollinated plant with strong taproot, erect stems. The stems are usually angular, furrowed, branched, with woody interior, sometimes hollow in the internodes, and vary from 1 to 6 m in height. The branching is either opposite or alternate. The roots are advantageous, with branched taproot, generally 30–60 cm deep, up to 2.5 m in loose soils, very near to the surface, and more branched in wet soils. Leaves are green and palmate (seven lobes). However, the size and shape of the leaflets differs markedly, according to genetic origin. The leaf arrangement is either opposite, or alternate or spiral. The leaflets are 6–11 cm (length) and 2–15 mm (width). Leaf margins are coarsely serrated. The adaxial and abaxial surfaces are green, with scattered, resinous trichomes. Inflorescences consist of numerous flower heads that can be found on long, leafy stems from each leaf axil. The staminate (male flower) consists of five pale-green, hairy sepals about 2.5–4 mm long, and five pendulous stamens, with slender filaments and stamen. The pistillate (female flowers) are almost sessile, and are in pairs. The fruit (seed), is an achene, contains a single seed with a hard shell tightly covered by the thin wall of the ovary, and it is ellipsoid, slightly compressed, smooth, about 2–5 mm long, generally brownish and mottled.

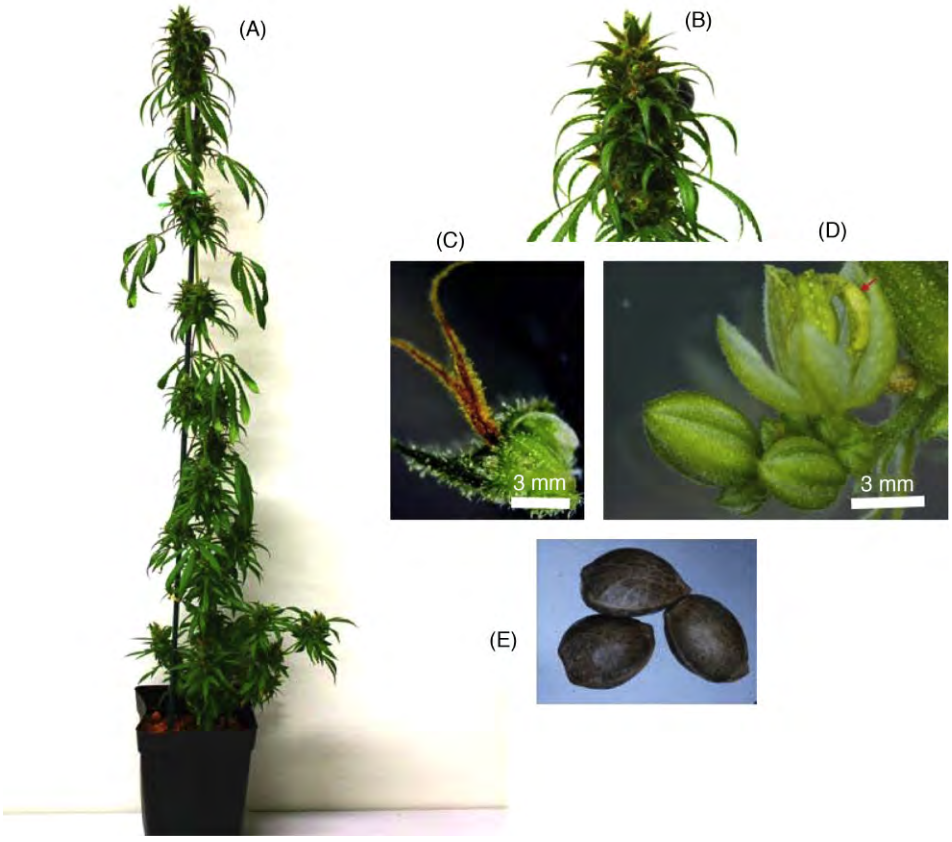


FIGURE 1.1 (A) female *C. sativa*; (B) portion of the female flowers; (C) pistillate female flower (stigmas, style, perigonal bract, and stipule); (D) portion of the female flowers show anther; (E) mature seed.

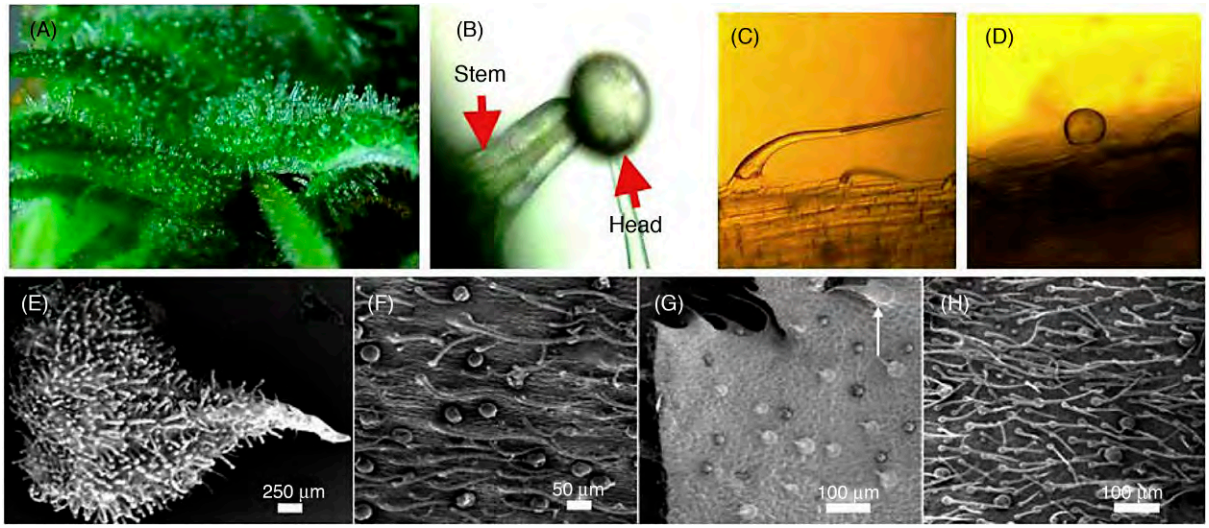


FIGURE 1.2 Microscopic photographs of *C. sativa* trichomes. (A) Trichomes on the flower; (B) capitate-stalked trichome; (C) capitate-sessile trichome; (D) bulbous trichome; (E) trichomes on the bract; (F) trichomes on the stem; (G) trichomes on the adaxial surface of a floral leaf. A big capitate-sessile trichome is indicated with an arrow; (H) trichomes on the abaxial surface of a leaf. Present abundant small capitate-sessile and bulbous trichomes. *Source: Adapted from Happyana et al. (2013).*

Microscopical Features

In general, *Cannabis* trichomes comprise a diverse set of structures and different types of trichomes (eg, glandular and nonglandular) on a single leaf, when viewed through a hand lens (Fig. 1.2). *Cannabis* trichome researchers have commonly described two types of the nonglandular trichome that have not been associated with terpenoid development (Table 1.1). Three types of glandular trichome have been described on female plants, namely bulbous, sessile, and capitate stalked (Happyana et al., 2013). Male plants have been found to exhibit a fourth type—the antherial glandular trichome, which has only been found on anthers (Fairbairn, 1972). Glandular trichomes are made from a series of differentiated cells with different functional properties, namely the secretory cells, and stalk cells (Kim & Mahlberg, 1991).

Classification of Cannabis

The first official publication which recorded the use of Latin binomials is Linnaeus's *Species Plantarum*, and it can be dated back to the year 1753. Afterward, the international community acknowledged it as the starting point for modern botanical nomenclature. The species name *Cannabis* means "*cane-like*," while the genus name "*sativa*" has the meaning "*planted or sown*," and signifies that the plant is propagated from seed, and not from perennial roots (Raman, 1998). According to the modern system of classification, *Cannabis* belongs to the family of *Cannabaceae*, along with the *Humulus* genus (hops) (Turner, Elsohly, & Boeren, 1980a,b). Different varieties of *Cannabis* have been developed over the course of many centuries, as the result of breeding and selection. However, the *Cannabis* processed by these methods creates many debates about further botanical classification. So far, there is no general agreement about the taxonomic rank of various groups within the genus *Cannabis*, and consequently its monospecific or polyspecific character, since the time of Linnaeus (late 18th century) (Hazekamp, Justin, Lubbe, & Ruhaak, 2010). UNODC (1956) divided domesticated *Cannabis* into three different groups:

- fiber hemp, long, unbranched plants, with poor seed production
- oil seed hemp, short, early maturing plants, with rich seed production
- drug hemp, short, strongly branched plants, with small dark green leaves.

Schultes, Klein, Plowman, & Lockwood (1974) distinguished three species within the genus: *C. sativa* L., *C. indica* Lam., and *C. ruderalis*. Other authors referred to the same taxa only at subspecific level within one single species, *C. sativa* (Hoffmann, 1961). Small and Cronquist (1976) divided the single species *C. sativa* into the subspecies *sativa* and *indica*, each consisting of a domesticated (Table 1.2) and wild varieties. Within the subspecies *sativa*, the domesticated and the wild varieties are *C. sativa* subsp. *sativa* var. *sativa* (domesticated), *C. sativa* subsp. *sativa* var. *spontama* (wild), *C. sativa* subsp. *indica* var. *indica* (domesticated), and *C. sativa* subsp. *indica* var. *kafiristanica* (wild). However, it is commonly accepted that *Cannabis* is monotypic, and consists only of a single species: *C. sativa* (Brenneisen, 1983; Beutler & Dermarderosian, 1978).

TABLE 1.1 A Summary of *Cannabis* Trichomes Classification, Structure, Distribution, Timing of Development, and Lifespan

Trichomes					
Classification	**Structure**	**Distribution**	**Timing of development/ density**	**Lifespan**	**References**
Nonglandular trichomes[a]	(1) *Noncystolithic trichomes*: long, unicellular, smooth, curved, covering trichomes (2) *Cystolithic trichomes*: more squat, unicellular, claw shape, cystolith covering trichomes, containing calcium carbonate	Lower side of vegetative leaves and pistillate bracts	Decreases with age	The viability and functioning secretion is correlated with senescence of epidermal cells	(Fairbairn, 1972; Hammond & Mahlberg, 1977; Turner et al., 1977, 1980b, 1981; Croteau, 1988; Werker, 2000; Guy & Stott, 2005; Happyana et al., 2013)
Glandular trichomes[b]	(1) *Bulbous*: with smallest gland	Vegetative leaves and pistillate bracts			
	(2) *Capitate-sessile (unstalked)*: the structure is commonly simple, and the trichomes head connected directly to the mesophyll cells.				
	(3) *Capitate-stalked*: the structure more complex, they developed resin head (also known as the glandular head) that resembles a golf ball sitting on a tee (the trichome's stalk).	Bracts and floral leaves	Increases with age		
Antherial sessile trichomes[c]	Large size, with a diameter of approximately 70–80 μm	Underside of the anther lobes			

[a] *Nonglandular trichomes lack cannabinoids.*
[b] *Glandular trichomes are the principal or sole site of storage of most cannabinoids, the content of Δ^9-THC in pistillate flowers ranged between 10 and 12%, and in leaves ranged between 1 and 2%.*
[c] *Male plants are of no consequence in medicine production because they develop few glandular trichomes and, consequently, produce few cannabinoids or terpenes.*

TABLE 1.2 Synopsis of C. *sativa* Sectional Species, Subspecies, and Varieties Recognized Based on Chemical, Genetic, and Morphological Variation

Section *sativa*	**Section *spontanea***
C. sativa (L.)[a] *C. chinensis* (Delile) *C. gigantea* (Delile) *C. americana* (Houghton) *C. sativa* subsp. *Intersita* (So.) subsp. *culta* (Serebr) subsp. *Sativa* (L.) var. *sativa* var. *praecox* var. *monoica* var. *gigantea* var. *Chinensis* var. *pedemontana*	*C. ruderalis* (L)[a] *C. sativa* subsp. *spontanea* (Serebr.) var. *spontanea* var. *ruderalis* *Section indica* *C. indica* (Lam.)[a] *C. macrosperma* (Stokes) *C. sativa* subsp. *indica* (Lam.) var. *indica* var. *kif* var. *afghanica* var. *kafiristanica*

[a] *Includes the endemic and domesticated populations (Raman, 1998; Sytsma et al., 2002; Hillig, 2005).*

The current scientific classification of *Cannabis* (Sytsma et al., 2002)

Class	Hamamelidae
Subclass	Rosales
Order	Cannabaceae
Family	*Cannabis*
Genus	*sativa*
Species	

Other Recent Taxonomic Studies

CHEMOTAXONOMIC CLASSIFICATION

Recently, chemotaxonomic classification splits the phenotypes based on the quantitative differences in the cannabinoid ratio of tetrahydrocannabinolic acid (THC), cannabinol (CBN), and cannabidiol (CBD), in the ratio of [THC] + [CBN]/[CBD]. If the ratio exceeded 1, plants are classified as "chemo-type," otherwise as "fiber-type," and this was the first study to differentiate between the drug- and fiber-type, by Fetterman et al. (1971). Therefore, this ratio was subsequently used to discriminate chemotype, intermediate type, and fiber-type (Turner, Cheng, Lewis, Russell, & Sharma, 1979). Hillig and Mahlberg (2004) split *Cannabis* into putative species and subspecies, using multivariate data analysis. Moreover, it was reported that, depending on age, the *Cannabis* plant can be classified into different morphotypes, at different time points of its development. Although this classification was not comprehensive enough to elucidate infrageneric taxonomic structure, and does not define the contents of cannabinoids for each chemotype, it provides a usable tool for classification (Hazekamp et al., 2010).

MOLECULAR CLASSIFICATION

Several molecular techniques have been evaluated to establish the genetic relationship among different varieties of *Cannabis* plants. Some recent studies have classified and identified *C. sativa* samples that cannot be differentiated by HPLC analysis alone, by using genomic DNA, random amplified polymorphic DNA (RAPD), and restriction fragment polymorphisms (RFLP) analysis, but little work appears to have been conducted with marker types that would be usable for breeding (Gillan, Cole, Linacre, Thorpe, & Watson, 1995; Faeti, Mandolino, & Ranalli, 1996). Recently, Kojoma, Iida, Makino, Sekita, and Satake (2002) reported that different *Cannabis* were identified by means of inter simple sequence repeat (ISSR). ISSR is a technique offering the reproducibility and simplicity of RAPDs with high reliability (Galvan, Bornet, Balatti, & Branchard, 2003).

Current *Cannabis* Varieties

Recently, *Cannabis* growers have become more aware to create variations between different strains for developing new varieties. Newly hybrid varieties have been developed as a result of the crossbreds, such as, "*super-sativa*" (Clarke & Watson, 2002; de Meijer, 2004). Recently, varieties of *Cannabis* have been licensed to GW Pharmaceuticals Ltd, as part of indoor breeding programs (de Meijer & Hammond, 2005). In the United States, the majority of *Cannabis* cultivars were selected from single landrace sources, or from multihybrid progenies made from different landraces (de Meijer, 2004). The marijuana potency monitoring project at the University of Mississippi (USA) is breeding Sinsemilla, Skunk 1, Four Way, Four Way-F, Thai/Skunk, Terbag W1, K2, and MX *Cannabis* of hybrid varieties (ElSohly, Holley, & Turner, 1985; Elsohly, Holley, Lewis, Russell, & Turner, 1984). In the Netherlands, there are three different *Cannabis* varieties from *sativa*: Bedrocan, Bedrobinol, and Bediol, and one variety from *C. indica* is Bedica – all studied and registered by Bedrocan BV (Fischedick, Hazekamp, Erkelens, Choi, & Verpoorte, 2010). Nowadays, many *Cannabis* hybrid cultivars (Table 1.3) and some selected pure strains have been commercialized in many private companies, and there are up to 20 more or less well defined strains for either indoor or outdoor cultivation, in The Netherlands, but a sufficient data set is not available, due to illegal cultivation. Today, the cultivation and production of hemp is restricted and controlled because of its association with narcotic use. Most of the hemp breeders cultivate fiber hemp with the ultimate goal to reduce THC content below 0.2%, or even to get noncannabinoid plants by breeding and crossing experiments (de Meijer, 1995).

Genetics of *Cannabis*

Genome of Cannabis sativa

The genome of *Cannabis* ($2n = 18 + XX$ for female, and $2n = 18 + XY$ for male) has a karyotype composed of nine autosomes and a pair of sex chromosomes (X and Y). Sex chromosomes changes during the developmental stages are claimed to occur in many dioecious plants, as a strategy for survival (Flemming et al., 2007). The genome was measured in both female (XX) and male plants (XY) (Vyskot & Hobza, 2015). The estimated haploid genome sizes are 818 Mb for female plants, and 843 Mb for males (Sakamoto et al., 1998). The genomic resources available for *Cannabis* are mainly confined to transcriptome information: the NCBI database contains 12,907 ESTs and 23 unassembled RNA-Seq datasets of Illumina reads (Marks et al., 2009). The genetic basis of cannabinoid variation in *C. sativa* showed that the amount of THC versus CBD is likely governed by one locus with two codominant alleles, *B*(*d*) and *B*(*t*) (de Meijer et al., 2003). One possible explanation for these results is that the two alleles encode either THCA or CBDA synthase so that homozygous plants would contain either tetrahydrocannabinolic

TABLE 1.3 Origin of Hemp Varieties Were Reported in Literature[a]

Variety	Country
Finola	Finland
Glukhov 33, Kuban, Uso 11, Zenica, USO 13, USO 15, USO 31, YUSO 14, YUSO 16	Ukraine
Asso, Carmagnola, CS (Carmagnola Selezionata), Carmono, Carma, Codimono, Eletta Campana, Ferrara, Ermes, Fibrimor Fibranova	Italy
Fasamo, Ferimon	Germany
Santhica 27, Epsilon 68, Fedora 17, Fedora 19, Fedrina 74, Felina 32, Felina 34, Fibrimon 21, Fibrimon 24, Fibrimon 56, Futura, Futura 77, Futura 75, Santhica 23, Dioica 88	France
Kompolti Sargaszaru, Kinai unisexualis, Kompolti, Kompolti Hybrid TC, Kompolti Hyper, Elite, Fibriko	Hungary
Fibramulta 151, Irene, Lovrin 110, Moldovan, Secuieni 1	Romania
Beniko, Bialobrzeskie, LKCSD, Dolnoslaskie	Poland
Chamaeleon, Dutch "Yellow" line	Netherlands
Ermakovskaya Mestnaya	Russia
Delta 405, Delta-llosa	Spain
Kenvir	Turkey
Swissmix	Swiss
Ratslaviska	Czech
Silistrensi, Mecnaja copt	Bulgaria
Pesnica	Slovenia
Flajsmanova, Novosadksa, Novosadska plus, Novosadska konoplja	Former Yugoslavia
Kinai Egylaki, Kinai Ketlaki	China
Kozuhura Zairai	Japan

[a] *Low THC cultivars, less than 0.2% dry weight.*

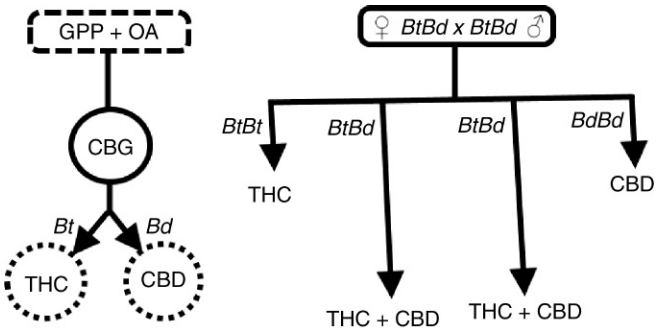


FIGURE 1.3 **Inheritance of chemical phenotype in *C. sativa* "co-dominant monogenic control," homozygous THC producing *BtBt* genotypes are typically selected for recreational use.** *Source: From de Meijer et al. (2003).*

acid (THCA) or cannabidiolic acid (CBDA) as the major cannabinoid, and heterozygotes would have an approximately equal mixture of the two (Fig. 1.3). Another explanation is that THCA and CBDA synthases are closely linked genes, perhaps produced as a result of a gene duplication event. A recent study analyzed the THCA synthase sequences from drug (high-THC) and fiber (low-THC) varieties, and found that the amino acid sequence of THCA synthase from high-THC varieties differed by 37 major substitutions, compared to low-THC varieties (Kojoma, Seki, Yoshida, & Muranaka, 2006).

Geographical Distribution

Small and Cronquist (1976) state that genus *Cannabis* geographically grows to the north of latitude 30°N and south of latitude 60°N (Hillig, 2005). The genus is believed to have originated in the Northwest Himalayas, and occurs widely in Africa.

Agricultural Status

Nowadays, fiber hemp is cultivated in a number of countries around the world, and China represents the largest producer of hemp with focus on fiber-type (Mediavilla, Bassetti, & Leupin, 1999). Nevertheless, cultivation of medicinal *Cannabis* is prohibited in most of countries, except by permission for purposes of research and pharmaceutical uses.

Conservation Initiatives

Cannabis populations are facing the threat of genetic drift—which has a direct effect on the changes to the phenotype and chemical profile, due to allogamous (de Meijer & Vansoest, 1992). The conservation of *Cannabis* germplasm is divided into two main strategies: in situ and ex situ.

Ex Situ Conservation in Gene Banks

The *Cannabis* gene bank at Vavilov Research Institute of Plant Industry (St. Petersburg, Russia) maintained about 200 accessions, for more than 50 years (de Meijer, 1998). In addition, the Hungarian gene bank at the Research Center for Agrobiodiversity (Tápiószele, Hungary) maintained about 70 accessions. Collections of up to 20 accessions are preserved in other depositories in Germany, Turkey, and Japan. In comparison with other crops, the available number of well-documented *Cannabis* accessions is limited (de Meijer & Vansoest, 1992). Nowadays, several accessions are maintained by the United States Department of Agriculture (USDA)/National Plant Germplasm System (NPGS), and associated data can be accessed from the Germplasm Resources Information Network (GRIN) database.

In Situ Conservation as In Vitro Gene Banks

In vitro conservation of encapsulated microcuttings of *Cannabis* shootlets was attempted under slow growth conditions between 5 and 15°C (Lata, Chandra, Khan, & ElSohly, 2008; Lata, Chandra, Mehmedic, Khan, & ElSohly, 2012), but adaptation to in vitro conditions could induce mutants of the offspring plants, causing genetic and chemical drift (Larkin & Scowcroft, 1981).

Cultivation Techniques of *Cannabis*

Outdoor Cultivation

Cannabis plant can be propagated from seeds, and the life cycle is completed within 4–6 months, depending on the time of the plantation and the variety. It can reach up to 5 m (16 ft.) in height, in a 4–6 months growing season (Raman, 1998; Clarke & Watson, 2002). Hermaphroditic varieties of this plant have been bred for industrial hemp production, as this allows more uniform crops (Leggett, 2006). The process of germination is usually completed in 3–7 days (Clarke & Watson, 2002). The seedling stage is completed within 2–3 months. Later, the plant is characterized by increased biomass and total growth under long day time lengths (vegetative growth). It is easy to recognize the male and female sex at this stage. Later in summer, the reproductive phase of *Cannabis* begins when the plant is exposed to short day time lengths (less light per day than darkness) of 12–14 h or less, depending on its latitude and genetic origin (Brenneisen, 1983). Once the male flowers ripened and pollinated, the female flowers died directly. The produced seeds after flowering have combinations of traits from two parents, as a result of cross fertilization (Clarke & Watson, 2002). This method is mostly used for the cultivation of *Cannabis* for hemp fiber, or *Cannabis* seed with less than 0.2% THC.

Indoor Cultivation

This method of breeding is used for increasing resin potency, and avoiding unwanted male plants (Chandra, Lata, Khan, & ElSohly, 2010). The complete growth cycle, quality, and quantity of biomass can be regulated under controlled environmental conditions (6–8 weeks). The successful indoor system requires an effective hydroponic system to deliver nutrients and oxygen, and support the plants' growth (Fig. 1.4). However, there is a number of different techniques that have been proposed for the indoor horticulture of *Cannabis*, for example, the standing aerated technique, nutrient film technique, and aeroponics technique. In hydroponic growing, the nutrient solution is best at a pH within a certain range (5.5–6.5) for maximum uptake and good plant growth (Argo & Fischer, 2002). Indoor *Cannabis* crop cultivation needs artificial light and compressed CO_2 gas for photosynthesis, and for controlling flowering and plant biomass (Jones, 1997). Here, selective vegetative female plants are used for making clones. Later, all clones are kept under standard environmental conditions (light, temperature, RH, and CO_2 concentration) in a growing room for vegetation (18 h/day photoperiod) and for flowering (12 h/day photoperiod).

In vitro Micropropagation

The micropropagation system offers a number of clear advantages, including (1) human-controlled method with fast propagation in a comparably short time, due to high potential multiplication rates, (2) it is independent of seasonal factors like climate and geography, and (3) the produced plants are usually free from microorganism-borne diseases (Zafar, Aeri, & Datta, 1992). On the other hand, in vitro propagation of *C. sativa* through the seed is possible in most of cultivars, although the greatest problem with such a method is the

FIGURE 1.4 **Indoor cultivation of *C. sativa*.** *Source: Photo provided from Bedrocan BV, the Netherlands.*

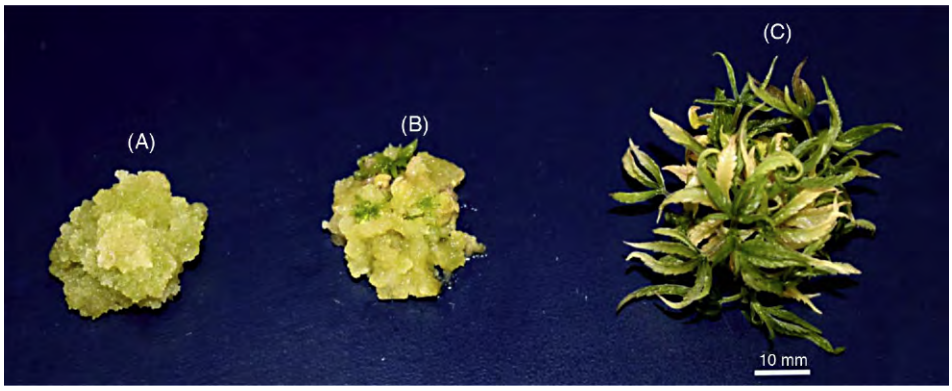


FIGURE 1.5 **In vitro micropropagation of leaf-derived calli from *C. sativa* L.** (A) Callus culture, (B) meristemoid formation, (C) shootlets multiplication on Gamborg's B5 medium supplemented with various combinations of auxins and cytokinins. *Source: Photos provided from Sayed Farag PhD project, Technische Universität Dortmund.*

high level of heterozygosity that could lead to a rapid and dramatic profile shift of secondary metabolites from one generation to the next (Chandra et al., 2010). In fact, in vitro propagation using explants or somatic embryogenesis has been reported (Lata et al., 2002). Besides the progress in the field of plant biotechnology, very little progress has been made to date toward developing an in vitro regeneration from *C. sativa*. Previous reports on de novo organogensis of *C. sativa* emerged in early 1980s (Fisse, Braut, Cosson, & Paris, 1981), and subsequently from callus of different genotypes and explant sources, including cotyledons and stem (Wielgus, Luwanska, Lassocinski, & Kaczmarek, 2008), young leaves (Lata, Chandra, Khan, & ElSohly, 2010), internodes, and axillary buds and petioles (Slusarkiewicz-Jarzina, Ponitka, & Kaczmarek, 2005), and roots (Ranalli & Mandolino, 1999). Alternatively, the use of meristematic callus for micropropagation was studied recently (Farag & Kayser, unpublished results, Fig. 1.5).

Recommendations for Future Action

Given the high therapeutic and commercial value of *Cannabis*, legal indoor breeding started in some pharmaceutical companies. The biotechnological research for genetic improvement has been minimal to date. Researches on transgenic *Cannabis* is still needed to facilitate the metabolic engineering of cannabinoids and agronomic traits.

MINI-DICTIONARY

Encapsulation In vitro technique for the production of synthetic seeds (Ca-alginate beads) for long-term storage of germplasm.
Genome The complete set of chromosomal and extra-chromosomal DNA/RNA of an organism, a cell, an organelle, or a virus.
Inter simple sequence repeats (ISSR) A molecular technique for evolutionary biology. Its simple sequence repeats (SSR), also known as microsatellites, are tandem repeats of a few base pairs distributed throughout the genome.
Micropropagation In vitro technique for multiplying plant tissues through in vitro culture, either indirectly (with intervening callus stage) or directly (without an intervening proliferative stage). This is achieved by altering the concentration of growth regulators, mainly auxins and cytokinins.
Random amplified polymorphic DNA (RAPD) A molecular technique for the rapid assignation of DNA-based character states for phylogenetic analysis. The technique uses the polymerase chain reaction (PCR) to amplify any genomic region containing single primer of nucleotide arbitrary sequence.
Restriction fragment length polymorphism (RFLP) A molecular technique for genome mapping, and variation analysis (genotyping, forensics, paternity tests, hereditary disease diagnostics, etc.). The technique uses restriction of endonucleases to cut DNA at specific (generally 4–6 bp) recognition sites.
Trichome Defined as hair-like structures that extend from the epidermis of aerial tissues; are present on the surface of most terrestrial plants.

References

Appendino, G., Chianese, G., & Taglialatela-Scafati, O. (2011). Cannabinoids: occurrence and medicinal chemistry. *Current Medicinal Chemistry*, *18*(7), 1085–1099.
Argo, W. R., Fischer, P. R. (2002). Understanding pH management for container-grown crops. Meister, Willoughby, Ohio.
Beutler, J. A., & Dermarderosian, A. H. (1978). Chemotaxonomy of Cannabis .1. Cross breeding between *Cannabis sativa* and *Cannabis ruderalis*, with analysis of Cannabinoid content. *Economic Botany*, *32*(4), 387–394.
Brenneisen, R. (1983). Psychotropic drugs. I. *Cannabis sativa* L. (Cannabinaceae). *Pharmaceutica Acta Helvetiae*, *58*(11), 314–320.
Brenneisen, R. (2007). Chemistry and analysis of phytocannabinoids and other Cannabis constituents. In M. A. Elsohly (Ed.), *Marijuana and the cannabinoids* (pp. 17–49). Totowa: Humana Press.
Chandra, S. H., Lata, I., Khan, A., & ElSohly, M. A. (2010). Propagation of elite *Cannabis sativa* for the production of Δ^9-Tetrahydrocannabinol (THC) using biotechnological tools. In A. Rajesh (Ed.), *Medicinal Plant Biotechnology* (pp. 98–114). UK: CABI.
Clarke, R. C. (1981). *Marijuana botany. An advanced study: the propagation and breeding of distinct cannabis*. Berkeley, CA: And/Or Press.
Clarke, R. C., & Watson, D. P. (2002). Botany of natural *Cannabis* medicines. In F. Grotenhermen, & E. Russo (Eds.), *Cannabis and cannabinoids: pharmacology, toxicology, and therapeutic potential* (pp. 3–13). New York: The Haworth Press.
Croteau, R. (1988). Catabolism of monoterpenes in essential oil plants. *Developments in Food Science*, *18*, 65–84.

de Meijer, E. P. M., & Vansoest, L. J. M. (1992). The CPRO *Cannabis* germplasm collection. *Euphytica*, *62*(3), 201–211.

de Meijer, E. P. M. (1995). Fibre hemp cultivars: a survey of origin, ancestry, availability and brief agronomic characteristics. *Journal of the International Hemp Associaiton*, *2*(2), 66–73.

de Meijer, E. (1998). Cannabis germplasm resources. In P. Ranalli (Ed.), *Advances in hemp research*. New York: Haworth Food Products Press, p. 272.

de Meijer, E. P., Bagatta, M., Carboni, A., Crucitti, P., Moliterni, V. M., Ranalli, P., & Mandolino, G. (2003). The inheritance of chemical phenotype in *Cannabis sativa* L. *Genetics*, *163*(1), 335–346.

de Meijer, E. (2004). The breeding of cannabis cultivars for pharmaceutical end uses. In G. W. Guy, B. A. Whittle, & P. Robson (Eds.), *Medicinal uses of cannabis and cannabinoids* (pp. 55–70). London: Pharmaceutical Press.

de Meijer, E. P. M., & Hammond, K. M. (2005). The inheritance of chemical phenotype in *Cannabis sativa* L. (II): cannabigerol predominant plants. *Euphytica*, *145*, 189–198.

Elbatsh, M. M., Moklas, M. A., Marsden, C. A., & Kendall, D. A. (2012). Antidepressant-like effects of delta(9)-tetrahydrocannabinol and rimonabant in the olfactory bulbectomised rat model of depression. *Pharmacology Biochemistry and Behavior*, *102*(2), 357–365.

ElSohly, M. A. (2002). Chemical constituents of *Cannabis*. In F. Grotenhermen, & E. Russo (Eds.), *Cannabis and cannabinoids: pharmacology, toxicology, and therapeutic potential* (pp. 27–36). New York, NY: The Haworth Press.

Elsohly, M. A., Holley, J. H., Lewis, G. S., Russell, M. H., & Turner, C. E. (1984). Constituents of *Cannabis sativa* L. 24. The potency of confiscated marijuana, hashish, and hash oil over a 10-year period. *Journal of Forensic Science*, *29*(2), 500–514.

ElSohly, M. A., Holley, J. H., & Turner, C. E. (1985). Constituents of Cannabis sativa L. XXVI. The delta-9-tetrahydrocannabinol content of confiscated marijuana, 1974–1983. In D. J. Harvey (Ed.), *Marijuana '84'* (p. 37). Oxford: IRL Press.

ElSohly, M. A., & Slade, D. (2005). Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life Sciences*, *78*(5), 539–548.

Faeti, V., Mandolino, G., & Ranalli, P. (1996). Genetic diversity of *Cannabis sativa* germplasm based on RAPD markers. *Plant Breeding*, *115*(5), 367–370.

Fairbairn, J. W. (1972). Trichomes and glands of *Cannabis Sativa* L. *Bulletin on Narcotics*, *24*(4), 29–33.

Fetterman, P., Keith, E. S., Waller, C. W., Guerrero, O., Doorenbo, N. J., & Quimby, M. W. (1971). Mississippi-grown *Cannabis sativa* L - preliminary observation on chemical definition of phenotype and variations in tetrahydrocannabinol content versus age, sex, and plant part. *Journal of Pharmaceutical Sciences*, *60*(8), 1246–1249.

Fischedick, J. T., Hazekamp, A., Erkelens, T., Choi, Y. H., & Verpoorte, R. (2010). Metabolic fingerprinting of *Cannabis sativa* L., cannabinoids and terpenoids for chemotaxonomic and drug standardization purposes. *Phytochemistry*, *71*(17-18), 2058–2073.

Fisse, J., Braut, F., Cosson, L., & Paris, M. (1981). Étude in vitro des capacités organogénétiques de tissus de *Cannabis sativa* L.; Effet de différentes substances de croissance. *Plantes Médicinales et Phytotherapie*, *15*, 217–223.

Flemming, T., Muntendam, R., Steup, C., & Kayser, O. (2007). Chemistry and biological activity of tetrahydrocannabinol and its derivatives. In M. T. H. Khan (Ed.), *Topics in heterocyclic chemistry: Bioactive heterocycles IV* (pp. 1–42). (Vol. 10). Berlin: Springer-Verlag.

Galvan, M. Z., Bornet, B., Balatti, P. A., & Branchard, M. (2003). Inter simple sequence repeat (ISSR) markers as a tool for the assessment of both genetic diversity and gene pool origin in common bean (*Phaseolus vulgaris* L.). *Euphytica*, *132*(3), 297–301.

Gillan, R., Cole, M. D., Linacre, A., Thorpe, J. W., & Watson, N. D. (1995). Comparison of *Cannabis sativa* by random amplification of polymorphic DNA (RAPD) and HPLC of cannabinoids: a preliminary study. *Science and Justice*, *35*(3), 169–177.

Guy, G. W., & Stott, C. (2005). The development of Sativex®- a natural cannabis based medicine. In R. Mechoulam, & J. Bruinrels (Eds.), *Cannabinoids as therapeutics milestones in drug therapy* (pp. 231–263). Berlin: Springer.

Hammond, C. T., & Mahlberg, P. G. (1977). Morphogenesis of capitate glandular hairs of *Cannabis sativa* (Cannabaceae). *American Journal of Botany*, *64*(8), 1023–1031.

Happyana, N., Agnolet, S., Muntendam, R., Van Dam, A., Schneider, B., & Kayser, O. (2013). Analysis of cannabinoids in laser-microdissected trichomes of medicinal *Cannabis sativa* using LCMS and cryogenic NMR. *Phytochemistry*, *87*, 51–59.

Hazekamp, A., Justin, T. F., Lubbe, A., & Ruhaak, R. L. (2010). 3.24 - Chemistry of *Cannabis*. *Comprehensive Natural Products II*, *3*, 1033–1084.

Hillig, K. W. (2005). Genetic evidence for speciation in *Cannabis* (Cannabaceae). *Genetic Resources and Crop Evolution*, *52*(2), 161–180.

Hillig, K. W., & Mahlberg, P. G. (2004). A chemotaxonomic analysis of cannabinoid variation in *Cannabis* (Cannabaceae). *American Journal of Botany*, *91*(6), 966–975.

Hoffmann, W. (1961). *Hanf,* Cannabis sativa *L. Handbuch der Pflanzenzüchtung* (vol 5). Berlin: Paul Parey.

Jones, J. B. (1997). *Hydroponics: a practical guide for the soilless grower*. Bocan Raton: CRC Press.

Kim, E. S., & Mahlberg, P. G. (1991). Secretory cavity development in glandular trichomes of *Cannabis sativa* L (Cannabaceae). *American Journal of Botany*, *78*(2), 220–229.

Kim, E. S., & Mahlberg, P. G. (1997). Immunochemical localization of tetrahydrocannabinol (THC) in cryofixed glandular trichomes of *Cannabis* (Cannabaceae). *American Journal of Botany*, *84*(3), 336–342.

Kojoma, M., Iida, O., Makino, Y., Sekita, S., & Satake, M. (2002). DNA fingerprinting of *Cannabis sativa* using inter-simple sequence repeat (ISSR) amplification. *Planta Medica*, *68*(1), 60–63.

Kojoma, M., Seki, H., Yoshida, S., & Muranaka, T. (2006). DNA polymorphisms in the tetrahydrocannabinolic acid (THCA) synthase gene in "drug-type" and "fiber-type" *Cannabis sativa* L. *Forensic Science International*, *159*(2-3), 132–140.

Larkin, P., & Scowcroft, W. (1981). Somaclonal variation a novel source of variability from cell cultures for plant improvement. *Theoretical and Applied Genetics*, *60*, 197–214.

Lata, H., Bedir, E., Hosick, A., Ganzera, M., Khan, I., & Moraes, R. M. (2002). *In vitro* plant regeneration from leaf-derived callus of *Cimicifuga racemosa*. *Planta Medica*, *68*(10), 912–915.

Lata, H., Chandra, S., Khan, I. A., & ElSohly, M. A. (2008). Propagation of *Cannabis sativa* L. using synthetic seed technology. *Planta Medica*, *74*(3), 328–1328.

Lata, H., Chandra, S., Khan, I. A., & ElSohly, M. A. (2010). High frequency plant regeneration from leaf derived callus of high delta(9)-tetrahydrocannabinol yielding *Cannabis sativa* L. *Planta Medica*, *76*(14), 1629–1633.

Lata, H., Chandra, S., Mehmedic, Z., Khan, I. A., & ElSohly, M. A. (2012). *In vitro* germplasm conservation of high Delta(9)-tetrahydrocannabinol yielding elite clones of *Cannabis sativa* L. under slow growth conditions. *Acta Physiologiae Plantarum*, *34*(2), 743–750.

Leggett, T. (2006). A review of the world cannabis situation. *Bulletin on Narcotics*, *58*(1–2), 1–155.

Marks, M. D., Tian, L., Wenger, J. P., Omburo, S. N., Soto-Fuentes, W., He, J., Gang, D. R., Weiblen, G. D., & Dixon, R. A. (2009). Identification of candidate genes affecting Δ^9-tetrahydrocannabinol biosynthesis in *Cannabis sativa*. *Journal of Experimental Botany*, *60*(13), 3715–3726.

Mediavilla, V., Bassetti, P., & Leupin, M. (1999). Agronomic characteristics of some hemp genotypes. *Journal of the International Hemp Association*, *6*, 45–53.

Potter, D. J. (2004). Growth and morphology of medicinal Cannabis. In G. W. Guy, B. A. Whittle, & P. J. Robson (Eds.), *The medicinal uses of Cannabis and cannabinoids* (pp. 17–54). London: Pharmaceutical Press.

Raman, A. (Ed.), (1998). The *Cannabis* plant: botany, cultivation, and processing for use. In *Cannabis*: *The Genus Cannabis* (pp. 29–54). Amsterdam: Harwood Academic Publishers.

Ranalli, P., & Mandolino, G. (1999). Advances in hemp research. In P. Ranalli (Ed.), *Advances in biotechnological approaches for hemp breeding and industry* (pp. 185–208). New York: The Haworth Press.

Ross, S. A., & ElSohly, M. A. (1996). The volatile oil composition of fresh and air-dried buds of *Cannabis sativa*. *Journal of Natural Products*, *59*(1), 49–51.

Sakamoto, K., Akiyama, Y., Fukui, K., Kamada, H., & Satoh, S. (1998). Characterization: genome sizes and morphology of sex chromosome in hemp (*Cannabis sativa* L.). *Cytologia*, *63*, 459–464.

Schultes, R. E., Klein, W. M., Plowman, T., & Lockwood, T. E. (1974). *Cannabis*: an example of taxonomic neglect. *Harvard University Botanical Museum Leaflets*, *23*, 337–367.

Slusarkiewicz-Jarzina, A., Ponitka, A., & Kaczmarek, Z. (2005). Influence of cultivar, explant source and plant growth regulator on callus induction and plant regeneration of *Cannabis sativa* L. *Acta Biologica Cracoviensia Series Botanica*, *47*(2), 145–151.

Small, E., & Cronquist, A. (1976). A practical and natural taxonomy for *Cannabis*. *Taxon*, *25*, 405–435.

Sytsma, K., Morawetz, J., Pires, J., Nepokroeff, M., Conti, E., Zjhra, M., Hall, J., & Chase, M. (2002). Urticalean rosids: circumscription, rosid ancestry, and phylogenetics based on rbcL, trnL-F, and ndhF sequences. *American Journal of Botany*, *89*(9), 1531–1546.

Turner, C. E., & Elsohly, M. A. (1976). Anhydrocannabisativine, a new alkaloid isolated from *Cannabis sativa*. *Lloydia*, *39*(6), 474–1474.

Turner, J. C., Hemphill, J. K., & Mahlberg, P. G. (1977). Gland distribution and cannabinoid content in clones of *Cannabis sativa* L. *American Journal of Botany*, *64*(6), 687–693.

Turner, C. E., Cheng, P. C., Lewis, G. S., Russell, M. H., & Sharma, G. K. (1979). Constituents of *Cannabis sativa* .15. Botanical and chemical profile of Indian variants. *Planta Medica*, *37*(3), 217–225.

Turner, C. E., Elsohly, M. A., & Boeren, E. G. (1980a). Constituents of *Cannabis sativa* L .17. a review of the natural constituents. *Journal of Natural Products*, *43*(2), 169–234.

Turner, J. C., Hemphill, J. K., & Mahlberg, P. G. (1980b). Trichomes and cannabinoid content of developing leaves and bracts of *Cannabis sativa* L (Cannabaceae). *American Journal of Botany*, *67*(10), 1397–1406.

Turner, J. C., Hemphill, J. K., & Mahlberg, P. G. (1981). Interrelationships of glandular trichomes and cannabinoid content. 2. Developing vegetative leaves of *Cannabis sativa* L (Cannabaceae). *Bulletin on Narcotics*, *33*(3), 63–71.

UNODC, United Nations, (2009). Recommended methods for identification and analysis of cannabis and cannabis products, New York. http://www.unodc.org/documents/scientific/ST-NAR-40-Ebook.pdf

UNODC, United Nations (1956). Problems of modern hemp breeding, with particular reference to the breeding of varieties of hemp containing little or no hashish, New York. http://www.unodc.org/unodc/en/data-and-analysis/bulletin/bulletin_1956-01-01_3_page007.html

Vanhoenacker, G., Van Rompaey, P., De Keukeleire, D., & Sandra, P. (2002). Chemotaxonomic features associated with flavonoids of cannabinoid-free cannabis (C*annabis sativa* subsp *sativa* L.) in relation to hops (*Humulus lupulus* L.). *Natural Product Letters*, *16*(1), 57–63.

Vyskot, B., & Hobza, R. (2015). The genomics of plant sex chromosomes. *Plant Science*, *236*, 126–135.

Werker, E. (2000). Trichome diversity and development. *Advances in Botanical Research*, *31*, 1–35.

Wielgus, K., Luwanska, A., Lassocinski, W., & Kaczmarek, Z. (2008). Estimation of *Cannabis sativa* L. tissue culture conditions essential for callus induction and plant regeneration. *Journal of Natural Fibers*, *5*(3), 199–207.

Zafar, R., Aeri, V., & Datta, A. (1992). Application of plant tissue and cell culture for production of secondary metabolites. *Fitoterapia*, *63*, 33–43.

CHAPTER

2

The Biosynthesis of Cannabinoids

F. Degenhardt, F. Stehle, O. Kayser

Laboratory of Technical Biochemistry, Department of Biochemical and Chemical Engineering, TU Dortmund University, Dortmund, Germany

SUMMARY POINTS

- This chapter focuses on the pathway which leads to the biosynthesis of phytocannabinoids in *C. sativa* L.
- CBGA is the central precursor of phytocannabinoid biosynthesis in *Cannabis*.
- CBGAS, only three enzymes—THCAS, CBDAS, and CBCAS—are involved in the biosynthesis of phytocannabinoids in *Cannabis* plants.
- Sequences of CBDAS and THCAS are known.
- The carboxyl group in CBGA seems to be essential for the enzymatic reactions catalyzed by CBDAS, CBCAS, and THCAS.
- The diversity of more than 60 cannabinoids is the result of nonenzymatic modifications.
- Propyl cannabinoids occur by the prenylation of divarinic acid (DA) with geranyl diphosphate (GPP).

KEY FACTS OF PHYTOCANNABINOIDS—BESIDES *C. SATIVA*

- Phytocannabinoids are plant-derived natural compounds that act as ligands to cannabinoid receptors (CB_1 and CB_2) or share chemical similarity with cannabinoids.
- *C. sativa* L. is intensively investigated for the presence of phytocannabinoids. To date, only a few plants are discovered that contain phytocannabinoids other than the ones known from *Cannabis*.
- The New Zealand liverwort *Radula marginata* and Japanese liverwort *Radula perrottetii* contain perrottetinene, a naturally occurring bibenzyl cannabinoid.
- Two cannabigerol-like compounds were detected in the aerial parts of *Helichrysum umbraculigerum* Less., a plant common in the eastern parts of South Africa.
- *N*-alkyl amides (cannabinomimetics), found in the medicinal plants *Echinaceae angustifolia* and *Echinaceae purpurea* (purple cornflower), are known to interact with the CB_2 receptor.

LIST OF ABBREVIATIONS

AAE	Acyl-activating enzyme
BBE	Berberine bridge enzyme
CBC	Cannabichromene
CBCA	Cannabichromenic acid
CBCAS	Cannabichromenic acid synthase
CBCVA	Cannabichrovarinic acid
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBDAS	Cannabidiolic acid synthase
CBDV	Cannabidivarin
CBDVA	Cannabidivarinic acid
CBG	Cannabigerol
CBGA	Cannabigerolic acid (3-geranyl olivetolate)
CBGAS	Cannabigerolic acid synthase
CBGVA	Cannabigerovarinic acid
CBN	Cannabinol
CBNRA	Cannabinerolic acid (*cis*-CBGA)
CHS	Chalcone synthase
CsAAE1	*C. sativa* hexanoyl-CoA synthetase 1
CsAAE3	*C. sativa* hexanoyl-CoA synthetase 2
CsHCS1	*C. sativa* hexanoyl-CoA synthetase 1
CsHCS2	*C. sativa* hexanoyl-CoA synthetase 2
DA	Divarinic acid
DABB	Dimeric α+β barrel
DMAPP	Dimethylallyl diphosphate

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00002-8
Copyright © 2017 Elsevier Inc. All rights reserved.

DOXP	1-Deoxy-D-xylulose-5-phosphate
GOT	Geranylpyrophosphate:olivetolate geranyltransferase
GPP	Geranyl diphosphate
HTAL	Hexanoyltriacetic acid lactone
IPP	Isopentenyl diphosphate
MEP	2C-methyl-D-erythritol-4-phosphate
MVA	Mevalonate
NPP	Neryl diphosphate
OA	Olivetolic acid
OAC	Olivetolic acid cyclase
OLS	Olivetol synthase
PKS	Polyketide synthase
SNP	Single nucleotide polymorphism
STS	Stilbene synthase
THC	Tetrahydrocannabinol
THCA	Tetrahydrocannabinolic acid
THCAS	Tetrahydrocannabinolic acid synthase
THCV	Tetrahydrocannabivarin
THCVA	Tetrahydrocannabivarinic acid

INTRODUCTION

Cannabis sativa L. (hemp) is one of the oldest domestic plants in the history of mankind, and has been cultivated for at least 10,000 years (Schultes, Klein, Plowman, & Lockwood, 1974). Together with *Humulus lupulus* (hop), *C. sativa* belongs to the small family of Cannabaceae. *Cannabis* is an annual, usually dioecious, wind-pollinated herb, with both male and female flowers growing on separate plants. The plant is well known for the biosynthesis of cannabinoids, the terpenophenolic constituents that show psychoactive effects. But since other plants also have secondary metabolites that interact with the human cannabinoid receptors, a new definition had to be made. Hence, phytocannabinoids are now defined as any plant-derived natural compound that can act as a ligand to human cannabinoid receptors (CB_1 and CB_2) or share chemical similarity with cannabinoids (Gertsch, Pertwee, & Di Marzo, 2010). Interestingly, all parts of the *Cannabis* plant, with the exception of seeds, can contain cannabinoids, but they mainly accumulate in the glandular trichomes of female flowers (Gagne et al., 2012; van Bakel et al., 2011).

The following chapter focuses on the pathway that leads to the enzymatic biosynthesis of cannabinoids. For a long time, it was postulated that the key intermediate is cannabidiol (CBD) or cannabidiolic acid (CBDA), both resulting from a condensation of a monoterpene, and olivetol or olivetolic acid (OA), respectively. In 1964, Gaoni and Mechoulam postulated cannabigerol (CBG) as the key intermediate, the condensation product of geranyl diphosphate (GPP), and olivetol or OA. Based on this, they concluded that the cannabinoids CBD, tetrahydrocannabinol (THC) and cannabinol (CBN) are all derived from CBG, and just differ in the way of cyclization (Gaoni & Mechoulam, 1964). Finally, incorporation studies with ^{13}C-labeled glucose have shown that GPP and OA are indeed the precursors for formation of cannabigerolic acid (CBGA). Thus, the general structure of cannabinoids is assembled by two parts: (1) a diphenol (resorcin) carrying an alkyl chain (OA); and (2) a monoterpene moiety (GPP) (Fig. 2.1). Subsequently, Fellermeier and

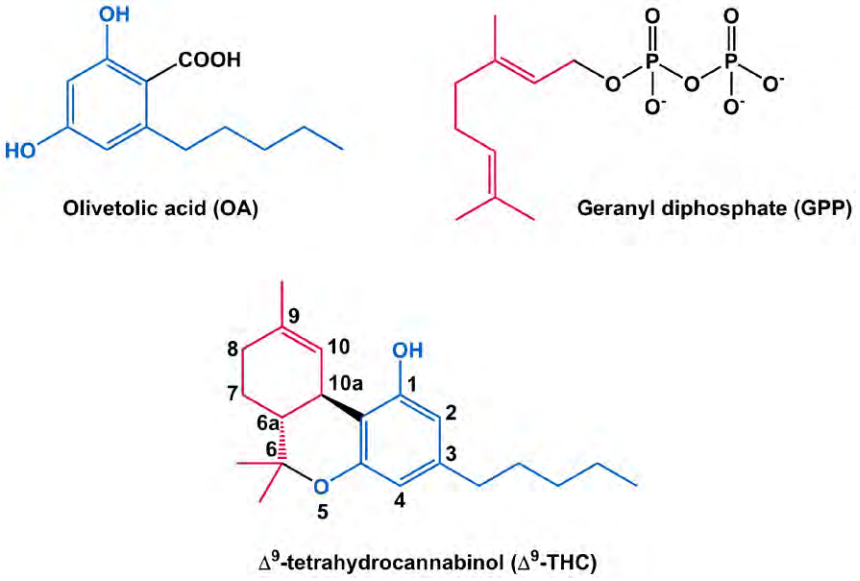


FIGURE 2.1 General structure of cannabinoids and their precursors, olivetolic acid, and geranyl diphosphate. Cannabinoids are composed of two parts: a cyclic monoterpene part *(red)*, and a diphenol (resorcin) part, carrying an alkyl chain *(blue)*. The dibenzopyran-numbering system is used.

coworkers postulated CBGA as the central cannabinoid precursor (Fellermeier, Eisenreich, Bacher, & Zenk, 2001; Fellermeier & Zenk, 1998). Interestingly, free OA has never been detected in Cannabis plant material until now.

It is worthy to note that, although more than 60 cannabinoids are known, only three enzymes, besides cannabigerolic acid synthase (CBGAS), namely tetrahydrocannabinolic acid synthase (THCAS), cannabidiolic acid synthase (CBDAS), and cannabichromenic acid synthase (CBCAS), are involved in cannabinoid biosynthesis. The resulting acidic cannabinoids are the most abundant ones accumulating in *Cannabis*. The neutral and psychoactive forms are the results of nonenzymatic decarboxylation during storage, heat or sunlight; explaining the heating of plant material (ie, smoking or baking), during *Cannabis* consumption (Fischedick, Hazekamp, Erkelens, Choi, & Verpoorte, 2010; Taura et al., 2007a). Thus, the broad diversity of the different cannabinoids is mainly due to nonenzymatic transformation or degradation of both acidic and neutral cannabinoids by the effects of light (UV irradiation) and auto-oxidation (Crombie, Ponsford, Shani, Yagnitinsky, & Mechoulam, 1968; Razdan, Puttick, Zitko, & Handrick, 1972). It is still unclear if all these forms are present in living plants as natural or artefacts, due to storage and sample preparation (ElSohly & Slade, 2005).

CANNABINOID PRECURSOR BIOSYNTHESIS

Polyketide Pathway Toward Olivetolic Acid

The origin of hexanoate in trichomes has not been elucidated so far. Suzuki, Kurano, Esumi, Yamaguchi, and Doi (2003) showed that the side-chain moiety of alkylresorcinols is formed by fatty acid units, but it remains unclear if the moiety is the result of biosynthesis or degradation of fatty acids. Studies regarding the incorporation of ^{13}C-labels into cannabinoids indicate that hexanoate is synthesized from acetyl-CoA as a starter unit, and five molecules of malonyl-CoA. These building blocks are precursors of the fatty acid biosynthesis (Fellermeier et al., 2001).

Based on this, two pathways are feasibly possible, after analysis of a cDNA/EST library generated from female flowers (glands) of *C. sativa*. First, the hexanoyl residue could be obtained by an early termination of the fatty acid biosynthesis. Subsequently, the hexanoyl moiety of the resulting hexanoyl-ACP would be cleaved by a thioesterase or transferred to CoA by an ACP-CoA transacylase. Finally, acyl-CoA synthetase would catalyze the conversion of the obtained n-hexanol to hexanoyl-CoA (Marks et al., 2009). Second, n-hexanol could be produced by the breakdown of C18 unsaturated fatty acids via the lipoxygenase pathway (Marks et al., 2009; Stout, Boubakir, Ambrose, Purves, & Page, 2012). Nevertheless, further studies are necessary to clarify the origin of the hexanol moiety.

Hexanoyl-CoA is a medium-chain fatty acyl-CoA that can be detected in high amounts in *Cannabis* flowers (Stout et al., 2012). It is synthesized by an acyl-activating enzyme (AAE) called hexanoyl-CoA synthetase (Marks et al., 2009; Page & Stout, 2013). AAEs can use short, medium, long as well as very long-chain fatty acids as carboxylic acid substrates. Two novel enzymes were identified, *C. sativa* hexanoyl-CoA synthetase 1 (CsHCS1 or CsAAE1) and *C. sativa* hexanoyl-CoA synthetase 2 (CsHCS2 or CsAAE3) that are capable of producing hexanoyl-CoA using hexanoate and CoA as substrates. Based on transcript levels, CsHCS1 seems to be trichome-specific. Although CsHCS2 exhibits lower transcript levels, in comparison to CsHCS1, it is abundant in all tissues. The gene of CsHCS1 consists of a 2163-nucleotide open reading frame, and encodes a 720-amino acid polypeptide chain. The gene of CsHCS2 is composed of a 1632-nucleotide open reading frame, and encodes a 543-amino acid polypeptide chain. Both CsHCSs generally require divalent cations for activity. This was shown by adding Mg^{2+}, Mn^{2+}, and Co^{2+} to the enzyme assays. Thus, CsHCS1 preferentially accepts Mg^{2+}, and CsHCS2 Co^{2+}. The highest enzyme activity was detected at 40°C and pH 9 for both enzymes. Furthermore, both enzymes can be inhibited by high concentrations of CoA (Page & Stout, 2013; Stout et al., 2012).

Taken together, the published data suggest that CsHCS1 is the enzyme involved in the biosynthesis of cannabinoids: (1) it is the most abundant AAE in trichomes; (2) it is highly specific for short-chain fatty acyl-CoA, particularly hexanoate (K_M value in the nM range); and (3) it is localized in the cytosol, as suggested for the olivetol synthase (see later). In contrast, CsHCS2 is localized in the peroxisomes and accepts a broad range of substrates, while showing a K_M value for hexanoate in the mM range (Page & Stout, 2013; Stout et al., 2012).

The alkylresorcinol moiety of cannabinoids is derived from OA, the product of polyketide synthases (PKSs) that catalyze the aldol condensation of hexanoyl-CoA with three molecules of malonyl-CoA (Fellermeier et al., 2001; Raharjo, Chang, Choi, Peltenburg-Looman, & Verpoorte, 2004) (Fig. 2.2). The second precursor malonyl-CoA is predominantly derived from acetyl-CoA by carboxylation. The ATP-dependent reaction is catalyzed by an acetyl-CoA carboxylase (EC 6.4.1.2). The enzyme utilizes the first step in the fatty acid biosynthesis (Chen, Kim, Weng, & Browse, 2011; Konishi, Shinohara, Yamada, & Sasaki, 1996). Taura et al. (2009) discovered a plant type III PKS in flowers and rapidly expanding leaves of *C. sativa*. The gene of olivetol synthase (OLS) encodes a 385-amino acid polypeptide chain that does

TABLE 2.1 Enzymes Involved In Cannabinoid Biosynthesis in C. *sativa* L

Enzyme		Accession no.[a]	EC no.	References
Olivetol synthase	OLS	AB164375	2.3.1.206	Taura et al. (2009)
Olivetolic acid cyclase	OAC	AFN42527.1	4.4.1.26	Gagne et al. (2012)
Cannabigerolic acid synthase	CBGAS	US2012/0144523 A1[b]	2.5.1.102	Fellermeier and Zenk (1998); Page and Boubakir (2012)
Cannabichromenic acid synthase	CBCAS		1.3.3.-	Morimoto et al. (1998)
Cannabidiolic acid synthase	CBDAS	AB292682	1.21.3.8	Taura et al. (2007a)
Tetrahydrocannabinolic acid synthase	THCAS	AB057805	1.21.3.7	Sirikantaramas et al. (2004)

The table lists the enzymes and the corresponding GenBank accession numbers involved in biosynthesis of *C. sativa* phytocannabinoids.
[a]*GenBank.*
[b]*Patent number.*

not contain a signal peptide (Table 2.1). The OLS protein has a theoretical molecular mass of 43 kDa, as confirmed by SDS-PAGE analysis. However, size-exclusion chromatography experiments revealed a molecular mass of about 89 kDa, indicating a homodimeric enzyme (Gagne et al., 2012; Taura et al., 2009). OLS (PKS-1) was preliminarily crystallized by Taguchi et al. (2008) and the structure was finally published by Yang et al. (2016). It is of interest that the enzyme does not produce OA, but olivetol, triketide pyrone, and tetraketide pyrone. Analysis of the amino acid sequence displayed a high similarity with those of *Medicago sativa* chalcone synthase (CHS), and other plant PKSs (60–70%). Additionally, the catalytic triade residues of CHS (Cys^{164}-His^{303}-Asn^{336}) are conserved (Taura et al., 2009). Since CHSs catalyze intramolecular C6 → C1 Claisen condensations, Raharjo, and coworkers were the first to suggest in 2004 (Raharjo et al., 2004) that OLS could be a stilbene synthase (STS). These enzymes catalyze C2 → C7 aldol condensations, followed by a decarboxylation step. Additionally, studies by Austin, Bowman, Ferrer, Schröder, & Noel (2004) showed that the cyclization reaction can be changed from a Claisen-type (CHS) to an aldol-type (STS) by substitution of a few amino acids in CHS (= aldol switch).

Nevertheless, since OLS alone is not capable to form OA, another enzyme/PKS might be involved in the biosynthesis. The missing enzyme should catalyze a C2 → C7 intramolecular aldol condensation upon which the carboxylate moiety is preserved. This is important since CBGAS does not accept olivetol as a prenyl donor (Fellermeier & Zenk, 1998). Gagne et al. (2012) isolated a gene encoding a 101-amino acid polypeptide chain. This small protein (12 kDa) shows similarities to a polyketide cyclase that belongs to the dimeric α + β barrel (DABB)-type protein family. Furthermore, the identified gene exhibits high expression levels in glandular trichomes. Together, this made the polyketide cyclase a promising candidate for the missing olivetolic acid cyclase (OAC).

Finally, using both OLS and OAC with hexanoyl-CoA and malonyl-CoA in one assay, the formation of OA, pentyldiacetic acid (triketide pyrone), and hexanoyltriacetic acid lactone (HTAL; tetraketide pyrone) could be demonstrated (Page & Gagne, 2013) (Fig. 2.2). It is assumed that OLS catalyzes the formation of an intermediate that is subsequently converted into OA by OAC (Gagne et al., 2012; Taguchi et al., 2008).

Biosynthesis of Geranyl Diphosphate

The monoterpene moiety of cannabinoids (Fig. 2.2) is derived from GPP. Its precursors, isopentenyl diphosphate (IPP), and dimethylallyl diphosphate (DMAPP), are predominantly (>98%) biosynthesized via the 2C-methyl-D-erythritol-4-phosphate (MEP) pathway [also termed as nonmevalonate pathway or 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway] (Fellermeier et al., 2001). These results are supported by Marks et al. (2009). They isolated RNA from the glands of a tetrahydrocannabinolic acid (THCA)-producing Cannabis strain and generated a cDNA library. After sequencing, they were able to identify all but one enzyme involved in the MEP pathway. Additionally, Stout et al. (2012) found high expression of MEP pathway genes in *Cannabis* flowers. Furthermore, in higher plants the MEP pathway, mainly involved in secondary metabolism, is localized in plastids (described in detail elsewhere, for example, Eisenreich, Bacher, Arigoni, & Rohdich, (2004), or Hunter (2007), whereas the mevalonate (MVA) pathway, predominantly contributing to primary metabolism, is localized in the cytosol. The compartmental separation between these two pathways is not absolute. The metabolites of both pathways can be transported bidirectionally across the plastid membranes (Eisenreich et al., 2004).

Subsequently, the head-to-tail condensation of IPP and DMAPP to form GPP is catalyzed by geranyl diphosphate synthase (Fig. 2.2) (Burke et al., 1999).

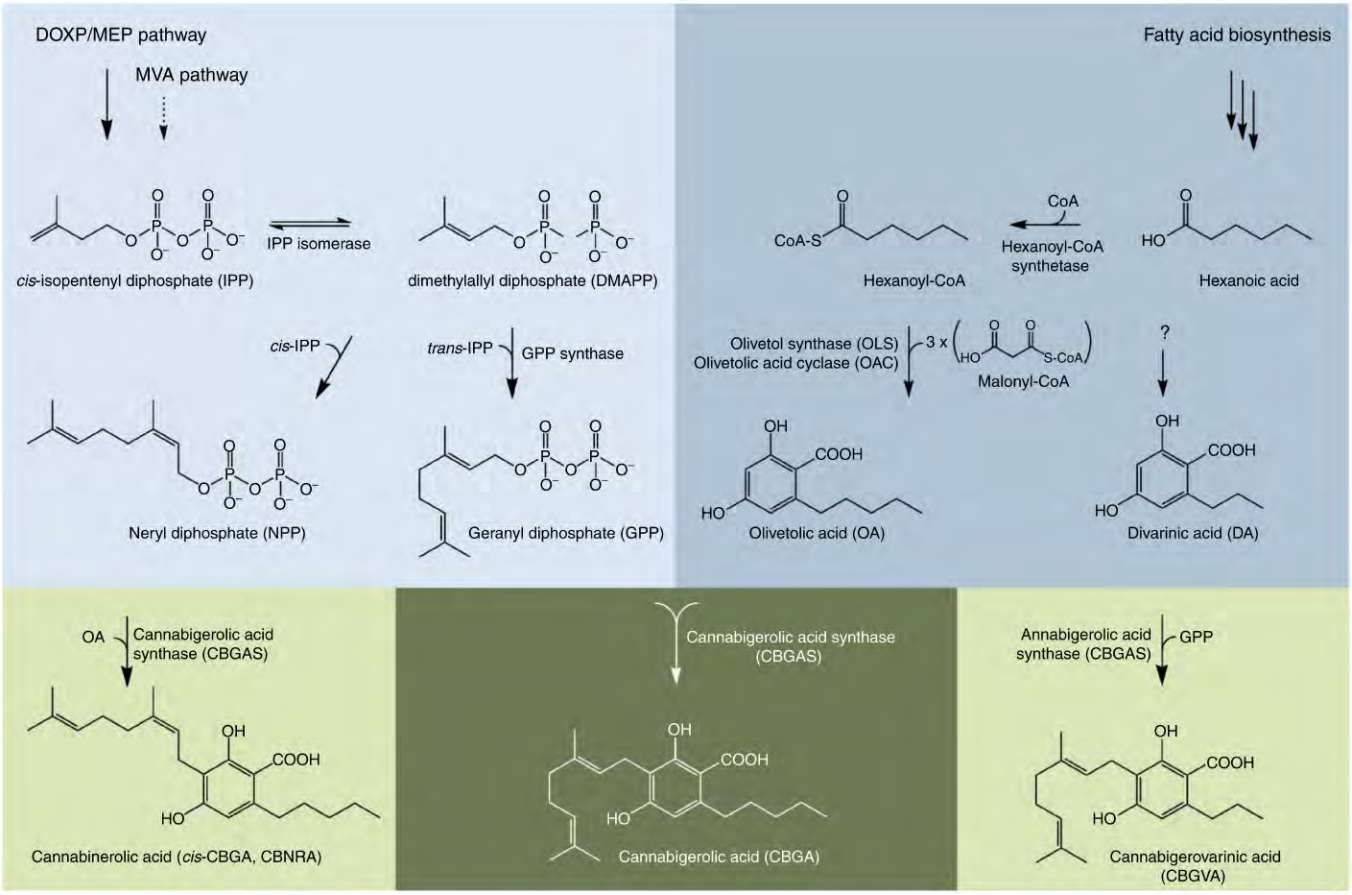


FIGURE 2.2 **Biosynthesis of cannabigerolic acid (CBGA).** The biosynthesis of the central intermediate CBGA is colored in dark green. The minor products CBNRA and CBGVA are shaded in light green. The precursor pathways are highlighted in light blue *(GPP)* and blue *(OA)*. MEP, 2C-methyl-D-erythritol-4-phosphate; DOXP, 1-deoxy-D-xylulose-5-phosphate; MVA, mevalonate (Burke, Wildung, & Croteau, 1999; de Meijer et al., 2009; Fellermeier & Zenk, 1998; Page & Gagne, 2013; Taura et al., 2009).

Cannabigerolic Acid Biosynthesis

Cannabigerolic acid synthase (CBGAS) or geranylpyrophosphate:olivetolate geranyltransferase (GOT) predominantly catalyzes the C-prenylation of OA by GPP to form CBGA (Fig. 2.2). CBGA is presumed to be the central precursor for cannabinoid biosynthesis, since different cyclization of the prenyl moiety leads to THCA or its isomers cannabichromenic acid (CBCA) and CBDA (Page & Boubakir, 2012; Sirikantaramas, Morimoto, & Shoyama, 2007).

Fellermeier and Zenk (1998) detected the enzyme in crude homogenates of rapidly expanding young leaves of *C. sativa*. This part of the plant contains the later enzymes of the THCA biosynthetic pathway (Morimoto, Komatsu, Taura, & Shoyama, 1997; Taura et al., 1995a). There are indications that CBGAS, like other prenyltransferases, is a membrane-bound prenyltransferase (Yamamoto, Kimata, Senda, & Inoue, 1997). However, Fellermeier and Zenk (1998) could not detect any enzyme activity in particulate fractions, but in the soluble fraction of the crude extract. Two major products were identified by mass spectrometry (MS) measurements as CBGA and its *cis*-isomer cannabinerolic acid (CBNRA; Fellermeier and Zenk, 1998, used CBNA instead of CBNRA). The enzyme activity was found to be Mg^{2+}-dependent. CBGAS seems to be specific for OA as a prenyl acceptor, but also accepts different prenyl donors like GPP and, to a lesser degree, neryl diphosphate (NPP) (Fig. 2.2). The production ratio of CBGA/CBNRA changes from 2:1 to 1:1 when NPP is used as a prenyl donor instead of GPP.

However, the aromatic prenyltransferase CBGAS seems to be a soluble enzyme, but Fellermeier and Zenk (1998) could not completely exclude a membrane-bound activity. Besides, two soluble hop prenyltransferases, involved in the biosynthesis of hop bitter acids, are described by Zuurbier, Fung, Scheffer, & Verpoorte (1998). Nevertheless, these are the only descriptions of soluble plant C-prenylating enzymes; until now, it was not possible to get the sequence information or to isolate the corresponding genes or enzymes.

Contradictorily, all known sequences of plant aromatic prenyltransferases belong to membrane-bound

enzymes (Yamamoto et al., 1997; Yamamoto, Senda, & Inoue, 2000; Zhao, Inoue, Kouno, & Yamamoto, 2003). This is in accordance with the second report dealing with the CBGAS (Page & Boubakir, 2012). They published a sequence of CBGAS that was mainly expressed in glandular trichomes of female flowers and young leaves of *Cannabis* plants. The gene encodes a 395-amino acid polypeptide chain showing a membrane-bound type of prenyltransferases. They were able to express the recombinant CBGAS in S*f*9 insect as well as in *Saccharomyces cerevisiae* cells, and verified the CBGAS activity in the microsomal fractions. Using MS measurements, CBGA (3-geranyl olivetolate; comparison with CBGA standard) was identified as the major product, and 5-geranyl olivetolate (identification only by LC-MS analysis) as the minor product. Furthermore, Page and Boubakir (2012) showed that CBGAS is specific only to GPP as a prenyl donor, and approves OA, olivetol, phlorisovalerophenone, naringenin, and resveratrol as prenyl acceptor. Additionally, the enzyme reaction is dependent on divalent cations, whereas the highest enzyme activity was obtained by using Mg^{2+} (Page & Boubakir, 2012).

CANNABINOID PATHWAY

CBGA, the central precursor of cannabinoid biosynthesis, is converted by three enzymes (Fig. 2.3): CBDAS, CBCAS, and THCAS. They predominantly use CBGA as substrate, and catalyze the stereoselective, oxidative cyclization of the monoterpene moiety of CBGA to CBDA, CBCA, or THCA, respectively. The THCAS and CBDAS reactions are oxygen-dependent, producing hydrogen peroxide proportional to either CBDA or THCA (Sirikantaramas et al., 2004; Taura et al., 2007b). Remarkably, the CBCAS reaction is oxygen independent, and can be inhibited by hydrogen peroxide. Thus, the enzyme seems not to be an oxygenase or a peroxidase (Morimoto, Komatsu, Taura, & Shoyama, 1998). Furthermore, all three enzymes also convert CBNRA, the

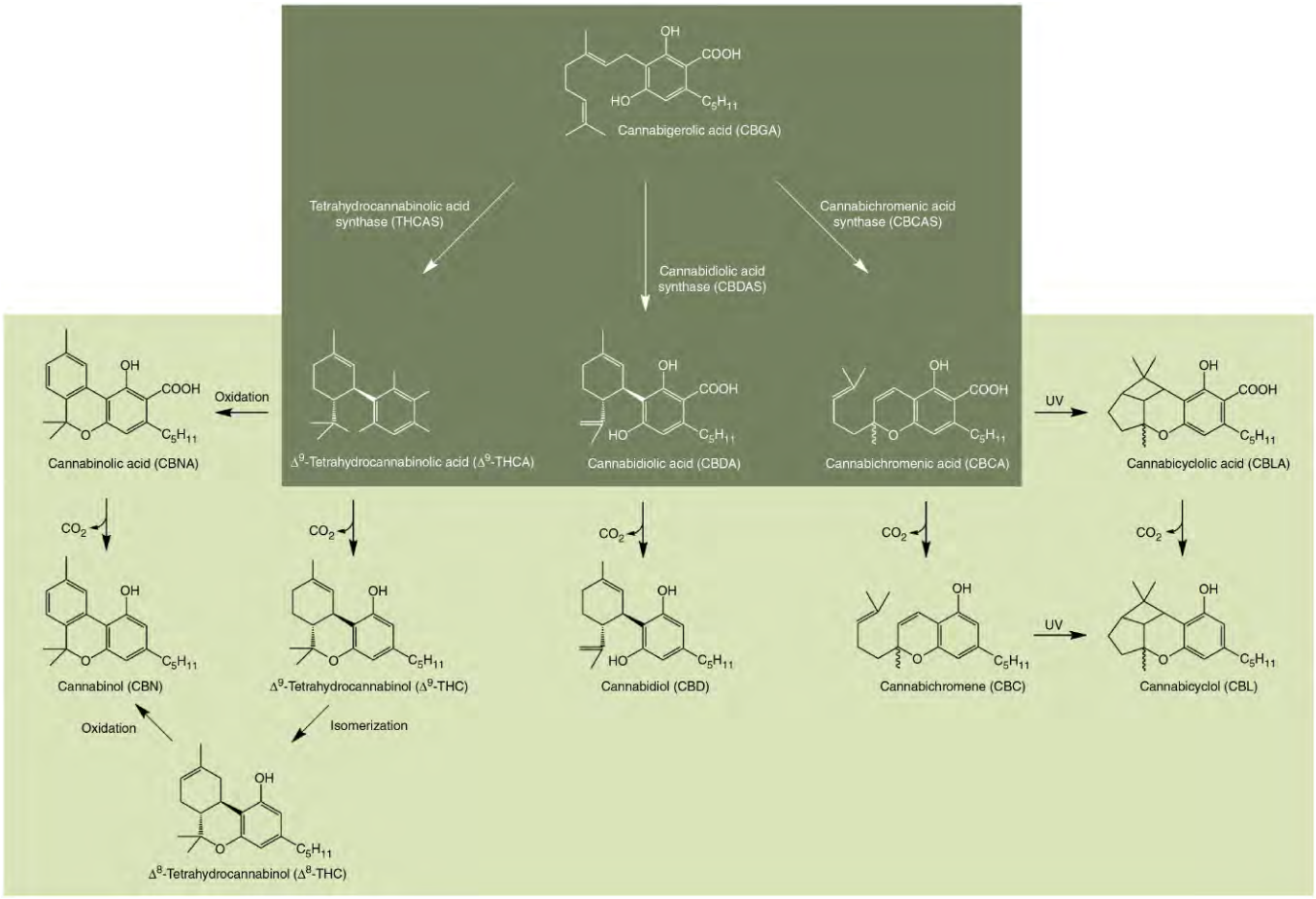


FIGURE 2.3 **Biosynthesis of cannabinoids.** The enzymatically catalyzed reactions are highlighted in dark green. All nonenzyme-dependent modifications reactions are colored in light green. Biosynthesis of C3-cannabinoids starting from cannabigerovarinic acid (CBGVA) is carried out by the same enzymes and for better clarity not shown (Crombie et al., 1968; de Meijer, 2011; Morimoto et al., 1998; Shoyama, Fujita, Yamauchi, & Nishioka, 1968; Shoyama, Oku, Yamauchi, & Nishioka, 1972; Taura et al., 1995a; 1996).

cis-isomer of CBGA, with a lower specificity (Morimoto et al., 1998; Shoyama et al., 2012; Sirikantaramas, Morimoto, & Shoyama, 2007; Taura et al., 1995b; Taura, Morimoto, & Shoyama, 1996). Since no enzymatic activity was detectable using the neutral cannabinoid CBG, the carboxyl group in CBGA seems to be essential for the enzymatic reactions catalyzed by THCAS, CBDAS, and CBCAS (Morimoto et al., 1997; Taura et al., 1995a; Taura et al., 1996).

Besides, it was postulated that THCA is biosynthesized and stored in the storage cavity of the glandular trichomes of Cannabis plants (Sirikantaramas, Morimoto, & Shoyama, 2007).

Tetrahydrocannabinolic Acid Synthase

The THCAS gene encodes a 545-amino acid polypeptide chain (Table 2.1). According to Sirikantaramas et al. (2004), a 28 amino acid long signal peptide is cleaved in the processed THCAS, leading to a protein of 517 amino acids. The mature THCAS has a theoretical molecular mass of 59 kDa. An actual mass of about 75 kDa was detected using SDS-PAGE (Taura et al., 1995b). This could be explained by posttranslational modifications, since eight possible Asn glycosylation sites were confirmed (Sirikantaramas et al., 2004). Furthermore, deglycosylated THCAS indeed showed a molecular mass of 59 kDa, and remained fully active (Taura et al., 2007b). THCAS is a monomeric enzyme with the highest activity between pH 5.5 and pH 6.0 (Taura et al., 1995b). Sequence comparison identified similarities to the berberine bridge enzyme (BBE) of *Eschscholzia californica* (Shoyama et al., 2012). BBE belongs to the family of oxidoreductases and has a covalently bound FAD (Kutchan & Dittrich, 1995). The THCAS amino acid sequence revealed a flavinylation consensus sequence (Arg^{110}-Ser-Gly-Gly-His^{114}) in which His^{114} is probably the FAD-binding site (Sirikantaramas et al., 2004). This could be confirmed by X-ray crystallography (PDB ID: 3VTE) at a resolution of 2.75Å. The results show that the enzyme is composed of two domains and one FAD binding pocket present in between. Besides His^{114}, a second residue, Cys^{176}, could be identified to be covalently bound to the FAD (Shoyama et al., 2012). Based on X-ray structure data and mutational analysis of THCAS, a possible catalytic reaction mechanism of THCAS was proposed by Shoyama et al. (2012), assigning a central role to Tyr^{484} in the catalytic mechanism (Fig. 2.5). Nevertheless, since the crystal structure was published without substrate analoga, further studies are necessary to verify the suggested mechanism.

Cannabis plants can be divided into two groups: "fiber-type" and "drug-type" plants (see dictionary). Alignment of THCAS coding sequences from "fiber-type" and "drug-type" plants showed 37 major amino acid substitutions. These substitutions seem to be the reason for decreased THCAS activity in "fiber-type" strains (Kojoma, Seki, Yoshida, & Muranaka, 2006). Minisequencing of samples from both types of *Cannabis* plants showed three different single nucleotide polymorphism (SNP) genotypes. "Fiber-type" plants are homozygous for the inactive THCAS form. "Drug-type" plants are either homozygous or heterozygous for the active form of THCAS. It seems that only a single copy of the gene encoding the active THCAS form is necessary for the biosynthesis of THCA (Rotherham & Harbison, 2011).

Cannabidiolic Acid Synthase

The gene of the wildtype CBDAS encodes a 544-amino acid polypeptide (Table 2.1). According to Taura et al. (2007b), processed CBDAS consists of 517 amino acids following cleavage of the 28 amino acid long signal peptide. The mature CBDAS has a theoretical molecular mass of 59 kDa. An actual mass of about 74 kDa was detected by SDS-PAGE that is possibly caused by posttranslational glycosylation of seven Asn residues (Taura et al., 1996; Taura et al., 2007b). CBDAS is a monomeric enzyme with a pH optimum of 5.0 (Taura et al., 1996). A comparison between THCAS and CBDAS revealed a sequence similarity of 84% (Taura et al., 2007b) (Figs. 2.4 and 2.5). Like THCAS, CBDAS is a flavinated enzyme in which His^{114} and Cys^{176} are most likely the FAD-binding sites. Since CBDAS exhibits structural and biochemical properties related to those of THCAS, it is probable that the reaction mechanism of CBDAS is similar to that of THCAS (Taura et al., 2007b).

Cannabichromenic Acid Synthase (CBCAS)

The sequence of CBCAS is still unknown. Morimoto et al. (1998) purified the enzyme to apparent homogeneity, but its sequence is not yet available in public databases. According to van Bakel et al. (2011), possible CBCAS candidates are currently analyzed biochemically.

However, CBCAS was isolated and partially purified from young leaves of *C. sativa* (Morimoto et al., 1997; 1998). In contrast to CBDAS and THCAS, CBCAS seems to be homodimer with a determined native molecular mass of 136 kDa and a maximum activity at pH 6.5. A molecular mass of 71 kDa was estimated for the monomers using SDS-PAGE. According to kinetic data, CBCAS has a higher affinity for CBGA than THCAS and CBCAS (Morimoto et al., 1998). CBCA and its neutral form CBC are both racemic. Studies of Morimoto et al. (1997) suggested that both enantiomers of CBCA are formed by a CBCAS catalyzed reaction with a molar ratio of 5:1. But it is still unknown which of the two isomers is the major product (Gaoni & Mechoulam, 1971; Morimoto et al., 1997; Taura et al., 2007a).

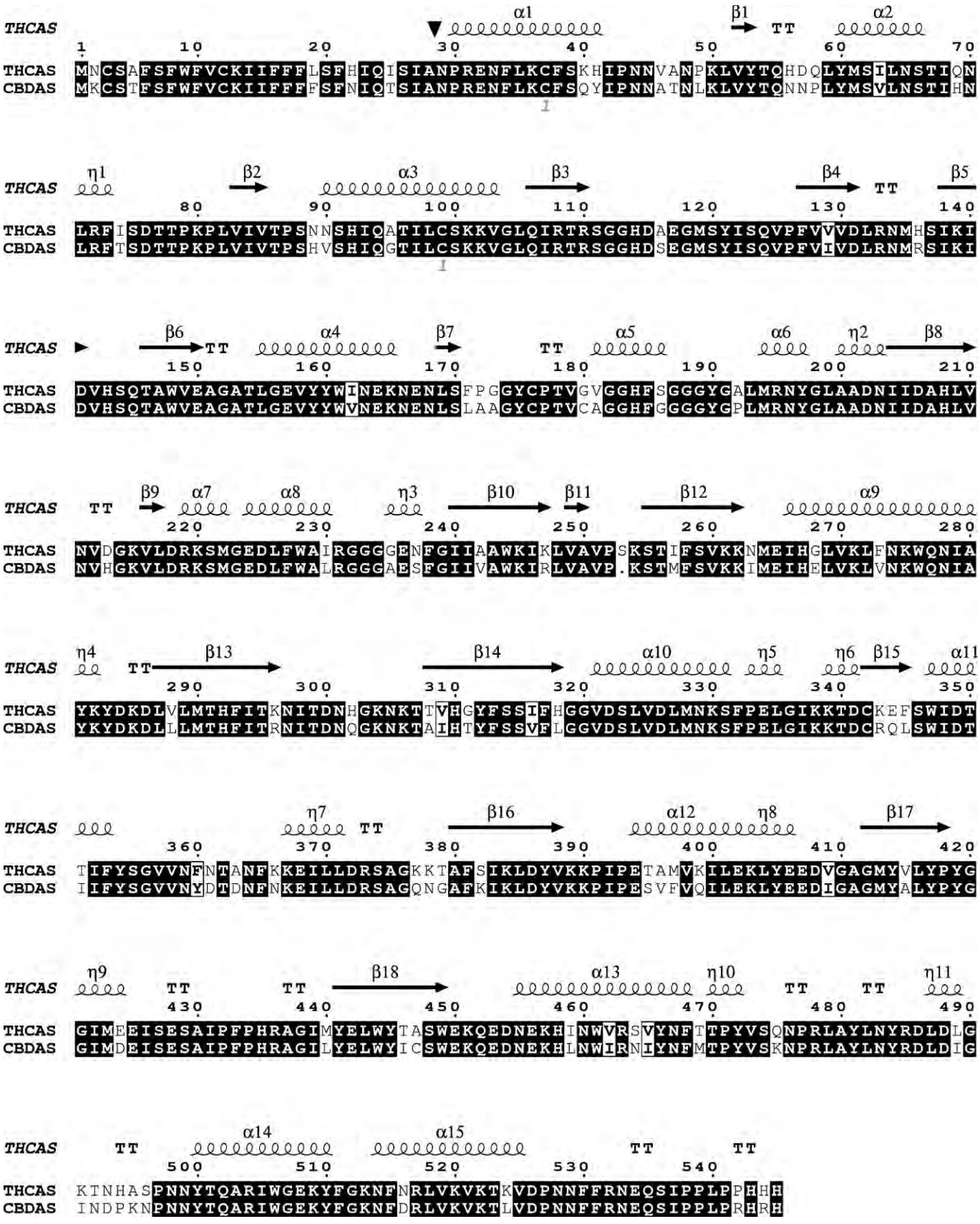


FIGURE 2.4 Alignment of amino acid sequences of THCA synthase and CBDA synthase. The alignment was performed with CLUSTAL W using the BLOSUM 62 matrix (Henikoff & Henikoff, 1992; Larkin et al., 2007). The signal peptide cleavage sites are indicated by a triangle. Secondary elements (α-alpha-helices; β-beta-sheets; TT-turns; η-3_{10} helix) are shown for the THCAS. Fully conserved residues are shaded in black. The sequences show an overall identity of 84%. The figure was made with ESPript 3.0 (Robert & Gouet, 2014). THCAS, tetrahydrocannabinolic acid synthase; CBDAS, cannabidiolic acid synthase.

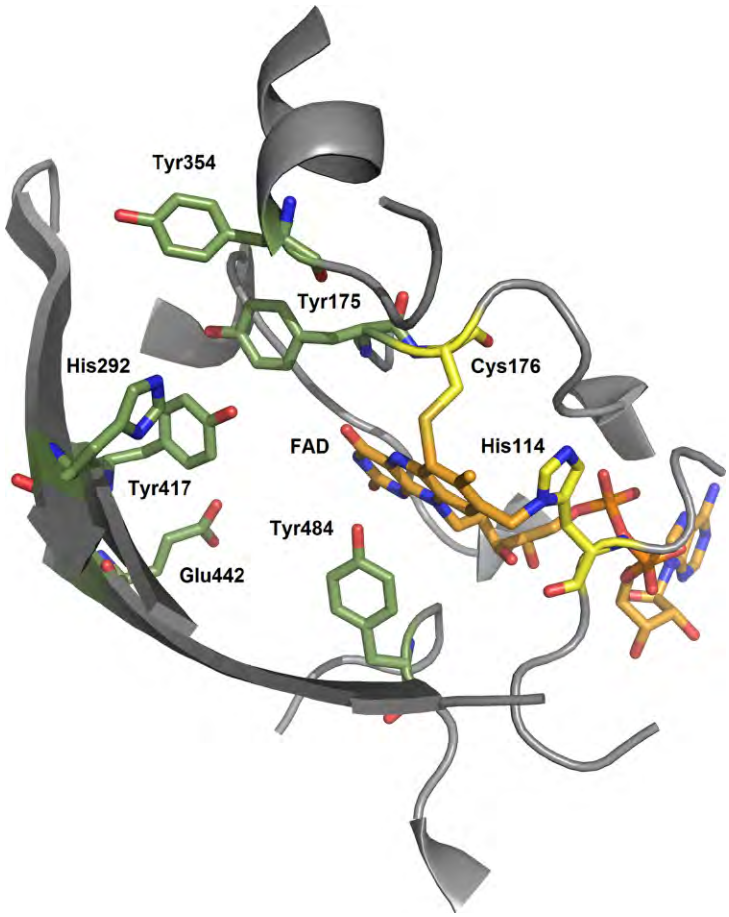


FIGURE 2.5 **X-ray structure of the active center of THCAS.** The backbone is shown as cartoon diagram (PDB ID: 3VTE). The FAD molecule *(orange)* is covalently attached to His^{114} and Cys^{176} *(yellow)*. The active site residues are highlighted in green (Shoyama et al., 2012). The close-up of active center was prepared with PyMOL. THCAS, tetrahydrocannabinolic acid synthase.

Cannabinoids with Propyl Side Chains

In contrast to the classic C5-phytocannabinoids, which contain an n-pentyl side chain, cannabinoids with an n-propyl side chain are called C3-phytocannabinoids or propyl cannabinoids. The C-prenylation of divarinic acid (DA) instead of OA by GPP yields in cannabigerovarinic acid (CBGVA) (Fig. 2.2) (de Meijer, Hammond, & Micheler, 2009). The formation of propyl cannabinoids does not occur by shortening the side chain of pentyl cannabinoids (Kajima & Piraux, 1982). CBGVA is the central branch-point intermediate in the biosynthesis of C3-cannabinoid acids, like CBGA for pentyl cannabinoid acids. The enzymes CBDAS, CBCAS, and THCAS are not selective for the length of the alkyl side chain, and can use both as a substrate. The resulting cannabinoids are called cannabidivarinic acid (CBDVA), cannabichrovarinic acid (CBCVA), and tetrahydrocannabivarinic acid (THCVA). The diverse amount of 2-carboxylic acids in different *Cannabis* strains is caused by dissimilar enzyme specificities at the level of CBGA or CBGA-analogs formation (de Meijer et al., 2009; Shoyama, Hirano, & Nishioka, 1984). Relatively high amounts of tetrahydrocannabivarin (THCV) and/or cannabidivarin (CBDV) are usually only detectable in *Cannabis indica* (de Zeeuw, 1972).

MINI-DICTIONARY

Cannabinoids Cannabinoids are a group of terpenophenolic compounds. They show affinities to cannabinoid receptors (CB_1, CB_2) or are structurally related to tetrahydrocannabinol (THC). Cannabinoids can be differentiated into phytocannabinoids, synthetic cannabinoids, and endocannabinoids.

CBGA Cannabigerolic acid (CBGA) is the central precursor of phytocannabinoid biosynthesis. It is nonpsychoactive.

"Drug-type" plants These are THCA-rich plants. The THCA is converted into psychoactive Δ^9-THC by a nonenzymatic decarboxylation that enables the plants to be labelled as THC-rich.

"Fiber-type" plants "Fiber-type" plants are also known as "nondrug" plants. These plants have a low (<0.2%) or no THCA content, but they contain a high amount of cannabidiolic acid (CBDA).

Phytocannabinoids Phytocannabinoids are a unique group of secondary metabolites (cannabinoids) occurring naturally in plants. Other names include natural cannabinoids or herbal cannabinoids (see Key facts).

Δ^8-THC In *Cannabis* plants, Δ^8-tetrahydrocannabinol (Δ^8-THC) is detectable in low amounts (<1% of the present Δ^9-THC). Like

Δ^9-THC, it is also psychoactive. Maybe Δ^8-THC is an artefact of extraction and/or analysis process. The term THC includes a combination of Δ^8-THC and Δ^9-THC.

Δ^9-THC Δ^9-Tetrahydrocannabinol (Δ^9-THC) and Δ^1-THC describe the same compound, differing in the numbering system used (dibenzopyran-numbering and monoterpene-numbering system, respectively). Δ^9-THC is responsible for the psychoactive effects of *Cannabis* products. It binds to the human cannabinoid receptors located in the central and peripheral nervous system. Misleadingly, Δ^9-THC is termed as the main component of drug-type *Cannabis* plants (see THCA).

Δ^9-THCA Δ^9-Tetrahydrocannabinolic acid (THCA), not THC, is the main component of drug-type *Cannabis* plants. It is nonpsychoactive. THCA is converted into neutral psychoactive Δ^9-THC by a nonenzymatic decarboxylation during heating or storage.

Acknowledgment

We gratefully acknowledge Parijat Kusari for critically reading this manuscript.

References

Austin, M. B., Bowman, M. E., Ferrer, J. L., Schröder, J., & Noel, J. P. (2004). An aldol switch discovered in stilbene synthases mediates cyclization specificity of type III polyketide synthases. *Chemistry and Biology*, *11*, 1179–1194.

Burke, C. C., Wildung, M. R., & Croteau, R. (1999). Geranyl diphosphate synthase: Cloning, expression, and characterization of this prenyltransferase as a heterodimer. *Proceedings of the National Academy of Sciences of the United States of America*, *96*, 13062–13067.

Chen, H., Kim, H. U., Weng, H., & Browse, J. (2011). Malonyl-CoA synthetase, encoded by *ACYL ACTIVATING ENZYME13*, is essential for growth and development of Arabidopsis. *The Plant Cell*, *23*, 2247–2262.

Crombie, L., Ponsford, R., Shani, A., Yagnitinsky, B., & Mechoulam, R. (1968). Hashish components. Photochemical production of cannabicyclol from cannabichromene. *Tetrahedron Letters*, *9*, 5771–5772.

de Meijer, E. (2011). *Cannabis sativa* plants rich in cannabichromene and its acid, extracts thereof and methods of obtaining extracts therefrom. U.S. Patent No. 2011/0098348 A1

de Meijer, E., Hammond, K., & Micheler, M. (2009). The inheritance of chemical phenotype in *Cannabis sativa* L.(III): variation in cannabichromene proportion. *Euphytica*, *165*, 293–311.

de Zeeuw, R. A., Wijsbek, J., Breimer, D. D., Vree, T. B., van Ginneken, C. A., & van Rossum, J. M. (1972). Cannabinoids with a propyl side chain in Cannabis: occurrence and chromatographic behaviour. *Science*, *175*, 778–779.

Eisenreich, W., Bacher, A., Arigoni, D., & Rohdich, F. (2004). Biosynthesis of isoprenoids via the non-mevalonate pathway. *Cellular and Molecular Life Sciences*, *61*, 1401–1426.

ElSohly, M., & Slade, D. (2005). Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life Sciences*, *78*, 539–548.

Fellermeier, M., Eisenreich, W., Bacher, A., & Zenk, M. H. (2001). Biosynthesis of cannabinoids. Incorporation experiments with ^{13}C-labeled glucoses. *European Journal of Biochemistry*, *268*, 1596–1604.

Fellermeier, M., & Zenk, M. H. (1998). Prenylation of olivetolate by a hemp transferase yields cannabigerolic acid, the precursor of tetrahydrocannabinol. *FEBS Letters*, *427*, 283–285.

Fischedick, J. T., Hazekamp, A., Erkelens, T., Choi, Y. H., & Verpoorte, R. (2010). Metabolic fingerprinting of *Cannabis sativa* L., cannabinoids and terpenoids for chemotaxonomic and drug standardization purposes. *Phytochemistry*, *71*, 2058–2073.

Gagne, S. J., Stout, J. M., Liu, E., Boubakir, Z., Clark, S. M., & Page, J. E. (2012). Identification of olivetolic acid cyclase from *Cannabis sativa* reveals a unique catalytic route to plant polyketides. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 12811–12816.

Gaoni, Y., & Mechoulam, R. (1964). Isolation, structure, and partial synthesis of an active constituent of hashish. *Journal of the American Chemical Society*, *86*, 1646–1647.

Gaoni, Y., & Mechoulam, R. (1971). The isolation and structure of Δ^1-tetrahydrocannabinol and other neutral cannabinoids from hashish. *Journal of the American Chemical Society*, *93*, 217–224.

Gertsch, J., Pertwee, R. G., & Di Marzo, V. (2010). Phytocannabinoids beyond the Cannabis plant – do they exist? *British Journal of Pharmacology*, *160*, 523–529.

Henikoff, S., & Henikoff, J. G. (1992). Amino acid substitution matrices from protein blocks. *Proceedings of the National Academy of Sciences of the United States of America*, *89*, 10915–10919.

Hunter, W. N. (2007). The non-mevalonate pathway of isoprenoid precursor biosynthesis. *Journal of Biological Chemistry*, *282*, 21573–21577.

Kajima, M., & Piraux, M. (1982). The biogenesis of cannabinoids in *Cannabis sativa*. *Phytochemistry*, *21*, 67–69.

Kojoma, M., Seki, H., Yoshida, S., & Muranaka, T. (2006). DNA polymorphisms in the tetrahydrocannabinolic acid (THCA) synthase gene in "drug-type" and "fiber-type" *Cannabis sativa* L. *Forensic Science International*, *159*, 132–140.

Konishi, T., Shinohara, K., Yamada, K., & Sasaki, Y. (1996). Acetyl-CoA carboxylase in higher plants: Most plants other than gramineae have both the prokaryotic and the eukaryotic forms of this enzyme. *Plant and Cell Physiology*, *37*, 117–122.

Kutchan, T. M., & Dittrich, H. (1995). Characterization and mechanism of the berberine bridge enzyme, a covalently flavinylated oxidase of benzophenanthridine alkaloid biosynthesis in plants. *Journal of Biological Chemistry*, *270*, 24475–24481.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., & Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, *23*, 2947–2948.

Marks, M. D., Tian, L., Wenger, J. P., Omburo, S. N., Soto-Fuentes, W., He, J., Gang, D. R., Weiblen, G. D., & Dixon, R. A. (2009). Identification of candidate genes affecting Δ^9-tetrahydrocannabinol biosynthesis in *Cannabis sativa*. *Journal of Experimental Botany*, *60*, 3715–3726.

Morimoto, S., Komatsu, K., Taura, F., & Shoyama, Y. (1997). Enzymological evidence for cannabichromenic acid biosynthesis. *Journal of Natural Products*, *60*, 854–857.

Morimoto, S., Komatsu, K., Taura, F., & Shoyama, Y. (1998). Purification and characterization of cannabichromenic acid synthase from *Cannabis sativa*. *Phytochemistry*, *49*, 1525–1529.

Page, J.E. & Boubakir, Z. (2012). Aromatic prenyltransferase from Cannabis. U.S. Patent No. 2012/0144523 A1.

Page, J.E. & Gagne, S. (2013). Genes and proteins for aromatic polyketide synthesis. U.S. Patent No. 2013/0067619 A1.

Page, J.E. & Stout, J.M. (2013). Genes and proteins for alkanoyl-CoA synthesis. Patent No. WO 2013/006953 A1.

Raharjo, T. J., Chang, W. -T., Choi, Y. H., Peltenburg-Looman, A. M. G., & Verpoorte, R. (2004). Olivetol as product of a polyketide synthase in *Cannabis sativa* L. *Plant Science*, *166*, 381–385.

Razdan, R. K., Puttick, A. J., Zitko, B. A., & Handrick, G. R. (1972). Hashish VI[1]: Conversion of (-)-$\Delta^{1(6)}$-tetrahydrocannabinol to (-)-$\Delta^{1(7)}$-tetrahydrocannabinol. Stability of (-)-Δ^1 and (-)-$\Delta^{1(6)}$-tetrahydrocannabinols. *Experientia*, *28*, 121–122.

Robert, X., & Gouet, P. (2014). Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Research*, *42*, W320–W324.

Rotherham, D., & Harbison, S. A. (2011). Differentiation of drug and non-drug Cannabis using a single nucleotide polymorphism (SNP) assay. *Forensic Science International*, *207*, 193–197.

Schultes, R., Klein, W., Plowman, T., & Lockwood, T. (1974). Cannabis: An example of taxonomic neglect. *Botanical Museum Leaflets, Harvard University*, *23*, 337–367.

Shoyama, Y., Fujita, T., Yamauchi, T., & Nishioka, I. (1968). Cannabichromenic acid, a genuine substance of cannabichromene. *Chemical and Pharmaceutical Bulletin*, *16*, 1157–1158.

Shoyama, Y., Hirano, H., & Nishioka, I. (1984). Biosynthesis of propyl cannabinoid acid and its biosynthetic relationship with pentyl and methyl cannabinoid acids. *Phytochemistry*, *23*, 1909–1912.

Shoyama, Y., Oku, R., Yamauchi, T., & Nishioka, I. (1972). Cannabis. VI. Cannabicyclolic acid. *Chemical and Pharmaceutical Bulletin*, *20*, 1927–1930.

Shoyama, Y., Tamada, T., Kurihara, K., Takeuchi, A., Taura, F., Arai, S., Blaber, M., Shoyama, Y., Morimoto, S., & Kuroki, R. (2012). Structure and function of Δ^1-tetrahydrocannabinolic acid (THCA) synthase, the enzyme controlling the psychoactivity of *Cannabis sativa*. *Journal of Molecular Biology*, *423*, 96–105.

Sirikantaramas, S., Morimoto, S., Shoyama, Y., Ishikawa, Y., Wada, Y., & Taura, F. (2004). The gene controlling marijuana psychoactivity: molecular cloning and heterologous expression of Δ^1-tetrahydrocannabinolic acid synthase from *Cannabis sativa* L. *Journal of Biological Chemistry*, *279*, 39767–39774.

Sirikantaramas, S. T. S., Morimoto, S., & Shoyama, Y. (2007). Recent advances in *Cannabis sativa* research: biosynthetic studies and its potential in biotechnology. *Current Pharmaceutical Biotechnology*, *8*, 237–243.

Stout, J. M., Boubakir, Z., Ambrose, S. J., Purves, R. W., & Page, J. E. (2012). The hexanoyl-CoA precursor for cannabinoid biosynthesis is formed by an acyl-activating enzyme in *Cannabis sativa* trichomes. *The Plant Journal*, *71*, 353–365.

Suzuki, Y., Kurano, M., Esumi, Y., Yamaguchi, I., & Doi, Y. (2003). Biosynthesis of 5-alkylresorcinol in rice: Incorporation of a putative fatty acid unit in the 5-alkylresorcinol carbon chain. *Bioorganic Chemistry*, *31*, 437–452.

Taguchi, C., Taura, F., Tamada, T., Shoyama, Y., Tanaka, H., Kuroki, R., & Morimoto, S. (2008). Crystallization and preliminary X-ray diffraction studies of polyketide synthase-1 (PKS-1) from *Cannabis sativa*. *Acta Crystallographica. Section F, Structural biology and crystallization communications*, *64*, 217–220.

Taura, F., Dono, E., Sirikantaramas, S., Yoshimura, K., Shoyama, Y., & Morimoto, S. (2007). Production of Δ^1-tetrahydrocannabinolic acid by the biosynthetic enzyme secreted from transgenic *Pichia pastoris*. *Biochemical and Biophysical Research Communications*, *361*, 675–680.

Taura, F., Morimoto, S., & Shoyama, Y. (1995a). Cannabinerolic acid, a cannabinoid from *Cannabis sativa*. *Phytochemistry*, *39*, 457–458.

Taura, F., Morimoto, S., & Shoyama, Y. (1995b). First direct evidence for the mechanism of Δ^1-tetrahydrocannabinolic acid biosynthesis. *Journal of the American Chemical Society*, *117*, 9766–9767.

Taura, F., Morimoto, S., & Shoyama, Y. (1996). Purification and characterization of cannabidiolic acid synthase from *Cannabis sativa* L. *Journal of Biological Chemistry*, *271*, 17411–17416.

Taura, F., Sirikantaramas, S., Shoyama, Y., Shoyama, Y., & Morimoto, S. (2007a). Phytocannabinoids in *Cannabis sativa*: recent studies on biosynthetic enzymes. *Chemistry and Biodiversity*, *4*, 1649–1663.

Taura, F., Sirikantaramas, S., Shoyama, Y., Yoshikai, K., Shoyama, Y., & Morimoto, S. (2007b). Cannabidiolic-acid synthase, the chemotype-determining enzyme in the fiber-type *Cannabis sativa*. *FEBS Letters*, *581*, 2929–2934.

Taura, F., Tanaka, S., Taguchi, C., Fukamizu, T., Tanaka, H., Shoyama, Y., & Morimoto, S. (2009). Characterization of olivetol synthase, a polyketide synthase putatively involved in cannabinoid biosynthetic pathway. *FEBS Letters*, *583*, 2061–2066.

van Bakel, H., Stout, J., Cote, A., Tallon, C., Sharpe, A., Hughes, T., & Page, J. (2011). The draft genome and transcriptome of *Cannabis sativa*. *Genome Biology*, *12*, R102.

Yamamoto, H., Kimata, J., Senda, M., & Inoue, K. (1997). Dimethylallyl diphosphate: Kaempferol 8-dimethylallyl transferase in *Epimedium diphyllum* cell suspension cultures. *Phytochemistry*, *44*, 23–28.

Yamamoto, H., Senda, M., & Inoue, K. (2000). Flavanone 8-dimethylallyltransferase in *Sophora flavescens* cell suspension cultures. *Phytochemistry*, *54*, 649–655.

Yang, X., Matsui, T., Kodama, T., Mori, T., Zhou, X., Taura, F., Noguchi, H., Abe, I., & Morita, H. (2016). Structural basis for olivetolic acid formation by a polyketide cyclase from *Cannabis sativa*. *FEBS Journal*, *283*, 1088–1106.

Zhao, P., Inoue, K., Kouno, I., & Yamamoto, H. (2003). Characterization of leachianone G 2″-dimethylallyltransferase, a novel prenyl side-chain elongation enzyme for the formation of the lavandulyl group of sophoraflavanone G in *Sophora flavescens* Ait. cell suspension cultures. *Plant Physiology*, *133*, 1306–1313.

Zuurbier, K. W. M., Fung, S. -Y., Scheffer, J. J. C., & Verpoorte, R. (1998). In-vitro prenylation of aromatic intermediates in the biosynthesis of bitter acids in *Humulus lupulus*. *Phytochemistry*, *49*, 2315–2322.

CHAPTER

3

Increasing Plant Concentrations of THC and Implications on Health Related Disorders

V. Vindenes, J. Mørland

Department of Drug Abuse Research, Division of Forensic Sciences, Norwegian Institute of Public Health, Oslo, Norway

SUMMARY POINTS

- Increased cannabis-related problems have been reported in several countries.
- Several studies have shown that the herbal cannabis market has changed worldwide in the later years toward increasing THC content (ie, increased potency).
- Cannabis potency varies widely between and within countries, and between different products.
- The literature does not support the theory that cannabis users titrate the dosage of cannabis according to their experience of THC-related "high." As a consequence, the THC exposure seems to increase when more potent cannabis is available.
- Increased concentrations of THC over the last decade have been found in blood samples from apprehended drivers and victims of fatal aviation accidents.
- In addition to THC, the content of other cannabinoids, like CBD, might influence the THC-effects.
- High-potency cannabis with low concentrations of CBD might be the reason for the increase in cannabis-related problems, but the causality has not been proven.
- The increase in cannabis-related problems might also be due to the earlier onset of cannabis use, leading to both exposure during a possible more vulnerable period, and increased life-time exposure to THC.

KEY FACTS OF EMCDDA

- The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) is an agency of the European Union.
- EMCDDA was established in 1993 and is located in Lisbon, Portugal.
- The role is to gather, analyze, and disseminate "factual, objective, reliable and comparable information" on drugs and drug addiction.
- EMCDDA provides evidence-based knowledge regarding the drug situation in Europe

KEY FACTS OF PANSS

- Positive and negative syndrome scale (PANSS) is used for measuring the symptoms associated with schizophrenia.
- Positive symptoms include paranoid and grandiose delusions, suspiciousness, fragmented thinking, conceptual disorganization, and perceptual alterations.
- Negative symptoms include lack of spontaneity, blunted affect, emotional withdrawal, and psychomotor retardation.
- The rating of the patients symptoms, using PANSS, is conducted through a brief interview.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00004-1
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

CBD	Cannabidiol
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
PANSS	Positive and negative syndrome scale
THC	Tetrahydrocannabinol

INTRODUCTION

Cannabis is the most popular illicit drug worldwide, with high rates of use among adolescents and young adults (Copeland, Rooke, & Swift, 2013). In the United Kingdom, it has been reported that a higher number of the 16- to 18-year-olds have smoked cannabis, compared to those who have not (Morgan, Schafer, Freeman, & Curran, 2010). A stable or decreasing trend in cannabis use, especially among young adults, has been reported in Western and Central Europe (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 2011). Cannabis is mainly used for recreational purposes, and is widely considered to be a safe drug, with relaxing and euphoric effects. There are, however, several specific adverse effects; for example, cognitive impairment (D'Souza et al., 2004; Bocker et al., 2010; Morgan et al., 2011), psychomotor impairment (Robbe, 2008; Schwope, Bosker, Ramaekers, Gorelick, & Huestis, 2012), anxiety attacks and paranoia (Bonn-Miller, Zvolensky, Bernstein, & Stickle, 2008), as well as development of psychosis (D'Souza, Sewell, & Ranganathan, 2009; Luzi, Morrison, Powell, Di, & Murray, 2008).

Different types of preparations are derived from the *Cannabis sativa* plant, and the main types are marijuana, resin (hash), and sinsemilla (skunk). Δ^9-tetrahydrocannabinol (THC) is the main psychoactive substance in all the different types. The plant contains almost 500 compounds, including 70 cannabinoids (McLaren, Swift, Dillon, & Allsop, 2008; Radhakrishnan, Wilkinson, & D'Souza, 2014). One of the other psychoactive cannabinoids is cannabidiol (CBD). The exact mechanisms by which CBD exerts its effects are not known (Niesink & van Laar, 2013), but it has been suggested it acts as a functional antagonist for some of the effects of THC, and may have anxiolytic and antipsychotic effects (McLaren et al., 2008; Morgan et al., 2011). CBD might thus offset some of the psychoactive effects of THC, thereby modulating the effects of cannabis intake.

Concerns have been raised regarding the currently available cannabis that is stronger than in the past. Higher-potency cannabis products have been claimed to cause increased exposure of THC and, accordingly, to have more negative health implications for the users. A higher number of unexpected adverse effects after cannabis consumption has been reported, and the number of people seeking treatment for dependence or cannabis-related problems has increased (Hall, 2009; Morgan et al., 2010; European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 2011).

CANNABIS PLANTS: HAS THE POTENCY CHANGED?

From the *Cannabis sativa* plant, different types of preparations are derived, and the main types are:

- Marijuana
- Resin (hash)
- Sinsemilla (skunk)

Previous studies have shown that the content of THC and other major cannabinoids varies widely between different illicit cannabis preparations (Potter, Clark, & Brown, 2008; ElSohly et al., 2000).

THC content in different plants varies according to a number of factors:

- part of the plant utilized (in decreasing order regarding THC potency: buds, leaves, stems, and seeds)
- the preparation and sample storage
- genetic factors
- cultivation techniques (eg, growing female plants isolated, resulting in seedless cannabis, and hence the name sinsemilla)
- outdoor versus indoor cultivation—indoor cultivation controls light, humidity, and temperature.

Several studies have shown that the herbal cannabis market has changed worldwide toward an increasing level of THC content (Mehmedic et al., 2010; Pijlman, Rigter, Hoek, Goldschmidt, & Niesink, 2005; Swift, Wong, Li, Arnold, & McGregor, 2013; ElSohly et al., 2000). The increase seen in THC concentration in some cannabis preparations is partly due to advances in growing techniques and cultivation methods, selection of the seeds, and also the domestic indoor production.

When cannabis potency is discussed, the main focus has been on the THC content in the different *Cannabis* plants, but since other substances might influence the THC effects, the concentrations of these substances must also be taken into account. CBD acts as a functional antagonist for some of the THC effects, and might have anxiolytic and antipsychotic effects (Morgan et al., 2010; Henquet & Kuepper, 2010; Luzi et al., 2008). The THC/CBD ratio is thus of major interest, when health-related cannabis effects are studied, and a large variation in the ratio between different plants has been found. CBD appears in cannabis resin, but rarely in herbal cannabis (Niesink & van Laar, 2013).

US data have indicated that herbal cannabis contained an average of 3.4% THC and 0.3% CBD in 1993, while, in 2008, THC levels more than doubled to 8.8%. CBD remained however low (0.4%) during this period (Mehmedic et al., 2010). The cannabinoid content of

Cannabis plants in Australia (2010–11) showed the same trends as expected in other countries toward the use of high-potency cannabis with very low CBD content (Swift et al., 2013). The average potency of the material in the United Kingdom has risen steadily in recent years (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 2011). In recent years, the average THC content in a cannabis cigarette of the type sinsemilla (skunk) is reported to be between 42 and 61 mg in Western Europe (Bocker et al., 2010), and 63 mg in a cigarette in the United States of America (ElSohly et al., 2000).

From the studies reporting THC potency, it can be seen that there are large variations in THC content in the *Cannabis* plants within a given year, and also from sample to sample. There are also large variations in the content of CBD between different samples of cannabis products (Cascini, Aiello, & Di, 2012; Zamengo, Frison, Bettin, & Sciarrone, 2014). The THC/CBD ratio is, however, not examined in most of the publications. The results are mainly based on occasional analyses of cannabis seizures, and not systematical testing over years.

From the existing literature, the protective effects of CBD in real life seem, however, to be uncertain, and it is not known for sure how the THC/CBD ratio has changed over the years. Further discussions will thus focus on the concentrations of THC in the different cannabis preparations.

DOES HIGHER CANNABIS POTENCY LEAD TO INCREASED EXPOSURE TO THC?

A critical point in the discussion is if the current available higher-potency cannabis leads to increased exposure to THC. It has been suggested that cannabis users titrate the THC dosage according to their experienced "high" during smoking and, as a consequence, the plasma THC concentrations, and thus the exposure of THC, would not increase—even though more potent cannabis is smoked. There are divergent results as to whether cannabis users modify the amount of THC inhaled when smoking cannabis (Hall & Swift, 2000). It is difficult to know the precise dosing of THC during cannabis smoking, due to individual differences in smoking behavior, and most of the studies investigating this issue have not measured THC concentrations achieved in plasma.

Higher-Potency Cannabis and Plasma THC Levels

Azorlosa et al. have shown that seven moderate marijuana users who smoked 4, 10, or 25 puffs from cigarettes containing either 1.75 or 3.55% THC, obtained greater plasma THC levels with increased dosing (Azorlosa, Heishman, Stitzer, & Mahaffey, 1992). A dose-concentration relationship has also been shown for higher doses of cannabis (cigarettes containing 29.3, 49.1, and 69.4 mg THC) (Hunault et al., 2008).

Perez-Reyes et al. found no significant difference in plasma THC-concentrations when subjects smoked cigarettes containing 1.32 versus 1.97% THC (peak levels ± SEM of 100 ± 10, and 120 ± 11 ng/mL, respectively), whereas peak plasma levels of 163 ± 19 ng/mL was seen after smoking cigarettes containing 2.54% THC (Perez-Reyes, Di, Davis, Schindler, & Cook, 1982).

Titration of THC Intake During Smoking

Heishman et al. demonstrated that subjects adjusted their smoking of marijuana cigarettes according to different THC content (cigarettes with 1.3 and 2.7% THC) (Heishman, Stitzer, & Yingling, 1989). Subjects smoking the high-potency cigarette inhaled a smaller volume due to shorter duration of inhalation of each puff. Concentrations of THC in plasma were however not measured, so the actual exposure is not known. From a survey-based study, where cannabis users from Amsterdam and San Francisco were asked if they adjusted the amount smoked according to the potency of the cannabis, 70% reported that they used less cannabis when the cannabis preparations were stronger (Reinarman, 2009).

Chait (1989) instructed ten participants to smoke as much or little marijuana of low, medium, and high THC content as they wanted during 30-min smoking periods. To investigate if the dose was regulated in regard to the different marijuana products with different THC levels, heart rate, carbon monoxide levels, and the number of cigarettes were measured. All the measured parameters showed linear trends as a function of cigarette potency, and the study revealed that the participants failed to regulate their intake in response to the different THC content and, accordingly, were more exposed to THC when smoking high-potency marijuana. Plasma concentrations of THC were however not measured.

Freeman et al. (2014) investigated if cannabis users, smoking their own cannabis in a naturalistic setting,

TABLE 3.1 Changes in Concentrations of THC in Blood Samples

	Canfield et al. (2010) USA	Fatal aviation accidents	Vindenes et al. (2013) Norway	Drivers apprehended by the police; suspected of being under the influence of drugs
	Mean conc THC ± SD (ng/mL)	Post mortem whole blood samples	Mean conc. THC ± SEM (ng/mL)	Whole blood samples from living subjects
Total number		*N* = 88		*N* = 1748
Other drugs	Yes in 38% of the cases other drugs detected		No other drugs detected.	
1997	1.3 ± 2.6	9		
1998	0.9 ± 1.9	7		
1999	3.1 ± 4.3	7		
2000	3.9 ± 4.7	8	4.0 ± 0.3	167
2001	4.2 ± 7.3	9	3.7 ± 0.3	151
2002	1.0 ± 2.0	6	3.6 ± 0.3	116
2003	7.8 ± 16.3	19	4.5 ± 0.4	153
2004	9.9 ± 15.4	7	5.5 ± 0.4	127
2005	2.7 ± 2.4	7	5.6 ± 0.6	139
2006	14.8 ± 20.5	9	5.0 ± 0.5	116
2007			5.5 ± 0.4	167
2008			5.3 ± 0.4	161
2009			5.3 ± 0.3	190
2010			6.6 ± 0.4	258

Only two studies have investigated the changes of concentrations of THC in blood samples over time. In contrast to the experimental studies, where THC concentrations have been measured in serum/plasma, both these studies have reported THC-concentrations in whole blood. The plasma/blood ratio is approximately 2, and should thus be taken into account when these concentrations are compared with other studies.

titrated the amount of smoked THC/CDB according to the potency of their joint. A significant correlation was seen between higher potency products and the amount THC smoked, but not in regard to CBD. Concentrations of THC and CBD were measured in the cannabis products. Another naturalistic study where the cannabis users rolled their own joints, and the exposure of THC was measured by analyzing the THC concentrations, and the inhaling volume during smoking, revealed that the cannabis users titrate the THC intake by inhaling lower volumes of strong joints (van der Pol et al., 2014). The lower inhaled volume, however, did not fully compensate for the higher cannabis doses per joint, and the authors concluded that smoking potent cannabis will lead to increased THC exposure. Plasma concentrations of THC were not analyzed in any of the naturalistic studies, and the actual exposure to THC could not be determined.

Another study showed that cannabis smokers preferred to smoke high-potency cannabis more often than low-potency cannabis (Chait & Burke, 1994), but no concentrations were measured to reveal if the plasma THC concentrations differed after smoking the different products.

THC Concentrations in Biological Samples

Changes in THC-concentrations in biological samples, over the years, have only been measured in two studies; in whole blood samples from fatal aviation accidents, and from drivers apprehended by the police for suspicion of impaired driving (Canfield et al., 2010; Vindenes, Strand, Kristoffersen, Boix, & Morland, 2013) (Table 3.1).

The mean THC concentrations each year in whole blood samples from fatal aviation accidents were 1.3 ng/mL in 1997, and 14.8 ng/mL in 2006 (Canfield et al., 2010). Considerable fluctuations were seen, however, between the different years. The total number of the aviation accidents during the 10 years period investigated was low (between 6 and 19 each year, with a total number of 88 diseased) and, in 38% of the cases, other drugs were found concomitantly. Presence of other drugs of abuse could likely affect the amount of THC taken, and cases with other drugs are thus less suitable for monitoring changes in cannabis use patterns over time. The small number of cases investigated also indicates cautious interpretation.

A study from Norway found a significant increase in the mean THC concentrations in blood samples from

drivers apprehended by the police, suspected of driving under the influence of drugs and/or alcohol over a 10 years period (Vindenes et al., 2013). The total number of cases was 1748, with THC as the only substance detected in the blood samples. A screening of around 40 different drugs of abuse was performed in all the cases. Using the gradient of the THC concentration curve, a 58% increase in mean THC concentrations was found. The mean age of the cannabis users did not change during the study period, and the increase in THC concentrations could not be explained by a shorter time between driving and collection of blood samples or analytical changes. The THC cases were compared with cases where only alcohol or amphetamines were detected. Neither the concentration of alcohol nor that of amphetamines showed a similar increase in concentrations as THC (3% for ethanol and 16% for amphetamines during the same period).

A generalization of the results from the fatal aviation accidents and the impaired car drivers to other groups of cannabis users must be done with great caution. The drivers apprehended by the police could represent, for example, a selected group as indicated by the majority having a drug abuse problem, and a large part driving without a license (Christophersen, Beylich, Bjornboe, Skurtveit, & Morland, 1996).

The reason for the increased THC concentrations should however be discussed, or if these are just random findings. Changes in smoking habits over the years, and a desire to achieve an increased "high" after cannabis smoking might lead to increased exposure, but if this was a widespread trend, such a trend would also be expected to be seen for other drugs of abuse. Such changes were not seen, either for amphetamines or ethanol, in the study of apprehended drivers. Another explanation might be that the cannabis smoking population have become older over the years, and tolerance to the THC effects might explain the increase in THC concentrations due to higher exposure to achieve the same "high." The mean age among the impaired drivers did, however, not change over the years. The steadily increase in THC concentration in blood over the years might indicate that this finding is not random, but a probable consequence of the increase in cannabis potency seen during the investigated time period.

DOES HIGHER EXPOSURE TO THC INCREASE THE RISK OF ADVERSE HEALTH EFFECTS?

To study cannabis effects on health, and if higher exposure might increase adverse effects, the effects can be divided into those related to *short-term* use (Table 3.2) and *long-term/heavy* use (Table 3.3).

Effects of Higher Exposure to THC on Effects of Short-Term Use

- Impaired cognitive effects
- Impaired psychomotor effects
- Increased risk of psychotomimetic symptoms

A substantial number of publications have reported disruption of cognitive and psychomotor effects after cannabis exposure (Ramaekers et al., 2006; Ranganathan & D'Souza, 2006; Bocker et al., 2010; D'Souza et al., 2004), and dose response effects have been seen for several of the acute cannabis effects. Heishman et al. (1989) have showed significant memory impairment and a trend toward a dose and plasma concentration response relation (Robbe & O'Hanlon, 1993). To explore if the dose response relationship was seen also for cigarettes with high content of THC, Hunault investigated the effects of cigarettes containing approximately 60 mg THC per cigarette, corresponding to 20% THC (Hunault et al., 2008). Pharmacokinetics of THC and the metabolites were reported, in addition to heart rate, blood pressure, and subjective experiences of feeling "high," and drowsiness. No psychomotor tests were performed, however. The study revealed a trend to linear association between THC dose and THC serum concentrations, and the pharmacological effects increased with increasing

TABLE 3.2 Literature Regarding Impairment of Acute Cannabis Effects

Acute effects	Type of effect	Literature supporting impairment	Dose response relationship
Cognitive effects	Memory	Consistent	Yes
	Learning	Consistent	Yes
	Attention/time perception	Consistent	Yes
Psychomotor effects	Reaction time	Consistent	Yes
	Divided attention	Consistent	Yes
	Driving skills/increased risk of traffic accidents	Consistent	Yes
Paranoia/psychotomimetic	Psychotomimetic symptoms, for example, measured by PANSS	Consistent for high doses	Yes

The table shows if there is literature supporting a dose–response relationship for different acute cannabis effects.

TABLE 3.3 Literature Regarding Impairment After Long-Term Use of Cannabis

Long-term or heavy use	Type of effect	Literature supporting impairment	Dose–response relationship
Cognitive impairment	Downregulation of receptors, activity in different brain regions	Some	a
	Altered brain development	Some	a
	Learning deficits	Not conclusive if this is due to the acute or residual cannabis effects	
	Reduction in IQ	Some	
Psychosis	Earlier onset with increased use	Consistent	a
Addiction	According to specific criteria	Some	a

The table shows if there is literature supporting evidence for impairment of different long-term effects, and if a dose–response relationship was observed.
[a]*Higher frequency among regular cannabis users and cannabis, smoking of high-potency cannabis and early onset of cannabis use, but classical dose–response relationships have not been reported, as such relationships are difficult to study for chronic effects.*

doses of THC. Another study investigated psychomotor impairment after smoking cannabis with THC content of approximately 69.4 mg per cigarette, and found that increasing THC doses caused slower reaction time, and more variance and an increased amount of errors (Bocker et al., 2010). Due to cognitive and psychomotor impairment, in addition to altered judgment, cannabis use has been associated with increased risk of traffic accidents (Grotenhermen et al., 2007; Laumon, Gadegbeku, Martin, & Biecheler, 2005). An increased risk of accidents has been shown with increasing THC concentrations (Ramaekers, Berghaus, van Laar & Drummer, 2004).

D'Souza et al. (2004) were the first to characterize positive psychotomimetic symptoms, negative symptoms, and cognitive effects in healthy individuals after intravenous administration of THC. A dose-dependent relationship was seen when a seven-item positive symptom subscale of Positive and Negative Syndrome Scale (PANSS) was used. PANSS is used to measure the symptoms associated with schizophrenia.

A consistent body of literature supports that increased doses of THC lead to increasing serum concentrations of THC, and further to more severe short-term effects of cannabis.

Effects of Higher Exposure of Cannabis on Effects of Long-Term or Heavy Use of Cannabis

- Cognitive impairment
- Psychotic disorders (including schizophrenia)
- Addiction

Several studies have investigated the cognitive impairment after long-term use of cannabis, and the most consistently reported findings are deficits in verbal learning, and reduced memory and attention (Hall & Degenhardt, 2014). It is not clear, however, if these findings might be attributed to the acute cognitive effects of cannabis, the residual effects of chronic use, or the effects of cumulative THC exposure on brain functioning. Meier et al. (2012) have found that persistent cannabis use was the most likely explanation of reduction in IQ among cannabis users, and that the effects are more pronounced with higher exposure. There have been reported structural changes in the hippocampus, prefrontal cortex, and cerebellum in chronic cannabis users, but it has not been determined if cognitive impairments in long-term cannabis users are correlated with structural changes in brain areas implicated in memory and emotion.

Cannabis use is associated with an earlier age of onset of lasting psychosis, but the reason for this is not understood (Volkow, Baler, Compton, & Weiss, 2014; Luzi et al., 2008). Even though millions of people use cannabis, only a minor group experiences persisting psychotic symptoms, and even fewer develop a psychotic disorder. From a study conducted in the Swedish army, only 3% of heavy cannabis users developed schizophrenia (Casadio, Fernandes, Murray, & Di, 2011). Increased frequency of psychoses was associated with higher exposure of cannabis. A number of studies have reported that those who consistently consume large amounts of cannabis have an increased risk of later developing schizophrenia-like psychoses (Potter et al., 2008; Radhakrishnan, Wilkinson, & D'Souza, 2014; Kuepper et al., 2011; Moore et al., 2007). Di Forti et al. (2009) investigated 280 cases presenting a first episode of psychosis, compared with 174 healthy controls recruited from the local population, and found that the first episode of psychosis was not more likely to occur in subjects who had ever taken cannabis or had started doing so earlier than the control group. However, psychosis was associated with more frequent and long lasting use of cannabis. Their most striking finding was that patients with a first episode of psychosis preferentially had used high-potency cannabis preparations: sinsemilla. Cannabis users with their first episode of psychosis were more likely to have taken cannabis for a longer time, and every day, compared to healthy controls from the general population (Di Forti et al., 2009). Di et al. (2014) have recently

shown that subjects who had smoked high-potency *Cannabis* cannabis every day had the earliest onset of psychosis, compared to the group of subjects who had never used cannabis. The daily users of high-potency cannabis had an average onset of psychosis 6 years before the non-cannabis users. Several studies have suggested that the younger the age when first exposed to cannabis, the greater the risk of a psychotic outcome (Arseneault et al., 2002; Di et al., 2014; Sewell, Ranganathan, & D'Souza, 2009; Casadio et al., 2011). Bonn-Miller et al. (2008) have reported that marijuana users who demonstrated high use frequency and high coping motives reported the highest levels of anxious arousal, worry, and agoraphobic cognition, across the anxiety-relevant criterian variables.

The demand for treatment of cannabis dependence has increased over the years (Volkow et al., 2014; Hall, 2009). It has been investigated whether THC increases dopamine in striatum, which is a characteristic for addictive drugs (Hyman & Malenka, 2001). Such a dopamine increase has been found for THC, and it has thus been concluded that THC shares the potentially addictive properties with other drugs of abuse (Bossong et al., 2009). Among the overall cannabis users, about 9% have been classified as dependent, according to the DSM-4 criteria (Hall, 2009). In the group with adolescence onset, the percentage was 17, and it increased to 25–50% among the daily users (Volkow et al., 2014).

A dose–response effect between cannabis intake and complications has been suggested, but there are a number of limitations to this assessment. The THC content varies considerably, and, when smoking cannabis, a joint may be inhaled over several sessions or shared with others.

To be able to quantify the total lifetime exposure of THC, several studies have used estimates of numbers of episodes of smoking cannabis as proxy for the THC amount. Such estimates must be considered as a rough estimate regarding the amount of THC exposure, due to the large difference seen in THC concentrations in the various available cannabis products.

Smoking higher potency cannabis is one cause that might lead to increased total exposure of THC during the lifetime, and yet another cause is earlier onset of cannabis use—due to a longer time period with cannabis use and, thus, a higher total exposure (Casadio et al., 2011; Sewell, Ranganathan, & D'Souza, 2009).

DIFFERENT EXPLANATIONS FOR INCREASED RISK OF ADVERSE HEALTH EFFECTS

It is difficult to investigate if higher-potency *Cannabis* leads to an increased risk of adverse health effects, since large variations in potency in different *Cannabis* plants, within a given year and from sample to sample, have been found. Information regarding the THC/CBD ratio could also be important in these investigations but, as long as this ratio is mainly unknown, it is thus not possible to take into account in epidemiologic studies reporting frequencies of implications in different populations.

Age at first exposure to cannabis appears to be a key factor for vulnerability to many of the harmful effects of cannabis (Arseneault et al., 2002; Stefanis et al., 2013; Casadio et al., 2011). Greater risk of psychotic experiences in those who started cannabis use early might be due to the higher cumulative exposure to cannabis of early users. Adolescence might, on the other hand, represent a period with increased vulnerability to THC during critical phases of brain maturation, such as, in early puberty, and the specific association between psychotic experiences and young initial age of THC exposure might be reflected by this vulnerability (Casadio et al., 2011; Copeland et al., 2013). During adolescence, levels of endocannabinoids and cannabinoid receptors increase, peaking at puberty (Schneider, Schomig, & Leweke, 2008). The "window of vulnerability" hypothesis has thus been suggested; this is a critical period during early adolescence where the brain is particularly susceptible to the psychosis-inducing effects of cannabis, during a critical period of development and maturation (Radhakrishnan et al., 2014). Different processes take place during this period, like neuronal migration and differentiation, synaptogenesis, axon formation, and dendritic proliferation, myelination, pruning, apoptosis, and activity dependent changes—these begin in utero, but continue into the early 20s, or even later (Molina-Holgado et al., 2002). Cannabis may disrupt one or more of these processes.

The hypothesis that there is a particular critical period during early adolescence is, however, not supported by a retrospective study by Stefanis et al. (2013). The association between age of onset of cannabis use and worse outcomes could simply reflect that earlier use is more likely to become longstanding, thus increasing the overall exposure to cannabis. Other causes that might increase the risk of cannabis-related problems due to early onset, is that the risk of developing addiction is increased, and it has been reported that there is an increased risk of loss of intelligence, leading to a behavior that might pursue behaviors that increase the health risk (Meier et al., 2012).

CONCLUSIONS

The two studies which have measured THC in blood samples over a time period indicate that at least some cannabis users have increased their THC exposure during the later years. The limited number of studies and selected groups investigated makes it, thus, difficult to conclude if this is representative for the "general" cannabis users worldwide.

From the existing literature, the causality between increase in THC-concentrations in cannabis preparations and the increase in cannabis-related problems cannot be claimed. There are, however, several findings indicating that cannabis smokers are not able to titrate the THC concentration to achieve a certain wanted THC concentration, and a trend toward increase in THC exposure is seen, with the use of more potent products. Such an increase in THC in blood might have consequences for the health-related disorders that cannabis users might experience. However, more studies on the risks related to the use of more potent cannabis products are needed before firm conclusions can be drawn.

MINI-DICTIONARY

Cannabis potency The concentration of THC in cannabis preparations.

CBD (Cannabidiol) Acts as a functional antagonist for some of the effects of THC, and might have anxiolytic and antipsychotic effects. CBD might offset some of the psychoactive effects of THC, thereby modulating the effects of cannabis. CBD binds weakly to the CB receptors, and the exact mechanism by which CBD exerts its effects is not known.

Sinsemilla A cannabis preparation named after the Spanish word "sin semilla," meaning "without seeds." Sinsemilla is also known as "skunk," "buds," or "netherweet". It has been grown with hydroponic cultivation, with artificial control of daylight length, propagation of female cuttings, and prevention of fertilization, all this leading to cannabis with a higher potency.

References

Arseneault, L., Cannon, M., Poulton, R., Murray, R., Caspi, A., & Moffitt, T. E. (2002). Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. *British Medical Journal*, *325*(7374), 1212–1213.

Azorlosa, J. L., Heishman, S. J., Stitzer, M. L., & Mahaffey, J. M. (1992). Marijuana smoking: effect of varying delta 9-tetrahydrocannabinol content and number of puffs. *Journal of Pharmacology and Experimental Therapeutics*, *261*(1), 114–122.

Bocker, K. B., Gerritsen, J., Hunault, C. C., Kruidenier, M., Mensinga, T. T., & Kenemans, J. L. (2010). Cannabis with high delta9-THC contents affects perception and visual selective attention acutely: an event-related potential study. *Pharmacology Biochemistry and Behavior*, *96*(1), 67–74.

Bonn-Miller, M. O., Zvolensky, M. J., Bernstein, A., & Stickle, T. R. (2008). Marijuana coping motives interact with marijuana use frequency to predict anxious arousal, panic related catastrophic thinking, and worry among current marijuana users. *Depression and Anxiety*, *25*(10), 862–873.

Bossong, M. G., van Berckel, B. N., Boellaard, R., Zuurman, L., Schuit, R. C., Windhorst, A. D., et al. (2009). Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. *Neuropsychopharmacology*, *34*(3), 759–766.

Canfield, D. V., Dubowski, K. M., Whinnery, J. E., Lewis, R. J., Ritter, R. M., & Rogers, P. B. (2010). Increased cannabinoids concentrations found in specimens from fatal aviation accidents between 1997 and 2006. *Forensic Science International*, *197*(1–3), 85–88.

Casadio, P., Fernandes, C., Murray, R. M., & Di, F. M. (2011). Cannabis use in young people: the risk for schizophrenia. *Neuroscience and Biobehavior Reviews*, *35*(8), 1779–1787.

Cascini, F., Aiello, C., & Di, T. G. (2012). Increasing Delta-9-Tetrahydrocannabinol (Delta-9-THC) content in herbal Cannabis over time: systematic review and meta-analysis. *Current Drug Abuse Reviews*, *5*(1), 32–40.

Chait, L. D. (1989). Delta-9-tetrahydrocannabinol content and human marijuana self-administration. *Psychopharmacology (Berlin)*, *98*(1), 51–55.

Chait, L. D., & Burke, K. A. (1994). Preference for high- versus low-potency marijuana. *Pharmacology Biochemistry and Behavior*, *49*(3), 643–647.

Christophersen, A. S., Beylich, K. M., Bjorneboe, A., Skurtveit, S., & Morland, J. (1996). Recidivism among drunken and drugged drivers in Norway. *Alcohol and Alcoholism*, *31*(6), 609–611.

Copeland, J., Rooke, S., & Swift, W. (2013). Changes in cannabis use among young people: impact on mental health. *Current Opinion in Psychiatry*, *26*(4), 325–329.

D'Souza, D. C., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y. T., et al. (2004). The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology*, *29*(8), 1558–1572.

D'Souza, D. C., Sewell, R. A., & Ranganathan, M. (2009). Cannabis and psychosis/schizophrenia: human studies. *European Archives of Psychiatry and Clinical Neuroscience*, *259*(7), 413–431.

Di Forti, M., Morgan, C., Dazzan, P., Pariante, C., Mondelli, V., Marques, T. R., et al. (2009). High-potency cannabis and the risk of psychosis. *British Journal of Psychiatry*, *195*(6), 488–491.

Di Forti, M., Sallis, H., Allegri, F., Trotta, A., Ferraro, L., Stilo, S. A., et al. (2014). Daily use, especially of high-potency Cannabis, drives the earlier onset of psychosis in Cannabis users. *Schizophrenia Bulletin*, *40*(6), 1509–1517.

ElSohly, M. A., Ross, S. A., Mehmedic, Z., Arafat, R., Yi, B., & Banahan, B. F., III (2000). Potency trends of delta9-THC and other cannabinoids in confiscated marijuana from 1980-1997. *Journal of Forensic Science*, *45*(1), 24–30.

European Monitoring Centre for Drugs and Drug Addiction., 2011. Cannabis http://www.emcdda.europa.eu/publications/drug-profiles/cannabis. In EMCDDA Insight 6.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). 2011. Annual Report: The State of the Drugs Problem in Europe, EMCDDA, EMCDDA, Lisbon,Portugal. Retrieved from: http://www.emcdda.europa.eu/attachements.cfm/att_143743_EN_EMCDDA_AR2011_EN.pdf

Freeman, T. P., Morgan, C. J., Hindocha, C., Schafer, G., Das, R. K., & Curran, H. V. (2014). Just say 'know': how do cannabinoid concentrations influence users' estimates of cannabis potency and the amount they roll in joints? *Addiction*, *109*(10), 1686–1694.

Grotenhermen, F., Leson, G., Berghaus, G., Drummer, O. H., Kruger, H. P., Longo, M., et al. (2007). Developing limits for driving under cannabis. *Addiction*, *102*(12), 1910–1917.

Hall, W. (2009). The adverse health effects of cannabis use: what are they, and what are their implications for policy? *International Journal of Drug Policy*, *20*(6), 458–466.

Hall, W., & Degenhardt, L. (2014). The adverse health effects of chronic cannabis use. *Drug Testing and Analysis*, *6*(1–2), 39–45.

Hall, W., & Swift, W. (2000). The THC content of cannabis in Australia: evidence and implications. *Australian and New Zealand Journal of Public Health*, *24*(5), 503–508.

Heishman, S. J., Stitzer, M. L., & Yingling, J. E. (1989). Effects of tetrahydrocannabinol content on marijuana smoking behavior, subjective reports, and performance. *Pharmacology Biochemistry and Behavior*, *34*(1), 173–179.

Henquet, C., & Kuepper, R. (2010). Does cannabidiol protect against the negative effects of THC? *British Journal of Psychiatry*, *197*(4), 259–260.

Hunault, C. C., Mensinga, T. T., de Vries, I., Kelholt-Dijkman, H. H., Hoek, J., Kruidenier, M., et al. (2008). Delta-9-tetrahydrocannabinol (THC) serum concentrations and pharmacological effects in males

after smoking a combination of tobacco and cannabis containing up to 69 mg THC. *Psychopharmacology (Berlin)*, *201*(2), 171–181.
Hyman, S. E., & Malenka, R. C. (2001). Addiction and the brain: the neurobiology of compulsion and its persistence. *Nature Reviews Neuroscience*, *2*(10), 695–703.
Kuepper, R., van Os, J., Lieb, R., Wittchen, H. U., Hofler, M., & Henquet, C. (2011). Continued cannabis use and risk of incidence and persistence of psychotic symptoms: 10 year follow-up cohort study. *British Medical Journal*, *342*, d738.
Laumon, B., Gadegbeku, B., Martin, J. L., & Biecheler, M. B. (2005). Cannabis intoxication and fatal road crashes in France: population based case-control study. *British Medical Journal*, *331*(7529), 1371.
Luzi, S., Morrison, P. D., Powell, J., Di, F. M., & Murray, R. M. (2008). What is the mechanism whereby cannabis use increases risk of psychosis? *Neurotoxicity Research*, *14*(2–3), 105–112.
McLaren, J., Swift, W., Dillon, P., & Allsop, S. (2008). Cannabis potency and contamination: a review of the literature. *Addiction*, *103*(7), 1100–1109.
Mehmedic, Z., Chandra, S., Slade, D., Denham, H., Foster, S., Patel, A. S., et al. (2010). Potency trends of Delta9-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. *Journal of Forensic Science*, *55*(5), 1209–1217.
Meier, M. H., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R. S., et al. (2012). Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(40), E2657–E2664.
Molina-Holgado, E., Vela, J. M., Arevalo-Martin, A., Almazan, G., Molina-Holgado, F., Borrell, J., et al. (2002). Cannabinoids promote oligodendrocyte progenitor survival: involvement of cannabinoid receptors and phosphatidylinositol-3 kinase/Akt signaling. *Journal of Neuroscience*, *22*(22), 9742–9753.
Moore, T. H., Zammit, S., Lingford-Hughes, A., Barnes, T. R., Jones, P. B., Burke, M., et al. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet*, *370*(9584), 319–328.
Morgan, C. J., Gardener, C., Schafer, G., Swan, S., Demarchi, C., Freeman, T. P., et al. (2011). Sub-chronic impact of cannabinoids in street cannabis on cognition, psychotic-like symptoms and psychological well-being. *Psychological Medicine*, *42*(2), 391–400.
Morgan, C. J., Schafer, G., Freeman, T. P., & Curran, H. V. (2010). Impact of cannabidiol on the acute memory and psychotomimetic effects of smoked cannabis: naturalistic study: naturalistic study. *British Journal of Psychiatry*, *197*(4), 285–290.
Niesink, R. J., & van Laar, M. W. (2013). Does Cannabidiol protect against adverse psychological effects of THC? *Frontiers in Psychiatry*, *4*, 130.
Perez-Reyes, M., Di, G. S., Davis, K. H., Schindler, V. H., & Cook, C. E. (1982). Comparison of effects of marihuana cigarettes to three different potencies. *Clinical Pharmacology and Therapeutics*, *31*(5), 617–624.
Pijlman, F. T., Rigter, S. M., Hoek, J., Goldschmidt, H. M., & Niesink, R. J. (2005). Strong increase in total delta-THC in cannabis preparations sold in Dutch coffee shops. *Addiction Biology*, *10*(2), 171–180.
Potter, D. J., Clark, P., & Brown, M. B. (2008). Potency of delta 9-THC and other cannabinoids in cannabis in England in 2005: implications for psychoactivity and pharmacology. *Journal of Forensic Science*, *53*(1), 90–94.
Radhakrishnan, R., Wilkinson, S. T., & D'Souza, D. C. (2014). Gone to Pot—a review of the association between Cannabis and psychosis. *Frontiers in Psychiatry*, *5*, 54.
Ramaekers, J. G., Berghaus, G., van Laar, M., & Drummer, O. H. (2004). Dose related risk of motor vehicle crashes after cannabis use. *Drug and Alcohol Dependence*, *73*(2), 109–119.
Ramaekers, J. G., Moeller, M. R., van Ruitenbeek, P., Theunissen, E. L., Schneider, E., & Kauert, G. (2006). Cognition and motor control as a function of Delta9-THC concentration in serum and oral fluid: limits of impairment. *Drug and Alcohol Dependence*, *85*(2), 114–122.
Ranganathan, M., & D'Souza, D. C. (2006). The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berlin)*, *188*(4), 425–444.
Reinarman, C. (2009). Cannabis policies and user practices: market separation, price, potency, and accessibility in Amsterdam and San Francisco. *International Journal of Drug Policy*, *20*(1), 28–37.
Robbe HWJ. 2008. Marijuana's effects on actual driving performance. Retrieved from http://www.druglibrary.org/schaffer/misc/driving/s1p2.htm
Robbe, H. W. J., & O'Hanlon, J. F. (1993). *Marijuana and actual driving performance*. Washington, DC: National Highway Traffic Safety Administration, US Department of Transportation.
Schneider, M., Schomig, E., & Leweke, F. M. (2008). Acute and chronic cannabinoid treatment differentially affects recognition memory and social behavior in pubertal and adult rats. *Addiction Biology*, *13*(3–4), 345–357.
Schwope, D. M., Bosker, W. M., Ramaekers, J. G., Gorelick, D. A., & Huestis, M. A. (2012). Psychomotor performance, subjective and physiological effects and whole blood Delta(9)-tetrahydrocannabinol concentrations in heavy, chronic cannabis smokers following acute smoked cannabis. *Journal of Analytical Toxicology*, *36*(6), 405–412.
Sewell, R. A., Ranganathan, M., & D'Souza, D. C. (2009). Cannabinoids and psychosis. *International Review of Psychiatry*, *21*(2), 152–162.
Stefanis, N. C., Dragovic, M., Power, B. D., Jablensky, A., Castle, D., & Morgan, V. A. (2013). Age at initiation of cannabis use predicts age at onset of psychosis: the 7- to 8-year trend. *Schizophrenia Bulletin*, *39*(2), 251–254.
Swift, W., Wong, A., Li, K. M., Arnold, J. C., & McGregor, I. S. (2013). Analysis of cannabis seizures in NSW, Australia: cannabis potency and cannabinoid profile. *PLoS One*, *8*(7), e70052.
van der Pol, P., Liebregts, N., Brunt, T., van Amsterdam, J., de Graaf, R., Korf, D. J., van den Brink, W., & van Laar, M. (2014). Cross-sectional and prospective relation of cannabis potency, dosing and smoking behaviour with cannabis dependence: an ecological study. *Addiction*, *109*(7), 1101–1109.
Vindenes, V., Strand, D. H., Kristoffersen, L., Boix, F., & Morland, J. (2013). Has the intake of THC by cannabis users changed over the last decade? Evidence of increased exposure by analysis of blood THC concentrations in impaired drivers. *Forensic Science International*, *226*(1–3), 197–201.
Volkow, N. D., Baler, R. D., Compton, W. M., & Weiss, S. R. (2014). Adverse health effects of marijuana use. *New England Journal of Medicine*, *370*(23), 2219–2227.
Zamengo, L., Frison, G., Bettin, C., & Sciarrone, R. (2014). Variability of cannabis potency in the Venice area (Italy): a survey over the period 2010-2012. *Drug Testing and Analysis*, *6*(1–2), 46–51.

CHAPTER

4

Age as a Predictor of Cannabis Use

D. Bergen-Cico, R.D. Cico***

***Department of Public Health, Addiction Studies, Syracuse University, Syracuse, NY, United States**
****Columbia University, New York, NY, United States**

SUMMARY POINTS

- This chapter examines the prevalence of cannabis use among people across all age groups.
- The largest percentage of cannabis users are people under the age of 20.
- People are most likely to first use cannabis prior to age 20; however, the number of people over the age of 30 who first initiate cannabis use has steadily increased over the past decade.
- Cannabis use spans the full life course.
- Although people are most likely to first use cannabis during their youth, their use may decline during early adulthood, and intermittently resurface in mid-to-late adulthood.
- Cannabis use among older adults may be becoming normative with the increasing prevalence of cannabis using peer cohorts who came of age during and following the 1960s.
- Cannabis use among adults over the age of 50 may reflect recreational and medicinal use.

KEY FACTS

Key Facts of *Cannabis*

Cannabis is a genus of flowering plants (*Cannabis indica*, *Cannabis ruderalis*, *C. sativa*) and psychoactive preparations derived from *C. sativa* that are commonly known as cannabis, hash, hashish, marihuana, or marijuana. *Cannabis* may be used in several forms. It is most commonly used in dried leaf form that can be smoked, vaporized, and eaten when prepared in food products such as specialty baked goods and chocolates. Extraction of resin from the *Cannabis* plant may be used to produce hashish (hash), typically a brown sticky oil or solid form that can be smoked or prepared into specialty foods. Most cannabis is psychoactive and produces a mild sense of euphoria and relaxation, at moderate doses.

Key Facts of Measuring Cannabis Use

The measurement of recreational drug use, including cannabis, is quantified with standard questions that ask respondents' to report if they have: (1) ever used cannabis (this indicates lifetime prevalence); (2) used cannabis in the past year (this indicates recent use); and (3) used cannabis in the past 30 days (this indicates current use). These recall and reporting measures are consistently used across most countries for national longitudinal trend tracking and cross-national comparison. Cannabis use or users are characterized in one of seven categories: (1) abstainers, (2) adolescent only users, (3) persistent users, (4) early adulthood decliners, (5) episodic users, and (6) late starters (Geller, Loftis & Brink, 2004; Juon et al., 2011; Patrick et al., 2011).

Key Facts of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)

The EMCDDA was established in 1993 to provide the European Union and its member states with regular monitoring data of drug use problems and trends in European countries. The mission of the EMCDDA is to provide practitioners, public health officials, and policymakers with objective data to support informed drug laws and strategies. The EMCDDA scientifically monitors and routinely collects country-based data using consistent methodology to enable reliable comparative data across nations and over time.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00005-3
Copyright © 2017 Elsevier Inc. All rights reserved.

Key Facts of Medical Cannabis

Medical cannabis, also known as medical marijuana, refers to the use of cannabis for the treatment, or symptom reduction, of a range of illnesses and conditions. It is recognized as a treatment for glaucoma, wasting disease, nausea, some forms of pain, and some seizure disorders. Medicinal cannabis may be used in several forms, and the method of ingestion depends on the condition being treated, and the preference of the use. It may be extracted into an oil or tincture that can be consumed directly, orally or mixed into beverages or foods. It can also be consumed in dried whole leaf form, which may be smoked, vaporized, and eaten when prepared in specialty food products, such as baked goods and chocolates. Most forms of medicinal cannabis produce some psychoactive effects. As such, some specialty forms of cannabis are cultivated to have very low levels of psychoactive compounds.

LIST OF ABBREVIATIONS

CCSA	Canadian Centre on Substance Abuse
CUD	Cannabis use disorder
EMCDDA	European Monitoring Center on Drugs and Drug Addiction
NSDUH	National Survey on Drug Use and Health
SAMHSA	Substance Abuse Mental Health Services Administration
THC	Tetrahydrocannabinol
UNODC	United Nations Office for Drugs and Crime

INTRODUCTION

This chapter examines cannabis (marijuana, hashish) use by age group, across the lifespan with an emphasis on current (past 30 days) and recent (past year) cannabis use in Europe and the United States. We have focused on these countries where cannabis use is more prevalent, and reliable data is routinely collected to track trends over time. Data from Australia, Canada, and New Zealand is included where available. Lifetime cannabis use is virtually nonexistent in Asian countries, whereas it is most prevalent in the Czech Republic, Denmark, France, New Zealand, Spain, and the United States (EMCDDA, 2012; Degenhardt et al., 2008). Moreover, there is no singular pattern or trajectory of cannabis use initiation or continuation within these countries.

Although there are some differences in survey methodology between countries, there are well-established standard cannabis use questions that enable comparative analysis. Specifically, such questions that ask if the respondent has ever used cannabis (indicates lifetime prevalence), used cannabis in the past year (recent use), and/or has used cannabis in the past 30 days (current use). Furthermore, cannabis use is generally characterized within one of seven categories: abstainers, adolescent only users, persistent users, early adulthood decliners, episodic users, and late starters (Geller, Loftis, & Brink, 2004; Juon, Fothergill, Green, Doherty, & Ensminger, 2011; Patrick, Schulenberg, O'Malley, Johnston, & Bachman, 2011). Although this chapter examines age as a predictor of cannabis use among people in countries in Europe and North America, within the age groups and populations in each country there are unique patterns of use, particularly when further analyzed by gender and race/ethnicity. Case in point, examination of cannabis use among youth in the United States shows that black youths consistently use cannabis at lower rates than their white peers (Johnston, O'Malley, Bachman, & Schulenberg, 2010; Wallace, 1999); however, rates of cannabis use among black adults are equal to or higher than usage rates among white adults (age matched peers), indicating a "crossover effect" (Juon et al., 2011). What this means for the data presented here is that, although there are distinct patterns of use within age groups in each country, age alone does not indicate likelihood of cannabis use. Furthermore, there are notable differences in the prevalence of cannabis use by age group from one country to the next.

Initiation of Cannabis Use

Individuals begin using psychoactive substances for reasons that reflect individual history, as well as social, familial, environmental, and educational influences (Bachman et al., 2008; Eaton et al., 2010). Therefore, it is necessary to examine current and historical usage patterns of cannabis among people of all age groups. Much of the literature and monitoring data on cannabis use has focused on new initiates (age of first use) and use among youth and emerging adults because use has been highest among these age groups, in recent history. Moreover, the age of first alcohol intake has significant relevance to rates of initiation for cannabis use in many cultures (Arria, Dohey, Mezzich, Bukstein, & Van Thiel, 1995; Bergen-Cico and Lape, 2013; Eaton et al., 2010; Johnston et al., 2010; Kokkevi et al., 2006). Although there is no set age that marks the development or establishment of a cannabis use disorder (CUD), the younger a person is when they begin using, the greater the maturational deviation and psychological dysregulation, thereby increasing the risk of early age onset for CUDs (Kosterman, Hawkins, Guo, Catalano, & Abbott, 2000). It is noteworthy that initiation of cannabis use increases rapidly during adolescence, through age 18–20, with sharp declines after the age of 20 (SAMHSA, 2013; Schulenberg et al.,

2005). Research has shown an association between delaying the onset of first use until age 20 or later with a lower risk of cannabis abuse and dependence (Behrendt, Wittchen, Höfler, Lieb, & Beesdo, 2009; Winters & Lee, 2008). Although cannabis has relatively low addiction potential, compared to stimulant and depressant drugs, people who use cannabis frequently, particularly cannabis with increasingly high THC levels, can develop cannabis dependence (Inaba & Cohen, 2014). Research has also found that persistent regular use of cannabis is associated with higher rates of mental health problems among susceptible persons (Fergusson, Horwood, & Swain-Campbell, 2002).

Historically, research has shown that people who began using cannabis at an early age were less likely to graduate high school and go to college (Grunbaum et al., 2004); however, more recent studies have found cannabis use among college students to be on a par with peers not attending college (Bergen-Cico & Lape, 2013; Johnston et al., 2010). Despite early and continued use of cannabis, a substantial percentage of youths who use cannabis are capable of graduating from high school and gaining entry to college; however, the frequency and intensity of cannabis (and other drug) use is a significant determinant of academic retention and success in college (Bergen-Cico, 2000; Bergen-Cico, Urtz, & Baretto, 2004; Horwood et al., 2010).

Age of first use is related to cannabis use disorder risk, whereby the younger the age of first use, the higher the risk of cannabis abuse and dependence (Behrendt et al., 2009; Bergen-Cico & Lape, 2013; Chen, O'Brien, & Anthony, 2005; Lopez-Quintero et al., 2011; Winters & Lee, 2008). Early age of first use for cannabis follows similar patterns for the development of substance dependence for alcohol (Dawson, Goldstein, Chou, Ruan, & Grant 2008; DeWit, Adlaf, Offord, & Ogborne, 2000) and nicotine (Behrendt et al., 2009), with some evidence of temporal ordering whereby early onset of alcohol use may be associated with subsequent development of cannabis abuse (Bergen-Cico & Lape, 2013; Kirisci et al., 2013). Moreover, research has found a significant relationship between the age of first cannabis use and drinking alcohol by oneself (Bergen-Cico & Lape, 2013). This is of concern because use of alcohol or cannabis when alone (solitary use) is not an indicator of social use, but rather a marker of social isolation and potential indicator of dependency. However, as noted in the chapter *How Age Leads to Cannabis Use Disorders*, Dr Behrendt notes there is limited documentation of higher risk of escalating cannabis use among people who begin using at younger ages, which produces a void in important data that may pertain to the progression from early age of fist cannabis use to the development of CUDs later in life (Behrendt, 2015). Moreover, delaying cannabis use until after age 20 does not in and of itself inoculate people from associated mental health problems. A study by Washburn and Capaldi (2014) found that patterns of cannabis use among men during their 20s was associated with diagnoses of cannabis disorders in their mid-20s, and predicted antisocial behavior and deviant peer association in their mid-30s, thus concluding that differential patterns of marijuana use in early adulthood may be associated with psychopathology toward midlife (Washburn & Capaldi, 2014).

Trends in Cannabis Use Across the Lifespan

Fig. 4.1 illustrates the percentage of past year and past month cannabis use across all age groups (age 12 and older) among people in the United States, based on national monitoring data collected in 2012 (SAMHSA, 2013). The major graph lines illustrate the higher prevalence of use through about age 20. The trend lines show a general decrease in usage as people get older, with a moderate convergence between past year and past month use, as age increases. This figure illustrates a mountainous peak around age 20, with substantial use following this peak in a gradual downward slope through age 25, followed by sharp declines. Thus, it indicates that use generally decreases as people age, and their responsibilities (work, family) increase (Fig. 4.2) (Tables 4.1 and 4.2).

A 30-year longitudinal study of cannabis use among adolescents in the United States found that social norms and attitudes regarding cannabis use vary and cluster within birth cohorts. This clustering directly affects cannabis use among teens, even after controlling for individual attitudes and perceptions of norms (Keyes et al. 2011). This proclivity and peer social influence may also pertain to use among adults, as evidenced by the increasing rates of current cannabis use among older populations, most notably people over the age of 50 (Fig. 4.3). People in birth cohorts that are disapproving of cannabis use are less likely to use, independent of their personal attitudes toward cannabis use, and vice versa. Age alone does not predict cannabis use, social norms, attributes of low self-control, sensation seeking, and externalizing behavior have strong long-term predictive power with regard to distinct trajectories of marijuana use over time (Brook, Zhang & Brook, 2011).

Initiation of cannabis use continues well beyond adolescence, and people begin using across their lifespan. The data in Table 4.3 illustrate the mean age of first use for US adults aged 26–49 who began using during the corresponding phase of adulthood. Over the 10-year time period covered in this table, the mean age increases from 31.2 to 33.1, while the numbers of new users in this age group concomitantly increased more than 50% from 90,000 in 2002, to 139,000 in 2012. Moreover, people may initiate cannabis use during their teens or early adulthood, and then discontinue use in midadulthood

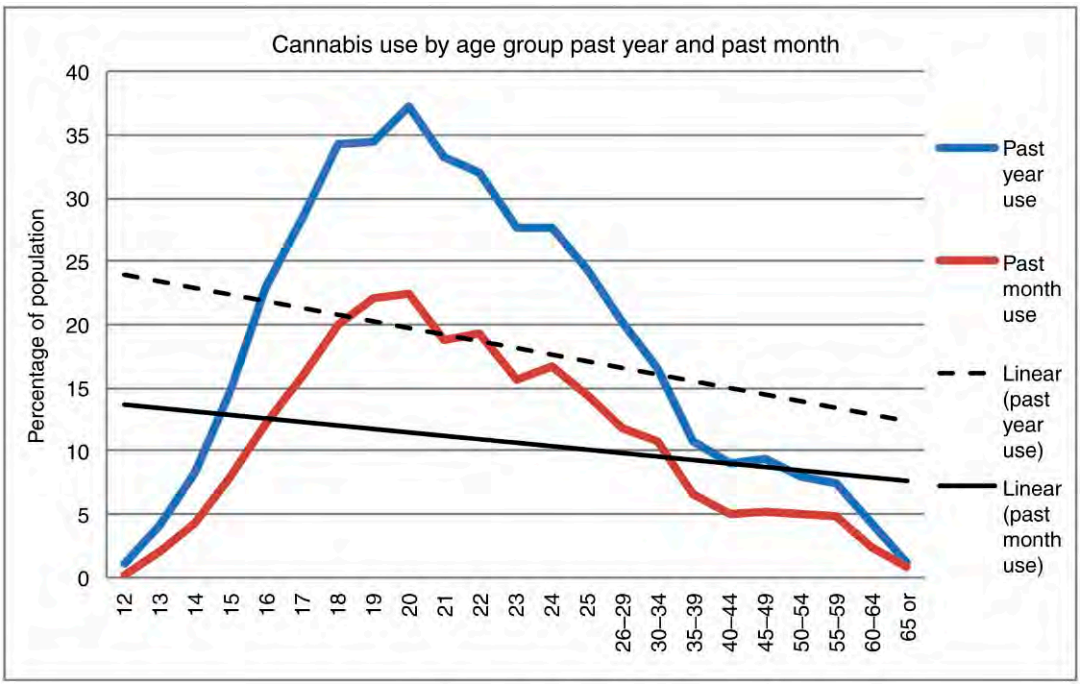


FIGURE 4.1 **Cannabis use across age groups.** This graph shows the percentage of past year and past month cannabis use across ages and age groups (age 12 and older) among people in the United States, based on national monitoring data collected in 2012 by the US Substance Abuse Mental Health Services Administration (SAMHSA, 2013). The blue and red graph lines illustrate the higher prevalence of use peaking through ~age 20. The black trend lines show a general decrease in usage as people get older, with a moderate convergence between past year and past month use as age increases (55 +).

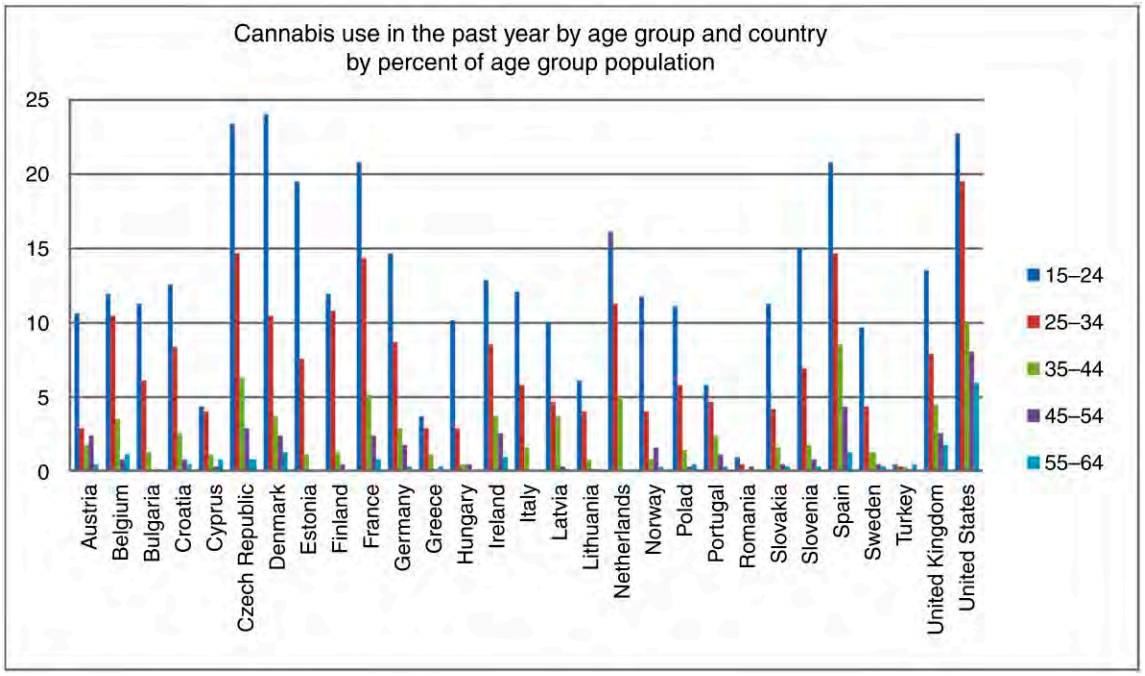


FIGURE 4.2 **Percentage of past year cannabis use by age group and country.** European data is from the European Monitoring Center on Drugs and Drug Addiction (EMCDDA, 2012), US data is from NSDUH (SAMHSA, 2013). Past year use is highest among 15–24 year olds across all countries. The highest prevalence of use among people in the 45–54 and 55–64 age group is in the United States.

TABLE 4.1 Percentage of Current Age Cohorts and Corresponding Age of First Use for Cannabis

Age of first use	Current age cohort (years)				
	12–17	**18–25**	**26–34**	**35–49**	**50 ≥**
≤ 13	6.5	7.8	7.9	7.2	1.9
14–15.	**7.3**	12.4	12.6	9.9	4.7
16–17	3.1	**15.4**	**15.4**	14	8.2
18–20	0	13.7	12.1	**10.8**	**10.7**
21–24	0	2.9	5.3	4.4	5
25 ≥	0	0	1.8	3.6	6.2

US NSDUH 2012 (SAMHSA, 2013). Note: bold data indicates highest prevalence for age of first use by current age cohort. The data in this table show that regardless of the current age group and cohort, people are most likely to first use cannabis by the age of 20.

TABLE 4.2 Cannabis Use by Age Group in US (Percentage)

Cannabis Use	12–17 (years)	18–25 (years)	26–34 (years)	35–49 (years)	50≥ (years)
Ever use cannabis	17	52.2	45.2	50	36.7
Used past year	**13.5**	**31.4**[a]	**19.5**	**10**	**4.6**
Past 30 day use	7.3	18.5[a]	11.5	5.7	3
1–5 days use past year	3.8	7.4[a]	4.5	2.6	1.2
6–10 days use past year	0.9	1.6[a]	1	0.5	0.2
11–25 days use past year	1.9	3.4[a]	1.7	1.1	0.5
26–100 days use past year	2.8	5[a]	2.8	1.8	0.8
Over 100 days of use past year	4	14[a]	8.6	4	1.9

US NSDUH 2012 (SAMHSA, 2013). Note: bold data indicates highest prevalence for use by current age cohort, when excluding lifetime use (ever used). Past year use is most prevalent.
[a]*Indicates age group with greatest prevalence by usage frequency. 18–25 year olds have the highest rate of chronic use.*

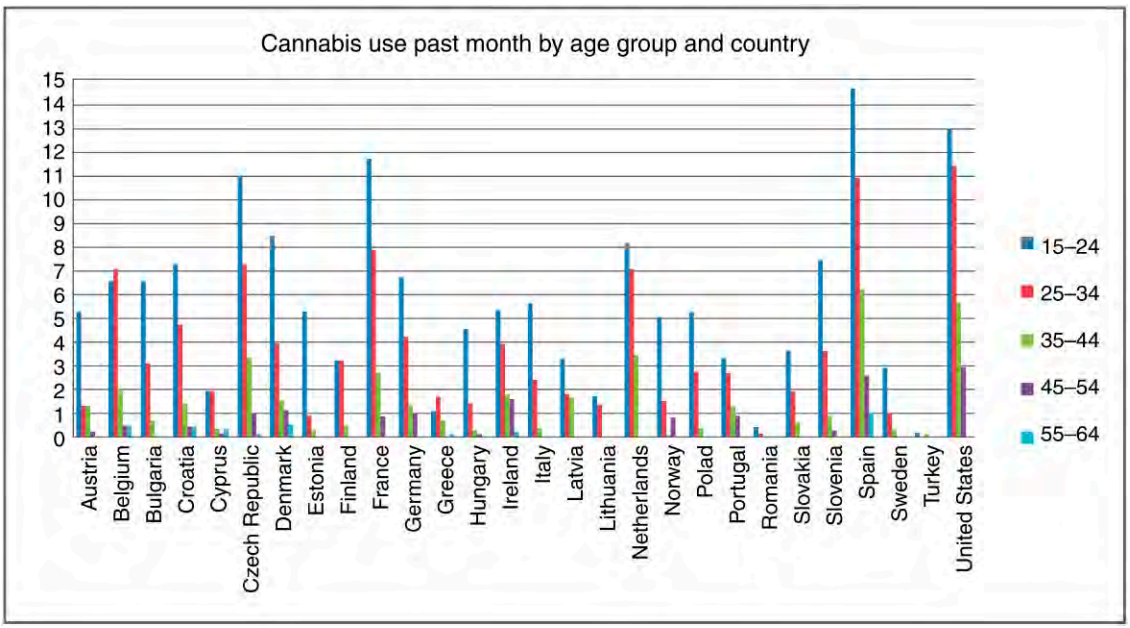


FIGURE 4.3 **Past month cannabis use by age group and country.** European data is from the European Monitoring Center on Drugs and Drug Addiction (EMCDDA, 2012), US data is from NSDUH (SAMHSA, 2013). Past month use is highest among 15–24 year olds across all countries, except Belgium, where it is greater among 25–34 year olds. The highest prevalence of use among people in the 45–54 age group is in the United States.

TABLE 4.3 Past Year Initiates Cannabis Use among US Adults Aged 26–49, 2002–2012

Mean age at first cannabis use among past year initiates in the United States, aged 26–49											
Age	31.2	29.6	29.5	30.4	29.1	32.4	32.6	32.2	36.3	29.5	33.1
Numbers	90,000	56,000	127,000	122,000	126,000	121,000	155,000	49,000	210,000	138,000	139,000
Year	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012

SAMHSA (2013); US NSUDH, 2002–2012.

as responsibilities (work, family) increase, and return to using again later in life (Kotarba, 2012). The mean age of first use of cannabis in Australia for persons aged 12 and older was 18.5 in 2010, indicating that youth are delaying their age of first use and/or there is an increase in older populations initiating cannabis use (AIHW, 2011).

Cannabis Use, Social Norms, and Legal Status

Over the past decade, many countries have reduced the severity of penalties for simple cannabis use or possession (Bergen-Cico, 2013; EMCDDA, 2014). European emphasis on cannabis control has focused on targeting drug supply and trafficking, rather than personal use and minor possession, and yet the number of offences related to cannabis personal possession and use have steadily increased over the past decade (EMCDDA, 2014). The legalization of cannabis in Uruguay, Colorado State and Washington State, where recreational cannabis use has been legalized with oversight and control, have been accompanied by increasing support for decriminalization and legalization of cannabis across the United States, Mexico, Central and South America, and many European countries (Uruguay, 2014; State of Washington, 2011; Amendment 64 Use and Regulation of Marijuana, 2012). Portugal and Netherlands have decriminalized cannabis use, and Switzerland is experimenting with legal recreational use. The United States reported a major decrease (~77%) in cannabis cultivation site eradication from 2011 to 2012, in conjunction with declining law enforcement efforts and increasing licit cultivation in the states of Colorado and Washington. What the change in licit supply will mean for usage across age groups is not yet known, as legal recreational use has been in place for less than a year, as of the writing of this chapter. The states of Washington and Colorado have established 21 as the minimum legal age for purchasing cannabis; how this may affect usage rates across age groups, notably people who were previously deterred by the illicit status of cannabis, has yet to be measured.

With the change in legal status comes a need to revisit the ways in which we have traditionally assessed consequences of cannabis use, and how legal consequences have delineated CUDs. Legal status aside, there is a need to examine potential risks associated with cannabis use across age groups, in the context of current use, frequency of use, and well-being. Decreases in legal consequences and increasing legal access to cannabis are reportedly part of the reason for increasing usage among older populations. Furthermore, cannabis use among older adults may be becoming normative, with increasing prevalence of cannabis using peer cohorts, as discussed in the latter part of this chapter.

Global Use

In 2012, between 125 million and 227 million people were estimated to have used cannabis, corresponding to about 3–5% of the global population aged 15–64 years (UNODC, 2014). According to the United Nations Office of Drugs and Crime (UNODC), the regions with the highest prevalence rates are West and Central Africa, Western and Central Europe, North America, and Oceania. Current cannabis usage epidemiological data is not available from Asia although the World Drug Report suggests use is increasing in Asia.

Among European nations, past year usage rate is estimated at 18.1 million, or 5.3% of the population aged 15–64 years. Whereas the past year usage rate for the United States was 7.3% of the population aged 12 and older, in 2012, this represents a notable increase from a rate of 6.2% in the United States in 2002. A closer look at this data within age groups provides some divergent trends. For example, among people aged 12–17 in the United States, the usage rates for cannabis dropped to 7.2% in 2012, from 8.2% in 2002. In fact, over this 10 year period, cannabis usage actually decreased among all age groups, for people under the age of 18 in the United States, while it increased among 18–25 year olds: from 17.3 in 2002 to 18.3% in 2012; from 7.7 to 11.3% among 26–34 year olds; and from 3.1 to 3.9% among those over the age of 35 (NCHS, 2014).

Table 4.4 presents data on current cannabis use by age group for 30 countries, and Table 4.5 includes data for 28 countries. The data illustrates the percentage of the population who currently use cannabis, where current use is defined in Table 4.4 as past year, and in Table 4.5 covers data on use in the past month. There are notable variations in the prevalence of use reported by country and by age group. It is important to note such variation because when we examine age as a predictor of use, use is contextual to the country, as well as age group. Variations

TABLE 4.4 Past Year Cannabis Use by Age Group: Europe, Canada, and the United States

Age groups	15–24	25–34	35–44	45–54	55–64
Austria	10.6	2.8	1.8	2.4	0.4
Belgium	11.9	10.5	3.5	0.7	1.0
Bulgaria	11.2	6.0	1.3	0.0	0.1
Canada[a]	20.3	8.4			
Croatia	12.6	8.4	2.5	0.7	0.5
Cyprus	4.3	4.0	1.0	0.2	0.7
Czech Republic	23.3	14.6	6.2	2.9	0.7
Denmark	23.9	10.4	3.6	2.3	1.2
Estonia	19.4	7.6	1.1	0.0	0.0
Finland	11.8	10.7	1.2	0.4	0.0
France	20.8	14.3	5.1	2.4	0.8
Germany	14.7	8.7	2.9	1.7	0.2
Greece	3.6	2.8	1.1	0.1	0.2
Hungary	10.1	2.9	0.4	0.4	0.0
Ireland	12.9	8.5	3.7	2.6	0.9
Italy	12.1	5.7	1.5	N.A.	N.A.
Latvia	10.0	4.6	3.7	0.3	0.1
Lithuania	6.1	4.0	0.7	0.1	0.1
Netherlands	16.1	11.3	4.9	N.A.	N.A.
Norway	11.7	4.0	0.7	1.5	0.3
Poland	11.0	5.7	1.4	0.3	0.4
Portugal	5.8	4.6	2.3	1.0	0.2
Romania	0.9	0.4	0.0	0.3	0.0
Slovakia	11.2	4.1	1.5	0.5	0.3
Slovenia	15.0	6.9	1.7	0.8	0.2
Spain	20.7	14.6	8.5	4.3	1.3
Sweden	9.7	4.3	1.2	0.4	0.3
Turkey	0.5	0.3	0.2	0.1	0.5
United Kingdom	13.5	7.9	4.4	2.6	1.7
Age Groups	**12–25**	**26–34**	**35–49**	**50–54**	**55–64**
United States[b]	22.7	19.5	10	8	5.9[c]

Note: some US age groups differ between EMCDDA, Canada and the United States. *N.A.*, data not available.
[a]*CCSA (2014).*
[b]*SAMHSA (2013).*
[c]*Estimate from NSDUH 2012 data.*
EMCDDA 2008–2012 unless noted.

between the 15–24 years age group and the 25–34 age group for past year cannabis use are quite notable for some countries, such as, Austria (10.6 and 2.8, respectively) and Hungary (10.1 and 2.9, respectively), yet negligible for others, such as, Belgium (11.9 vs 10.5 respectively) and Finland (11.8 vs 10.7 respectively) (EMCDDA, 2012).

Cannabis Use Among Older Adults

Until recently, cannabis use and CUD among older individuals was considered rare (Compton, Grant, Colliver, Glantz, & Stinson, 2004). However, as the generation who came of age in the 1960s began to reach age 50, we have

TABLE 4.5 Past Month Cannabis Use by Age Group, Europe and the United States

Age groups	15–24	25–34	35–44	45–54	55–64
Austria	5.3	1.4	1.4	0.3	0.0
Belgium	6.6	7.1	1.9	0.6	0.6
Bulgaria	6.6	3.2	0.8	0.0	0.0
Croatia	7.3	4.8	1.5	0.5	0.5
Cyprus	2.0	2.0	0.4	0.2	0.4
Czech Republic	11.0	7.3	3.4	1.1	0.2
Denmark	8.5	4.0	1.6	1.2	0.6
Estonia	5.3	1.0	0.4	0.0	0.0
Finland	3.3	3.3	0.6	0.0	0.0
France	11.8	7.9	2.8	1.0	0.2
Germany	6.8	4.3	1.5	1.1	0.0
Greece	1.2	1.8	0.8	0.0	0.2
Hungary	4.6	1.5	0.4	0.2	0.0
Ireland	5.4	4.0	1.9	1.7	0.3
Italy	5.7	2.5	0.5	N.A.	N.A.
Latvia	3.4	1.9	1.8	0.1	0.0
Lithuania	1.8	1.5	0.1	0.0	0.0
Netherlands	8.2	7.1	3.5	N.A.	N.A.
Norway	5.1	1.6	0.2	0.9	0.0
Poland	5.3	2.8	0.5	0.0	0.0
Portugal	3.4	2.8	1.4	1.0	0.1
Romania	0.5	0.2	0.0	0.0	0.0
Slovakia	3.7	2.0	0.7	0.1	0.0
Slovenia	7.5	3.7	1.0	0.4	0.1
Spain	14.7	11.0	6.3	2.7	1.1
Sweden	3.0	1.1	0.4	0.1	0.1
Turkey	0.2	0.1	0.2	0.1	N.A.
Age Groups	**12–25**	**26–34**	**35–49**	**50+**	
United States[a]	13	11.5	5.7	3	N.A.

Note: Some US age groups differ between EMCDDA. *N.A.*, data not available.
[a]*(SAMHSA, 2013)*
EMCDDA 2008–2012 unless noted.

seen an increase in current cannabis users among older people (Blazer & Wu, 2009; Han et al., 2009a,b). This generational shift in drug use is evident in the US National Survey on Drug Use and Health (NSDUH) data that shows increasing rates of current recreational drug use among persons aged 50–64 (SAMHSA, 2013). The current 50–64 year old cohort, comprised of baby boomers born after 1950, had higher rates of recreational drug use among youth, as compared to previous generations. By 2014, the 50–64 year old cohort consisted entirely of baby boomers who now account for the increase in cannabis use among older adults. A decade ago, researchers predicted that, by 2020, there would be a threefold increase in substance abuse problems among adults aged 50 and older (Gfroerer, Penne, Pemberton, & Folsom, 2003; Gfroerer, Wu, & Penne, 2002). In 2012, the percentage of persons in the age groups 50–54, 55–59, and 60–64 who reported using marijuana in the past year increased significantly over the prior decade (since 2002). In the United States, in 2012, 8% of those aged 50–54 said they had used

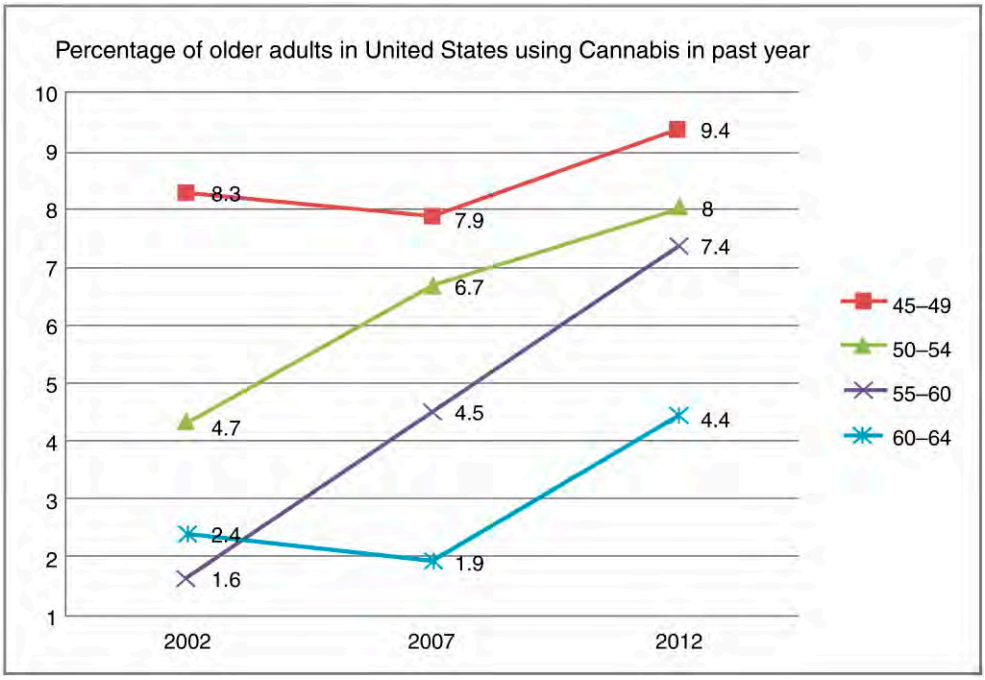


FIGURE 4.4 **Percentage of older adults in United States using cannabis in the past year.** These graphs show strong and steady upward trends in the percentage of older adults who recently used cannabis. The data represent people who came of age during and following the 1960s and, therefore, belong to a peer cohort in which cannabis use has been more normative than previous generations. *Source: NSDUH 2002, 2007, and 2012 (SAMHSA, 2013).*

marijuana in the past year, nearly double the percentage from 2002. Users aged 55–59 more than quadrupled, from 1.6% in 2002 to 7.4% in 2012. Among Americans aged 60–64, use nearly doubled, from 2.4 to 4.4% in the same period. Fig. 4.4 shows the rates of marijuana use in the previous year, by 5-year age groups (45–49; 50–54; 55–59; 60–64) for the years 2002, 2007, and 2012. Use among the older adults and the elderly varies from country to country and, upon further analysis, there are notable differences within age groups in a given country. For example, in England, current cannabis use among older adults varies widely between those in the 50–64 age group (1.8% past year use) and those over 65 (0.4%), and people over 50 living in London use cannabis at a rate 4 times higher than England (Fahmy, Hatch, Hotopf, & Stewart, 2012).

Survey data alone does not provide much context regarding current cannabis use among older adults, although they increasingly report using cannabis medically for nausea and pain (Sacco & Kuerbis, 2013). In addition to recreational use of cannabis, there is increasing use of cannabis for medical purposes. Medical use of cannabis is legal and controlled in Canada, the Netherlands, Switzerland, and several states in the United States.

Cannabis abuse or dependence is reported by very few older adults, with only 0.004% of US adults aged 50–64 reporting cannabis abuse or possible dependence in the current year. This suggests that current cannabis use among older populations may not be problematic, provided the individuals have the ability to cope with stressors, and self-regulate their emotions in the absence of cannabis and other psychoactive substances. The extent to which their use may be problematic depends on factors, such as, emotional coping strategies in the absence of cannabis use, interpersonal and social skills, and the extent to which they have been able to be active contributing members of society, regardless of their use.

MINI-DICTIONARY

Age of first use; age of initiation; age of onset These terms may be used interchangeably. The term refers to the age at which a person first used a drug. In this chapter, the drug is cannabis.
Cannabis A drug derived from certain plants in the Cannabaceae/hemp family. *Cannabis* is also commonly known as *Cannabis indica*, *Cannabis sativa*, hash, hashish, marihuana, or marijuana. *Cannabis* may be used in several forms, but is most commonly used in dried leaf form that can be smoked, vaporized, and eaten when prepared in food products such as specialty baked goods and chocolates.
Current use This term refers to use of a drug within the past 30 days and indicates a person who currently uses that drug. In this chapter, the drug refers to cannabis.
Lifetime use Reported use of cannabis in the 30 days or past month preceding the survey or similar means of data collection.
Medicinal cannabis Medical cannabis, also known as medical marijuana, refers to the use of cannabis for the treatment, or symptom reduction, of a range of illnesses and conditions. It is

recognized as a treatment for glaucoma, wasting disease, nausea, some forms of pain, and some seizure disorders.

Past 30-day use Reported use of cannabis in the 30 days or past month preceding the survey or similar means of data collection.

Past year use Reported use of cannabis in the 12 months preceding the survey or similar means of data collection.

Prevalence The proportion (usually expressed as a percentage) of a defined group (eg, age group, gender, race) or population reporting a specified behavior or outcome. In this chapter, the behavior is cannabis use.

Recent use This term refers to use of a drug at a recent point in time in a person's life, defined here as within the previous year. In this chapter, the drug refers to cannabis.

THC is the acronym for tetrahydrocannabinol, the psychoactive ingredient in cannabis. The higher the level of THC the more potent the cannabis is. Chronic use of high potency THC increases likelihood of CUD and dependency. Levels of THC vary from across types of cannabis.

References

Amendment 64 Use and Regulation of Marijuana, Constitution of the State of Colorado. Article XVIII, Available from: http://www.fc-gov.com/mmj/pdf/amendment64.pdf

Arria, A. M., Dohey, M. A., Mezzich, A. C., Bukstein, O. G., & Van Thiel, D. H. (1995). Self-reported health problems and physical symptomatology in adolescent alcohol abusers. *Journal of Adolescent Health, 16*(3), 226–231.

Australian Institute of Health and Welfare (AIHW) (2011) National Drug Strategy Household Survey 2010.;1; Drug Statistics Series No. 25. Cat. no. PHE 145. Canberra: AIHW.

Bachman, J. G., O'Malley, P. M., Schulenberg, J. E., Johnston, L. D., Freedman-Doan, P., & Messersmith, E. E. (2008). *The education-drug use connection: How successes and failures in school relate to adolescent smoking, drinking, drug use, and delinquency*. New York: Francis Taylor.

Bergen-Cico, D. (2000). Substance abuse and attrition among first-year college students. *Journal of the First-Year Experience & Students in Transition, 12*(1), 61–76.

Bergen-Cico, D. (2013). Perspectives of US drug policy: observations and advice from drug policy administrators in emerging democracies in Central and Eastern Europe. *World Medical & Health Policy, 5*(4), 362–389.

Bergen-Cico, D., & Lape, M. (2013). Age of first use as a predictor of current alcohol and marijuana use among college-bound adolescents. *Journal of Child and Adolescent Substance Abuse, 22*, 235–253.

Bergen-Cico, D., Urtz, A., & Barretto, C. (2004). Longitudinal assessment of the effectiveness of environmental management and enforcement strategies on college student substance abuse behaviors. *Journal of Student Affairs Research and Practice, 41*(2), 235–262.

Behrendt, S. (2015). How age leads to cannabis use disorders. In Victor Preedy (Ed.), *The comprehensive handbook of cannabis related pathology. Biology, diagnosis, treatment, and pharmacology*. Philadelphia: Academic Press.

Behrendt, S., Wittchen, H. -U., Höfler, M., Lieb, R., & Beesdo, K. (2009). Transitions from first substance use to substance use disorders in adolescence: is early onset associated with a rapid escalation? *Drug and Alcohol Dependence, 99*(1), 68–78.

Blazer, D. G., & Wu, L. T. (2009). The epidemiology of substance use and disorders among middle aged and elderly community adults: national survey on drug use and health. *American Journal of Geriatric Psychiatry, 17*(3), 237–245.

Brook, J. S., Zhang, C., & Brook, D. W. (2011). Developmental trajectories of marijuana use from adolescence to adulthood: personal predictors. *Archives of Pediatrics & Adolescent Medicine, 165*(1), 55–60.

Canadian Centre on Substance Abuse (CCSA). (2014). Canadian drug summary: cannabis ISBN 9781771781244.

Chen, C. Y., O'Brien, M. S., & Anthony, J. C. (2005). Who becomes cannabis dependent soon after onset of use? Epidemiological evidence from the United States: 2000-2001. *Drug and Alcohol Dependence, 79*, 11–22.

Compton, W. M., Grant, B. F., Colliver, J. D., Glantz, M. D., & Stinson, F. S. (2004). Prevalence of marihuana use disorders in the United States. 1991–1992 and 2001–2002. *Journal of the American Medical Association, 291*(17), 2114–2121.

Dawson, D. A., Goldstein, R. B., Chou, S. P., Ruan, W. J., & Grant, B. F. (2008). Age at first drink and the first incidence of adult-onset DSM-IV alcohol use disorders. *Alcoholism: Clinical and Experimental Research, 32*(12), 1–12.

Degenhardt, L., Chiu, W. T., Sampson, N., Kessler, R. C., Anthony, J. C., Angermeyer, M., & Wells, J. E. (2008). Toward a global view of alcohol, tobacco, cannabis, and cocaine use: findings from the WHO World Mental Health Surveys. *PLoS Medicine, 5*(7), e141.

DeWit, D. J., Adlaf, E. M., Offord, D. R., & Ogborne, A. C. (2000). Age at first alcohol use: a risk factor for the development of alcohol disorders. *American Journal of Psychiatry, 157*(5), 745–750.

Eaton, D. K., Kann, L., Kinchen, S., Shanklin, S., Ross, J., Hawkins, J., … & Wechseler, H. (2010). Youth risk behavior surveillance—United States, 2009. MMWR Surveillance Summaries, *59*(SS-5), 1–148.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). (2012). *Annual report [2010:] the state of the drugs problem in Europe*. Luxembourg: Publications Office of the European Union.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). (2014). European drug report 2014 trends and developments. Lisbon Portugal. Luxembourg: Publications Office of the European Union.

Fahmy, V., Hatch, S. L., Hotopf, M., & Stewart, R. (2012). Prevalences of illicit drug use in people aged 50 years and over from two surveys. *Age and Ageing, 41*(4), 553–556.

Fergusson, D. M., Horwood, L. J., & Swain-Campbell, N. R. (2002). Cannabis use and psychosocial adjustment in adolescence and young adulthood. *Addiction, 97*(9), 1123–1135.

Geller, T., Loftis, L., & Brink, D. S. (2004). Cerebellar infarction in adolescent males associated with acute marijuana use. *Pediatrics, 113*(4), e365–e370.

Gfroerer, J., Penne, M., Pemberton, M., & Folsom, R. (2003). Substance abuse treatment need among older adults in 2020: the impact of the aging baby-boom cohort. *Drug and Alcohol Dependence, 69*(2), 127–135.

Gfroerer, J.C., Wu, L.-T., & Penne, M.A. (2002). Initiation of marijuana use: trends, patterns, and implications (Analytic Series: A-17, DHHS Publication No. SMA 02-3711). Rockville, MD: Substance Abuse and Mental Health Services Administration (SAMHSA), Office of Applied Studies.

Grunbaum, J. A., Kann, L., Kinchen, S., Ross, J., Hawkins, J., Lowry, R., … & Collins, J. (2004). Youth risk behavior surveillance--United States, 2003. MMWR. Surveillance Summaries. *53*(2), 1–96.

Han, B., Gfroerer, J. C., Colliver, J. D., & Penne, M. A. (2009a). Substance use disorder among older adults in the United States in 2020. *Addiction, 104*(1), 88–96.

Han, K. H., Lim, S., Ryu, J., Lee, C. W., Kim, Y., Kang, J. H., Kang, S. S., Ahn, Y.K., Park, C.S. & Kim, J. J. (2009b). CB1 and CB2 cannabinoid receptors differentially regulate the production of reactive oxygen species by macrophages. *Cardiovascular Research*, cvp240.

Horwood, L. J., Fergusson, D. M., Hayatbakhsh, M. R., Najman, J. M., Coffey, C., Patton, G. C., & Hutchinson, D. M. (2010). Cannabis use and educational achievement: findings from three Australasian cohort studies. *Drug and Alcohol Dependence, 110*(3), 247–253.

Inaba, D., & Cohen, W. E. (2014). *Uppers, downers, all rounders: Physical & mental effects of psychoactive drugs* (7th ed.). Medford, OR: CNS Productions.

Johnston, L.D., O'Malley, P.M., Bachman, J.G., & Schulenberg, J.E. (2010). Monitoring the Future: National Survey Results on Drug Use, 1975-2009. Volume II: College Students and Adults Ages 19–50. NIH Publication No. 10-7585. National Institute on Drug Abuse.(NIDA).

Juon, H. S., Fothergill, K. E., Green, K. M., Doherty, E. E., & Ensminger, M. E. (2011). Antecedents and consequences of marijuana use trajectories over the life course in an African American population. *Drug and Alcohol Dependence*, *118*(2), 216–223.

Keyes, K. M., Schulenberg, J. E., O'Malley, P. M., Johnston, L. D., Bachman, J. G., Li, G., & Hasin, D. (2011). The social norms of birth cohorts and adolescent marijuana use in the United States, 1976–2007. *Addiction*, *106*(10), 1790–1800.

Kirisci, L., Tarter, R., Ridenour, T., Zhai, Z. W., Fishbein, D., Reynolds, M., & Vanyukov, M. (2013). Age of alcohol and cannabis use onset mediates the association of transmissible risk in childhood and development of alcohol and cannabis disorders: evidence for common liability. *Experimental and Clinical Psychopharmacology*, *21*(1), 38–45.

Kokkevi, A., Gabhainn, S. N., & Spyropoulou, M. (2006). Early initiation of Cannabis use: a cross-national European perspective. *Journal of Adolescent Health*, *39*(5), 712–719.

Kosterman, R., Hawkins, D., Guo, J., Catalano, R. E., & Abbott, R. D. (2000). The dynamics of alcohol and marijuana initiation: patterns and predictors of first use in adolescence. *American Journal of Public Health*, *90*(3), 360–366.

Kotarba, J. A. (2012). *Baby boomer rock'n'roll fans: the music never ends*. Lanham, MD: Scarecrow Press.

Lopez-Quintero, C., Perez de los Cobos, J., Hasin, D. S., Okuda, M., Wang, S., Grant, B. F., & Blanco, C. (2011). Probability and predictors of transition from first use to dependence on nicotine, alcohol, cannabis, and cocaine: results of the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC). *Drug and Alcohol Dependence*, *115*(1), 120–130.

National Center for Health Statistics (NCHS) (2014) Health, United States, 2013: Special feature on prescription drugs. Hyattsville, MD.

Patrick, M. E., Schulenberg, J. E., O'Malley, P. M., Johnston, L. D., & Bachman, J. G. (2011). Adolescents' reported reasons for alcohol and marijuana use as predictors of substance use and problems in adulthood. *Journal of Studies on Alcohol and Drugs*, *72*(1), 106–116.

Sacco, P., & Kuerbis, A. (2013). Older adults. In M. G. Vaughn, & B. E. Perron (Eds.), *Social work practice in the addictions, contemporary social work practice* (pp. 213–227). New York: Springer.

Schulenberg, J. E., Merline, A. C., Johnston, L. D., O'Malley, P. M., Bachman, J. G., & Laetz, V. B. (2005). Trajectories of marijuana use during the transition to adulthood: the big picture based on national panel data. *Journal of Drug Issues*, *35*(2), 255–280.

Substance Abuse and Mental Health Services Administration (SAMHSA), (2013) Results from the 2012 National Survey on Drug Use and Health (NSDUH): Summary of National Findings, NSDUH Series H-46, HHS Publication No. (SMA) 13-4795. Rockville, MD: Substance Abuse and Mental Health Services Administration.

State of Washington. (2011). Initiative Measure No. 502. Accessed at http://lcb.wa.gov/publications/Marijuana/I-502/i502.pdf

United Nations Office on Drugs and Crime (UNODC). (2014). World Drug Report 2014. Vienna, Austria.

Uruguay. (2014). Law No. 19.172.

Wallace, J. M., Forman, T. A., Guthrie, B. J., Bachman, J. G., O'Malley, P. M., & Johnston, L. D. (1999). The epidemiology of alcohol, tobacco and other drug use among black youth. *Journal of Studies on Alcohol*, *60*(6), 800–809.

Washburn, I. J., & Capaldi, D. M. (2014). Heterogeneity in men's marijuana use in the 20s: adolescent antecedents and consequences in the 30s. *Development and Psychopathology*, 1–13.

Winters, K. C., & Lee, C. -Y. S. (2008). Likelihood of developing an alcohol and-cannabis use-disorder during youth: association with recent use and age. *Drug and Alcohol Dependence*, *92*(1–3), 239–247.

CHAPTER

5

Lifetime Cannabis Use and Cognition in Psychosis Spectrum Disorders

M.J. Cuesta, A.M. Sánchez-Torres, R. Lorente-Omeñaca, L. Moreno-Izco

Department of Psychiatry, IdiSNA, Navarra Institute for Health Research, Pamplona, Spain

SUMMARY POINTS

- Research concerning the association among cannabis use, cognition, and psychosis is not conclusive.
- Most of the research points to better cognitive functioning among patients using cannabis, but there are also findings supporting similar or even worse performance in cannabis users, compared with nonusers.
- Better cognition has been related to better social skills and premorbid IQ in cannabis users, as well as lower vulnerability to psychosis.
- It has also been proposed that cannabis may have neuroprotective or cognitive enhancing properties.
- Possible confounders in study designs should be controlled to obtain more robust findings.

KEY FACTS OF COGNITION AND PSYCHOSIS

- Psychotic disorders are characterized by the presence of positive, negative, disorganized, and/or cognitive symptoms.
- Cognitive deficits in psychosis are mainly related to processing speed, verbal memory, working memory, executive functions, attention, and social cognition.
- Cognitive deficits are qualitatively similar among psychotic disorders, being more severe in schizophrenia, and milder in affective psychoses.

KEY FACTS OF CANNABIS AND PSYCHOSIS

- Cannabis use is more prevalent among patients with psychosis, than in the general population.
- It is related to poorer treatment compliance, higher rates of relapse, and worse psychosocial outcomes.
- It has been associated with an increased risk of developing psychosis, and with an earlier age of onset.

INTRODUCTION

Psychosis spectrum disorders include those diagnoses that involve nonaffective psychoses, such as, schizophrenia, schizophreniform or brief psychotic disorders, and also affective psychoses, such as, bipolar and schizoaffective disorders. They are characterized by the presence of a wide range of symptoms, which may include delusions, hallucinations, disorganized behavior, blunted affect, and abulia, among others. Moreover, cognitive deficits are considered core features of psychotic disorders, and represent an important target for research because of their impact on functional outcome, treatment adherence, and risk of relapse (Carter et al., 2008).

The prevalence of cannabis use disorders among patients with a psychotic disorder is higher than in the

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00006-5
Copyright © 2017 Elsevier Inc. All rights reserved.

TABLE 5.1 Cognitive Effects of Cannabis Use in Healthy Subjects

Studies with healthy subjects	Findings related to cognitive performance
Fried et al. (2005)[a] Pure cannabis users	Current users (24 h abstinence) impairments in: *Episodic memory (immediate and delayed)* *Visual processing speed* Lower *IQ* Former users (3 months abstinence): None
Medina et al. (2007)[b] Adolescent users after 30 days of abstinence	Impairments in: *Spatial processing* *Processing speed* *Episodic memory* *Selective attention* *Planning*
Meier et al. (2012) Dunedin cohort, 20 years of persistent use	*Global cognitive impairment* Decline in *IQ* Worse results in adolescent-onset users Impact on everyday functioning
Tait et al. (2011)[c]	Improved performance in an *immediate recall task* related to sustained abstinence
Grant et al. (2012)[d]	Impaired *executive planning* and *decision making*
Fernández-Serrano et al. (2011)	*Episodic memory* and *planning* deficits persist after mid-term abstinence No deficits after long-term abstinence
Pope et al. (2001)[e] Heavy cannabis users assessed after 0, 1, 7, and 28 days of abstinence	*Episodic memory* deficits after 0, 1, and 7 days of abstinence No deficits after 28 days of abstinence

[a]*Fried, P.A., Watkinson, B., & Gray, R. (2005). Neurocognitive consequences of marihuana-a comparison with pre-drug performance.* Neurotoxicology and Teratology, 27*(2), 231–239.*
[b]*Medina, K.L., Hanson, K.L., Schweinsburg, A.D., Cohen-Zion, M., Nagel, B.J., & Tapert, S.F. (2007). Neuropsychological functioning in adolescent marijuana users: subtle deficits detectable after a month of abstinence.* Journal of the International Neuropsychological Society, 13*(5), 807–820.*
[c]*Tait, R.J., Mackinnon, A., & Christensen, H. (2011). Cannabis use and cognitive function: 8-year trajectory in a young adult cohort.* Addiction, 106*(12), 2195–2203.*
[d]*Grant, J.E., Chamberlain, S.R., Schreiber, L., & Odlaug, B.L. (2012). Neuropsychological deficits associated with cannabis use in young adults.* Drug and Alcohol Dependence, 121*(1–2), 159–162.*
[e]*Pope, H.G. Jr., Gruber, A.J., Hudson, J.I., Huestis, M.A., & Yurgelun-Todd, D. (2001). Neuropsychological performance in long-term cannabis users.* Archives of General Psychiatry, 58*(10), 909–915.*

general population. Specifically, there is a median lifetime prevalence of 27.1% for cannabis use disorder in patients with schizophrenia (Koskinen, Lohonen, Koponen, Isohanni, & Miettunen, 2010). In addition, cannabis use has been demonstrated to be a risk factor for the development of psychosis (Henquet, Murray, Linszen, & van Os, 2005; Moore et al., 2007).

Both acute administration and long-term cannabis use have been related to cognitive impairments in healthy subjects (Fernández-Serrano, Perez-Garcia, & Verdejo-García, 2011; Verdejo-García, 2011). Global cognitive decline, which implies a lower IQ and a negative impact on everyday functioning, has been reported after 20 years of persistent cannabis use, especially among adolescent-onset users (Meier et al., 2012) (Table 5.1).

However, research on the relationship between cognition and cannabis use in patients with psychosis has produced counterintuitive results. Most studies have reported improved cognitive performance among psychotic patients who use cannabis, compared with those patients who do not. Therefore, several hypotheses have been proposed to explain these a priori striking results. In this chapter, we review the main findings regarding the relationship between lifetime cannabis use and cognition in psychosis spectrum disorders, and the hypothesis that could explain the mechanisms underlying this relationship.

COGNITIVE EFFECTS OF LONG-TERM USE OF CANNABIS IN PSYCHOTIC PATIENTS

The relationship between cannabis and cognition must be considered in regard to two main effects: the effects of acute administration, and those arising from chronic use. Here, we focus on the cognitive effects of chronic use in patients diagnosed with a psychotic spectrum disorder.

TABLE 5.2 Summary of Cognitive Areas Studied in Psychosis Related to Cannabis Use

Evidence of better performance in cannabis users	Evidence of worse performance in cannabis users
Memory	Premorbid IQ
Verbal fluency	Social cognition
Visuospatial construction	Working memory
Sequencing	Decision-making
Face recognition	Attention
Psychomotor/visuomotor speed	Verbal and visual learning
Verbal skills	Executive functions
Executive functions	
Working memory	

The effects of long-term cannabis use on cognition depend on several factors, such as, the continuity of cannabis use over time, the amount and frequency of use, the age at consumption onset and, of course, the premorbid cognitive functioning of the patient.

One might think that clinical populations would be more vulnerable to the adverse effects of cannabis on cognition. However, research on the relationship between psychotic illness and cognitive performance points to better cognitive functioning in cannabis-using patients, compared with those who do not present comorbid use (Potvin, Joyal, Pelletier, & Stip, 2008; Yucel et al., 2012). In addition, higher frequency of use and younger age at onset have been associated with higher cognitive functioning (Schnell, Koethe, Daumann, & Gouzoulis-Mayfrank, 2009).

Most longitudinal studies have reported better cognitive functioning in cannabis using patients, both in short follow-up periods (Rodríguez-Sánchez et al., 2010) and in long-term follow-up periods (Stirling, Lewis, Hopkins, & White, 2005). Specifically, Stirling, Lewis, Hopkins, and White (2005) followed a sample of patients with a first-episode of psychosis over 10–12 years, and reported that those with a history of cannabis use outperformed those who had never used cannabis in measures of memory, verbal fluency, visuospatial construction, sequencing, and face recognition. In contrast, we also followed a group of patients with a psychosis spectrum disorder and one of their siblings, over 10 years, and did not find a relationship between longitudinal patterns of cannabis use and cognitive outcomes in the patient group. Further, patients reporting longitudinal cannabis use showed poorer social cognition, and the combination of low premorbid IQ, current cannabis use, and earlier age at illness onset resulted in worse performance in the working memory domain (Sánchez-Torres et al., 2013). Cross-sectional studies have also found worse performance of some cognitive functions in patients with a history of cannabis use. Smith et al. (2014) reported cannabis-related striatal and thalamic shape abnormalities, also associated with poorer working memory performance and earlier age of cannabis use onset, both in schizophrenic patients and healthy controls who had a history of cannabis use. Mata et al. (2008) explored the influence of cannabis abuse prior to psychosis onset on executive functioning and decision-making tasks, and found that those patients who had abused cannabis showed worse performance on the decision-making task, but not in executive functioning (Table 5.2).

To interpret the results of this field, it is also important to have a healthy control group of cannabis users, because differential effects of cannabis in patients with schizophrenia and healthy subjects have been reported (Jockers-Scherubl et al., 2004). Abstinent schizophrenic patients with a history of chronic cannabis use showed better cognitive function than nonusers. However, abstinent healthy controls who were cannabis users showed impaired cognitive function compared with nonusers (Jockers-Scherubl et al., 2004, 2007).

Methodological variability in studies approaching the relationships between cannabis use and cognition in psychosis must be taken into account when interpreting the results. Factors, such as, sample size, diagnostic groups included, instruments used to assess drug use, and parameters associated with consumption, such as time since onset of consumption, frequency, and recency, should be taken into account when considering the effects of cannabis on cognition. For example, some of the studies have small samples, and this factor may reduce the statistical power by increasing type II statistical errors or false negatives (Loberg & Hugdahl, 2009) (Table 5.3).

Regarding diagnosis, most studies have focused on schizophrenia and nonaffective psychosis, and also on first-episode psychosis. Patients with bipolar disorder

TABLE 5.3 Methodological Issues to Take Into Account When Interpreting Results of the Association Between Cannabis and Cognition in Psychosis

- Sample size
- Instruments used to assess cannabis consumption
- Diagnosis included
- Variability in the duration of use
- Comorbid drug use
- Pharmacological treatments
- Illness related variables:
  - Age at psychosis onset
  - Premorbid IQ
  - Premorbid adjustment
  - Psychopathology
- History of cannabis use:
  - Age at onset
  - Frequency and amount of cannabis use
  - Potency of the substance
  - Time since last use
- Data analysis: causality of associations

have shown better performance in measures of attention, processing speed, set-shifting, and working memory (Braga, Burdick, Derosse, & Malhotra, 2012). Ringen et al. (2010) reported differential associations between cannabis and cognition in patients with schizophrenia and bipolar disorders. While schizophrenic patients who used cannabis underperformed nonusers in attention, executive functioning, and verbal memory measures, bipolar cannabis-using patients showed better verbal fluency than those patients who did not use cannabis.

How the patients were grouped according their consumption habits is another matter for discussion. They are often classified into dichotomic groups, and cognitive functioning is then compared between the two groups. The criterion to be included in these groups varies between studies, and often the frequency or amount of cannabis consumption, which should be taken into account in order to investigate dose-response relationships, is not considered (Rabin, Zakzanis, & George, 2011). Indeed, few studies use standardized instruments to assess cannabis use. Most studies establish a cutoff point to determine a positive or negative history of cannabis use, or include as consumers those patients who meet the criteria for a comorbid drug use disorder. As an example, in previous research we considered cannabis use as a continuous variable, based on information recollected with an adaptation of the Composite International Diagnostic Interview (CIDI; WHO, 1993) and explored the influence of lifetime cannabis consumption on cognition in chronic schizophrenia spectrum disorders (Sánchez-Torres et al., 2013). Rabin, Zakzanis, Daskalakis, and George (2013) also explored cumulative cannabis exposure and cognitive performance. They found an association between more years of cannabis exposure and poorer performance in tasks of attention, verbal and visual learning, and executive functions in patients with current cannabis use. Interestingly, these findings were only present in patients with current cannabis use, which has led the authors to suggest that abstinence may result in some cognitive improvement. This study also explored the differences in cognitive functioning caused by lifetime cannabis use by comparing current and former users with patients who had never used cannabis, and found that lifetime cannabis users showed better psychomotor speed. The combination of these apparently contradictory results may suggest that patients who have never used cannabis represent an etiologically different subgroup of patients, presenting worse cognitive functioning even in the absence of drug use.

One study published recently highlighted the importance of the frequency and the potency of the substance in the onset of psychotic illness (Di Forti et al., 2014). Although the latter has not been explored regarding cognitive function, it seems to be an important issue for future studies.

Another source of variability in research is the duration of cannabis consumption that is labeled as "chronic." Some studies include patients after 1 year of continued use (Rentzsch, Buntebart, Stadelmeier, Gallinat, & Jockers-Scherubl, 2011), while others include patients who have used cannabis for more than 10 years (Sánchez-Torres et al., 2013). In most studies, abstinence for at least 4 weeks (Rentzsch et al., 2011) or even as long as 6 months (Smith et al., 2014) is an inclusion criterion, to avoid the possible acute effects of consumption on cognition. For example, Mata et al. (2008) excluded patients with a current drug dependence diagnosis, and considered as patients with a history of cannabis consumption those who had at least weekly use during the year prior to illness onset.

The inclusion of patients who use other drugs may also influence the results of the cognitive assessment. Potvin et al. (2008) analyzed a pool of studies that addressed cognitive functioning in schizophrenic patients with different comorbid drug use. They concluded that this type of patient did not represent a homogeneous group, and the two factors that determined cognitive functioning were the preferred substance consumed, and the age at the assessment. In addition, substance use showed differential effects on cognition. For example, exclusive use of alcohol was related to global cognitive functioning, similar to that of schizophrenic patients without comorbid substance use, while mixed consumption including cannabis resulted in better cognitive functioning. Thus, drug interaction may have cognitive effects not comparable with the ones related to exclusive consumption of cannabis or other types of drugs. Moreover, the cognitive effects of nicotine are not considered in most studies because it is difficult to find cannabis users who do not use tobacco. However, tobacco smoking has an important impact on cognition because it has been related to enhancement of attention, working memory, and information processing (Sacco et al., 2005; Segarra et al., 2011). It has also been suggested that nicotine may interact with genes related to the risk of developing schizophrenia (Quednow et al., 2012). To avoid these confounders, Rabin et al. (2011) performed a meta-analysis including only those studies with groups of patients without current comorbid use of other drugs, but they did not include tobacco among these drugs. Their results further supported better cognitive performance in patients using cannabis, compared with nonusing patients, but the effect sizes were only in the small to medium range.

It is also of interest to account for the effects of pharmacological treatments on cognition associated with cannabis use. Most of the studies report antipsychotic doses, and some of them take this into account when analyzing the differences between cannabis users and nonusers. However, patients with psychosis usually received other

treatments, such as, anticholinergics, antidepressants, benzodiazepines, or mood stabilizers, which may also affect cognitive function, either improving or worsening performance. However, few studies report these treatments and account for them when assessing cognition (Coulston, Perdices, & Tennant, 2007).

Researchers have focused their efforts on minimizing the effect of confounding variables on the relationship between cognition and cannabis in psychotic illness. DeRosse, Kaplan, Burdick, Lencz, and Malhotra (2010) tried to control the possible clinical and demographic confounders in a large sample of schizophrenia and schizoaffective disorder patients (n = 455; 175 cannabis-using patients and 280 nonusing). Patients with comorbid cannabis abuse or dependence showed better performance on measures of processing speed and verbal skills, even after controlling for gender and global functioning, which were the only features that differed between groups. These authors emphasized the importance of studying homogeneous groups of patients to avoid the influence of possible confounders. However, this study rated lifetime clinical symptoms as present, subthreshold, and absent, which may obscure the complexity of the psychopathology in these disorders. They did not explain whether the patients used other drugs, or the amount and frequency of cannabis use.

Schnell et al. (2009) also noted the importance of understanding the history of cannabis use, including age at onset of use, time since last use, and lifetime average, as well as maximum frequency of use. Even after controlling for these variables and others such as psychopathological symptoms, education, and nicotine use, patients with schizophrenia and comorbid cannabis use disorder outperformed patients with schizophrenia without a history of cannabis use in visuomotor speed, executive functioning, and working memory tasks.

A recent study on a large sample of schizophrenic patients, conducted as part of the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) project, assessed the relationship between cognition and drug use (cannabis, cocaine, and methamphetamine), obtaining data of drug use from radioimmunoassay of hair. This test provided data on drug consumption in the last 90 days. Groups of consumers and nonconsumers were created according to these data. A key strength of this study, in addition to the implementation of the radioimmunoassay to confirm consumption, is the statistical analysis used. While most of the studies analyze the relationship between cannabis and cognition with association measures, which do not allow for the study of cause-effect relationships, this study applied moderator models to study the effects of drugs on cognition, taking into account multiple possible confounders. According to this study, patients who tested positive for cannabis use did not show better cognitive function, compared with those who tested negative. These results were consistent in nondeficit patients, for men and women, in patients in the middle and chronic phases of illness, and for those treated with lower and higher antipsychotic doses (Bahorik et al., 2014). These authors suggest that the finding of better cognitive functioning in cannabis users, compared with nonusers, may be a consequence of including first-episode patients with cannabis-induced psychosis, the heterogeneity of diagnoses, and the absence of laboratory testing to confirm drug consumption. Patients with schizophrenia tend to under-report drug use, so it would be desirable to contrast self-reports with laboratory testing to improve reliability (Bahorik et al., 2013).

In summary, most research points towards better cognitive function associated with cannabis use in psychotic patients. However, the results are inconclusive because of the methodological variability of the studies, the limited sample sizes, and the amount of research that has found no differences or poorer cognitive functioning in patients with comorbid cannabis use.

SUPERIOR COGNITIVE FUNCTIONING OF PSYCHOTIC PATIENTS WHO USE CANNABIS: EXPLICATIVE HYPOTHESIS

Findings supporting the cognitive superiority of psychotic patients who use cannabis, compared with nonusers, are inconclusive due to the methodological variability already discussed, but, to date, the majority of research points in this direction. Therefore, several hypotheses have been proposed to explain these counterintuitive findings (Table 5.4).

TABLE 5.4 Explicative Hypothesis of Cognitive Functioning Related to Cannabis Use in Psychotic Disorders

Hypothesis supporting *worse* cognitive functioning in psychotic patients using cannabis
• Cannabis use has neurotoxic effects, which are added to the brain abnormalities associated with psychosis • Sustained cannabis use is associated with striatal and thalamic shape abnormalities, which augment the brain abnormalities associated with psychosis • Animal models have demonstrated that cannabis abuse alter dopaminergic modulation in prefrontal cortex
Hypothesis supporting *better* cognitive functioning in psychotic patients using cannabis
• Cannabis users have higher premorbid IQ than nonusers • Cannabis users have superior social skills than nonusers • Cannabis induces psychosis in subjects with low vulnerability to develop a psychotic disorder • Cannabis has neuroprotective effects • Cannabis enhances cognition by stimulating prefrontal neurotransmission

One hypothesis suggests that patients with a history of cannabis use have better premorbid IQ than those who have never consumed cannabis. Leeson, Harrison, Ron, Barnes, and Joyce (2011) attributed the differences between cannabis-using and non-cannabis using first-episode patients to higher premorbid intellectual functioning in the cannabis users, prior to psychosis onset. Patients who used cannabis reported better cognitive functioning in current IQ, verbal memory, working memory span, and planning ability in the first episode. Cannabis users also showed higher premorbid IQ than nonusers; however, when controlling for premorbid IQ, the only differences in planning ability remained significant. These authors explain their results in the context of the cognitive reserve hypothesis. Higher cognitive reserve has a neuroprotective effect in patients with psychosis, which results in a later onset of psychosis, and has a positive impact on the course of the illness. Therefore, cannabis use may undermine this protective effect, anticipating the onset of psychosis. This hypothesis is also supported by the fact that good premorbid functioning is necessary to initiate and sustain illicit drug use (Rodríguez-Sánchez et al., 2010).

Better cognition in patients with comorbid cannabis use has also been related to superior social skills, compared with those patients without drug use. This hypothesis is based on the idea that the onset and maintenance of illegal drug consumption requires a set of social and personal skills. Moreover, higher interpersonal skills have been related to better cognitive functioning in schizophrenia (Harvey, Keefe, Patterson, Heaton, & Bowie, 2009), so this may be the link between cannabis use and better cognitive function. However, this idea contrasts with the findings that support poorer outcomes and a higher presence of psychotic symptoms in cannabis users with psychosis (Grech, Van Os, Jones, Lewis, & Murray, 2005; Linszen, Dingemans, & Lenior, 1994), leading to a worse course of illness. As Cohen, Solowij, and Carr (2008) proposed, the contradictory findings regarding clinical, psychosocial, and cognitive outcomes related to cannabis consumption in psychosis may be due to the differential response of brain areas responsible for psychotic, negative and cognitive symptoms to cannabis consumption.

Another hypothesis states that cannabis triggers psychosis in subjects with low vulnerability to develop a psychotic disorder. Therefore, these subjects may be more cognitively preserved than those psychotic patients who are genetically more vulnerable to psychosis and, thus, develop the illness even in absence of accumulative adverse environmental events. In fact, early onset of consumption and higher frequency of use have been related to an increased probability of developing psychosis (Di Forti et al., 2014; Moore et al., 2007). van Os, Bak, Hanssen, Bijl, de Graaf, and Verdoux (2002) demonstrated that cannabis was linked to the emergence of psychosis in up to 50% of subjects without genetic liability to psychosis. Thus, many psychotic patients would not have developed the illness if they had not used cannabis.

Relatives of psychotic patients represent a population with increased vulnerability to psychosis, but research concerning cognition and cannabis use in relatives of psychotic patients is scarce. It has been suggested that genetic risk for psychosis is associated with sensitivity to cannabis. This is demonstrated by studies of unaffected siblings who report increased psychotomimetic effects of cannabis, both regarding current and lifetime use (GROUP Investigators, 2011; van Winkel & Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011). Our study of a group of psychotic patients and their siblings, which has already been discussed (Sánchez-Torres et al., 2013), revealed a greater influence of cannabis on cognitive performance in siblings, compared with controls. Siblings showed poorer processing speed associated with cannabis use 10 years prior, consumption over 10 years, current use, and an earlier age at cannabis use onset. Cannabis use 10 years prior and current use were also associated with poorer declarative memory. In controls, cannabis use did not predict cognitive performance independently, but was associated with other variables such as earlier age at cannabis use onset, lower IQ, and tobacco use. These results could be explained by a greater vulnerability to the effects of cannabis in siblings. On the contrary, Meijer, Simons, Quee, and Verweij (2012) did not find a differential association between cannabis use and cognitive functioning between psychotic patients, siblings, and controls. They found an association of current cannabis use with worse performance on immediate verbal learning, processing speed, and working memory in the three groups. However, current cannabis use was associated with better performance on acquired knowledge, affect recognition, and face identity recognition in all three groups. Although they used a structured interview to assess drug use, frequency of consumption was highly variable in the lifetime cannabis use group, ranging from 5 to more than 100 times in a subject's lifetime. Additionally, it is noteworthy that cannabis frequency referred only to the preceding year, which may have led to a dilution of dose-response cannabis effects.

The supposed neuroprotective effects of cannabis, which would counteract the putative neurotoxic process related to psychosis have also arisen as a possible explanation for the better cognition associated with cannabis. Many studies have attributed antioxidant, antiexcitotoxic and antiinflammatory properties to cannabinoids at the cellular level, as well as neuroprotective or neurotoxic effects in vitro and in vivo (Sarne & Mechoulam, 2005). This hypothesis is supported by studies with animal models of Parkinson's and Alzheimer's

disease (Ramírez, Blázquez, Gómez del Pulgar, Guzmán, & de Ceballos, 2005). Furthermore, studies with rodents suggest that chronic cannabis administration causes behavioral deficits in healthy animals, but also restore some functions in animals that present brain abnormalities similar to those seen in psychotic patients (Seillier, Advani, Cassano, Hensler, & Giuffrida, 2010). Thus, better cognitive functioning in cannabis-using patients may reflect the neuroprotective effect of cannabinoids in an injured brain, as this association is not found in healthy subjects.

Some authors suggest that cannabinoids may enhance cognition by stimulating prefrontal neurotransmission (Coulston et al., 2007), because the distribution of CB1 endogenous cannabinoid receptors is dense in the prefrontal cortex, specifically in the cingulated and superior frontal gyri. Cannabis use, by mediating neurotransmitter release in these brain areas, may have the capacity to enhance cognitive performance and reduce negative symptoms. However, in most studies reporting better cognitive function related to cannabis use, patients were abstinent for at least 3–4 weeks, so the acute effects of cannabis may be discarded as an explicative hypothesis.

It has been highlighted that although cannabis-using patients perform better in cognitive tasks than nonusing patients, it does not mean that cannabis improves cognition in schizophrenia (Rabin et al., 2011). On the contrary, some authors contend that better cognitive functioning represents a risk factor for cannabis abuse in schizophrenia (DeRosse et al., 2010).

CONCLUSIONS

The relationship between cannabis use, psychosis, and cognition is still a matter of debate. Research on healthy subjects has demonstrated that cannabis use entails adverse cognitive effects. Thus, it is unlikely that cannabis can improve cognition in psychotic patients who also present core cognitive deficits associated with the mental disorder. In addition, cannabis use involves worse clinical outcomes in psychotic patients, such as, higher rates of relapse, worse psychosocial outcomes, and worse adherence to treatment.

The methodological limitations already exposed should be overcome to better characterize this relationship. Prospective studies that perform exhaustive assessments of cannabis use and additional laboratory testing and control for confounding variables may help to elucidate whether cannabis is related to better cognitive functioning. In addition, statistical procedures, such as, moderation and mediation analyses would help to disentangle the cause-effect relationship between cannabis, cognition, and other confounding variables.

MINI-DICTIONARY

Comorbid Refers to a disorder that occurs simultaneously with another.
Dose-response effect The change in response (eg, higher or lower processing speed) caused by the amount of exposure to an agent, in this case, to cannabis.
Effect size A statistical concept, which refers to an index used to assess the magnitude of an event. Here, it refers to the magnitude of the differences in cognitive performance between groups (eg, patients who use cannabis and those who do not).
Executive functions The cognitive mechanisms that control the cognitive processes needed to understand complex or novel situations. This concept encompasses functions, such as, abstraction and planning, decision-making skills, initiation, self-monitoring, mental flexibility, and inhibition of immediate responses in pursuit of longer term goals.
Nonaffective psychosis Psychotic disorders in which affective or mood symptoms are not predominant, such as, schizophrenia, schizophreniform disorder, delusional disorder, brief psychotic disorder, and psychosis not otherwise specified.
Radioimmunoassay drug testing A laboratory procedure which evaluates drug metabolites deposited into growing hair shafts. This technique provides a 30-day surveillance window for detecting use for each 0.5 in. segment of hair.
Set-shifting The ability to change the focus of attention between two or more external stimuli.
Social cognition The ability to recognize, manipulate, and behave with respect to socially relevant information. It includes domains such as emotion processing, social perception, social knowledge, theory of mind, and attributional biases.
Working memory An active system for the storage and processing of information for short periods of time that is essential for other complex functions, such as, language comprehension, reading, or reasoning.

References

Bahorik, A. L., Newhill, C. E., & Eack, S. M. (2014). Neurocognitive functioning of individuals with schizophrenia: using and not using drugs. *Schizophrenia Bulletin*, *40*(4), 856–867.
Bahorik, A. L., Newhill, C. E., Queen, C. C., & Eack, S. M. (2013). Under-reporting of drug use among individuals with schizophrenia: prevalence and predictors. *Psychological Medicine*, *44*(1), 61–69.
Braga, R. J., Burdick, K. E., Derosse, P., & Malhotra, A. K. (2012). Cognitive and clinical outcomes associated with cannabis use in patients with bipolar I disorder. *Psychiatry Research*, *200*(2–3), 242–245.
Carter, C. S., Barch, D. M., Buchanan, R. W., Bullmore, E., Krystal, J. H., & Cohen, J. (2008). Identifying cognitive mechanisms targeted for treatment development in schizophrenia: an overview of the first meeting of the Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia Initiative. *Biological Psychiatry*, *64*(1), 4–10.
Chung, Y. C., Bok, E., Huh, S. H., Park, J. Y., Yoon, S. H., & Kim, S. R. (2011). Cannabinoid receptor type 1 protects nigrostriatal dopaminergic neurons against MPTP neurotoxicity by inhibiting microglial activation. *Journal of Immunology*, *187*(12), 6508–6517.
Cohen, M., Solowij, N., & Carr, V. (2008). Cannabis, cannabinoids and schizophrenia: integration of the evidence. *Australian and New Zealand Journal of Psychiatry*, *42*(5), 357–368.
Coulston, C. M., Perdices, M., & Tennant, C. C. (2007). The neuropsychology of cannabis and other substance use in schizophrenia: review of the literature and critical evaluation of methodological issues. *Australian and New Zealand Journal of Psychiatry*, *41*(11), 869–884.

DeRosse, P., Kaplan, A., Burdick, K. E., Lencz, T., & Malhotra, A. K. (2010). Cannabis use disorders in schizophrenia: effects on cognition and symptoms. *Schizophrenia Research*, *120*(1-3), 95–100.

Di Forti, M., Sallis, H., Allegri, F., Trotta, A., Ferraro, L., & Stilo, S. A. (2014). Daily use, especially of high-potency cannabis, drives the earlier onset of psychosis in cannabis users. *Schizophrenia Bulletin*, *40*(6), 1509–1517.

Fernández-Serrano, M. J., Perez-Garcia, M., & Verdejo-García, A. (2011). What are the specific vs. generalized effects of drugs of abuse on neuropsychological performance? *Neuroscience and Biobehavioral Reviews*, *35*(3), 377–406.

Genetic Risk and Outcome of Psychosis (GROUP) Investigators. (2011). Evidence that familial liability for psychosis is expressed as differential sensitivity to cannabis: an analysis of patient-sibling and sibling-control pairs. *Archives of General Psychiatry*, *68*(2), 138–147.

Grech, A., van Os, J., Jones, P. B., Lewis, S. W., & Murray, R. M. (2005). Cannabis use and outcome of recent onset psychosis. *European Psychiatry*, *20*(4), 349–353.

Harvey, P. D., Keefe, R. S., Patterson, T. L., Heaton, R. K., & Bowie, C. R. (2009). Abbreviated neuropsychological assessment in schizophrenia: prediction of different aspects of outcome. *Journal of Clinical and Experimental Neuropsychology*, *31*(4), 462–471.

Henquet, C., Murray, R., Linszen, D., & van Os, J. (2005). The environment and schizophrenia: the role of cannabis use. *Schizophrenia Bulletin*, *31*(3), 608–612.

Jockers-Scherubl, M. C., Danker-Hopfe, H., Mahlberg, R., Selig, F., Rentzsch, J., & Schurer, F. (2004). Brain-derived neurotrophic factor serum concentrations are increased in drug-naive schizophrenic patients with chronic cannabis abuse and multiple substance abuse. *Neuroscience Letters*, *371*(1), 79–83.

Jockers-Scherubl, M. C., Wolf, T., Radzei, N., Schlattmann, P., Rentzsch, J., & Gomez-Carrillo de Castro, A. (2007). Cannabis induces different cognitive changes in schizophrenic patients and in healthy controls. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *31*(5), 1054–1063.

Koskinen, J., Lohonen, J., Koponen, H., Isohanni, M., & Miettunen, J. (2010). Rate of cannabis use disorders in clinical samples of patients with schizophrenia: a meta-analysis. *Schizophrenia Bulletin*, *36*(6), 1115–1130.

Leeson, V. C., Harrison, I., Ron, M. A., Barnes, T. R., & Joyce, E. M. (2011). The effect of cannabis use and cognitive reserve on age at onset and psychosis outcomes in first-episode schizophrenia. *Schizophrenia Bulletin*, *38*(4), 873–880.

Linszen, D. H., Dingemans, P. M., & Lenior, M. E. (1994). Cannabis abuse and the course of recent-onset schizophrenic disorders. *Archives of General Psychiatry*, *51*(4), 273–279.

Loberg, E. M., & Hugdahl, K. (2009). Cannabis use and cognition in schizophrenia. *Frontiers in Human Neuroscience*, *3*, 53.

Mata, I., Rodríguez-Sánchez, J. M., Pelayo-Teran, J. M., Perez-Iglesias, R., Gonzalez-Blanch, C., & Ramirez-Bonilla, M. (2008). Cannabis abuse is associated with decision-making impairment among first-episode patients with schizophrenia-spectrum psychosis. *Psychological Medicine*, *38*(9), 1257–1266.

Meier, M. H., Caspi, A., Ambler, A., Harrington, H., Houts, R., & Keefe, R. S. (2012). Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(40), E2657–2664.

Meijer, J., Simons, C. J., Quee, P. J., & Verweij, K. (2012). Cognitive alterations in patients with non-affective psychotic disorder and their unaffected siblings and parents. *Acta Psychiatrica Scandinavica*, *125*(1), 66–76.

Moore, T. H., Zammit, S., Lingford-Hughes, A., Barnes, T. R., Jones, P. B., & Burke, M. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet*, *370*(9584), 319–328.

Potvin, S., Joyal, C. C., Pelletier, J., & Stip, E. (2008). Contradictory cognitive capacities among substance-abusing patients with schizophrenia: a meta-analysis. *Schizophrenia Research*, *100*(1–3), 242–251.

Quednow, B. B., Brinkmeyer, J., Mobascher, A., Nothnagel, M., Musso, F., & Grunder, G. (2012). Schizophrenia risk polymorphisms in the TCF4 gene interact with smoking in the modulation of auditory sensory gating. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(16), 6271–6276.

Rabin, R. A., Zakzanis, K. K., & George, T. P. (2011). The effects of cannabis use on neurocognition in schizophrenia: a meta-analysis. *Schizophrenia Research*, *128*(1–3), 111–116.

Rabin, R. A., Zakzanis, K. K., Daskalakis, Z. J., & George, T. P. (2013). Effects of cannabis use status on cognitive function, in males with schizophrenia. *Psychiatry Research*, *206*(2–3), 158–165.

Ramírez, B. G., Blazquez, C., Gomez del Pulgar, T., Guzman, M., & de Ceballos, M. L. (2005). Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *Journal of Neuroscience*, *25*(8), 1904–1913.

Rentzsch, J., Buntebart, E., Stadelmeier, A., Gallinat, J., & Jockers-Scherubl, M. C. (2011). Differential effects of chronic cannabis use on preattentional cognitive functioning in abstinent schizophrenic patients and healthy subjects. *Schizophrenia Research*, *130*(1–3), 222–227.

Ringen, P. A., Vaskinn, A., Sundet, K., Engh, J. A., Jonsdottir, H., & Simonsen, C. (2010). Opposite relationships between cannabis use and neurocognitive functioning in bipolar disorder and schizophrenia. *Psychological Medicine*, *40*(8), 1337–1347.

Rodríguez-Sánchez, J. M., Ayesa-Arriola, R., Mata, I., Moreno-Calle, T., Perez-Iglesias, R., & Gonzalez-Blanch, C. (2010). Cannabis use and cognitive functioning in first-episode schizophrenia patients. *Schizophrenia Research*, *124*(1–3), 142–151.

Sacco, K. A., Termine, A., Seyal, A., Dudas, M. M., Vessicchio, J. C., & Krishnan-Sarin, S. (2005). Effects of cigarette smoking on spatial working memory and attentional deficits in schizophrenia: involvement of nicotinic receptor mechanisms. *Archives of General Psychiatry*, *62*(6), 649–659.

Sánchez-Torres, A. M., Basterra, V., Rosa, A., Fananas, L., Zarzuela, A., & Ibanez, B. (2013). Lifetime cannabis use and cognition in patients with schizophrenia spectrum disorders and their unaffected siblings. *European Archives of Psychiatry and Clinical Neuroscience*, *263*(8), 643–653.

Sarne, Y., & Mechoulam, R. (2005). Cannabinoids: between neuroprotection and neurotoxicity. *Current Drug Target*, *4*(6), 677–684.

Schnell, T., Koethe, D., Daumann, J., & Gouzoulis-Mayfrank, E. (2009). The role of cannabis in cognitive functioning of patients with schizophrenia. *Psychopharmacology (Berlin)*, *205*(1), 45–52.

Segarra, R., Zabala, A., Eguiluz, J. I., Ojeda, N., Elizagarate, E., & Sanchez, P. (2011). Cognitive performance and smoking in first-episode psychosis: the self-medication hypothesis. *European Archives of Psychiatry and Clinical Neuroscience*, *261*(4), 241–250.

Seillier, A., Advani, T., Cassano, T., Hensler, J. G., & Giuffrida, A. (2010). Inhibition of fatty-acid amide hydrolase and CB1 receptor antagonism differentially affect behavioural responses in normal and PCP-treated rats. *International Journal of Neuropsychopharmacology*, *13*(3), 373–386.

Smith, M. J., Cobia, D. J., Wang, L., Alpert, K. I., Cronenwett, W. J., & Goldman, M. B. (2014). Cannabis-related working memory deficits and associated subcortical morphological differences in healthy individuals and schizophrenia subjects. *Schizophrenia Bulletin*, *40*(2), 287–299.

Stirling, J., Lewis, S., Hopkins, R., & White, C. (2005). Cannabis use prior to first onset psychosis predicts spared neurocognition at 10-year follow-up. *Schizophrenia Research*, *75*(1), 135–137.

van Os, J., Bak, M., Hanssen, M., Bijl, R. V., de Graaf, R., & Verdoux, H. (2002). Cannabis use and psychosis: a longitudinal population-based study. *American Journal of Epidemiology*, *156*(4), 319–327.

van Winkel, R. Genetic Risk and Outcome of Psychosis (GROUP) Investigators. (2011). Family-based analysis of genetic variation underlying psychosis-inducing effects of cannabis: sibling analysis and proband follow-up. *Archives of General Psychiatry*, *68*(2), 148–157.

Verdejo-García, A. (2011). Efectos neuropsicológicos del consumo de cannabis. *Trastornos adictivos*, *13*(3), 97–101.

World Health Organization. (1993). *Composite international diagnostic interview, version 1.1*. Geneva, Switzerland: WHO.

Yucel, M., Bora, E., Lubman, D. I., Solowij, N., Brewer, W. J., & Cotton, S. M. (2012). The impact of cannabis use on cognitive functioning in patients with schizophrenia: a meta-analysis of existing findings and new data in a first-episode sample. *Schizophrenia Bulletin*, *38*(2), 316–330.

CHAPTER

6

A Profile of Synthetic Cannabinoid Users

A.N. Sanders, J.M. Stogner

Department of Criminal Justice and Criminology, University of North Carolina at Charlotte, Charlotte, NC, United States

SUMMARY POINTS

- The most commonly reported reasons for synthetic cannabinoid use are legal status, accessibility, avoiding detection in drug screening, and drug effects.
- While many demographic studies reveal that synthetic cannabinoid use may be concentrated among teenagers and young adults, use has been reported across all demographics.
- Synthetic cannabinoid users typically fall into one of four categories: the replacement user, the experimental user, the drug aficionado, or the habitual user.
- The replacement user wants to avoid legal consequences and drug detection traditionally associated with cannabis, by way of substitution.
- The experimental user wants to try synthetic cannabinoids out of curiosity, partially fueled by the media, or to adhere to a deviant social milestone.
- The drug aficionado user takes pride in their drug use repertoire, and desires the novelty of the drug experience.
- The habitual user prefers synthetic cannabinoids as their drug of choice, in comparison to other substances, for both reasons associated with other types of users but, more importantly, for synthetic cannabinoids' own unique merits.

KEY FACTS OF SYNTHETIC CANNABINOID USERS

- Synthetic cannabinoids emerged as early as 2004, but gained popularity in 2008.
- Synthetic cannabinoids are substances that imitate the effects of cannabis.
- Synthetic cannabinoid users typically want to avoid a consequence that comes with cannabis use.
- Features most appealing to users about synthetic cannabinoids include the substances' legal status, accessibility, detection in drug screening, and drug effects.
- While synthetic cannabinoid users may tend to be teenagers or young adults, demographics alone cannot describe a synthetic cannabinoid user, as reported use has been seen across age, gender, race, education, employment, and prior substance use.

LIST OF ABBREVIATIONS

DEA	Drug Enforcement Agency
EMCDDA	European Monitoring Center for Drugs and Drug Addiction
GPA	Grade point average
LGBT	Lesbian, gay, bisexual, transgender

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00007-7
Copyright © 2017 Elsevier Inc. All rights reserved.

INTRODUCTION

During a brief 5-year window, synthetic cannabinoids became popular within the United States and several other countries. The popularity of these compounds can largely be attributed to their initial status as a "legal high" and somewhat desirable effects. Synthetic cannabinoid use was not restricted to any one demographic, and many different types of substance users experimented with or habitually used synthetic cannabinoids. Their reported reasons for use typically included a desire to experience the psychopharmacological effects of a cannabinoid while avoiding the potential consequences associated with traditional cannabis use. Users typically reported that synthetic cannabinoids' legal status, availability in retail stores and online, and noninclusion on drug screens made it an appealing alternative to marijuana in many instances, assuming the drugs' effects were near equivalent. In addition to these highly visible reasons for engaging in synthetic cannabinoid use, users may be motivated by other factors, such as social desirability, excitement, and curiosity. Reports of user distinctive demographics, personal characteristics, and use motivations enables researchers to further develop a profile of synthetic cannabinoid users that may serve as a framework for future research and policy efforts.

This chapter presents a typology of synthetic cannabinoid users based on theory, and recent research on reasons for use, and user demographics. The presented typology divides synthetic cannabinoid users into four categories: those who use the substance as a replacement or substitute, those who use to experiment or try the substance as a social milestone, those who regularly use various substances in order to extend the breadth of their drug use experience, and lastly, those who are habitual users. Each element of the presented typology reflects common reasons for synthetic cannabinoid use, without limiting the profile to demographic characteristics. This information, in sum, suggests that the recent bans on synthetic cannabinoids will reduce use, likely redirecting would-be users to cannabis, but not eliminate synthetic cannabinoid use completely.

Reasons for Synthetic Cannabinoid Use

A common reason for synthetic cannabinoid use is that such products were at one time legal and, despite after-regulations and bans, were marketed as legal alternatives to cannabis in retail stores and online (EMCDDA, 2009; Schifano et al., 2009; Vardakou, Pistos, & Spiliopoulou, 2010; Vandrey et al., 2012; Lindigkeit et al., 2009; Barratt, Cakic, & Lenton, 2013; Fattore & Fratta, 2011). Though their intended use is readily apparent to potential consumers, synthetic cannabinoid compounds are typically labeled as "not for human consumption." Such packaging details made the drug nature of the synthetic cannabinoids less obvious. Further, the products may have been labeled "incense" or "herbal mixtures," and referred to as a "herbal smoking blend" (Auwärter, Dresen, Weinmann, Müller, Pütz, & Ferreirós 2009; Schifano et al., 2009). Many labels list herbal plants, such as, "Indian Warrior" (*Pedicularis densiflora*) or "Lion's Tail" (*Leonotis leonurus* or "wild dagga"), which are unregulated plants known to have minor psychoactive effects when smoked (EMCDDA, 2009). Despite the "not for human consumption" warning labels, these synthetic cannabinoid products were frequently purchased for use as a psychoactive substance (Vandrey et al., 2012). Some labels actually advertised products as a "legal high." Others claimed the product was solely a "herbal high" in an attempt to insinuate legal status, and that the product was primarily a natural compound (Lindigkeit et al., 2009; Zimmermann et al., 2009). The illusion that they are herbal appears to appeal to many users, as they assume that natural compounds are safer to use (Every-Palmer, 2011; Fattore & Fratta, 2011). Products were also colorfully decorated, possibly as a tool to market to younger users (Schifano et al., 2009; Fattore & Fratta, 2011) or appear legitimate. Schifano et al. (2009) points out that many of these advertising features may have made it possible for synthetic cannabinoids to be mistaken for an incense product by consumers, but this is unlikely due to the cost, packaging, and retail placement of the product. This concern, however, may be somewhat founded in that it may have assisted teenagers attempting to evade detection or suspicion from less drug-savvy adults (Table 6.1).

Synthetic cannabinoid products, either completely or seemingly legal (as some packages actually contained banned substances), were easily obtained. Products were regularly sold in head shops, convenience stores, gas stations, and over the Internet (Auwärter et al., 2009; Schifano et al., 2009; Barratt et al., 2013; Every-Palmer, 2011; Fattore & Fratta, 2011). The price of such products, while sometimes more expensive than cannabis, were affordable and sometimes accompanied by discounts, especially online (Vardakou et al., 2010; Schifano et al., 2009). Such products, especially those sold over the Internet, were sold without age restrictions (Auwärter et al., 2009; Vardakou et al., 2010; Fattore & Fratta, 2011). This enabled young adults and teenagers access to a psychoactive substance without the regulations linked to alcohol and tobacco possession/use. The Internet also seemed to help perpetuate use as several blogs and websites were available in which users could describe their intoxicating experiences, and discuss different brands and methods of use (Lindigkeit et al., 2009; Auwärter et al., 2009; Vardakou et al., 2010; Fattore & Fratta, 2011). There were also instances of Youtube videos documenting use of such products (Lindigkeit

TABLE 6.1 Various Brand Names of Synthetic Cannabinoids

Popular brand names of synthetic cannabinoids				
Spice	K2	Spice Diamond	Spice Gold	Spice Silver
Chronic Spice	Arctic Spice	Spice of Life	Spicylicious	2Spicy
Spice Mojo	Red X Dawn	Zohai	Mr. Nice Guy	Aroma
Earthquake	Space Truckin	Solar Flare	Moon Rocks	Galaxy Gold
Kamikaze	Chernobyl	Yucatan Fire	Black Mamba	Earth Impact
Dream	Stinger	Scene	Skunk	Smoke
Sence	Scence	Aroma	Bliss	ChillX
Mystery	Genie	Blue Lotus	Bombay Blue	Scope
Pulse	Kronic	Gorillaz		

This list displays frequently reported brand names of synthetic cannabinoid products. Many names reflect specific spice related products (Spice Diamond, Spicylicious), an exciting experience (Earthquake, Kamikaze), or a relaxing experience (Dream, Aroma). This list is not exhaustive, as new brands can easily emerge. Data are from the EMCDDA, as well as compiled from works referenced throughout the chapter.

et al., 2009). The Internet helped to spread knowledge of synthetic cannabinoid substances quickly, and may have fostered the false belief that experimentation was common or even normative, increasing popularity and curiosity as a result.

A common reason for individuals to choose synthetic cannabinoids over cannabis, and what allowed it to be so readily available, was its initial legal status, and the assumption that users would avoid the potential legal consequences associated with cannabis. Lindigkeit et al. (2009) discuss how a seemingly legal product can be desired by those who want to try cannabis, but are uneasy about its illegal nature and subsequent consequences. Lindigkeit et al. (2009), as well as Vardakou et al. (2010), purport that this reluctance could be particularly important for younger people. Though the actual likelihood of penalization for marijuana possession is low in many jurisdictions, individuals less familiar with drug use likely overestimate the threat and certainty of punishment. This makes products labeled "legal highs," and perceived to have few consequences, particularly appealing to novice users. The initial legal status was also important to those who expected to be subject to drug screens. Synthetic cannabinoids were not initially detectable in blood and urine tests (Schifano et al., 2009; Barratt et al., 2013) and, even if they were, carried limited penalties compared to marijuana. This makes synthetic cannabinoids desirable for people who may be subjected to such drug screens, such as, students, athletes, probationers, psychiatric patients, the military, and those in other careers subjected to workplace drug testing (Schifano et al., 2009; Dresen et al., 2010; Rosenbaum, Carreiro, & Babu, 2012; Johnson, Johnson, & Alfonzo, 2011). Many studies indicate that users engage in synthetic cannabinoid use specifically to avoid drug detection in screening tests (EMCDDA, 2009; Auwärter et al., 2009; Vardakou et al., 2010; Every-Palmer, 2011; Fattore & Fratta, 2011).

Further complicating the issues discussed previously is the "cat and mouse" game between manufacturers and policymakers. Khey et al. (2014) refer to an ongoing battle between these groups that includes a cyclical pattern of "regulate and reformulate." After a psychoactive product is manufactured and introduced, policymakers typically choose to ban that particular compound, though enacting policy is a time-consuming process. Following a ban, manufacturers tweak the chemical composition of their product in a way that leads it to retain its psychoactive properties but be chemically distinct enough to avoid regulation (often because products carrying the "not for human consumption" label are immune to regulation as an analog). For example, several US jurisdictions banned a limited list of synthetic cannabinoid compounds in 2010 and 2011. However, there was variability in which compounds were included, and none were exhaustive. On March 1, 2011, the DEA placed the five most common chemicals in the major brands (JWH-018, JWH-073, JWH-200, CP-47,497, and CP-47,497 C8 homolog) into Schedule I on an emergency basis. Other products quickly emerged and were followed by new regulation. Eventually, the broad sweeping Synthetic Drug Abuse Prevention Act of 2012 was signed into law on July 9, 2012, but even this regulation may fail to address all synthetic cannabinoids. The number of psychoactive synthetic cannabinoids is continually growing; to demonstrate the number of these compounds, a brief list follows: HU-210, O-806, JWH-122, CP-47,497, CP-47,497-C8, CP-55,950, JWH-018, JWH-200, JWH-091, JWH-250, AM694, SR-18, Anandanine, Olemide, WIN-55,212-2, WIN-49,098. The long-term efficacy of any synthetic cannabinoids regulation cannot be evaluated at this point (Tables 6.2 and 6.3).

Also helping to drive use was the positive purported experiences of users. Some synthetic cannabinoid

TABLE 6.2 Legal Responses to Synthetic Cannabinoids

Country	Date of regulation	Legal response
Australia (Queensland)	Sep. 2011 and Nov. 2011	Amends *Drugs Misuse Regulation (1987)* to include new drug analogs and substances
Austria	Jan. 2012	Passes act controlling new substances as listed in a regulation by the Minister for Health
Finland	Jun. 2011	Updates the *2008 Narcotics Act* to include new substances
Hungary	Apr. 2012	*Government Decree 66/2012* creates a new schedule of substances allowing 1 year for a drug to be risk-assessed
Ireland	Aug. 2010	*Criminal Justice (Psychoactive Substances) Act (2010)* makes it a criminal offence to advertise, sell, or supply psychoactive substances not specifically controlled under existing legislation
New Zealand	Jul. 2013	*Psychoactive Substances Act* regulates the availability of psychoactive substances
Portugal	Apr. 2013	*Decree-law 54/2013* prohibits the advertising and distribution of a new list of psychoactive substances
Romania	Nov. 2011	Passes a new law requiring a specific permit to sell products likely to provoke psychoactive effects similar to those caused by substances already controlled under drug laws
United States	Jul. 2012	*Synthetic Drug Abuse Prevention Act of 2012* amends the *Controlled Substances Act (1970)* and synthetic drugs in Schedule I
United Kingdom	Nov. 2011	*Misuse of Drugs Act* allows temporary control of new substances under drug laws for up to 1 year

Beginning in 2011, there was a wave of new legislation, particularity in Europe, in response to the growing popularity of synthetic cannabinoids. Many legal responses banned either specific synthetic cannabinoid compounds, or any related analogs. Data are from the EMCDDA, DEA, New Zealand Drug Foundation, and the National Cannabis Prevention and Information Centre.

TABLE 6.3 Timeline of Major Legal and Media Events in the United States

Major events affecting awareness of synthetic cannabinoids in the United States	
Date	**Event**
Jun. 2010	American teenager, David Mitchell Rozga, committed suicide after smoking synthetic cannabis
Jun. 2010	US Senator Chuck Grassley proposed the *David Mitchell Rozga Act* to ban the use and distribution of synthetic cannabinoids
Mar. 1, 2011	"Spice" and "K2" temporarily scheduled by the DEA
Oct. 20, 2011	Three Louisiana State University football players are suspended for testing positive for synthetic cannabinoid use
Jan. 2012	Actress Demi Moore is rushed to the hospital for issues related to synthetic cannabinoid use
Jul. 9, 2012	*Food and Drug Safety and Innovation Act* passed, permanently regulating many synthetic cannabinoids
May 16, 2013	DEA added three additional synthetic cannabinoid compounds to Schedule 1 under the *Food and Drug Safety Innovation Act*

In the United States, the first major publicized event regarding synthetic cannabinoids was the suicide of a teenager. This event prompted legal action temporarily banning such substances. More media coverage revealed popular athletes and an actress using synthetic cannabinoids, with publicized consequences. In 2012, many synthetic cannabinoids were permanently banned. As new compounds emerged after the initial ban, more compounds were subsequently added to the banned list. Data are from North Iowa Today news, CBS News, and the DEA.

users indicated experiencing more pleasurable effects than they had with cannabis. Many have even indicated a preference to the synthetic substances (Vandrey et al., 2012; Barratt et al., 2013, Every-Palmer, 2011; Fattore & Fratta, 2011), though this seems far from ubiquitous. As the effects of various cannabis strains are themselves variable and affected by setting and expectations, it is challenging to interpret users' subjective comparisons. Perhaps it is more important to stress the overall similarity noted by users, than to engage in relative ranking of experiences. Regardless, users have reported synthetic cannabinoid intoxication to be quite enjoyable, and note that it can sometimes be accompanied by pleasurable smells and taste (Fattore & Fratta, 2011; Schifano et al., 2009). Effects of synthetic cannabinoids often last for a shorter amount of time than cannabis intoxication would, an effect seemingly desirable by some users (Schifano et al., 2009; Every-Palmer, 2011). Other users have reported synthetic cannabinoid use in combination with other substances, such as, ketamine, kratom, and cannabis (Schifano et al., 2009) that alter their experience and assessment of the drug.

Additionally, some users are motivated by impulsivity and the quest to broaden their horizons. For example, as synthetic cannabinoids gained popularity and subsequent media attention in Germany, many young people characterized as "sensation-seekers" became interested in synthetic cannabinoids, due to the increased coverage (EMCDDA, 2009; Vardakou et al., 2010). This more general form of behavior is likely related to synthetic

cannabinoid use, but a more specific mindset also exists among recreational drug users, and is quite relevant. Some individuals highly value the breadth of their drug use experiences. Despite being content with cannabis, they may choose to branch out to other substances to fulfill a need to experience all that is available. The scope of their drug use may also be tied to their assumptions about respect within a drug-using group, whereby status is linked to being able to recount and reflect upon a diverse array of drug experiences. Others may simply be curious about a new substance, or use synthetic cannabinoids to partially satisfy their curiosity related to cannabis (Barratt et al., 2013). Khey et al. (2014) also note that experimental synthetic cannabinoid use may be a social, deviant milestone in some young subcultures. Such users are not acting with a specific intent, but rather for the novelty of trying a new, unknown substance, and to share an experience with the others in the group.

Demographics Associated with Cannabinoid Use

While it is critical to stress that synthetic cannabinoid use has occurred within virtually every segment of the population, use does appear to be concentrated in some groups more than others. Not surprisingly, many demographic studies indicate that young adults and teenagers are the most frequent synthetic cannabinoid users (Vardakou et al., 2010; Lindigkeit et al., 2009; Auwärter et al., 2009). National studies of substance use have recently added synthetic cannabinoids to their surveys, and have found that use is more concentrated in adults aged 19–28 (Johnston, O'Malley, Bachman, & Schulenberg, 2014a). Such studies also indicate that, among high school students, use is emerging and more frequent among male students (Johnston, O'Malley, Bachman, & Schulenberg, 2014b). While concentrated in younger populations, use is by no means exclusive to younger users.

Another developing demographic with emerging rates of synthetic cannabinoid use is the military (Walker, Neighbors, Walton, Pierce, Mbilinyi, Kaysen, & Roffman, 2014; Loeffler, Hurst, Penn, & Yung, 2012). As members of most militaries are subject to strict substance use policies, the challenges associated with synthetic cannabinoid detection makes use extremely appealing to service members (Walker et al., 2014). Military demographics associated with synthetic cannabinoid use include service members who are young, less educated, of lower income, and not married (Walker et al., 2014). For the same reason, synthetic cannabinoid use is arguably higher among probationers who have a particularly high stake in successfully passing a drug screen (Vardakou et al., 2010).

Other studies of synthetic cannabinoid users focus on university populations (Hu, Primack, Barnett, & Cook, 2011; Stephens, 2011; Stogner & Miller, 2014). Hu et al. (2011) and Stephens (2011) both found that males were more likely than females to have reported synthetic cannabinoid use, as were younger university students. Using a random sample of university students, Stogner and Miller (2014) supported prior research indicating that males were more frequent users than females, but expanded demographic findings in observing that Whites and Hispanics reported more use than other races, and that students with lower GPAs reported more use than those with higher GPAs. Use was also associated with students from higher income families and students who self-identified as LGBT. Stogner and Miller (2014) found no associations between synthetic cannabinoid use and age, class year, marital status, employment, Greek affiliation, and athlete status. As reported by *Monitoring the Future*, a United States-based national study of substance use, prevalence of synthetic cannabinoid use among college students and similarly aged young adults peaked in 2011, and have steadily decreased since then, likely in part due to federal regulation (Johnston et al., 2014a). Although providing a national look at prevalence of use, the study does not examine demographics of synthetic cannabinoid users.

Once again, case studies have revealed that synthetic cannabinoid use is not bound to any single demographic, but spans across demographics such as age, race, education, employment, and prior substance use history (Vandrey et al., 2012; Barratt et al., 2013). Specific case studies indicate that synthetic cannabinoid use has been identified in male teenagers, older adults, and women (Castellanos, Singh, Thornton, Avila, & Moreno, 2011; Mir, Obafermi, Young, & Kane, 2011; Young, Schwarz, Medina, Obafemi, Feng, Kane, & Kleinschmidt, 2012; Zimmermann et al., 2009; Barratt et al., 2013; Gunderson, Haughey, Ait-Daoud, Joshi, & Hart, 2012; Schneir, Cullen, & Ly, 2011). Furthermore, Rosenbaum et al. (2012) note that at-risk demographics are difficult to isolate due to the importance of the Internet in the spread of synthetic cannabinoid use, and its influence in both adolescents and adults. This suggests that, while use may be associated with certain characteristics, demographics alone cannot fully describe synthetic cannabinoid users.

A Profile of Synthetic Cannabinoid Users

Even among the demographic and case studies, the identified common reasons for synthetic cannabinoid use were evident among the studied populations. A clear typology of synthetic cannabinoid users, related to but not dependent on demographic factors, emerged. Recognizing that there is no single archetypal synthetic cannabinoid user, and that any framework will fail

TABLE 6.4 Summary of the Four Profiles

Profile	Characteristics of user
• The replacement user	• Uses synthetic cannabinoids as a substitute for another substance • May be attempting to avoid legal consequences • May want to avoid detection in drug screening • Typically has a history of cannabis use
• The experimental user	• Only wants to try the substance out of curiosity • May use in an attempt to adhere to perceived deviant social milestones • Uses for the sake of having used, and has no intent of using again
• The drug aficionado	• A rare group of individuals who are interested in the novelty of the experience, and adding to their drug use repertoire • May be respected among peers for their drug knowledge and experience
• The habitual user	• A frequent user of synthetic cannabinoids • Uses synthetic cannabinoids as their drug of choice, and may favor it over alternatives

In sum, the four distinct profiles of a synthetic cannabinoid user reflect the reasons for which a person engages in use. Each profile can include users from all demographics. The most common profile would be the replacement user, with the other three profiles representing a minority of synthetic cannabinoid users. Data summarize information presented in the chapter.

to encompass every user, we introduce a typology of synthetic cannabinoid user profiles that may have utility in describing, evaluating, and managing emerging drug issues. Our typology is represented by four distinct profiles: (1) those who use synthetic cannabinoids as a replacement or substitute for another substance, (2) those who experiment with synthetic cannabinoids as a social milestone or to have a single experience with a novel substance, (3) those who regularly use substances and use synthetic cannabinoids in order to extend the breadth of their drug use experiences, and (4) frequent, regular users of synthetic cannabinoids. Specific characteristics for each profile are explored in the following sections (Table 6.4).

The Replacement User

The first general category of synthetic cannabinoid user, and likely the most prevalent of the four, is composed of users who choose to substitute synthetic cannabinoids for another substance (typically cannabis). Users of synthetic cannabinoids typically have a history of cannabis use (Stogner & Miller, 2014; Hu et al., 2011), although such use is not always the case. This category is comprised of substance users who are attempting to skirt legal consequences, and turn to synthetic substances as a more legitimate alternative. Members of this group likely view marijuana more positively, but chose an alternative due to concerns regarding to the substances' psychoactive effects. While synthetics were certainly viewed as more legal prior to the bans, now in place in several countries, synthetic cannabinoids are still viewed as a substitute with fewer legal risks, by a number of individuals. Prior to recent legislation, cannabis users concerned about receiving a citation or arrest may have used synthetic cannabinoids to eliminate any risk of arrest. Probationers are one group particularly likely to have substituted due to recurrent drug screens that would identify cannabis as an illegally used drug. This same mindset likely affected the decisions of athletes and military personnel whose health is intently monitored by governing agencies (Khey et al., 2014). Following the bans, individuals may still have acknowledged risk, but still used synthetics due to perceiving that risk to be lower than what would be associated with cannabis.

Though being able to use a replacement substance without concern of arrest was critical in the spread of synthetic cannabinoid use, other factors affected its utility as a replacement. Convenience, prior to bans, also likely played a role. As synthetic cannabinoid products were available over-the-counter, consumers could directly purchase them at virtually any hour. Though the price may have been higher, individuals could obtain a product without being dependent on an illicit dealer, or having to arrange a meeting. Second, many users may have turned to synthetic cannabinoids in order to avoid detection in drug testing. Even after synthetic cannabinoids became illegal, they may not have been as easily identified on drug screens as cannabis. An individual expecting to be screened as part of their work may substitute to increase the chance of testing negative for recreational drugs. As bans were established, synthetic cannabinoids became more vulnerable to drug detections, as researchers, medical professionals, and law enforcement focused on such substances. Overall, the prevalence of this user profile is directly tied to the legal status of the drug. As synthetic cannabinoids are banned, their value as a legal alternative decreases. Once testing identifies synthetic cannabinoids as consistently as cannabis itself, the existence of this user profile will largely disappear.

The Experimental User

The second profile is that of the experimental synthetic cannabinoid user. Rather than engaging in synthetic cannabinoid as a substitute or replacement, some users possess more novel motives for use, and only want to try

a particular substance. Such experimental users act more out of curiosity than specific intent (Barratt et al., 2013). Khey et al. (2014) argue that some experimental users are attempting to simply adhere to perceived deviant social milestones. For example, using substances may be a rite of passage in some youth subcultures, in that the act of using the substance is more important than the effects. Khey et al. (2014) further postulate that these users merely want to try the substance simply for the sake of having used it, and have no intent of using again. The former legality of these substances facilitates this practice and, for some, it may have made synthetic cannabinoids an appealing substance with which to fulfill perceived peer expectations of substance use. Of course, many youth in this category may assume that both cannabis and synthetic cannabinoid experiences are expected by their peers, and experiment with both, rather than one or the other. It is possible that this mentality and practice is perpetuated by the Internet and the availability of websites, blogs, and videos depicting use of such products.

The Drug Aficionado

Within drug using groups, there seem to exist individuals that consider the scope and breadth of drug using experiences to be a valuable commodity. Individuals are respected for their ability to recount the experiences with a wide variety of drugs. While not dependent on any one substance, they claim to have experimented with substances in several categories, and selected a handful as drugs of choice. They are seen as a resource to those less experienced drug users. In Germany, there were many reports of people using synthetic cannabinoids simply because they were the new, unknown drug on the marker (EMCDDA, 2009; Vardakou et al., 2010). Khey et al. (2014) similarly describe "avid drug users" in their typology of *Salvia divinorum* users. These users are not necessarily attempting to avoid any negative consequence associated with traditional substance use, but are more interested in the novelty of the experience, and adding to their drug use repertoire. These individuals seem to exist in virtual peer networks (eg, message boards of popular drug use forums), and in local communities. For these individuals, use is about expanding their experience. Legal regulations are unlikely to dissuade them, since they typically engage in a variety of forms of substance use, both legal and illegal. Regulation is unlikely to have an effect on their experimentation, but, as noted previously, few of these individuals exist.

The Habitual User

The last profile represents the frequent synthetic cannabinoid user. Among some users, synthetic cannabinoids have become a drug choice, as opposed to a substitute or trial occurrence. While most synthetic cannabinoid users do not consider it their primary drug of choice, some users report aspects that lead them to report favoring it over alternatives. Some report experiencing stronger or more enjoyable effects from synthetic cannabinoids, as opposed to traditional cannabis (Vandrey et al., 2012; Fattore & Fratta, 2011; Barratt et al., 2013). The popularity of synthetic cannabinoid use among this group of users may be in part due to reasons discussed throughout this chapter, but are not completely encompassing, as the frequent user desires the synthetic substance over a traditional one based on the synthetics' merits alone. Overall, it does not seem to be the case that this profile describes a particularly large number. Bans likely have less effect on these individuals than the replacement users, but may decrease their occurrence if availability becomes an issue. Evidence with other synthetics suggests that a black market develops soon after a ban (Winstock, Mitcheson, & Marsden, 2010), but it has yet to be seen whether users will be able to continue to obtain a stream of the product long after the bans.

CONCLUSIONS

The categorization of substance users into a framework has utility for research, law enforcement, and policy development. A user rational-type typology allows us to better identify different types of users, than mere demographics alone. The intentions individuals have when selecting synthetic cannabinoid use are intimately tied to the ways in which they will react to legal changes. Harm reduction strategies must similarly consider that motives for use are distinct, and should be appropriately tailored to address all groups. This chapter provides four distinct profiles of a synthetic cannabinoid user not bounded simply by demographics, but rather encompassing common reasons for use. Such profiles are able to encompass a greater diversity of users, and provide insight into drug use overall.

MINI-DICTIONARY

Deviant social milestone Synthetic cannabinoid use perpetuated by a subcultural influence to meet deviant standards, in this case the use of synthetic cannabinoid products.
Instrumental drug use Substance use that is goal-oriented. This may include avoiding a specific consequence such as legal ramifications, or a positive drug test.
Recreational drug use Substance use that is for enjoyment. This type of use is for pure enjoyment and does not meet a particular goal.
Sensation seeker A person who engages in synthetic cannabinoid use as a thrill. This pattern can be perpetuated by media attention that attracts potential users with their memorable coverage of the substance.

Synthetic cannabinoid user A person who uses synthetic cannabinoid products.
The drug aficionado A person who uses synthetic cannabinoids for the novelty of the experience, and to add to their drug use repertoire. These users pride themselves on the various substances they have used, but are rare among substance users.
The experimental user A person who uses synthetic cannabinoids only to try. These users typically do not exhibit reoccurring use.
The habitual user A person who regularly engages in synthetic cannabinoid use. These users typically prefer synthetic cannabinoids as their drug of choice.
The replacement user A person whose uses synthetic cannabinoids to replace or substitute another substance, typically cannabis. These users typically want to avoid negative consequences associated with other substances.

References

Auwärter, V., Dresen, S., Weinmann, W., Müller, M., Pütz, M., & Ferreirós, N. (2009). "Spice" and other herbal blends: harmless incense or cannabinoid designer drugs? *Journal of Mass Spectrometry*, *44*(5), 832–837.

Barratt, M. J., Cakic, V., & Lenton, S. (2013). Patterns of synthetic cannabinoid use in Australia. *Drug and Alcohol Review*, *32*(2), 141–146.

Castellanos, D., Singh, S., Thornton, G., Avila, M., & Moreno, A. (2011). Synthetic cannabinoid use: A case series of adolescents. *Journal of Adolescent Health*, *49*(4), 347–349.

Dresen, S., Ferreirós, N., Pütz, M., Westphal, F., Zimmermann, R., & Auwärter, V. (2010). Monitoring of herbal mixtures potentially containing synthetic cannabinoids as psychoactive compounds. *Journal of Mass Spectrometry*, *45*(10), 1186–1194.

European Monitoring Centre for Drugs and Drug Addiction (2009). Understanding the "Spice" phenomenon Thematic papers, European Monitoring Centre for Drugs and Drug Addiction.

Every-Palmer, S. (2011). Synthetic cannabinoid JWH-018 and psychosis: an explorative study. *Drug and Alcohol Dependence*, *117*(2), 152–157.

Fattore, L., & Fratta, W. (2011). Beyond THC: the new generation of cannabinoid designer drugs. *Frontiers in Behavioral Neuroscience*, *5*(60), 1–12.

Gunderson, E. W., Haughey, H. M., Ait-Daoud, N., Joshi, A. S., & Hart, C. L. (2012). Spice" and "K2" herbal highs: A case series and systematic review of the clinical effects and biopsychosocial implications of synthetic cannabinoid use in humans. *American Journal on Addictions*, *21*(4), 320–326.

Hu, X., Primack, B. A., Barnett, T. E., & Cook, R. L. (2011). College students and use of K2: An emerging drug of abuse in young persons. *Substance Abuse Treatment, Prevention and Policy*, *6*, 16–19.

Johnson, L. A., Johnson, R. L., & Alfonzo, C. (2011). Spice: a legal marijuana equivalent. *Military Medicine*, *176*(6), 718–720.

Johnston, L. D., O'Malley, P. M., Bachman, J. G., & Schulenberg, J. E. (2014a). *Monitoring the future. National survey results on drug use, 1975-2013: Volume 2, college students and adults ages 19-50*. Ann Arbor, MI: Institute for Social Research, The University of Michigan.

Johnston, L. D., O'Malley, P. M., Bachman, J. G., & Schulenberg, J. E. (2014b). *Monitoring the future. National survey results on drug use, 1975-2013: Volume 1, secondary school students*. Ann Arbor, MI: Institute for Social Research, The University of Michigan.

Khey, D. N., Stogner, J., & Miller, B. L. (2014). *Emerging trends in drug use and distribution*. Cham, Switzerland: Springer International Publishing.

Lindigkeit, R., Boehme, A., Eiserloh, I., Luebbecke, M., Wiggermann, M., Ernst, L., & Beuerle, T. (2009). Spice: a never ending story? *Forensic Science International*, *191*(1), 58–63.

Loeffler, G., Hurst, D., Penn, A., & Yung, K. (2012). Spice, bath salts, and the U.S. Military: The emergence of synthetic cannabinoid receptor agonists and cathinones in the U. S. Armed Forces. *Military Medicine*, *177*(9), 1041–1048.

Mir, A., Obafemi, A., Young, A., & Kane, C. (2011). Myocardial infarction associated with use of the synthetic cannabinoid K2. *Pediatrics*, *128*(6), e1622–e1627.

Rosenbaum, C. D., Carreiro, S. P., & Babu, K. M. (2012). Here today, gone tomorrow... and back again? A review of herbal marijuana alternatives (K2, Spice), synthetic cathinones (bath salts), kratom, Salvia divinorum, methoxetamine, and piperazines. *Journal of Medical Toxicology*, *8*(1), 15–32.

Schifano, F., Corazzaa, O., Delucab, P., Daveyc, Z., Di Furiad, L., Farre'e, M., Fleslandf, L., Mannoneng, M., Paganid, S., Peltoniemig, T., Pezzolesia, C., Scherbaumh, N., Siemannh, H., Skutlef, A., Torrense, M., & Van Der Kreeft, P. (2009). Psychoactive drug or mystical incense? Overview of the online available information on Spice products. *International Journal of Culture and Mental Health*, *2*(2), 137–144.

Schneir, A. B., Cullen, J., & Ly, B. T. (2011). "Spice" girls: synthetic cannabinoid intoxication. *Journal of Emergency Medicine*, *40*(3), 296–299.

Stephens, J. L. (2011). *Synthetic cannabinoid usage among college students: the example of K2 and Spice*. Denton, TX: University of North Texas.

Stogner, J. M., & Miller, B. L. (2014). A spicy kind of high: a profile of synthetic cannabinoid users. *Journal of Substance Use*, *19*(1–2), 199–205.

Vandrey, R., Dunn, K. E., Fry, J. A., & Girling, E. R. (2012). A survey study to characterize use of Spice products (synthetic cannabinoids). *Drug and Alcohol Dependence*, *120*(1), 238–241.

Vardakou, I., Pistos, C., & Spiliopoulou, C. (2010). Spice drugs as a new trend: mode of action, identification and legislation. *Toxicology Letters*, *197*(3), 157–162.

Walker, D., Neighbors, C., Walton, T., Pierce, A., Mbilinyi, L., Kaysen, D., & Roffman, R. (2014). Spicing up the military: use and effects of synthetic cannabis in substance abusing army personnel. *Addictive Behaviors*, *39*(7), 1139–1144.

Winstock, A., Mitcheson, L., & Marsden, J. (2010). Mephedrone: still available and twice the price. *Lancet*, *376*(9752), 1537.

Young, A. C., Schwarz, E., Medina, G., Obafemi, A., Feng, S. Y., Kane, C., & Kleinschmidt, K. (2012). Cardiotoxicity associated with the synthetic cannabinoid, K9, with laboratory confirmation. *American Journal of Emergency Medicine*, *30*(7), 1320–1320e5.

Zimmermann, U. S., Winkelmann, P. R., Pilhatsch, M., Nees, J. A., Spanagel, R., & Schulz, K. (2009). Withdrawal phenomena and dependence syndrome after the consumption of "spice gold". *Deutsches Ärzteblatt International*, *106*(27), 464–467.

CHAPTER

7

Dual Disorders in Cannabis Misuse

*F. Arias-Horcajadas**, *N. Szerman***, *P. Vega*†, *I. Basurte***, *B. Mesías*†

**Psychiatric Department, Doce de Octubre Hospital, Madrid, Spain
**Gregorio Marañon Hospital, Madrid, Spain
†Instituto de Adicciones, Madrid, Spain

SUMMARY POINTS

- Over 75% of cannabis addicts in treatment have a dual diagnosis, and the prevalence of mood and anxiety disorders is particularly significant.
- About half of cannabis addicts have a personality disorder diagnosis.
- Cannabis-dependent subjects begin using other drugs, such as, alcohol, cocaine, and tobacco, at an earlier age than other addicts who do not use cannabis.
- Cannabis dependence is most associated with antisocial personality disorder, bipolar disorder, psychosis, and agoraphobia.
- The presence of these mental disorders is significantly associated with cannabis use beginning at an earlier age.

KEY FACTS

Key facts of eCB system as an element in emotional regulation:

- Regulation of the serotonin system (5-HT) and noradrenaline (NA).
- Regulation of hypothalamic-pituitary-adrenal axis (HPA) hyperactivity.
- Regulation of neurogenesis mechanisms in the hippocampus.

Key facts of mechanisms underlying the role of the eCB system in depression:

- CB1 agonists and FAAH inhibitors increase 5-HT.
- CB1 agonists increase NA in the locus coeruleus (LC) and the PFC.
- CB1 agonists diminish HPA axis hyperactivity, increase neurogenesis in the hippocampus, and increase the levels of brain-derived neurotrophic factor (BDNF) in the hippocampus, which have a neurotrophic role.

Key facts of eCB in PTSD:

- Alteration in eCB levels.
- Increase in CB1 density.
- Relationship with variants of the FAAH gene.

Key facts of chronic use of cannabis and cognitive effects:

- Alterations in memory.
- Alterations in executive functions, inhibitory processing, decision making.
- Alterations in attention.

Key facts of cannabis use and psychosis:

- Transitory psychotic symptoms in intoxication.
- Induced psychotic disorders after prolonged use.
- Schizophrenia after prolonged use and use at early age in vulnerable subjects (genetic vulnerability: COMT, AKT, CB1, CB2 genes; environmental factors: adverse experiences).

LIST OF ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
5-HT	Serotonin
ADHD	Attention deficit hyperactivity disorder
AEA	Anandamide
BD	Bipolar disorder

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00008-9
Copyright © 2017 Elsevier Inc. All rights reserved.

CB1, CB2	Cannabinoid receptors 1 and 2
CBD	Cannabidiol
COMT	Catechol-O-methyl transferase
ECA	Epidemiologic Catchment Area Project
eCB system	Endocannabinoid system
FAAH	Fatty acid amide hydrolase
GABA	Gamma-aminobutyric acid
HPA axis	Hypothalamic-pituitary-adrenal axis
MINI	Mini International Neuropsychiatric Interview
NA	Noradrenaline
NESARC	National Epidemiologic Survey on Alcohol and Related Conditions
OCD	Obsessive-compulsive disorder
PD	Personality disorder
PDQ4	Personality disorder questionnaire, version 4
PFC	Prefrontal cortex
PTSD	Posttraumatic stress disorder
SUD	Substance use disorder
THC	Δ^9-Tetrahydrocannabinol

INTRODUCTION

The comorbidity between a substance use disorder (SUD) and any other lifetime psychiatric disorder is known as dual pathology. It is a problem of great importance due to its high prevalence (in serious mental disorders, the prevalence of lifetime SUDs is over 50%), its clinical and prognostic implications (the presence of comorbidity modifies the clinical manifestations and the course), difficulties in the therapeutic approach (less scientific evidence and less adherence), and the associated healthcare costs. The underlying mechanisms of comorbidity are not well defined, and different explanatory hypotheses have been proposed: common vulnerability models, self-medication, and etiological hypotheses.

Many studies suggest that cannabis is associated with a wide variety of mental disorders. Psychiatric manifestations due to cannabis use have been extensively described in medical literature. In the 19th century, a French doctor, Moureau de Tours, was already suggesting that cannabis intoxication could mimic any psychiatric manifestation.

The description of a widely distributed cannabinoid system in our brains, and the knowledge of its implication in a broad variety of functions, such as emotional regulation, response to stress, cognitive functions, and brain development, suggest that a possible mechanism by which cannabis produces this wide variety of manifestations may be the modifications that Δ^9-tetrahydrocannabinol (THC) could produce in such system; one that may previously have been normal, or that had a dysfunction that contributed to such alterations.

Age when use begins, duration of use, and strength of the drug are relevant factors regarding the risk of developing mental disorders. Since the psychiatric manifestations only occur in a percentage of users, it is likely that there must be individual vulnerability to such development. The interaction between use, the said genetic or biological vulnerability factors, and other adverse environmental factors, such as stressful situations, seems to contribute to the development of these symptoms.

THE CANNABINOID SYSTEM IN PSYCHIATRIC DISORDERS

The Role of the Cannabinoid System in Emotional Regulation and Response to Stress

The eCB system is becoming established as a fundamental element in the regulation of our body's response to stress, and in emotional regulation. The eCB system is present in many regions of the brain that are implicated in these aspects. The eCB inhibits hyperactivation of the HPA system in response to stress, a hyperactivity related equally to depression and anxiety symptoms. It also regulates negative feedback of corticoids, reduces the release of NA, regulates the sympathetic response, and inhibits the secretion of cytokines, adopting the role of a buffer in this mechanism.

On the other hand, chronic states of stress alter the eCB system, and cause a downregulation of the CB1 receptors, which results in subsequent alteration of the response to stress. Another noteworthy aspect is that the eCB system modulates emotional response in a way that is very dependent on the context, therefore, environmental factors are relevant in the functioning of the system.

The modulation of the neurotransmission of NA, 5-HT, GABA, glutamate, and other neurotransmitters is crucial in these effects, but the implication in neuroplasticity, neurogenesis, and neurotrophic mechanisms may also be important.

The eCB System in Depression

It has been suggested that, in depression, there would be a hypofunctioning of the eCB system, based on various experimental data, although not all the information is consistent (Table 7.1). The interaction between different vulnerability factors of the eCB system and other neurotransmission systems, together with adverse environmental factors, are going to contribute to the appearance of depression. The interaction between the CNR1 polymorphisms with others of 5HTTLPR, and with environmental factors, has been described. On the other hand, stress factors related to the pathogenesis

TABLE 7.1 The eCB System is Implicated in the Pathogenesis of Depression

- Knock-out animals for the CB1 receptor (CB1KO) are more vulnerable to chronic stress and present HPA axis hyperactivity
- Chronic exposure to CB1 or THC agonists produces a depressive phenotype, and alterations in neuroplasticity. This phenomenon is found more when the exposure occurs during adolescence, with a depressive phenotype being observed in adult animals. So, adolescence is a critical period, in part because CB1 receptor activation affects the monoaminergic system
- CB1 antagonists induce depression both in animals and in humans
- Studies of genetic polymorphisms of the CB1 receptor in humans indicate that certain variants contribute to the risk of depression in response to stressful events, and have an influence on the response to antidepressants
- A reduction in the levels of AEA and 2-AG in women with depression.
- Inhibitors of the FAAH show an antidepressant effect in animal models
- Animal models of depression usually present changes in receptor expression of the eCB system, generally an increase in CB1 expression in the PFC, and a reduction in the subcortical regions
- An increase in CB1 receptor density in the PFC postmortem in suicidal depressives
- Antidepressants modify the eCB system, increase the system's limbic signal, and possibly contribute to neurogenesis through the eCB system
- Animal models with CB2 receptor overexpression also implicate this receptor in depression

of depression, such as early maternal separation, cause changes in the eCB system.

The eCB System in Anxiety

The role of the eCB system in anxiety seems to be even more complex than in depression. Attempts have been made to explain the discrepancies between the different experimental results by a biphasic effect, so low doses of cannabinoid agonists would be anxiolytic, and high doses, anxiogenic. This effect has been explained by the influence of different brain regions (PFC, amygdala, and hippocampus) or by differences in the effects of different receptors, mainly CB1 and TRPV1.

Biphasic effects of cannabinoids have been shown in processes, such as, feeding behavior, motor activity, motivational processes, and anxiety responses. Some authors found that the anxiolytic-like effects of the low dose of cannabinoids are mediated via the CB1 receptor on cortical glutamatergic terminals. On the contrary, the CB1 receptor on the GABAergic terminals is required to induce an anxiogenic-like effect under a high-dose treatment (Rey, Purrio, Viveros, & Lutz, 2012).

In addition, the response of the eCB system is very influenced by context, and that dependence on environmental factors could also explain the different results in the studies.

The CB1 receptor is highly expressed in GABA interneurons. One model suggests that the eCB system in the PFC regulates the response to stress through the GABA interneurons, which regulate regions such as the basolateral amygdala (BLA), and monoamines such as in the raphe nuclei, and this would regulate the HPA axis. Also, other neurotransmitters have been implicated, such as, the opioid system, the 5-HT1A receptors, nitric oxide, and the glutamatergic system. Likewise, other data support the role of the eCB system in anxiety (Table 7.2).

Equally, it may have a role in the pathogenesis of OCD, as can be seen in an animal model (marble burying behavior), while CBD improves compulsive behaviors in such model.

The eCB System in Bipolar Disorder

In the case of BD, the link with the endogenous cannabinoid system could be due to the modulation of GABA and serotonin neurotransmitters. In this respect, the manic episodes seem to be exacerbated by cannabis use, therefore, is it considered that a high endocannabinoid tone would cause these episodes (Agrawal, Nurnberger, & Lynskey, 2011). On the other hand, low activity of the CB1 receptors could contribute to causing depression, as we have described; however, the results have not been replicated.

TABLE 7.2 Role of eCB System in Anxiety

- The CB1 receptor is highly expressed in GABA interneurons
- FAAH enzyme inhibitors have an anxiolytic effect in animal models
- Genetic variants of FAAH in humans regulate the habituation of the amygdala to threatening situations
- The eCB system modulates the amygdala response to stress. The increase in the levels of AEA in the basolateral amygdala facilitates long-term fear extinction
- CB1 antagonists, such as, rimonabant are anxiogenic
- Anxiolytic effects of nabilone and CBD have been described. The latter possibly acts through the 5-HT1A receptors, although it may act through other mechanisms, such as, facilitation of neurogenesis in the hippocampus by boosting AEA, activation of the TRPV1 receptors, CB2 agonism, GPR55 antagonism, or PPAR-gamma agonism

In studies on genetic association, different polymorphisms have been found to be associated with bipolar disorder within the CNR1, CNR2, and FAAH genes.

The eCB System in Psychosis

In experimental studies, D'Souza et al. (2004, 2005) have found how the administration of THC to healthy subjects reproduced both the positive and negative symptoms of psychosis in a dose-dependent manner, and how it exacerbated them when it was administered to patients with psychosis. So, the agonists increase the positive symptoms, induce negative and cognitive symptoms, and induce a deficit in the PPI in the same way as seen in psychosis.

Many animal model studies confirm the role of the eCB system in psychosis, the most noteworthy being the work done with the phencyclidine model that shows the presence of positive and negative symptoms, and in which changes in the CB1 receptor and eCB levels are described.

In humans, pharmacological experimental studies, genetic studies of the eCB system, postmortem studies that analyze CB1 receptor density, studies on endocannabinoid levels, and cognitive and neurophysiological studies that compare the alterations in psychosis and those caused by the use of cannabis support the view that the eCB system has an important role in psychosis (Arias, 2007).

On one hand, THC modifying a previously normal eCB system may contribute to the appearance of psychosis. Another possible view is the role of dysregulation of the eCB system in vulnerability to psychosis, independent of cannabis use. It is possible that the consumption of THC in subjects with a vulnerable eCB system could contribute to the development of psychosis in addicts.

From the point of view of alterations in the eCB system in patients with schizophrenia, who do not use cannabis, there are several interesting findings. One is the discovery of high levels of AEA in the cerebrospinal fluid (CSF) in patients who are not taking medication, or who are being treated with atypical antipsychotics (Leweke, Giuffrida, Wurster, Emrich, & Piomelli, 1999). This increase in levels is not found in patients treated with typical antipsychotics. This seems to relate AEA levels with the D2 receptor, the main target of action for first generation antipsychotics. At the same time, and in keeping with the aforementioned, the ability of THC to reduce the activity of the D2 receptor has been observed, as well as the selective blocking of CB1 that boosts the D1 and D2 receptors. Consequently, an increase in AEA could be an indication of a compensatory mechanism for reducing D2 hyperactivity and, therefore, alleviating psychotic symptoms. On the other hand, cannabis use contributes to this increase in AEA not occurring, so this supposed protective mechanism would be lost (Leweke et al., 2007). Therefore, a dysregulation of AEA in psychosis has been suggested.

Other studies show a reduction in the levels of the CB1 receptor in the dorsolateral frontal cortex of patients. This CB1 receptor reduction has been interpreted as a compensation for the high levels of GABA. Consequently, a reduction in CB1 levels and its inhibitory action could have the effect of increasing dopaminergic transmission. Nonetheless, other studies found a high density of CB1 receptors in the anterior and posterior cingulate cortex and in the dorsolateral prefrontal cortex.

Although, once again, the studies are not consistent with each other, polymorphisms of the CB1 receptor gene have been described in relation to psychosis, but also those of the CB2 receptor that may be more related to neuroinflammatory processes. In any case, genetic variants of the dopaminergic system have been described (the COMT and AKT1 genes), or implicated in neurodevelopmental processes that could influence vulnerability to psychosis in cannabis users, as well as interact with other stress factors such as traumatic childhood experiences.

While the agonists have a propsychotic effect, CBD and rimonabant present atypical antipsychotic properties in animal models, and some clinical trials on humans have confirmed their possible clinical utility.

There are also data that suggest that the eCB system is implicated in the neurodevelopmental alterations in psychosis. The eCB system is important in this aspect, and it is considered that, in psychosis, there may be an alteration in neurodevelopment. Additionally, the fundamental role that the eCB system has in the regulation of the inflammatory response could be included. Therefore, the eCB system would be implicated in the development of schizophrenia, not only because of its regulatory function in other neurotransmitters, but also because of its function in neurodevelopment and neuroinflammatory processes.

CANNABIS DEPENDENCE AND MENTAL DISORDERS

Depression

Various cohort studies suggest that cannabis plays a moderate role in inducing depression. The possibility that the association between cannabis and depression may be due to common social or family factors, or other possible confounding factors, cannot be ruled out. The metaanalysis by Moore et al. (2007) concludes that the effects of cannabis on the subsequent appearance of

depression are moderate (odds ratio 1.3–1.6), and that such effects are dose-dependent. The most recent studies present more consistent data regarding that modest but positive effect between cannabis use and an increase in the risk of depression (Lev-Ran et al., 2014; Lev-Ran & Feingold, 2016). It is important to highlight the possible influence of the age when cannabis use began, so use during adolescence predicts subsequent depression.

In a study on twins, in each case one being a cannabis user and the other not, it was found that the twin that used cannabis had a higher risk of depression and suicide attempts (Lynskey et al., 2004). Also, cannabis use contributes to the persistence of depression and, on the other hand, the presence of depression hinders cessation of use.

Bipolar Disorder

Bipolar disorder is one of the conditions most related with cannabis dependence, as epidemiological studies on the general population confirm, such as the ECA or the NESARC, along with many clinical studies. On one hand, there is a high prevalence of use and dependence in bipolar patients. On the other, cannabis use inducing manic phases is also described. The increased risk of appearance of a bipolar disorder has been related to the dosage of cannabis and use beginning at an earlier age. Likewise, cannabis use brings forward the age of onset of bipolar disorder (Lagerberg et al., 2011), which supports the view that it has at least a role in precipitating the symptoms.

Cannabis use has been associated with clinical changes in bipolar disorder, such as more mixed episodes, rapid cycling, more suicide attempts, greater functional limitations, more psychotic symptoms, and an increase in the number of relapses. In the NESARC study, an association is indicated, and it is related to an earlier age of onset of bipolar disorder and more mixed, depressive and manic episodes (Lev-Ran, Le Foll, McKenzie, George, & Rehm, 2013a).

Anxiety Disorders

Anxiety symptoms are some of those most frequently found in cannabis users, both during periods of intoxication and abstinence. Although the majority of users mention a relaxing effect, one of the most frequently found adverse effects of intoxication is the appearance of anxiety and panic attacks. As in the disorders previously mentioned, panic disorder being induced following cannabis use has been outlined, both in longitudinal studies and in case descriptions. Cannabis use during adolescence predicted subsequent anxiety symptoms, as is the case with depression (Patton et al., 2002). Cannabis dependence has been associated with a greater prevalence of different anxiety disorders, such as, panic disorder, agoraphobia, and social phobia.

Social Phobia

It has been suggested that the relationship between cannabis and social phobia may be different from that which exists in other anxiety disorders. Social phobia usually appears earlier, therefore cannabis use as self-medication is considered to be significant. Also, the presence of both together increases functional limitations (Buckner et al., 2012).

Cannabinoid agonists are implicated in the improvement of the phobia, while rimonabant has shown a detrimental effect in experimental studies using a public speaking test. Likewise, CBD in such test produces an improvement.

Panic

The presence of cannabis use increases the risk of developing panic attacks and panic disorder, and of an earlier age of onset of this disorder (Zvolensky et al., 2008). A greater association has also been described with agoraphobia, although the relationship is not clear. In general, the relationship referred to is one in which cannabis induces panic, although occasionally the use is described as self-medication to reduce anxiety, this being influenced by the expectation that cannabis alleviates anxiety.

Posttraumatic Stress Disorder

The eCB system is implicated in the elimination of aversive memories. It regulates emotional response after a trauma, and the action of corticoids in response to the trauma, by mediating CB1 in the hippocampus and the basolateral amygdala. So, facilitation of the eCB signaling improves PTSD. In the same way, an association has been found between PTSD and cannabis use. Greater use in order to cope with aversive situations, more severe cannabis withdrawal symptoms, and more craving have been described.

Cannabis and Cognitive Deterioration

The presence of CB1 and the eCB in the hippocampus and the PFC suggest that the eCB system has a role in cognitive functioning. Acute use causes alterations in the hippocampus-PFC responsible for the processes of encoding and recuperating information. Acute cannabis use alters cognitive capacity (attention, memory, problem solving) and psychomotor performance. The effects include slowing of reaction speed, lack of motor coordination, alteration of short-term memory, concentration difficulties, and impairment of divided attention. These effects are dose-dependent.

Habitual cannabis use may cause more persistent neuropsychological deficits. Chronic use affects episodic memory, decision-making and response inhibition processes, and causes learning and executive function deficits, mainly in working memory. Above all, alteration in memory has been found.

There are discrepancies regarding the severity of the changes, and whether or not they are reversible. Longer duration of use and higher doses cause more cognitive alterations. The age when use began is also a relevant factor. Data seem to indicate that those deficits exist in the short term but, after prolonged abstinence, such changes do not persist. These are more serious when use has begun at an earlier age, implicating processes of brain maturation.

Amotivational Syndrome

Smith (1968) and McGlothlin and West (1968) described the amotivational syndrome. At that time, there was a rapid increase in descriptions of cases of apathy along with lack of concentration and memory. The majority improved with abstinence. Blunted intellectual function was described in a broad spectrum of patients in Egypt by Soueif (1976).

The description of these symptoms was widely circulated after the initial description, but nowadays they are a controversial matter. The condition is characterized by the presence of apathy, lack of motivation, lack of interest in work or education, and personal care. The majority of clinicians consider that they are symptoms that are observed in users, but what is not clear is whether they form a condition in itself, or whether they are the result of a chronic state of intoxication. In any event, they have been found to remit when use stops, although they may continue in a small percentage of users, despite withdrawal. The lack of improvement with antidepressants, and the frequent concurrence of lapses of memory and attention, suggests that the symptoms may be more related to the cognitive deterioration caused by cannabis, affecting motivation as well as other cognitive areas, rather than to a depressive disorder.

Cannabis and Psychosis

In the last few decades, various cohort studies have been carried out with the aim of investigating the relationship between cannabis use and schizophrenia. After the initial study by Andréasson, Allebeck, Engström, and Rydberg (1987), there followed others. These studies show that cannabis use at an early age is associated with a greater risk of suffering from psychosis as an adult. It has also been proved that such risk is greater than with other drugs, and that it is the only drug regarding which there is evidence that it brings forward the age of onset of psychosis.

However, this hypothesis has been criticized. It has been suggested that although cannabis use precedes the onset of psychotic symptoms, there could already be a greater susceptibility to cannabis dependence in subjects who are vulnerable to developing psychosis.

In general, the majority of authors consider that cannabis use would be a precipitating factor that would cause the development of schizophrenic disorder in individuals vulnerable to suffering from it. As such, the potential interaction between genetic vulnerability and cannabis use for the development of schizophrenia has been suggested in studies that found a higher risk of experiencing psychotic symptoms after cannabis use, among carriers of the 158 valine allele of the COMT gene, although other studies have not corroborated this. Nonetheless, other genes have been implicated, as well as the existence of epistasis between genes from different neurotransmission systems, or that are implicated in neurodevelopment and, most interestingly, the interaction between these genetic variants and environmental factors, such as, adverse experiences during childhood, or stressful life events.

Therefore, we can consider that psychosis is one of the most serious effects associated with cannabis use, and is possibly the adverse consequence about which we have the most scientific evidence. An effect influenced by the age when use began, possibly the amount and duration of use, the strength of the drug (perhaps in relation to the amount of CBD content), and for which a vulnerability that has not yet been established, is required. Likewise, it is described as an effect of synthetic cannabinoids, and psychotic symptoms have been outlined as an adverse effect in clinical studies focused on the therapeutic utility of cannabinoid agonists.

Considering that schizophrenia is a neurodevelopmental illness, and the extensive evidence that the eCB system modulates such neurodevelopment (cellular proliferation, neurogenesis, neuronal migration, axonal projections), cannabis use at an early age affecting this neurodevelopment could provide quite a plausible explanation for this adverse effect.

Personality Disorders

Personality disorders are highly prevalent in cannabis-dependent subjects. Approximately half the addicts in clinical samples have some personality disorder diagnosis, the most prevalent being antisocial, borderline, paranoid, depressive, or avoidant personality disorders. In the NESARC study, the high prevalence of these personality disorders is confirmed, above all, antisocial, dependent, and histrionic (Lev-Ran, Le Foll, McKenzie, George, & Rehm, 2013b).

MADRID STUDY ON THE PREVALENCE OF DUAL PATHOLOGY IN CANNABIS ADDICTS IN TREATMENT

The sample consisted of 837 subjects in treatment in the Community of Madrid mental health or drug network. Of the total of 837 patients assessed, 710 had some diagnosis of a lifetime SUD (including alcohol, and excluding tobacco). Regarding cannabis, 353 patients had a lifetime diagnosis (abuse or dependence), of which 133 misused at the time, and 357 had substance use disorders involving drugs other than cannabis. Only 20 subjects had a lifetime diagnosis of cannabis misuse without other associated SUDs, therefore, in the analysis, all subjects with a diagnosis of cannabis use disorder were included, even if they had other associated SUDs (Arias et al., 2013).The MINI interview was used to diagnose axis I mental disorders, and the PDQ4 questionnaire for the assessment of personality disorders.

Table 7.3 shows the distribution of lifetime comorbid mental disorders. It was considered that a dual pathology existed if there was a current mental disorder diagnosis established through the MINI and/or a personality disorder diagnosis according to the PDQ4. Table 7.4 indicates the distribution of personality disorders in patients with lifetime cannabis misuse. Table 7.5 shows the current diagnoses of axis I disorders assessed by the MINI in subjects with current cannabis misuse. 76.5% had an axis I diagnosis established through the MINI and/or a personality disorder. The high comorbidity in cannabis addicts concurs with other studies (Stinson, Ruan, Pickering, & Grant, 2006).

The most prevalent disorders were different mood and anxiety disorders. The high prevalence of manic

TABLE 7.3 Lifetime Mental Disorders (axis I) in Subjects With Lifetime Cannabis Use Disorder (*N* = 353)

	N	Percentage
Presence of dual pathology[a]	270	76.5
Presence of a current axis 1 disorder (without personality disorder)	226	64.0
Diagnosis of a previous mental disorder	101	28.6
Lifetime psychiatric diagnoses		
Major depressive episode	92	26.1
Dysthymic disorder	72	20.4
Suicide risk (assessed with the MINI)	116	32.9
Bipolar disorder	90	25.5
Anxiety disorder	89	25.2
Agoraphobia	44	12.5
Social phobia	39	11.0
Obsessive-compulsive disorder	34	9.6
Posttraumatic stress disorder	20	5.7
Psychotic disorders	43	12.2
Bulimia	6	1.7
Generalized anxiety disorder	77	21.8
Number of diagnoses or mental disorders 1.5 (d.e. = 1.7)		

[a]*It was considered to be dual pathology if there was a current mental disorder established by the MINI and/or a diagnosis of personality disorder via the PDQ4.*

TABLE 7.4 Personality Disorders in Patients With Lifetime Cannabis Use Disorder

	N	Percentage
Presence of personality disorders	180	51.0
Paranoid	69	19.5
Schizoid	22	6.2
Schizotypal	36	10.2
Antisocial	69	19.5
Borderline	70	19.8
Histrionic	22	6.2
Narcissistic	24	6.8
Avoidant	65	18.4
Dependent	25	7.1
Obsessive	53	15.0
Passive-aggressive	41	11.6
Depressive	71	20.1
Number of diagnoses 1.6 (d.e. = 2.3)		

TABLE 7.5 Current Axis I Mental Disorders in Subjects With Current Cannabis Abuse or Dependence (*n* = 135)

	N	Percentage
Current mental disorder (MINI)	88	66.2
Major depressive episode	26	19.5
Dysthymia	19	14.3
Suicide risk	40	30.1
Bipolar disorder	39	29.3
Anxiety disorder	31	23.3
Agoraphobia	21	15.8
Social phobia	14	10.5
Obsessive-compulsive disorder	11	8.3
Posttraumatic stress disorder	6	4.5
Psychosis	16	12
Bulimia	2	1.5
Generalized anxiety disorder	32	24.1

or hypomanic symptoms should be highlighted. It is possible that a high percentage was induced by use, as has been previously explained (Henquet, Krabbendam, de Graaf, ten Have, & van Os, 2006). Likewise, suicide risk, assessed by the MINI, was found in one third of the cannabis addicts. Cannabis use is considered to increase such risk (Lynskey et al., 2004). This suicide risk is higher in dual pathology patients than in those who do not present dual pathology (Szerman et al., 2011).

There were no significant differences in the prevalence of mental disorders between the cannabis addicts (n = 353) and the rest of the addicts (n = 357), although there tended to be a higher presence of dual diagnoses in the cannabis group (64% vs 57.7%, p = 0.09). There was a higher number of males in the cannabis group (86.7% vs 76.6%, p = 0.001), and they were younger (35.2 vs 39.3 years of age, p = 0.001). Antisocial personality disorder was more prevalent in the cannabis group (19.5% vs 9%, p = 0.001), and obsessive personality disorder was less prevalent (15% vs 21%, p = 0.04). When considering the subgroup of current cannabis addicts (n = 133), a greater prevalence of agoraphobia (16.5% vs 9.2%, p = 0.01), psychosis (16.5% vs 9.4%, p = 0.02), and bipolar disorder (29.3% vs 21.8%, p = 0.05) was found in comparison to the rest of the addicts.

Information regarding the age of onset of the mental disorders was not collected. The age when cannabis use began in the patients with the mental disorders mentioned was compared (Table 7.6).

Therefore, we cannot conclude that cannabis misuse or dependence is specifically associated with certain mental disorders, but we can highlight that polysubstance use in which cannabis use is included is associated with serious mental disorders, such as, psychosis and bipolar disorder. Given the nature of the study, we cannot determine what type of relationship there is with these disorders. In any case, we observed that the age at which cannabis use began is earlier in patients with these comorbid diagnoses, which suggests that cannabis use may have a role in causing or precipitating such disorders. Different, recent studies indicate that cannabis use is related to an earlier onset of psychosis, and a recent metaanalysis confirms this (Large, Sharma, Compton, Slade, & Nielssen, 2011), and that the earlier cannabis use begins, the higher the risk that a psychosis may appear (Dragt et al., 2012). Likewise it has been suggested that cannabis has a causal role in inducing mania (Henquet et al., 2006), and an earlier age of onset of bipolar disorder in cannabis users (Lagerberg et al., 2011). In a study conducted on adolescents, 62% of cannabis addicts were found to have comorbid disorders, and there was an association with mood disorders (depression and bipolar disorder) and anxiety disorders (especially with panic disorder) (Wittchen et al., 2007). We found a high prevalence of panic disorder in the cannabis addicts, although when the association was compared with the rest of the addicts, there was a greater association with agoraphobia. The close association of cannabis use with bipolar disorder and psychosis has been established in many studies (Agrawal et al., 2011; Arendt, Rosenberg, Foldager, Perto, & Munk-Jørgensen, 2007).

In addition, this subgroup of cannabis users seems to constitute a more severe subgroup, given that they also began using other drugs at an earlier age. In any event, it is necessary to consider the limitations of the method used to collect data with possible memory bias.

In summary it was considered that 76.5% of the cannabis addicts had a dual diagnosis. Likewise, this subgroup was different from the rest of the addicts because of its association with antisocial personality disorder, bipolar disorder, psychosis, and agoraphobia.

Therefore, the presence of dual pathology is very high in addicts in treatment who are cannabis dependent, and such use, possibly in the context of a polysubstance use pattern, is associated with serious mental disorders such as psychosis and bipolar disorder. Beginning cannabis

TABLE 7.6 Age When Cannabis Use Began and Presence of Mental Disorders and Age When Use of Other Substances Began in Cannabis Addicts

Age when cannabis use began in years (s.d.)			
	Presence of disorder	**Absence of disorder**	***p***
Agoraphobia	14.9 (3.3)	16.3 (4.4)	0.05
Bipolar disorder	15.5 (3.3)	16.4 (4.5)	0.05
Psychosis	14.6 (3.6)	16.4 (4.4)	0.02
Antisocial personality disorder	15.0 (4.25)	16.4 (4.3)	0.01
Age when use of other substances began in cannabis addicts (in years)			
	Cannabis addicts	**Addicts not addicted to cannabis**	
Alcohol	15.1 (4.1)	17.5 (6.9)	0.001
Cocaine	19.8 (5.5)	22.6 (7.4)	0.001
Tobacco	13.9 (2.9)	14.9 (3.6)	0.001

use at an earlier age is associated with a higher risk of presenting such mental disorders.

MINI-DICTIONARY

Cannabidiol Another component of the *Cannabis sativa*, but which has different effects from THC, and a different mechanism of action, which has been investigated for its therapeutic use in some mental disorders.
Dual disorder The comorbidity between a substance use disorder (SUD) and any other lifetime psychiatric disorder.
Endocannabinoid system A neurotransmission system, the fundamental role of which is as a modulator of other systems, such as, the glutamatergic or the GABAergic systems. Up to now, the CB1 and CB2 receptors and two endogenous compounds, anandamide and 2-AG, have been described, as well as the enzymes implicated in the synthesis and degradation of such compounds.
FAAH Fatty acid amide hydrolase, the main enzyme that degrades anandamide.
Δ^9-Tetrahydrocannabinol The main psychoactive component of the *Cannabis sativa*, implicated in the mental effects of cannabis use.

References

Agrawal, A., Nurnberger, J. I., & Lynskey, M. T. (2011). Cannabis involvement in individuals with bipolar disorder. *Psychiatry Research*, *185*, 459–461.

Andréasson, S., Allebeck, P., Engström, A., & Rydberg, U. (1987). Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. *Lancet*, *2*, 1483–1486.

Arendt, M., Rosenberg, R., Foldager, L., Perto, G., & Munk-Jørgensen, P. (2007). Psychopathology among cannabis-dependent treatment seekers and association with later substance abuse treatment. *Journal of Substance Abuse and Treatment*, *32*, 113–119.

Arias, F. (2007). Revisión sobre el consumo de cannabis como factor de riesgo de la esquizofrenia. *Adicciones*, *19*, 191–203.

Arias, F., Szerman, N., Vega, P., Mesias, B., Basurte, I., Morant, C., Ochoa, E., Poyo, F., & Babin, F. (2013). Abuso o dependencia al cannabis y otros trastornos psiquiátricos. Estudio Madrid sobre prevalencia de patología dual. *Actas Españolas de Psiquiatría*, *41*, 123–130.

Buckner, J. D., Heimberg, R. G., Schneier, F. R., Liu, S. M., Wang, S., & Blanco, C. (2012). The relationship between cannabis use disorders and social anxiety disorder in the National Epidemiological Study of Alcohol and Related Conditions (NESARC). *Drug and Alcohol Dependence*, *124*, 128–134.

Dragt, S., Nieman, D. H., Schultze-Lutter, F., van der Meer, F., Becker, H., & de Haan, L. (2012). Cannabis use and age at onset of symptoms in subjects at clinical high risk for psychosis. *Acta Psychiatrica Scandinavica*, *125*, 45–53.

D'Souza, D. C., Abi-Saab, W. M., Madonick, S., Forselius-Bielen, K., Doersch, A., Braley, G., Gueorguieva, R., Cooper, T. B., & Krystal, J. H. (2005). Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biological Psychiatry*, *57*, 594–608.

D'Souza, D. C., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y. T., Braley, G., Gueorguieva, R., & Krystal, J. H. (2004). The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology*, *29*, 1558–1572.

Henquet, C., Krabbendam, L., de Graaf, R., ten Have, M., & van Os, J. (2006). Cannabis use and expression of mania in the general population. *Journal of Affective Disorders*, *95*, 103–110.

Lagerberg, T. V., Sundet, K., Aminoff, S. R., Berg, A. O., Ringen, P. A., Andreassen, O. A., & Melle, I. (2011). Excessive cannabis use is associated with earlier age at onset in bipolar disorder. *European Archives of Psychiatry and Clinical Neurosciences*, *261*, 397–405.

Large, M., Sharma, S., Compton, M. T., Slade, T., & Nielssen, O. (2011). Cannabis use and earlier onset of psychosis: a systematic meta-analysis. *Archives of General Psychiatry*, *68*, 555–561.

Lev-Ran, S., & Feingold, D. (2016). Cannabis use and its association to mental illness: a focus on mood and anxiety disorders. In V.R. Preedy (Ed.), *Handbook of cannabis, related pathologies: Biology, diagnosis, treatment, pharmacology*. Chennai: Elsevier.

Lev-Ran, S., Le Foll, B., McKenzie, K., George, T. P., & Rehm, J. (2013a). Bipolar disorder and co-occurring cannabis use disorders: characteristics, co-morbidities and clinical correlates. *Psychiatry Research*, *209*, 459–465.

Lev-Ran, S., Le Foll, B., McKenzie, K., George, T. P., & Rehm, J. (2013b). Cannabis use and cannabis use disorders among individuals with mental illness. *Comprehensive Psychiatry*, *54*, 589–598.

Lev-Ran, S., Roerecke, M., Le Foll, B., George, T. P., McKenzie, K., & Rehm, J. (2014). The association between cannabis use and depression: a systematic review and meta-analysis of longitudinal studies. *Psychological Medicine*, *44*, 797–810.

Leweke, F. M., Giuffrida, A., Koethe, D., Schreiber, D., Nolden, B. M., Kranaster, L., Neatby, M. A., Schneider, M., Gerth, C. W., Hellmich, M., Klosterkötter, J., & Piomelli, D. (2007). Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: impact of cannabis use. *Schizophrenia Research*, *94*, 29–36.

Leweke, F. M., Giuffrida, A., Wurster, U., Emrich, H. M., & Piomelli, D. (1999). Elevated endogenous cannabinoids in schizophrenia. *Neuroreport*, *10*, 1665–1669.

Lynskey, M. T., Glowinski, A. L., Todorov, A. A., Bucholz, K. K., Madden, P. A., Nelson, E. C., Statham, D. J., Martin, N. G., & Heath, A. C. (2004). Major depressive disorder, suicidal ideation, and suicide attempt in twins discordant for cannabis dependence and early-onset cannabis use. *Archives of General Psychiatry*, *61*, 1026–1032.

McGlothlin, W. H., & West, L. J. (1968). The marijuana problem: an overview. *American Journal of Psychiatry*, *125*, 370–378.

Moore, T. H., Zammit, S., Lingford-Hughes, A., Barnes, T. R., Jones, P. B., Burke, M., & Lewis, G. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet*, *370*, 319–328.

Patton, G. C., Coffey, C., Carlin, J. B., Degenhardt, L., Lynskey, M., & Hall, W. (2002). Cannabis use and mental health in young people: cohort study. *British Medical Journal*, *325*, 1195–1198.

Rey, A. A., Purrio, M., Viveros, M. P., & Lutz, B. (2012). Biphasic effects of cannabinoids in anxiety responses: CB1 and GABA(B) receptors in the balance of GABAergic and glutamatergic neurotransmission. *Neuropsychopharmacology*, *37*, 2624–2634.

Smith, D. E. (1968). Acute and chronic toxicity in marihuana. *Journal of Psychedelic Drugs*, *2*, 37–47.

Soueif, M. I. (1976). Some determinants of psychological deficits associated with chronic cannabis consumption. *Bulletin on Narcotics*, *1*, 25–42.

Stinson, F. S., Ruan, W. J., Pickering, R., & Grant, B. F. (2006). Cannabis use disorders in the USA: prevalence, correlates and co-morbidity. *Psychological Medicine*, *36*, 1447–1460.

Szerman, N., Lopez-Castroman, J., Arias, F., Morant, C., Babín, F., & Mesías, B. (2011). Dual diagnosis and suicide risk in a Spanish outpatient sample. *Substance Use and Misuse*, *48*, 1–7.

Wittchen, H. U., Fröhlich, C., Behrendt, S., Günther, A., Rehma, J., & Zimmermann, P. (2007). Cannabis use and cannabis use disorders and their relationship to mental disorders: a 10-year prospective-longitudinal community study in adolescents. *Drug and Alcohol Dependence*, *88S*, S60–S70.

Zvolensky, M. J., Lewinsohn, P., Bernstein, A., Schmidt, N. B., Buckner, J. D., Seeley, J., & Bonn-Miller, M. O. (2008). Prospective associations between cannabis use, abuse, and dependence and panic attacks and disorder. *Journal of Psychiatry Research*, *42*, 1017–1023.

CHAPTER

8

Cannabis Use and Cognitive Function

C. Evren

Research, Treatment and Training Center for Alcohol and Substance Dependence (AMATEM), Bakirkoy Training and Research Hospital for Psychiatry, Neurology and Neurosurgery, Istanbul, Turkey

SUMMARY POINTS

- This chapter gives an overview of studies that evaluated the impairments in cognitive functions related with cannabis use.
- Risk factors that may mediate the relationship between cannabis use and impairments in cognitive functions are reviewed.
- Adolescents are more vulnerable than adults to neurocognitive abnormalities associated with chronic heavy cannabis use.
- Cognitive deficits may differ in severity, depending on the quantity, recency, age of onset, and duration of cannabis use.
- Although some studies reported recovery with prolonged abstinence, others reported persisting impairments in several cognitive domains, such as, attention, working memory, and executive functioning.
- Further research on cognitive deficits associated with cannabis use will enhance our understanding of underlying pathophysiology and improving treatments for substance abuse.
- Future studies must control the effects of potentially confounding axis I disorders and use of other substances, and consider gender differences.
- Recognition of cognitive problems related to cannabis use can help to select a proper treatment that will be beneficiial for the subject.
- Combinations of behavioral therapies and pharmacotherapies for cognitive impairments may be more effective than single approaches.

KEY FACTS OF COGNITIVE FUNCTIONS

- Cognitive functions involve all aspects of perception, thinking, reasoning, and remembering.
- The regions of the brain primarily involved with the cognitive functions include the hippocampus, prefrontal cortex, and cerebellum.
- Δ^9-Tetrahydrocannabinol is thought to be responsible for the cognitive effects of smoked cannabis.
- Cannabis use during critical developmental periods in the still maturing brain may induce persistent alterations in brain structure and brain function.
- Heavy cannabis users may develop tolerance to the impairing effects of this drug on cognitive functions.
- Cognitive deficits were found in working and episodic memory, as well as in executive and attentional functions.
- Cognitive impairments related with cannabis use predict poor treatment retention and outcome.

LIST OF ABBREVIATIONS

AT	Attention
CB	Cannabis
CBUrs	Cannabis users
CF	Cognitive functions
CU	Cannabis use
DM	Decision-making
EF	Executive functions
HCU	Heavy cannabis users
THC	Δ^9-Tetrahydrocannabinol
VF	Verbal fluency
VLM	Verbal learning and memory
WM	Working memory

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00009-0
Copyright © 2017 Elsevier Inc. All rights reserved.

INTRODUCTION

Prevalence rates for cannabis use (CBU) is increasing, particularly among adolescents and young adults, alongside a decreasing perception of harm, and less disapproval of its use (Johnston, O'Malley, Bachman, & Schulenberg, 2013). Unfortunately, cannabis (CB) has become the most widely used illicit substance in both developed and developing countries, and constitutes a major health concern.

Over the past two decades, an extensive, growing body of research has demonstrated that CBU adversely affects cognitive functions (CF) (Table 8.1). In particular, deficits were found among heavy cannabis users (HCU) in working memory (WM) and episodic memory, as well as in attention (AT) and executive functions (EF) (Bolla, Brown, Eldreth, Tate, & Cadet, 2002; Messinis, Kyprianidou, Malefaki, & Papathanasopoulos, 2006; Solowij et al., 2002).

TABLE 8.1 Examples of the Studies that Evaluated Different Domains of Cognitive Functions With Cannabis Use

Cognitive functions (CF)	Examples of the studies
Attention (AT)	Medina et al. (2007); Solowij et al. (2002)
Working memory (WM)	Day et al., 2013
Verbal fluency (VF)	Morrison et al. (2009)
Verbal learning and memory (VLM)	Hanson et al. (2010); Harvey et al. (2007); Lisdahl and Price (2012); Medina et al. (2007)
Executive functions (EF)	Gonzalez et al. (2012); Grant et al. (2012); Lisdahl and Price (2012); Ranganathan and D'Souza (2006); Schuster, Crane, Mermelstein, & Gonzalez (2012); Solowij et al. (2012)

Although some studies reported recovery with prolonged abstinence (Pope, Gruber, Hudson, Huestis, & Yurgelun-Todd, 2001, 2002), others reported persisting impairments in several CF, such as, AT, WM, and EF (Bolla et al., 2002; Grant, Gonzalez, Carey, Natarajan, & Wolfson, 2003; Solowij et al., 2002). Consistent with this, in a recent study we conducted, the scores of the Adult Memory and Information Processing Battery Form A and B increased during one month remission follow-up, compared to baseline evaluation (Fig. 8.1). The auditory reaction time of the sample decreased significantly after a month, whereas there was no significant difference in the visual reaction time (Fig. 8.2). These findings supported the previous literature suggesting that some impairment may last longer than a month (Umut et al., 2016).

Δ^9-Tetrahydrocannabinol (THC), is thought to be responsible for the cognitive effects of smoked CB (Thames, Arbid, & Sayegh, 2014). The endogenous cannabinoid system is involved in regulatory neural mechanisms that modulate processes underlying a range of CF that are impaired by CB (Solowij & Pesa, 2010). The regions of the brain primarily involved with the CF include the hippocampus, prefrontal cortex, and cerebellum, which are regions of the brain responsible for decision-making (DM), EF, and memory (Hanson et al., 2010).

EFFECTS OF CANNABIS ON COGNITION

Pope et al. (2001, 2002) have tested AT among current HCU, former HCU, and control subjects on days 0, 1, 7, and 28 of abstinence. On all four occasions, no significant differences between the groups were found in AT performance, despite impairments in VLM, which persisted

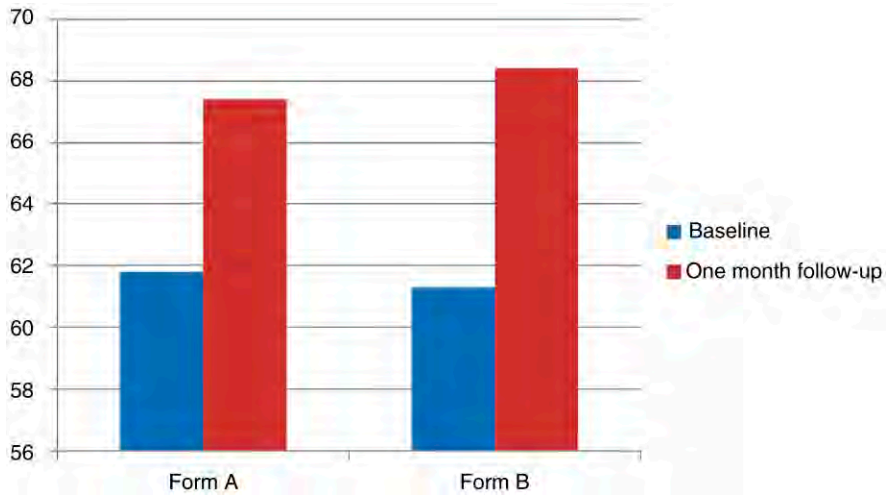


FIGURE 8.1 Comparing baseline and follow-up adult memory and information processing battery form A and form B scores. Adult Memory and Information Processing Battery (form A: z, = –2.31, p = 0.02; form B: z = –3.69, p = < 0.01).

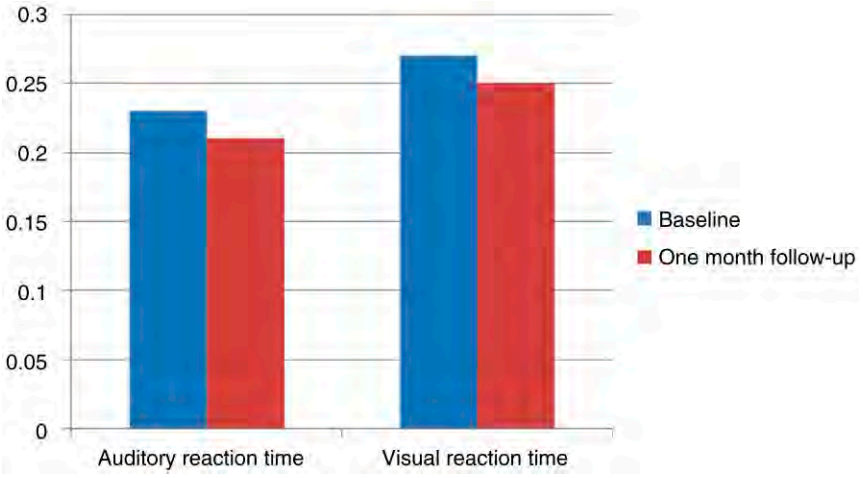


FIGURE 8.2 Comparing baseline and follow-up simple reaction time test (auditory and visual) scores. Auditory reaction time: z = –3.02, $p = 0.03$; visual reaction time: z = –1.78, $p = 0.07$).

up to 7 days of abstinence. After 28 days of abstinence, VLM impairments were no longer found. In a study that used a shorter abstinence period (ie, 24 h), cannabis users (CBUrs) showed both longer reaction times and delayed processing speed, compared with controls (Solowij et al., 2002).

Acute CB intoxication may interfere with time estimation, suggesting a "speeding up of an internal clock" (Hanson et al., 2010). Within several hours of intake, regular CBU in human adolescents or young adults appears to negatively affect VLM, AT, and spatial WM (Fried, Watkinson, & Gray, 2005; Harvey, Sellman, Porter, & Frampton, 2007). THC intoxication has been shown to impair CF on a number of levels, such as, the ability to plan, organize, solve problems, DM, remember, and control emotions and behavior (Crean, Crane, & Mason, 2011).

These impairments in CF can increase the likelihood of accidents, have been linked to the problematic risky behaviors (Romer et al., 2011; Day et al., 2013) and have been found to have hindered ability to make the best use of behavioral therapies, and trouble learning and applying the skills required for successful recovery, putting them at increased risk for relapse to CBU (Aharonovich, Brooks, Nunes, & Hasin, 2008; Crean et al., 2011).

There are reviews that compared cognitive consequences between CB and other substances (Table 8.2) and that considered CF among CBUrs (Table 8.3). These reviews generally support the adverse acute effects of CB on cognition; however, nonacute effects remain less clear.

Tolerance to Effects of Cannabis on Cognitive Functions

Some researchers have reported CB-related impairments in multiple CF (Hunault et al., 2009), while others have failed to observe such effects (Hart, van Gorp, Haney, Foltin, & Fischman, 2001; Ramaekers, Kauert, Theunissen, Toennes, & Moeller, 2009). One potential source of inconsistent findings is the type of tasks used to assess CF. Other source of the apparent incongruent findings may be the CBU histories of the research participants studied.

While Hart et al. (2001) demonstrated that HCU showed only minimal impairment on CF after acute CB administration, the lack of impairment may be due to the fact that subjects were still impaired in the placebo condition, as a result of residual effects. A plausible hypothesis is that infrequent CBUrs are more susceptible to the cognitive-impairing effects of CB than frequent users, who are tolerant to many CB-related performance

TABLE 8.2 Reviews that Compared Cognitive Consequences of Cannabis and Other Substances

Lundqvist (2005)	When compared with stimulants and heroin, CB induces loss of internal control and cognitive impairment, especially of AT and memory, for the duration of intoxication. HCU is associated with reduced function of the attentional system, as exhibited by decreased mental flexibility, increased perseveration, and reduced learning, to shift and/or sustain AT.
Holst & Schilt (2011)	This study reported that all substances of abuse, except CB, were associated with sustained deficits in EF, especially inhibition. Also, there was little evidence for sustained cognitive impairments in adult abstinent CBUrs.
Solowij et al. (2011)	This study suggested that adolescent CBUrs performed significantly worse than alcohol users and nonusers on all measures of learning and memory. They recalled significantly fewer words overall, demonstrating impaired learning, retention, and retrieval.

TABLE 8.3 Some Comprehensive Reviews for the Cannabis and Cognitive Functions

Gonzalez, Carey, & Grant (2002)	From the 40 articles, 22 reported at least some subtle impairments in CF.
Grant et al. (2003)	A metaanalysis identified selective deficits in VLM among chronic users with a range of abstinence, while other CF appeared unaffected.
Ranganathan and D'Souza (2006)	CB impairs all stages of memory, including encoding, consolidation, and retrieval.
Gonzalez (2007)	There are some methodological limitations across studies examining the nonacute effects of CB that include heterogeneous participant samples, and differ in factors, such as, severity of substance use, length of abstinence, and the presence of comorbid substance abuse and psychiatric disorders.
Schreiner and Dunn (2012)	Conducting two metaanalyses, they found that any negative residual effects on CF, attributable to either CB residue or withdrawal symptoms, are limited to the first 25 days of abstinence. Furthermore, there was no evidence for enduring negative effects of CBU.
Schoeler and Bhattacharyya (2013)	Relatively consistent findings have been reported regarding the acute impairments induced by a single dose of THC on verbal and WM. It is unclear whether they may persist beyond the intoxication state. In the long-term, these impairments seem particularly likely to manifest, and may also persist following abstinence, if regular and heavy use of CB strains high in THC is started at an early age.

impairing effects (Ramaekers et al., 2009; Hart et al., 2010; Theunissen et al., 2012). Nevertheless, although HCU develop tolerance to the impairing effects of THC on neurocognitive task performance, they do not develop cross-tolerance to the impairing effects of alcohol, and the presence of the latter even selectively potentiates THC effects on measures of divided AT (Ramaekers et al., 2011).

Duration, Frequency, and Amount of Cannabis Use

Duration, frequency, and amount of regular CBU have been associated with CB-related impairment, and recovery of CF in some studies (Grant et al., 2003). Studies have found that THC levels in urine are associated with the severity of cognitive impairment (Fried, Watkinson, James, & Gray, 2002; Pope et al., 2001). A study that evaluated the dose/frequency-effect relationship suggested that HCU is associated with persistent decrements in CF, even after 28 days of abstinence (Bolla et al., 2002). The other study demonstrated that the THC dose was linearly related to cognitive performance (Hunault et al., 2009), although it is unclear if these decrements will resolve with continued abstinence, or become progressively worse with continued HCU. Nevertheless, specific CF may deteriorate with increasing years of HCU (Messinis et al., 2006; Coullaut-Valera, Arbaiza-Diaz Del Río, de Arrúe-Ruiloba, Coullaut-Valera, & Bajo-Bretón, 2011). Together, these studies suggest that the magnitude of adverse effects of CB on cognition varies, depending upon the frequency of use, and length of abstinence.

Early Onset Cannabis Use

Adults who initiated use in early adolescence show greater dysfunction than those who began use later (Schweinsburg, Brown, & Tapert, 2008). Individuals who initiate CU at an early age, when the brain is still developing, might be more vulnerable to lasting neuropsychological deficits than individuals who begin use later in life. In a study conducted among 28-day abstinent CBUrs, it was shown that CU before the age of 17 has been associated with poor neurocognitive performance on tasks involving verbal ability (Pope et al., 2003). Adolescents who started using CB between the ages of 14–22 and stopped by the age of 22 had significantly more cognitive problems aged 27, than their nonusing peers (Brook, Stimmel, Zhang, & Brook, 2008). In a study examining the EF, individuals who started chronic CBU before the age of 15 showed significantly poorer cognitive performance on tasks assessing sustained attention, impulse control, and EF, compared with the controls and the late-onset group (Fontes et al., 2011). Finally, in a prospective study, individuals were followed from birth to the age of 38, and neuropsychological testing was conducted at the age of 13, before initiation of CU, and again at the age of 38, after a pattern of persistent CU had developed. Neuropsychological decline was concentrated among adolescent-onset CBUrs, with more persistent use associated with greater decline. Further, cessation of CBU did not fully restore neuropsychological functioning among adolescent-onset CBUrs (Meier et al., 2012). These suggest that, if regular CBU starts during early and middle adolescence, it might produce permanent or at least long lasting alterations in CF.

EFFECTS OF CANNABIS ON EXECUTIVE FUNCTIONING

A recent synthesis of studies on CU and EF suggest that findings vary substantially, depending on the specific measures employed, and whether CBUrs are tested while acutely intoxicated or when abstinent (Crean et al., 2011). Studies showing the greatest deficits in EF are the subjects who had been smoking heavy amounts of CB for long periods of time (Crean et al., 2011). Fried et al. (2005) suggested that residual CB effects are evident

beyond the acute intoxication period in current heavy users, after taking into account predrug performance, but similar deficits are no longer apparent 3 months after cessation of regular use, even among former heavy using young adults. Nevertheless, according to Crean et al. (2011) CB seems to continue to exert impairing effects in EF even after 3 weeks of abstinence and beyond.

In the following sections, acute effects denote 0–6 h after last CU, residual effects denote 7 h to 20 days after last CU, whereas long-term effects denote 3 weeks or longer after last CU.

Attention

In their review, Crean et al. (2011) suggested that evidence of the impairing effects of CB intoxication on AT is stronger in less experienced CBUrs, than those with established drug tolerance; AT in the latter group is disrupted more by acute abstinence than acute CB administration, probably as a function of neuroadaptation to chronic HCU. Investigations on the residual effects of CB on EF show that AT may or may not continue to be impaired during the interval associated with the elimination of THC and its metabolites from the brain. A recent study found that sustained CB abstinence for 3 weeks moderately improved critical tracking, and divided AT performance in chronic, daily CBUrs, but impairment was still observable compared to controls (Bosker et al., 2013). When long-term effects of CB were evaluated, basic attentional abilities are largely restored (Crean et al., 2011).

Planning and Decision-Making

Acute CBU has been generally found to impair aspects of planning and DM, for example, response speed, accuracy, and latency (Crean et al., 2011). A single study evaluating DM during the period of time from 7 h to 20 days since last use suggests that this ability is impaired (Whitlow et al., 2004). A study of Grant, Chamberlain, Schreiber, & Odlaug (2012) identified deficits on the quality of DM and executive planning in CBUrs, even in the absence of axis I disorders and a history of using other illicit drugs. A study conducted among nontreatment seeking young adult CBUrs, suggested that DM deficits may be more strongly associated with problems experienced from CBU, rather than solely being a consequence of CBU, per se (Gonzalez et al., 2012). When long-term effects of CB were evaluated, the most enduring and detectable deficits are seen in DM, concept formation, and planning (Crean et al., 2011).

Verbal Fluency

Morrison et al. (2009) studied verbal fluency (VF) among recreational CBUrs and, among this sample, compared those who were administered placebo or moderate (2.5 mg) THC, and found no impairing effects on VF abilities. The findings for VF are somewhat mixed for residual and long-term CB effects, but may be due, in part, to sample differences in the degree of CB exposure (Crean et al., 2011).

Working Memory

Although, according to some authors, there is mixed evidence regarding whether CBU leads to deficits in WM (Solowij & Pesa, 2010), Crean et al. (2011) suggested in their review that these impairments in aspects of WM are well established. In contrast to the acute effects of CB on WM, deficits resulting from residual and long-term CB effects have not been found.

Verbal Learning and Memory

VLM are two of the most extensively examined impaired CF in CBUrs. A growing number of studies suggest performance deficits in acutely intoxicated subjects, in terms of immediate and delayed recalls of words, intrusion, and learning (Solowij & Battisti, 2008; Gonzalez et al., 2012). A review focused on the long-term effect of CBU use on memory suggested that impairments are not dissimilar to those associated with acute intoxication, and have been related to the duration, frequency, dose, and age of onset of CBU (Solowij & Battisti, 2008). A study on CBU and VLM found significant associations between certain components of VLM and frequency of use, cumulative lifetime dose, and duration of regular use (Wagner, Becker, Gouzoulis-Mayfrank, & Daumann, 2010).

Finally, recent CBUrs demonstrated significantly worse performance than nonusers, across cognitive domains of AT/WM, information processing speed, and EF (Thames et al., 2014). There were no statistically significant differences between recent users and past users on neurocognitive performance. Frequency of CBU in the last 4 weeks, and the amount of daily CBU, were negatively associated with neurocognitive performance. Although some of these widespread adverse effects of CBU on neurocognitive functioning appear to attenuate with abstinence, past users' neurocognitive functioning was consistently lower than nonusers.

CANNABIS USE IN ADOLESCENTS

CB is a popular drug of abuse among adolescents, and previous reports show that fewer adolescents believe that regular CBU is harmful to health. Evidence from studies suggests that the severity of the effects

of CBU on cognitive development is dependent on the age when CBU begins. One possible explanation is that those who begin CBU early in adolescence are more likely to become heavily dependent. It is also plausible that chronic CBU will then interfere with educational and vocational training. From a more biological perspective, however, CBU during critical developmental periods in the still maturing brain may induce persistent alterations in brain structure and brain function (Shrivastava, Johnston, & Tsuang, 2011). Although fundamental CF evolve during childhood, existing CF refine throughout adolescence. A study suggested that CBU has a detrimental effect on prospective memory ability in young adults, but users may not be aware of these deficits (Bartholomew, Holroyd, & Heffernan, 2010).

A study suggested that, after 12 h of abstinence from CB, aspects of adolescent CF are independently related to the frequency of CBU, beyond acute intoxication (Harvey et al., 2007). A review reported that studies of adolescent heavy users have identified impairments in learning, memory, and WM up to 6 weeks after cessation, suggesting persisting effects, yet raise the possibility that abnormalities may remit with a longer duration of abstinence. Given ongoing neuromaturation during youth, adolescents may be more vulnerable to potential consequences of CBU than adults (Schweinsburg et al., 2008). As in adults, the degree of impairment on learning and memory, in adolescents, was associated with the duration, quantity, frequency, and age of onset of CU, but was unrelated to alcohol exposure or other drug use (Solowij et al., 2011).

The results of the study by Medina et al. (2007) suggested that, even after a month of monitored abstinence, adolescent CBUrs demonstrate subtle neuropsychological deficits, compared with nonusers. Since the number of lifetime CBU episodes was associated with poorer CF in this study, it is possible that frequent CBU during adolescence may negatively influence neuromaturation and cognitive development. A study conducted by Hanson et al. (2010) detected poorer VLM and verbal WM among adolescent CBUrs that improved during 3 weeks of abstinence, while AT deficits persisted. Another study found that impaired short-term recall memory distinguished adolescent CBUrs from nonusers (Dougherty et al., 2013). Nevertheless, a recent study suggested that abstinent (for at least 30 days) adolescents with CBU disorder history did not differ from the controls, according to CF, suggesting that previously reported deficits may be related to other factors, including residual drug effects, preexisting cognitive deficits, concurrent use of other substances (eg, nicotine), or psychopathology (Hooper, Woolley, & De Bellis, 2014).

ADDITIONAL EFFECT OF POLYSUBSTANCE USE AND PSYCHIATRIC COMORBIDITY

Additional to using different cognitive paradigms, other main limitations for the research of CBU and CF are recruitment of CBUrs with potentially confounding axis I disorders and/or with a history of using other illicit drugs (Hart et al., 2010; Solowij & Pesa, 2010).

Polysubstance Use

Nowadays, CBU as a single drug is becoming rare, whereas polysubstance use is becoming more common. A study that compared subjects only using CB and subjects using ecstasy and CB found no difference on any of the neuropsychological tests but, apart from speed of processing, where higher ecstasy consumption predicted slower processing, the deficits were more closely related to CB than ecstasy usage (Croft, Mackay, Mills, & Gruzelier, 2001). Another study that investigated the consequences of prolonged patterns of alcohol and CBU on CF in late adolescence suggested a poorer CF in alcohol users with HCU by the 18-month follow-up (Jacobus, Squeglia, Bava, & Tapert, 2013).

Psychiatric Comorbidity

The additive effects of CB dependency and depression may not extend to CF (Secora et al., 2010). Earlier initiation of CBU may be linked to poor cognitive outcomes and a significantly greater proportion of the attention deficit hyperactivity disorder patients began using CB before the age of 16 (Tamm et al., 2013).

One would expect that regular CBU will further worsen the impaired CF in patients with schizophrenia or bipolar disorder. Surprisingly, a number of recent studies reported similar or even better CF in CBUrs with schizophrenia (Shrivastava et al., 2011) or bipolar patients (Braga, Burdick, Derosse, & Malhotra, 2012), compared to patients without comorbid drug use. CB using patients who develop psychosis have less neurodevelopmental impairment, and better cognitive reserve than other psychotic patients—perhaps reflecting different etiological processes (Cunha et al., 2013).

Other Comorbidity

The patients with multiple sclerosis who smoke CB are more cognitively impaired than nonusers (Pavisian et al., 2014). Finally, although there is a minimal impact of CB on CF among uninfected individuals, or those at early stages of HIV infection, there is a synergistic effect (due to performance on memory task) of HIV and

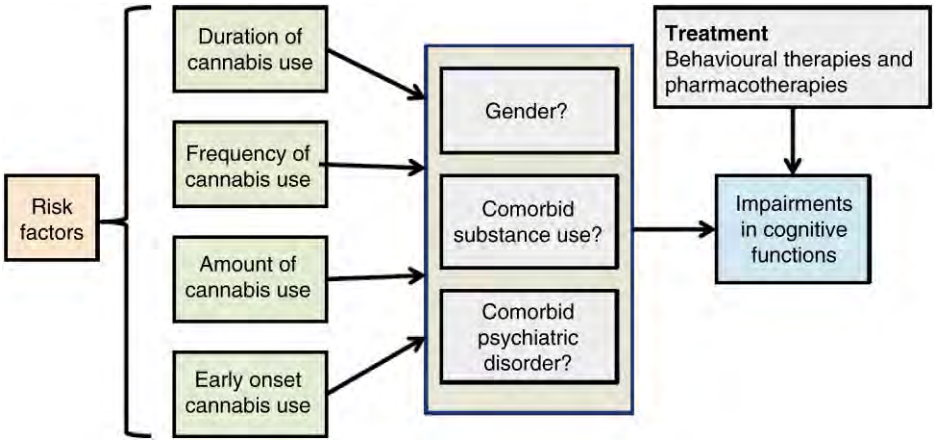


FIGURE 8.3 Risk factors and treatment of impairments in cognitive functions related with cannabis use. Duration, frequency, amount of cannabis use, and early onset cannabis use are related with impairments in cognitive functions. Effects of gender, comorbid psychiatric disorders, and use of other substances are not well known. Treatment of cognitive impairments should include both behavioral therapies and pharmacotherapies.

CBU in patients with advanced HIV disease (Cristiani, Pukay-Martin, & Bornstein, 2004).

GENDER DIFFERENCES FOR THE EFFECT OF CANNABIS USE ON COGNITIVE FUNCTIONS

As previous research identifying cognitive deficits in CBUrs employed samples that were largely male (Bolla et al., 2002; Messinis et al., 2006; Solowij et al., 2002), the CF of female CBUrs is largely unknown. Although Anderson, Rizzo, Block, Pearlson, and O'Leary, 2010 did not find gender differences in effects of CB on cognition, women requested to discontinue the smoking session more often than men, likely leading to an underestimation of differences. Lisdahl and Price (2012) examined how the amount of CBU may differentially affect CF in male and female CBUrs. Although they found that CBUrs had poorer immediate recall and interference inhibition than controls, sex did not moderate these relationships. Nevertheless, in another study, more CBU was more consistently associated with poorer episodic memory performance in females than males, whereas, conversely, it was associated with poorer DM performance in males than females (Crane, Schuster, & Gonzalez, 2013). These results suggest the importance of examining the impact of CB on neurocognition separately for males and females (Fig. 8.3).

TREATMENT

Among adults with CBU disorder, dropouts from combined motivational enhancement therapy and cognitive behavioral therapy scored significantly lower than completers on measures of abstract reasoning and processing accuracy, providing initial evidence that cognitive functioning plays a role in treatment retention in this population (Aharonovich et al., 2008). Abstinence rates following behavioral therapies have been modest, and there are no effective or approved pharmacotherapies for the treatment of CBU disorder. Therefore, targeting cognitive impairment associated with chronic CBU may be a promising novel strategy for the treatment of CB addiction (Sofuoglu, Sugarman, & Carroll, 2010). The bulk of the literature on treatments for addictive disorders suggests that combinations of behavioral therapies and pharmacotherapies may be more effective than single approaches. Thus, medications and behavioral approaches, including cognitive remediation, which target these CF may have utility for the treatment of CBU disorder. One potential area for the future research may be to evaluate specific CF that is most strongly related to improved treatment outcome.

MINI-DICTIONARY

Attention Attention is a process of selectively attending to one aspect of the environment while ignoring other things; includes divided and sustained attention to target stimuli, and it is mediated by the frontal lobes.
Cannabis Cannabis (marijuana) is a green or gray mixture of dried, shredded flowers and leaves of the hemp plant *Cannabis sativa*.
Cognitive functions An intellectual process by which one becomes aware of, perceives, or comprehends ideas. It involves all aspects of perception, thinking, reasoning, and remembering.
Decision-making A process of selecting a course of action among several alternatives.
Executive functions The term executive function describes a set of cognitive abilities that control and regulate other abilities and behaviors. They include the ability to initiate and stop actions, to monitor and change behavior as needed, and to plan future behavior when faced with novel tasks and situations.
Heavy cannabis users Usually defined as using cannabis on at least 10 occasions per month.

Memory The mental capacity or faculty of retaining and reviving facts, events, impressions, etc., or of recalling or recognizing previous experiences.
Δ^9-Tetrahydrocannabinol (THC) The primary psychoactive agent of the *Cannabis sativa* plant that is a partial agonist of the Cannabinoid Type-1 receptors.
Verbal fluency Generating multiple, verbal responses associated with a specified conceptual category.
Verbal learning The acquisition and retention of verbal information.
Working memory Working memory is an ability to hold and manipulate information, and remember it after a short delay.

References

Aharonovich, E., Brooks, A. C., Nunes, E. V., & Hasin, D. S. (2008). Cognitive deficits in marijuana users: effects on motivational enhancement therapy plus cognitive behavioral therapy treatment outcome. *Drug and Alcohol Dependence*, *95*, 279–283.

Anderson, B. M., Rizzo, M., Block, R. I., Pearlson, G. D., & O'Leary, D. S. (2010). Sex, drugs, and cognition: effects of marijuana. *Journal of Psychoactive Drugs*, *42*, 413–424.

Bartholomew, J., Holroyd, S., & Heffernan, T. M. (2010). Does cannabis use affect prospective memory in young adults? *Journal of Psychopharmacology*, *24*, 241–246.

Bolla, K. I., Brown, K., Eldreth, D., Tate, K., & Cadet, J. L. (2002). Dose-related neurocognitive effects of marijuana use. *Neurology*, *59*, 1337–1343.

Bosker, W. M., Karschner, E. L., Lee, D., Goodwin, R. S., Hirvonen, J., Innis, R. B., Theunissen, E. L., Kuypers, K. P., Huestis, M. A., & Ramaekers, J. G. (2013). Psychomotor function in chronic daily cannabis smokers during sustained abstinence. *PLoS One*, *8*, e53127.

Braga, R. J., Burdick, K. E., Derosse, P., & Malhotra, A. K. (2012). Cognitive and clinical outcomes associated with cannabis use in patients with bipolar I disorder. *Psychiatry Research*, *200*, 242–245.

Brook, J. S., Stimmel, M. A., Zhang, C., & Brook, D. W. (2008). The association between earlier marijuana use and subsequent academic achievement and health problems: a longitudinal study. *American Journal of Addiction*, *17*, 155–160.

Coullaut-Valera, R., Arbaiza-Diaz Del Río, I., de Arrúe-Ruiloba, R., Coullaut-Valera, J., & Bajo-Bretón, R. (2011). Cognitive deterioration associated with the use of different psychoactive substances. *Actas Espanolas de Psiquiatria*, *39*, 168–173.

Crane, N. A., Schuster, R. M., & Gonzalez, R. (2013). Preliminary evidence for a sex-specific relationship between amount of cannabis use and neurocognitive performance in young adult cannabis users. *Journal of the International Neuropsychological Society*, *19*, 1009–1015.

Crean, R. D., Crane, N. A., & Mason, B. J. (2011). An evidence based review of acute and long-term effects of cannabis use on executive cognitive functions. *Journal of Addiction Medicine*, *5*, 1–8.

Cristiani, S. A., Pukay-Martin, N. D., & Bornstein, R. A. (2004). Marijuana use and cognitive function in HIV-infected people. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *16*, 330–335.

Croft, R. J., Mackay, A. J., Mills, A. T., & Gruzelier, J. G. (2001). The relative contributions of ecstasy and cannabis to cognitive impairment. *Psychopharmacology (Berlin)*, *153*, 373–379.

Cunha, P. J., Rosa, P. G., Ayres Ade, M., Duran, F. L., Santos, L. C., Scazufca, M., Menezes, P. R., dos Santos, B., Murray, R. M., Crippa, J. A., Busatto, G. F., & Schaufelberger, M. S. (2013). Cannabis use, cognition and brain structure in first-episode psychosis. *Schizophrenia Research*, *147*, 209–215.

Day, A. M., Metrik, J., Spillane, N. S., & Kahler, C. W. (2013). Working memory and impulsivity predict marijuana-related problems among frequent users. *Drug and Alcohol Dependence*, *131*, 171–174.

Dougherty, D. M., Mathias, C. W., Dawes, M. A., Furr, R. M., Charles, N. E., Liguori, A., Shannon, E. E., & Acheson, A. (2013). Impulsivity, attention, memory, and decision-making among adolescent marijuana users. *Psychopharmacology (Berlin)*, *226*, 307–319.

Fontes, M. A., Bolla, K. I., Cunha, P. J., Almeida, P. P., Jungerman, F., Laranjeira, R. R., Bressan, R. A., & Lacerda, A. L. (2011). Cannabis use before age 15 and subsequent executive functioning. *British Journal of Psychiatry*, *198*, 442–427.

Fried, P. A., Watkinson, B., & Gray, R. (2005). Neurocognitive consequences of marihuana. A comparison with pre drug performance. *Neurotoxicology and Teratology*, *27*(2), 231–239.

Fried, P., Watkinson, B., James, D., & Gray, R. (2002). Current and former marijuana use: preliminary findings of a longitudinal study of effects on IQ in young adults. *Canadian Medical Association Journal*, *166*, 887–891.

Gonzalez, R. (2007). Acute and non-acute effects of cannabis on brain functioning and neuropsychological performance. *Neuropsychology Review*, *17*, 347–361.

Gonzalez, R., Carey, C., & Grant, I. (2002). Nonacute (residual) neuropsychological effects of cannabis use: a qualitative analysis and systematic review. *Journal of Clinical Pharmacology*, *42*(11 Suppl.), 48–57.

Gonzalez, R., Schuster, R. M., Mermelstein, R. J., Vassileva, J., Martin, E. M., & Diviak, K. R. (2012). Performance of young adult cannabis users on neurocognitive measures of impulsive behavior and their relationship to symptoms of cannabis use disorders. *Journal of Clinical and Experimental Neuropsychology*, *34*, 962–976.

Grant, I., Gonzalez, R., Carey, C. L., Natarajan, L., & Wolfson, T. (2003). Non-acute (residual) neurocognitive effects of cannabis use: a meta-analytic study. *Journal of the International Neuropsychological Society*, *9*, 679–689.

Grant, J. E., Chamberlain, S. R., Schreiber, L., & Odlaug, B. L. (2012). Neuropsychological deficits associated with cannabis use in young adults. *Drug and Alcohol Dependence*, *21*, 159–162.

Hanson, K. L., Winward, J. L., Schweinsburg, A. D., Medina, K. L., Brown, S. A., & Tapert, S. F. (2010). Longitudinal study of cognition among adolescent marijuana users over three weeks of abstinence. *Addictive Behaviours*, *35*, 970–976.

Hart, C. L., Ilan, A. B., Gevins, A., Gunderson, E. W., Role, K., Colley, J., & Foltin, R. W. (2010). Neurophysiological and cognitive effects of smoked marijuana in frequent users. *Pharmacology Biochemistry and Behavior*, *96*, 333–341.

Hart, C. L., van Gorp, W. G., Haney, M., Foltin, R. W., & Fischman, M. W. (2001). Effects of acute smoked marijuana on complex cognitive performance. *Neuropsychopharmacology*, *25*, 757–765.

Harvey, M. A., Sellman, J. D., Porter, R. J., & Frampton, C. M. (2007). The relationship between non-acute adolescent cannabis use and cognition. *Drug and Alcohol Review*, *26*, 309–319.

Hooper, S. R., Woolley, D., & De Bellis, M. D. (2014). Intellectual, neurocognitive, and academic achievement in abstinent adolescents with cannabis use disorder. *Psychopharmacology (Berlin)*, *231*, 1467–1477.

Hunault, C. C., Mensinga, T. T., Böcker, K. B., Schipper, C. M., Kruidenier, M., Leenders, M. E., de Vries, I., & Meulenbelt, J. (2009). Cognitive and psychomotor effects in males after smoking a combination of tobacco and cannabis containing up to 69 mg delta-9-tetrahydrocannabinol (THC). *Psychopharmacology (Berlin)*, *204*, 85–94.

Jacobus, J., Squeglia, L. M., Bava, S., & Tapert, S. F. (2013). White matter characterization of adolescent binge drinking with and without co-occurring marijuana use: a 3-year investigation. *Psychiatry Research*, *214*, 374–381.

Johnston, L. D., O'Malley, P. M., Bachman, J. G., & Schulenberg, J. E. (2013). *Monitoring the future. National survey results on drug use, 1975-2012. Volume I: secondary school students*. Ann Arbor, MI: Institute for Social Research, The University of Michigan, 604.

Lisdahl, K. M., & Price, J. S. (2012). Increased marijuana use and gender predict poorer cognitive functioning in adolescents and emerging adults. *Journal of the International Neuropsychological Society*, *18*, 678–688.

Lundqvist, T. (2005). Cognitive consequences of cannabis use: comparison with abuse of stimulants and heroin with regard to attention, memory and executive functions. *Pharmacology Biochemistry and Behavior*, *81*, 319–330.

Medina, K. L., Hanson, K. L., Schweinsburg, A. D., Cohen-Zion, M., Nagel, B. J., & Tapert, S. F. (2007). Neuropsychological functioning in adolescent marijuana users: subtle deficits detectable after a month of abstinence. *Journal of the International Neuropsychological Society*, *13*, 807–820.

Meier, M. H., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R. S., McDonald, K., Ward, A., Poulton, R., & Moffitt, T. E. (2012). Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 2657–2664.

Messinis, L., Kyprianidou, A., Malefaki, S., & Papathanasopoulos, P. (2006). Neuropsychological deficits in long-term frequent cannabis users. *Neurology*, *66*, 737–739.

Morrison, P. D., Zois, V., McKeown, D. A., Lee, T., Holt, D., Powel, J. F., Kapur, S., & Murray, R. M. (2009). The acute effects of synthetic intravenous delta9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. *Psychological Medicine*, *39*, 1607–1616.

Pavisian, B., Macintosh, B. J., Szilagyi, G., Staines, R. W., O'Connor, P., & Feinstein, A. (2014). Effects of cannabis on cognition in patients with MS: a psychometric and MRI study. *Neurology*, *82*, 1879–1887.

Pope, H. G., Jr., Gruber, A. J., Hudson, J. I., Cohane, G., Huestis, M. A., & Yurgelun-Todd, D. (2003). Early-onset cannabis use and cognitive deficits: what is the nature of the association? *Drug and Alcohol Dependence*, *69*, 303–310.

Pope, H. G., Jr., Gruber, A. J., Hudson, J. I., Huestis, M. A., & Yurgelun-Todd, D. (2002). Cognitive measures in long-term cannabis users. *Journal of Clinical Pharmacology*, *42*(11 Suppl), 41–47.

Pope, H. G., Jr., Gruber, A. J., Hudson, J. I., Huestis, M. A., & Yurgelun-Todd, D. (2001). Neuropsychological performance in long-term cannabis users. *Archives of General Psychiatry*, *58*, 909–915.

Ramaekers, J. G., Kauert, G., Theunissen, E. L., Toennes, S. W., & Moeller, M. R. (2009). Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. *Journal of Psychopharmacology*, *23*, 266–277.

Ramaekers, J. G., Theunissen, E. L., de Brouwer, M., Toennes, S. W., Moeller, M. R., & Kauert, G. (2011). Tolerance and cross-tolerance to neurocognitive effects of THC and alcohol in heavy cannabis users. *Psychopharmacology (Berlin)*, *214*, 391–401.

Ranganathan, M., & D'Souza, D. C. (2006). The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berlin)*, *188*, 425–444.

Romer, D., Betancourt, L. M., Brodsky, N. L., Giannetta, J. M., Yang, W., & Hurt, H. (2011). Does adolescent risk taking imply weak executive function? A prospective study of relations between working memory performance, impulsivity, and risk taking in early adolescence. *Developmental Science*, *14*, 1119–1133.

Schoeler, T., & Bhattacharyya, S. (2013). The effect of cannabis use on memory function: an update. *Substance Abuse Rehabilitation*, *4*, 11–27.

Schreiner, A. M., & Dunn, M. E. (2012). Residual effects of cannabis use on neurocognitive performance after prolonged abstinence: a meta-analysis. *Experimental and Clinical Psychopharmacology*, *20*, 420–429.

Schuster, R. M., Crane, N. A., Mermelstein, R., & Gonzalez, R. (2012). The influence of inhibitory control and episodic memory on the risky sexual behavior of young adult cannabis users. *Journal of the International Neuropsychological Society*, *18*, 827–833.

Schweinsburg, A. D., Brown, S. A., & Tapert, S. F. (2008). The influence of marijuana use on neurocognitive functioning in adolescents. *Current Drug Abuse Reviews*, *1*, 99–111.

Secora, A. M., Eddie, D., Wyman, B. J., Brooks, D. J., Mariani, J. J., & Levin, F. R. (2010). A comparison of psychosocial and cognitive functioning between depressed and non-depressed patients with cannabis dependence. *Journal of Addictive Diseases*, *29*, 325–337.

Shrivastava, A., Johnston, M., & Tsuang, M. (2011). Cannabis use and cognitive dysfunction. *Indian Journal of Psychiatry*, *53*, 187–191.

Sofuoglu, M., Sugarman, D. E., & Carroll, K. M. (2010). Cognitive function as an emerging treatment target for marijuana addiction. *Experimental and Clinical Psychopharmacology*, *18*, 109–119.

Solowij, N., & Battisti, R. (2008). The chronic effects of cannabis on memory in humans: a review. *Current Drug Abuse Reviews*, *1*, 81–98.

Solowij, N., & Pesa, N. (2010). Cognitive abnormalities and cannabis use. *Revista Brasileira de Psiquiatria.*, *32*(Suppl. 1), 31–40.

Solowij, N., Jones, K. A., Rozman, M. E., Davis, S. M., Ciarrochi, J., Heaven, P. C., Lubman, D. I., & Yucel, M. (2011). Verbal learning and memory in adolescent cannabis users, alcohol users and non-users. *Psychopharmacology (Berlin)*, *216*, 131–144.

Solowij, N., Jones, K. A., Rozman, M. E., Davis, S. M., Ciarrochi, J., Heaven, P. C. L., Pesa, N., Lubman, D. I., & Yucel, M. (2012). Reflection impulsivity in adolescent cannabis users: A comparison with alcohol-using and non-substance-using adolescents. *Psychopharmacology*, *219*, 575–586.

Solowij, N., Stephens, R. S., Roffman, R. A., Babor, T., Kadden, R., Miller, M., Christiansen, K., McRee, B., & Vendetti, J. Marijuana Treatment Project Research Group. (2002). Cognitive functioning of long-term heavy cannabis users seeking treatment. *JAMA*, *287*, 1123–1131.

Tamm, L., Epstein, J. N., Lisdahl, K. M., Molina, B., Tapert, S., Hinshaw, S. P., Arnold, L. E., Velanova, K., Abikoff, H., & Swanson, J. M. MTA Neuroimaging Group. (2013). Impact of ADHD and cannabis use on executive functioning in young adults. *Drug and Alcohol Dependence*, *133*, 607–614.

Thames, A. D., Arbid, N., & Sayegh, P. (2014). Cannabis use and neurocognitive functioning in a non clinical sample of users. *Addictive Behaviours*, *39*, 994–999.

Theunissen, E. L., Kauert, G. F., Toennes, S. W., Moeller, M. R., Sambeth, A., Blanchard, M. M., & Ramaekers, J. G. (2012). Neurophysiological functioning of occasional and heavy cannabis users during THC intoxication. *Psychopharmacology (Berlin)*, *220*, 341–350.

Umut, G., Atagun, M.I., Evren, C., Ozturk, H., Hısım, O., & Keskinkilic, C. (2016). The changes of the cognitive and psychomotor functions in the chronic cannabis users after a month remission. Bulleten of Clinical Psychopharmacology. (in press).

Van Holst, R. J., & Schilt, T. (2011). Drug-related decrease in neuropsychological functions of abstinent drug users. *Current Drug Abuse Reviews*, *4*, 42–56.

Wagner, D., Becker, B., Gouzoulis-Mayfrank, E., & Daumann, J. (2010). Interactions between specific parameters of cannabis use and verbal memory. *Prog Neuropsychopharmacol Biological Psychiatry*, *34*, 871–876.

Whitlow, C. T., Liguori, A., Livengood, L. B., Hart, S. L., Mussat-Whitlow, B. J., Lamborn, C. M., Laurienti, P. J., & Porrino, L. J. (2004). Long-term heavy marijuana users make costly decisions on a gambling task. *Drug and Alcohol Dependence*, *76*, 107–111.

CHAPTER

9

Cannabis, Migration, and Psychosis Onset

A. Kokona[*], *I. Tarricone*[*,**], *M. Di Forti*[†], *E. Carra*[†]

*Department of Medical and Surgical Sciences, Bologna University, Bologna, Italy
**Department of Mental Health, Bologna, Italy
†Department of Psychosis Studies, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom

SUMMARY POINTS

- Psychotic disorders have a multifactorial etiology, characterized by risk factors operating at various stages of life.
- The use of cannabis is associated with a risk of developing psychosis of about 2 times greater, and provokes also an earlier age of onset of schizophrenia.
- The best cannabis use related predictors of psychotic symptoms are: (1) early adolescence age at first use; (2) high-potency cannabis; (3) genetic vulnerability; (4) heavy use, and (5) individual psychosis vulnerability at baseline.
- Cannabis use can make antipsychotic treatment less effective, and the remission of psychotic symptoms more problematic, and is associated with a higher rate of relapse and greater number of hospitalizations, likely in combination with lower adherence to therapy.
- Considerable evidence indicates that the incidence of schizophrenia, and psychotic disorders in general, are higher in migrant populations and ethnic minorities, compared to the general population.
- There are substantial variations in incidence rates of psychotic disorders across both places and minority groups.
- Environmental factors at the individual and area levels play an important role in the genesis of psychotic disorders among migrant populations.
- The higher risk of psychosis in first generation migrants could not be explained by substance abuse, but some studies have observed a relationship between cannabis use and psychotic onset among second generation migrants.
- Further studies are needed to emit light on the correlation of migration and cannabis use on the development of psychotic disorders.

KEY FACTS OF CANNABIS

- Cannabis is the world's most widely used illicit substance.
- Cannabis contains several cannabinoids, the two best described are: Δ^9-tetrahydrocannabinol (D9-THC) and Cannabidiol (CBD).
- The main types of cannabis available on the "street" were marijuana (grass) and resin (hash), but, in recent years, a more potent variant termed sinsemilla or skunk has become available in many countries.
- The main difference between these substances is that skunk contains a very high concentration of D9-THC (12–18%) and virtually no CBD, while grass and hash are known to contain a similar percentage of D9-THC and CBD (2–4%).
- The use of cannabis is associated with a risk of developing psychosis of about 2 times greater, and the use provokes also an earlier age of onset of schizophrenia

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00010-7
Copyright © 2017 Elsevier Inc. All rights reserved.

KEY FACTS OF MIGRATION AND PSYCHOSIS ONSET

- There are about 232 million international migrants in the world.
- It is expected that the number of international migrants worldwide will increase in the future.
- The migratory movements are determined by many reasons, with demographic factors, economic disparities, and environmental change, continuing to be major drivers of migration.
- The intense urbanization of developed countries determines also *internal migration*: many people move from rural areas to urban areas in search of higher profits and better services.
- Rates of schizophrenia are higher among migrant and ethnic minority groups.

LIST OF ABBREVIATIONS

AC	African-Caribbean
AOP	Age of psychosis onset
CB1 receptor	Cannabinoid receptor type 1
CB2 receptor	Cannabinoid receptor type 2
CBD	Cannabidiol
CNS	Central nervous system
D9-THC	Δ^9-Tetrahydrocannabinol
DSM	Diagnostic and Statistical Manual of Mental Disorders
DUP	Duration of untreated psychosis
EU-GEI	European Network of National Schizophrenia Networks Studying Gene–Environment Interactions
FEP	First episode psychosis
RR	Relative risk
OR	Odds ratio

INTRODUCTION

The term "Psychosis" indicates a mental state characterized by a set of positive and negative "psychotic" symptoms. Positive symptoms are represented by delusions (false and immutable ideas characterized by extraordinary conviction and subjective certainty, not subject to criticism, and not arising from the cultural context of origin of the subject), hallucinations (perception disorders in any of the five sensory modalities), and disorganization of speech and thinking, described as thought disorders. Negative symptoms include flattening of affect, volition, and mental capacity. Other typical features are alogia (understood as poverty of speech) and apathy (loss of energy, interests, and initiative). The presence of all psychotic symptoms is not necessarily indicative of a disorder. These symptoms have been consistently reported as experienced even by individuals from nonclinical populations (van Os & Linscott, 2009). Why someone, who experiences psychotic symptoms, crosses over the threshold into frank disorder can depend on how frequent and intrusive the psychotic symptoms are, on how they interfere with the person's life, and on how they are tolerated by the person's social context (Johns & van Os, 2001).

The rates of schizophrenia range from 7.7 to 43.0 per 100,000 with a worldwide variation up to fivefold (McGrath, 2006). Schizophrenia is commoner in men than women (male to female ratio = 1.4; McGrath, 2006), with a peak incidence in males between the ages of 20 and 24 years, and in females between 29 and 32 years (Hafner, 2003).

Psychosis in general and schizophrenia in particular, have a multifactorial etiology. The strongest and most replicated risk is a family history of psychotic disorders. The heritability for schizophrenia has been calculated as between 66% and 85% (Cardno et al., 1999). Despite major progress, genetics have not been able to fully clarify the pathophysiology of psychotic disorders. Many authors have started to focus their attention on the role of environmental factors in determining the onset of psychotic disorders (Brietzke et al., 2012). Epidemiological studies have reported how rates of substance misuse, especially cannabis, are consistently higher in first episode psychosis patients, compared to the general population. Two metaanalyses have consistently indicated that cannabis use is associated with an increased risk (about twofold) of developing psychosis (Henquet & Murray, 2005; Moore & Zammit, 2007). Another very important predictor of psychosis is migration history. It has been widely replicated that rates of schizophrenia are higher among migrant and ethnic minority groups, with a mean weighted relative risk among migrants (first and second generation) of 2.7 (95% CI 2.3–3.2) (Cantor-Graae & Selten, 2005).

We aim to summarize, in this chapter, the available evidences about the role of cannabis use and migration in the onset of psychosis, and about their possible convergence in increasing the risk of psychosis.

CANNABIS AND PSYCHOSIS

What We Know About Cannabis

The name cannabis refers to the plant *Cannabis sativa*, used to obtain different preparations. Actually, cannabis has been known for about 4000 years for its effects over the mind, and is now the third most widely used substance in the world, after alcohol and tobacco (Murray & Morrison, 2007). Cannabis contains several cannabinoids,

TABLE 9.1 Physical Effects of Acute Cannabis Intoxication

Acute cannabis intoxication: physical effects
Decreased salivation
Dry mouth
Conjunctival redness
Accelerated pulse and tachycardia in acute dosage
Orthostatic hypotension
Reduction of intraocular pressure
Reduced motor skills and concentration
Appetite stimulation

TABLE 9.2 Psychological Effects of Acute Cannabis Intoxication

Acute cannabis intoxication: psychological effects
Intensification of perceptions • Visual perceptions are more acute in the contours, colors, and depth of the images • There is a greater extent of perception of the spatial separation of sound sources • Synesthesia: the sounds take visual and color quality • Perceptions of touch, taste, and smell are increased
Space–time references (may appear longer or shorter than normal)
Mental processes and emotions • Positive effect on mood: relaxation, serenity, tendency to laugh • Greater receptivity to the funny side of situations • Different and profound meanings in ordinary situations • Tendency to introspection • Wandering thoughts • Increased imagination and creativity • Slight tendency to disinhibition • Anxiety, panic, dysphoria • Paranoia • Depression, apathy • Auditory and visual hallucinations • Persecutory ideas
Behavioral effects • Impairment of driving ability • Motor incoordination • Impairment of depth perception • Impairment in the sense of time • Impairment in the peripheral vision • Damaged reaction time and attention span • Deficits in short-term memory • Difficulty concentrating • Deterioration of the essential skills of leadership • Altered judgment, with high risk sexual behaviors

the two best described are: Δ^9-tetrahydrocannabinol (D9-THC) and Cannabidiol (CBD) (Gul et al., 2008).

Until recently, the main types of cannabis available on the "street" were marijuana (grass) and resin (hash), but, in recent years, a more potent variant termed sinsemilla or skunk has become available in many countries. The main difference between these substances is that skunk contains a very high concentration of D9-THC (14–16%), and virtually no CBD, while grass and hash are known to contain a similar percentage of D9-THC and CBD (2–4%) (Potter, Clark, & Brown, 2008; Hardwick & King, 2008). The main effects of cannabis are summarized in Tables 9.1–9.3.

The Endocannabinoid Systems

D9-THC and CBD bind to the CB1 receptor, mainly expressed in the nervous system (especially in the hippocampus, amygdalae, cerebellum, basal ganglia, and prefrontal cortex) and in some peripheral tissues and CB2 receptor, expressed on cells of the immune system of mammals, and in CNS neurons, but at lower levels than the CB1 receptor (Onaivi et al., 2006). The CB1 and CB2 receptors are part of the endocannabinoid system (Piomelli, 2003) (Fig. 9.1). The endogenous agonists to the CB1 and CB2 receptors are anandamide (Devane et al., 1992) and 2-arachidonoylglycerol (2-AG) (Sugiura et al., 1995). Endocannabinoids act presynaptically to inhibit the release of amino-acid neurotransmitters on the terminals of neighboring GABAergic and glutamatergic neurons (Fig. 9.1). The endocannabinoids are involved in: (1) the regulation of cognitive functions in neuronal circuits of the cortex, memory in hippocampal neurons, and emotions in neurons of the amygdala; (2) the modulation of motor activity in striatal projections neurons; (3) the influence of central processing of pain; (4) the reinforcing effects of substance of abuse in the mesolimbic system (Di Marzo, Bifulco, & De Petrocellis, 2004; Piomelli, 2003).

TABLE 9.3 Chronic Cannabis Abuse Effects

Chronic cannabis abuse effects
Addiction
Altered brain development
Cognitive impairment: with lower IQ among those who are frequent users during adolescence
Early loss of memory
Amotivational syndrome • Loss of interest in activities and things in general, associated with apathy and passivity • Loss of desire to work and indifference to successes and/or failures at work • Loss of energy, fatigue, easy fatigability • Melancholy and irritability • Reduced concentration • Neglect in appearance and personal hygiene • A lifestyle that prioritizes how to obtain and consume cannabis
Decline in school and/or work performance
Decline in information processing
Onset of depressive and/or psychotic symptoms and/or disorders
Chronic bronchitis

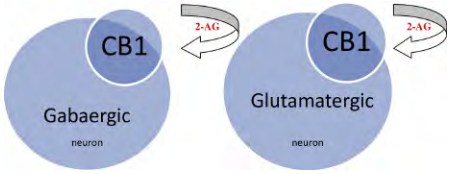


FIGURE 9.1 The endocannabinoid system. Endocannabinoids (2-AG) act presynaptically to inhibit the release of amino-acid neurotransmitters on the terminals of neighboring GABAergic and glutamatergic neurons.

The Epidemiology of Cannabis and Psychosis

The United Nations' 2012 report on drug abuse showed an annual prevalence of cannabis use, in 2010, ranging from 2.6% to 5% of the adult population (between 119 million and 224 million estimated users aged 15–64); cannabis remains the world's most widely used illicit substance (UNODC, 2012).

The use of cannabis is also associated with a risk of developing psychosis of about 2 times greater (Henquet & Murray, 2005; Moore & Zammit, 2007), and generally the use precedes the development of these disorders, and provokes also an earlier age of onset of schizophrenia: cannabis users are 2.7 years younger than noncannabis users (Donoghue et al., 2014), and 6 years younger, if we consider a daily use of high-potency cannabis (Di Forti et al., 2014). The earlier is the age of onset of cannabis use, the greater is the risk of developing a psychotic disorder (Arseneault & Cannon, 2002).

Comparing subjects with and without lifetime cannabis use, it results that the mean age at first psychotic symptoms for the first group is 25.7, while for the second group is 29.2 (Donoghue et al., 2014). The age of onset of psychotic symptoms for cannabis abusers is similar both in males and females, showing than a bigger impact on females than in males (Allegri et al., 2013).

Some authors have suggested that early age of onset of psychotic disorders (AOP) among cannabis users is not associated with the early age of use onset, but with a longer period of use (Stefanis et al., 2013). Daily use (frequency of use) and high-potency (skunk) cannabis have an independent effect both on a greater risk of psychosis onset, and in bringing further forward the AOP, compared to those who use infrequently and or low-potency (hash) cannabis (Di Forti et al., 2014).

The best cannabis use-related predictors of psychotic symptoms can be summarized as follows: (1) early adolescence age (15 years or younger) at first use; (2) frequent use and use of high-potency cannabis (skunk-type) (Di Forti et al., 2009, 2015); (3) genetic vulnerability (Henquet & Murray, 2005; Caspi et al., 2005; van Winkel, van Beveren, Simons, & Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011; Di Forti et al., 2014).

Finally, several evidences indicate that cannabis plays a causal role in the onset of psychosis (Ferdinand et al., 2005) Moreover, patients suffering from schizophrenia are more likely to use cannabis than the general population (Thornicroft, 1990). Cannabis use is also associated with poor outcome in individuals with established psychosis vulnerability (Tarricone et al., 2014). In fact, cannabis is associated with a higher rate of relapse, and greater number of hospitalizations, likely in combination with lower adherence to therapy (Caspari, 1999). In addition, these patients have a worse outcome in social-working terms (Barbeito et al., 2013), and a more difficult social reintegration: the rate of unemployment is twice as high in schizophrenics with abuse as nonabusers (Pencer, Addington, &, Addington, 2005).

Why Cannabis Causes Psychosis

Cannabis use is a risk factor for the development of schizophrenia-like psychotic disorders (Henquet & Murray, 2005; Moore & Zammit, 2007), but only around 3% of those who are regular consumers develop psychosis (Murray & Morrison, 2007).

The most useful model to explain how and why a subject develops schizophrenia seems to be the "gene x environment" (GxE) interaction model. This model includes genetic and environmental risk factors, which combine to influence the risk of developing schizophrenia and psychotic disorders (van Os, Rutten, & Poulton, 2008; Schizophrenia working group of the Psychiatric Genomics, 2014). Several drugs, such as, LSD, amphetamine, ketamine, and cannabis, have been studied to explain different actions of, respectively, serotonin, dopamine, and endocannabinoids actions in the development of psychotic symptoms (Paparelli, Di Forti, Morrison, & Murray, 2011).

Neuroimaging studies have suggested that the active ingredient in cannabis, D9-THC, might increase dopamine release in the nucleus accumbens, inhibiting, via CB1 activation, the release of glutamate onto GABAergic neurons that project from the nucleus accumbens to the ventral tegmental area. This lifts the inhibitory effect on the firing of dopaminergic neurons which project back to the nucleus accumbens (Pertwee, 2008). This model explains how D9-THC might affect dopamine levels in cerebral regions, including the striatum, implicated in the pathogenesis of psychotic symptoms, and offering a possible key to the understanding the biology of cannabis-induced psychosis (Morrison & Murray, 2009).

In addition, it seems that D9-THC can exert opposite effects, stimulating or inhibiting the central nervous system transmission via activation or blocking of CB1 (Berrendero et al., 1999).

This model fits with the evidence that genetic variation at the AKT1 gene, which influences post–D2 receptor signaling, renders some individuals more vulnerable to cannabis-induced psychosis (van Winkel, van

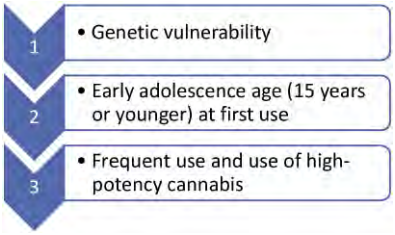


FIGURE 9.2 The best cannabis use related predictors of psychotic symptoms. **Fig. 9.2** shows the best three cannabis use-related predictors of psychotic symptoms. *Source: Henquet and Krabbendam (2005).*

Beveren, Simons, & Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011; Di Forti et al., 2012).

On the contrary, CBD seems to ameliorate the cognitive impairment and psychotogenic effects caused by D9-THC (Murray, Morrison, Henquet, & Di Forti, 2008). A recent study has shown that, if we pretreat healthy subjects with CBD, it reduces the THC-induced paranoia, and inhibits the detrimental effects of THC on episodic memory (Englund et al., 2013).This might explain why the use of skunk, high-potency cannabis, which contains high levels of D9-THC and almost no CBD, is associated with the highest risk of psychosis (Di Forti et al., 2009, 2015) (Fig. 9.2).

Among the protective factors, it has recently been investigates as well the role of anandamide, an endo which seems to be protective against psychotic-like symptoms, whom levels are lower in heavy cannabis users compared with light cannabis users (Morgan et al., 2013).

MIGRATION AND PSYCHOSIS

The Migration Phenomena

The term migration indicates the displacement of one or more individuals from one place to another. Migratory movements bring with them, from one continent to another, different cultural patterns, and they allow people to discover environments other than those of belonging, putting in touch with each other different kinds of populations. The 21st century has been called "the age of migration," because there are about 232 million international migrants in the world, and it is expected that the number of international migrants worldwide will increase in the future, with demographic factors, economic disparities, and environmental change continuing to be major drivers of migration (United Nations, 2013).

Types of Migrations

Only a small portion of migrants moved by choice. The migratory movements are determined by many reasons: occupational, family (marriage, reunions), health, study, political, ethnic and religious reasons, and catastrophic events. Surely, the main force that drives people to migrate is poverty, understood not as being poor by itself, but as the poverty of the context in which the individual lives. The model of migrant, outlined by researchers in the field, is that of an individual who has some economic availability and, at the same time, is in good physical condition, and has a high level of education. This is the so-called model of the "healthy migrant": any family or community "invests" on that member that is more likely to accomplish successfully the migratory venture. The intense urbanization taking place in the majority of developing countries acts in a decisive way on *internal migration*: many people move from rural areas to urban areas, in search of higher profits and better services. Also, the search for a better education leads many young people to leave their homeland and go elsewhere, or move within it. There are also many people who emigrate because, within their country, they wouldn't realize their own projects, and would not be able to carry out the activities for which they were formed (Table 9.4).

Epidemiology of Migration and Psychosis

Considerable evidence indicates that the incidence of schizophrenia and psychotic disorders in general, are higher in migrant populations and ethnic minorities, compared to the general population. The first study that has shown a relationship between psychotic disorders and migration was that of Ødegaard (1932). The results of this work have shown a marked increase in the incidence of schizophrenia in Norwegian immigrants in the United States, compared to nonimmigrant Norwegians. To give an explanation for these observations, Ødegaard hypothesized that people who travel bring with them a predisposition to psychotic disorders greater than nonmigrants. This theory was called "selective migration." The recent literature on the subject has confirmed this increased risk among migrant populations, but, by studying the incidence rates of psychotic disorders in second generation migrants, an unexpected subsequent increased risk of psychotic onset in the second generation migrants was shown, compared to those of the first generation (Cantor-Graae & Selten, 2005; Bourque, van der Ven, & Malla, 2011). In a metaanalysis of Cantor-Graae and Selten (2005), the RR of developing schizophrenia in first generation migrants is 2.9, and increases to 4.5 in the second generation migrants (Cantor-Graae & Selten, 2005). The work of Bourque, instead, shows a similar risk between the two generations of migrants: OR of 2.3 for the first generation, and 2.1 for the second generation of migrants (Bourque et al., 2011). From these considerations, the hypothesis of "selective migration" has been questioned, and it has been suggested that social and environmental factors could play an important role in the genesis of these disorders among migrant

TABLE 9.4 Classification of Types of Migrations

Migration types	
According to the extent	Mass migration: movement, both voluntary and forced, of ethnic groups
	Migration by infiltration: changes of residence of individuals, families or small groups
According to the voluntary nature	Voluntary: are determined by the free choices made by the people who move
	Organized: related to the intervention of political or administrative authorities aimed at stimulating or at least to regulate and control the movement
	Forced: made obligatory by the authorities
According to the duration	Permanent: the transfer is definitive
	Temporary: do not involve the permanent transfer of the individuals who, after a more or less long period, return to their place of origin
	Commuting: shifts on daily or weekly basis, related to the periodicity of certain activities
According to the distance	External: movements that occur from one state to another
	Internal: movements that take place within the political boundaries of a state
According to the place of birth	First generation: individuals born in the places of origin and immigrated in a new one
	Second generation: a naturally born citizen of a country, whose parents are foreign born
	Ethnic minorities: groups born in a country that stand for certain traditions, customs and costumes, consisting of a small number of people

populations, and may explain the so-called "excess of psychosis" among immigrants. Another point that supports the theory of socio-environmental factors, as discussed by van Os in 2010, is the substantial variation in the incidence rates of psychotic disorders across both places and minority groups (van Os, Kenis, & Rutten, 2010). Indeed, Bourque et al. (2011) have identified differences in the risk of psychosis related to the specific meeting between ethnicity and host community: in the United Kingdom, black minorities have shown a higher risk, while in the Netherlands a greater risk was associated to the North Africans immigrated recently, and with a lighter skin color (Bourque et al., 2011). In France, a recent study has identified migrants from Sub-Saharan Africa as the most vulnerable group in this country (IRR 7.1, CI 95% 2.3–21.8) (Tortelli et al., 2014).

Why Does Migration Cause Psychotic Disorders?

Although migration is now increasingly recognized as a risk factor for psychosis, the relationship between migration and psychotic disorders is still unclear.

Some studies have suggested a role of genetic susceptibility to the increased risk of first episode psychosis (FEP) in migrant populations. Two studies conducted in the United Kingdom and one in the Netherlands found no difference in risk between the parents and siblings of migrant patients, compared to those of natives (Sugarman & Craufurd, 1994; Hutchinson, Takei, & Fahy, 1996; Selten et al., 2008). This indicates a similar genetic susceptibility for migrants and native populations, where an important role in the onset of psychotic disorders is played by environmental factors (Morgan, Charalambides, Hutchinson, & Murray, 2010).

The hypothesis of a possible role of obstetric complications in the increased risk of development of FEP in migrants was excluded, finding in some cases even lower rates of obstetric complications among migrants, compared to native populations (Hutchinson et al., 1997; McKenzie & Murray, 1999).

The risk of developing a psychotic disorder is greater the earlier is the age of migration. The risk is greatest at the ages between 0 and 4 years, and gradually decreases with the increase of the age of migration (Veling, Hoek, Selten, & Susser, 2011).

Some social factors have been identified as risk factors for psychotic onset in migrants, such as, unemployment, living alone, separation from a parent in childhood, being single, poor education, and limited social networks (Mallett, Leff, Bhugra, Pang, & Zhao, 2002; Morgan et al., 2007, 2008).

A study has associated a higher risk of FEP for the cases where a history of migration was associated with a family dysfunction (RR = 4), compared to the cases with only a migration history (RR = 2) (Patino et al., 2005) (Table 9.5).

It is very interesting to understand what are the specific characteristics of each host society that interact with a specific type of migrant and ethnic minority to increase the risk of psychosis. Today, therefore, it is necessary to speak of "migrant status" indicating the special meeting between the migrant and the host country. The nature of this meeting could explain the increased risk of psychosis in migrants in different countries. Perceived disadvantage, perceptions of discrimination, negative ethnic identity (lack of affinity to own ethnic group), and less identification with the culture of origin are some social factors associated with the development of psychotic disorders among migrants (Cooper et al., 2008; Veling et al., 2008a; Veling, Hoek, Wiersma, & Mackenbach, 2009; Bhugra, Leff, Mallett, Morgan, & Zhao, 2010).

TABLE 9.5 Risk Factors for First Episode Psychosis in Migrants

Reason for migration: refugees
Early age of migration
Past history of migration before age of 15
Parental separation before age 16
Unemployment
Living condition (far from family, alone)
Low employment level
Low education
Loss of cultural identity
Perceived discrimination
Self-esteem and self-concept
Mismatch between expectation and achievement
Social defeat
Low ethnic density
Economic crisis and intolerance

(Adapted from Bhugra, D., 2000.)

The RR of schizophrenia in migrant and minority ethnic groups is highest in those areas where they form a smaller proportion of the local population. In other words, the less ethnically dense an area, the higher the RR (Boydell et al., 2003; Veling et al., 2008a,b). In a study conducted in the United States, acculturative stress has been proposed as a risk factor for the development of psychotic disorders in migrants (Devylder et al., 2013).

Almost all of the studies conducted so far on this topic have only dealt with the phenomenon of external migration. That is, they considered only the people who migrated from one state to another. Very few and very old are the studies that have considered the phenomenon of internal migration, meaning that people who migrate from one region to another within the confines of the same country (Tarricone et al., 2016). A more detailed study on the phenomenon of internal migration may be useful in elucidating more comprehensively the association between migration and psychotic onset, and giving additional elements to understand the risk factors that underlie this correlation.

MIGRATION, CANNABIS USE, AND PSYCHOSIS ONSET

The use of substances, particularly cannabis, and immigrant status are well known risk factors for psychotic onset. Yet little is known on the association between these two risk factors in the development of new-onset psychosis: how cannabis use affects the onset of psychotic disorders in immigrant populations and ethnic minorities groups. The studies that focus on specific risk factors of psychosis in migrants are growing in number. The first population-based study conducted in the United Kingdom on FEP patients is by Harrison et al. (1997) in Nottingham which found a strikingly high rate of schizophrenia in African-Caribbean (AC) first and second generation, compared to the general population. Studying the use of substances, a higher use of substances was shown in white patients, compared with AC (Harrison et al., 1997). Therefore, according to this study, the higher risk of psychotic onset in AC could not be explained by the use of substances. Another study conducted in the Netherlands has observed a similar cannabis use among patients with schizophrenia, regardless of whether they were native Dutch or different migrant groups with high rates of psychotic disorders, such as, Moroccans and Surinamese (Veen, Selten, & Hoek, 2002). In Italy, our previous study showed higher incidence rate of psychosis among migrants, compared to natives; migrants showed a lower substance abuse rate compared to natives (Tarricone et al., 2012). Even in these cases, the higher risk of psychosis in migrant populations could not be explained by substance abuse. By further investigating second generation migrants, some studies have observed a relationship between cannabis use and psychotic onset. In a study conducted in South London, a greater use of cannabis for the black migrants born in the United Kingdom was found, compared to the natives (OR = 4.8), and a minor use for black migrants born abroad (OR = 0.8) (Afuwape, Johnson, & Craig, 2006). Further studies are needed to shed light on the correlation of these two risk factors on the development of psychotic disorders, in order to refine the understanding of the etiology and pathogenesis of psychosis. We cannot rule out that the lack of association between cannabis use and immigrant status in the development of psychotic disorders up to this day is due to a lack of studies that focus on specific risk factors, such as, cannabis and other substance use, of psychosis in migrants. The EU-GEI study (European Community's Seventh Framework Program, grant agreement No. HEALTH-F2-2009-241909, Project EU-GEI: European Network of National Schizophrenia Networks Studying Gene–Environment Interaction) coordinated by the University of Maastricht, a multicenter study that investigates the interplay between genetic and environmental factors in causing schizophrenia and other psychotic disorders, will be crucial to enable a clearer understanding of the correlation of migration and cannabis use in psychosis onset.

MINI-DICTIONARY

Endocannabinoid system A signaling system of the brain composed by neuromodulator lipids and specific receptors, involved in the control of many functions, such as, the immune response, appetite, gastrointestinal function, pain sensation, mood and differentiation, proliferation, and apoptosis of cells.
Environmental risk factors Factors and circumstances (such as early life adversity, growing up in an urban environment, minority group position and cannabis use) that interact with genetic predisposition to influence the risk of psychosis development through life.
Ethnic density The amount of people belonging to the same ethnic group in a specific area, neighborhood, or city.
Ethnicity The fact of belonging to a social group that has in common traditions, customs, beliefs, and language.
Etiology The term derives from the Greek αἰτιολογία (aitiologia), "giving a reason for." In the medical field, it indicates the study of different genetic, environmental, and social factors that stand behind the different medical disorders.
First episode psychosis Presence of hallucinations, delusions, thought disorder, bizarre or distorted behavior, negative symptoms, mania, or clinical suspicion of psychosis; absence of an organic cause or profound learning disability; no previous contact with psychiatric services for psychotic symptoms.
Migration The process of going from one country (external migration), region or place (internal migration) of residence to settle in another, for the purposes of settling down either permanently or for a prolonged period. The migratory movements are determined by many reasons: pull factors, as educational or economic growth, or personal factors, and push factors, such as, political, poverty, terrorism, and war.
Psychosis Group of severe mental disorders characterized by impaired contact with reality, and the ability to critique and judge, by the presence of thought, perception, and affectivity disorders, and the impairment of skills and social relationships.
Psychosis onset The time between the first reported/observed change in mental state/behavior to the development of psychotic symptoms.
Psychosis vulnerability The genetic predisposition of developing a psychotic disorder, regardless of the presence or absence of environmental risk factors for psychosis.

References

Afuwape, S. A., Johnson, S., & Craig, T. J. K. (2006). Ethnic differences among a community cohort of individuals with dual diagnosis in South London. *Journal of Mental Health*, *15*(5), 551–567.

Allegri, F., Belvederi Murri, M., Paparelli, A., Marcacci, T., Braca, M., Menchetti, M., Michetti, R., Berardi, D., & Tarricone, I. (2013). Current cannabis use and age of psychosis onset: a gender-mediated relationship? Results from an 8-year FEP incidence study in Bologna. *Psychiatry Research*, *210*(1), 368–370.

Arseneault, L., & Cannon, M. (2002). Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. *British Medical Journal*, *325*(7374), 1212–1213.

Barbeito, S., Vega, P., de Azua S. R., Saenz, M., Martinez-Cengotitabengoa, M., Gonzalez-Ortega, I., Bermudez, C., Hernanz, M., de Corres, B. C., & Gonzalez-Pinto, A. (2013). Cannabis use and involuntary admission may mediate long-term adherence in first-episode psychosis patients: A prospective longitudinal study. *BMC Psychiatry*, *13*, ArtID 326.

Berrendero, F., Sepe, N., et al. (1999). Analysis of cannabinoid receptor binding and mRNA expression and endogenous cannabinoid contents in the developing rat brain during late gestation and early postnatal period. *Synapse*, *33*(3), 181–191.

Bhugra, D. (2000). Migration and schizophrenia. *Acta Psychiatrica Scandinava* (Suppl. 407), 68–73.

Bhugra, D., Leff, J., Mallett, R., Morgan, C., & Zhao, J. H. (2010). The culture and identity schedule-a measure of cultural affiliation: acculturation, marginalization and schizophrenia. *International Journal of Social Psychiatry*, *56*, 540–556.

Bourque, F., van der Ven, E., & Malla, A (2011). A meta-analysis of the risk for psychotic disorders among first- and second-generation immigrants. *Psychological Medicine*, *41*(5), 897–910.

Boydell, J., van Os, J., et al. (2003). Incidence of schizophrenia in south-east London between 1965 and 1997. *British Journal of Psychiatry*, *182*, 45–49.

Brietzke, E., Kauer Sant'anna, M., Jackowski, A., Grassi-Oliveira, R., Bucker, J., Zugman, A., Mansur, R. B., & Bressan, R. A. (2012). Impact of childhood stress on psychopathology. *Revista Brasileira de Psiquiatria*, *34*(4), 480–488Review.

Cantor-Graae, E., & Selten, J. P. (2005). Schizophrenia and migration: a meta-analysis and review. *American Journal of Psychiatry*, *162*(1), 12–24.

Cardno, A. G., Marshall, E. J., et al. (1999). Heritability estimates for psychotic disorders: the Maudsley twin psychosis series. *Archives of General Psychiatry*, *56*(2), 162–168.

Caspari, D. (1999). Cannabis and schizophrenia: results of a follow-up study. *European Archives of Psychiatry and Clinical Neuroscience*, *249*(1), 45–49.

Caspi, A., Moffitt, T. E., Cannon, M., McClay, J., Murray, R., Harrington, H., Taylor, A., Arseneault, L., Williams, B., Braithwaite, A., Poulton, R., & Craig, I. W. (2005). Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biological Psychiatry*, *57*(10), 1117–1127.

Cooper, C., Morgan, C., Byrne, M., et al. (2008). Perceptions of disadvantage, ethnicity and psychosis: results from the ÆSOP study. *British Journal of Psychiatry*, *192*, 185–190.

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., & Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*, *258*(5090), 1946–1949.

Devylder, J. E., Oh, H. Y., Yang, L. H., Cabassa, L. J., Chen, F. P., & Lukens, E. P. (2013). Acculturative stress and psychotic-like experiences among Asian and Latino immigrants to the United States. *Schizophrenia Research*, *150*, 223–228.

Di Forti, M., Morgan, C., Dazzan, P., Pariante, C., Mondelli, V., Marques, T. R., Handley, R., Luzi, S., Russo, M., Paparelli, A., Butt, A., Stilo, S. A., Wiffen, B., Powell, J., & Murray, R. M. (2009). High-potency cannabis and the risk of psychosis. *British Journal of Psychiatry*, *195*(6), 488–491.

Di Forti, M., Iyegbe, C., Sallis, H., Kolliakou, A., Falcone, M. A., Paparelli, A., Sirianni, M., La Cascia, C., Stilo, S. A., Marques, T. R., Handley, R., Mondelli, V., Dazzan, P., Pariante, C., David, A. S., Morgan, C., Powell, J., & Murray, R. M. (2012). Confirmation that the AKT1 (rs2494732) genotype influences the risk of psychosis in cannabis users. *Biological Psychiatry*, *72*, 811–816.

Di Forti, M., Sallis, H., Allegri, F., Trotta, A., Ferraro, L., Stilo, S. A., Marconi, A., La Cascia, C., Reis Marques, T., Pariante, C., Dazzan, P., Mondelli, V., Paparelli, A., Kolliakou, A., Prata, D., Gaughran, F., David, A. S., Morgan, C., Stahl, D., Khondoker, M., Maccabe, J. H., & Murray, R. M. (2014). Daily use, especially of high-potency cannabis, drives the earlier onset of psychosis in cannabis users. *Schizophrenia Bulletin*, *40*, 1509–1517.

Di Forti, M., Marconi, A., Carra, E., Fraietta, S., Trotta, A., Bonomo, M., Bianconi, F., Gardner-Sood, P., O'Connor, J., Russo, M., Stilo, S. A., Reis Marques, T., Mondelli, V., Dazzan, P., Pariante, C., David,

A. S., Gaughran, F., Atakan, Z., Iyegbe, C., Powell, J., Morgan, C., Lynskey, M., & Murray, R. M. (2015). Proportion of patients in south London with first-episode psychosis attributable to use of high potency cannabis: a case-control study. *Lancet Psychiatry*, *2*(3), 233–238.

Di Marzo, V., Bifulco, M., & De Petrocellis, L. (2004). The endocannabinoid system and its therapeutic exploitation. *Nature Reviews Drug Discovery*, *3*, 771–784.

Donoghue, K., Doody, G. A., Murray, R. M., Jones, P. B., Morgan, C., Dazzan, P., Hart, J., Mazzoncini, R., & Maccabe, J. H. (2014). Cannabis use, gender and age of onset of schizophrenia: data from the ÆSOP study. *Psychiatry Research*, *2153*(3), 528–532.

Englund, A., Morrison, P. D., Nottage, J., Hague, D., Kane, F., Bonaccorso, S., Stone, J. M., Reichenberg, A., Brenneisen, R., Holt, D., Feilding, A., Walker, L., Murray, R. M., & Kapur, S. (2013). Cannabidiol inhibits THC-elicited paranoid symptoms and hippocampal-dependent memory impairment. *Journal of Psychopharmacology*, *27*, 19.

Ferdinand, R. F., Sondeijker, F., van der Ende, J., Selten, J., Huizink, A., & Verhulst, F. C. (2005). Cannabis use predicts future psychotic symptoms, and vice versa. *Addiction*, *100*, 612–618.

Gul, W., Carvalho, P., et al. (2008). (6aR,10aR)-6,6,9-Trimethyl-3-pentyl-6a,7,8,10a-tetra-hydro-6H-benzo[c]-chromen-1-yl 4-methyl-benzene-sulfonate. *Acta Crystallographica Section E*, *64*(Pt 9), o1686.

Hafner, H. (2003). Gender differences in schizophrenia. *Psychoneuroendocrinology*, *28*(Suppl. 2), 17–54.

Hardwick, S., & King, L. (2008). *Home Office cannabis potency study*. Home Office. Available from http://drugs.homeoffice.gov.uk/publication-search/cannabis/potency

Harrison, G., Glazebrook, C., Brewin, J., Cantwell, R., Dalkin, T., Fox, R., Jones, P., & Medley, I. (1997). Increased incidence of psychotic disorders in migrants from the Caribbean to the United Kingdom. *Psychological Medicine*, *27*(4), 799–806.

Henquet, C., & Krabbendam, L. (2005). Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. *British Medical Journal*, *330*(7481), 11.

Henquet, C., & Murray, R. (2005). The environment and schizophrenia: the role of cannabis use. *Schizophrenia Bulletin*, *31*(3), 608–612.

http://www.unodc.org/unodc/data-and-analysis/WDR-2012.html

Hutchinson, G., Takei, N., & Fahy, T. A. (1996). Morbid risk of schizophrenia in first-degree relatives of white and African-Caribbean patients with psychosis. *British Journal of Psychiatry*, *169*, 776–780.

Hutchinson, G., Takei, N., Bhugra, D., et al. (1997). Increased rate of psychosis among African-Caribbeans in Britain is not due to an excess of pregnancy and birth complications. *British Journal of Psychiatry*, *171*, 145–147.

Johns, L. C., & van Os, J. (2001). The continuity of psychotic experiences in the general population. *Clinical Psychology Review*, *21*(8), 1125–1141.

Mallett, R., Leff, J., Bhugra, D., Pang, D., & Zhao, J. H. (2002). Social environment, ethnicity and schizophrenia. *Social Psychiatry and Psychiatric Epidemiology*, *37*, 329–335.

McGrath, J. J. (2006). Variations in the incidence of schizophrenia: data versus dogma. *Schizophrenia Bulletin*, *32*(1), 195–197.

McKenzie, K., & Murray, R. (1999). Risk factors for psychosis in the UK African-Caribbean population. In D. Bhugra, & V. Bahl (Eds.), *Ethnicity: An agenda for mental health* (pp. 48–59). London, UK: Gaskel.

Moore, T. H., & Zammit, S. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet*, *370*(9584), 319–328.

Morgan, C., Kirkbride, J., Leff, J., et al. (2007). Parental separation, loss and psychosis in different ethnic groups: a case-control study. *Psychological Medicine*, *37*, 495–503.

Morgan, C., Kirkbride, J., Hutchinson, G., et al. (2008). Cumulative social disadvantage, ethnicity and first-episode psychosis: a case-control study. *Psychological Medicine*, *38*, 1701–1715.

Morgan, C., Charalambides, M., Hutchinson, G., & Murray, R. M. (2010). Migration, ethnicity, and psychosis: toward a sociodevelopmental model. *Schizophrenia Bulletin*, *36*(4), 655–664.

Morgan, C. J., Page, E., Schaefer, C., Chatten, K., Manocha, A., Gulati, S., Curran, H. V., Brandner, B., & Leweke, F. M. (2013). Cerebrospinal fluid anandamide levels, cannabis use and psychotic-like symptoms. *British Journal of Psychiatry*, *202*(5), 381–382.

Morrison, P. D., & Murray, R. M. (2009). From real-world events to psychosis: the emerging neuropharmacology of delusions. *Schizophrenia Bulletin*, *35*, 668–674.

Murray, R. M., & Morrison, P. D. (2007). Cannabis, the mind and society: the hash realities. *Nature Reviews Neuroscience*, *8*(11), 885–895.

Murray, R. M., Morrison, P. D., Henquet, C., & Di Forti, M. (2008). Cannabis, the mind and society: the hash realities. *Nature Reviews Neuroscience*, *8*, 885–895.

Ødegaard, Ø. (1932). Emigration and Insanity. *Acta Psychiatrica et Neurologica Scandinavica Supplementum*.

Onaivi, E. S., Ishiguro, H., Gong, J. -P., Patel, S., Perchuk, A., Meozzi, P. A., Myers, L., Mora, Z., Tagliaferro, P., Gardner, E., Brusco, A., Akinshola, B. E., Liu, Q. -R., Hope, B., Iwasaki, S., Arinami, T., Teasenfitz, L., & Uhl, G. R. (2006). Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. *Annals of the New York Academy of Science*, *1074*, 514–536.

Paparelli, A., Di Forti, M., Morrison, P. D., & Murray, R. M. (2011). Drug-induced psychosis: how to avoid star gazing in schizophrenia research by looking at more obvious sources of light. *Frontiers in Neuroscience*, *5*, 1.

Patino, L. R., Selten, J. P., Van Engeland, H., Duyx, J. H., Kahn, R. S., & Burger, H. (2005). Migration, family dysfunction and psychotic symptoms in children and adolescents. *British Journal of Psychiatry*, *186*, 442–443.

Pencer, A., Addington, J., & Addington, D. (2005). Outcome of a first episode of psychosis in adolescence: a 2-year follow-up. *Psychiatry Research*, *133*(1), 35–43.

Pertwee, R. G. (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. *British Journal of Pharmacology*, *153*(2), 199–215.

Piomelli, D. (2003). The molecular logic of endocannabinoid signalling. *Nature Reviews Neuroscience*, *4*, 873–884.

Potter, D. J., Clark, P., & Brown, M. B. (2008). Potency of D9–THC and other cannabinoids in cannabis in England in 2005: implications for psychoactivity and pharmacology. *Journal of Forensic Science*, *53*, 90–94.

Schizophrenia Working Group of the Psychiatric Genomics C. (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature*, *511*(7510), 421–427.

Selten, J. P., Blom, J. D., Van der Tweel, I., Veiling, W., Leliefeld, B., & Hoek, H. W. (2008). Psychosis risk for parents and siblings of Dutch and Moroccan-Dutch patients with non-affective psychotic disorder. *Schizophrenia Research*, *104*, 274–278.

Stefanis, N. C., Dragovic, M., Power, B. D., Jablensky, A., Castle, D., & Morgan, V. A. (2013). Age at initiation of cannabis use predicts age at onset of psychosis: the 7- to 8-year trend. *Schizophrenia Bulletin*, *39*, 251–254.

Sugarman, P. A., & Craufurd, D. (1994). Schizophrenia in the Afro-Caribbean community. *British Journal of Psychiatry*, *164*, 474–480.

Sugiura T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A., Waku, K. (1995). 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain *Biochemical and Biophysical Research Communications*, *2151*(1), 89–97.

Tarricone, I., Boydell, J., Kokona, A., Triolo, F., Gamberini, L., Sutti, E., Marchetta, M., Menchetti, M., Di Forti, M., Murray, R. M., Morgan, C., & Berardi, D. (2016). Risk of psychosis and internal migration: Results from the Bologna First Episode Psychosis study. *Schizophrenia Research*, *173*(1–2), 90–93.

Tarricone, I., Boydell, J., Panigada, S., llegri, F., Marcacci, T., Minenna, M., Kokona, Triolo, F., Storbini, V., Michetti, Morgan, C., Di Forti, M., Murray, M., & Berardi, D. (2014). The impact of substance use at psychosis onset on First Episode Psychosis course: results from a 1 year follow-up study in Bologna. *Schizophrenia Research*, *153*(1–3), 60–63.

Tarricone, I., Mimmi, S., Paparelli, A., Rossi, E., Mori, E., Panigada, S., Carchia, G., Bandieri, V., Michetti, R., Minenna, G., Boydell, J., Morgan, C., & Berardi, D. (2012). First-episode psychosis at the West Bologna Community Mental Health Centre: results of an 8-year prospective study. *Psychological Medicine*, *7*, 1–10.

Thornicroft, G. (1990). Cannabis and psychosis. Is there epidemiological evidence for an association? *British Journal of Psychiatry*, *157*, 25–33.

Tortelli, A., Morgan, C., Szoke, A., Nascimento, A., Skurnik, N., de Caussade, E. M., Fain-Donabedian, E., Fridja, F., Henry, M., Ezembe, F., & Murray, R. M. (2014). Different rates of first admissions for psychosis in migrant groups in Paris. *Social Psychiatry and Psychiatric Epidemiology*, *49*(7), 1103–1109.

United Nations. (2013). Global Migration: Demographic aspects and its relevance for development. Technical Paper No. 2013/6. Department of Economic and Social Affairs, Population Division.

van Os, J., & Linscott, R. J. (2009). A systematic review and meta-analysis of the psychosis continuum: evidence for a psychosis proneness-persistence-impairment model of psychotic disorder. *Psychological Medicine*, *39*(2), 179–195.

van Os, J., Rutten, B. P. F., & Poulton, R. (2008). Gene-environment interactions in schizophrenia: review of epidemiological findings and future directions. *Schizophrenia Bulletin*, *34*(6), 1066–1082.

van Os, J., Kenis, G., & Rutten, B. P. (2010). The environment and schizophrenia. *Nature*, *468*, 203–212.

van Winkel, R., van Beveren, N. J., Simons, C., Genetic Risk, Outcome of Psychosis (GROUP), & Investigators (2011). AKT1 moderation of cannabis-induced cognitive alterations in psychotic disorder. *Neuropsychopharmacology*, *36*(12), 2529–2537.

Veen, N., Selten, J. -P., & Hoek, H. W. (2002). Use of illicit substances in a psychosis incidence cohort: a comparison among different ethnic groups in the Netherlands. *Acta Psychiatrica Scandinavica*, *105*(6), 440–443.

Veling, W., Hoek, H. W., & Mackenbach, J. P. (2008a). Perceived discrimination and the risk of schizophrenia in ethnic minorities: a case-control study. *Social Psychiatry and Psychiatric Epidemiology*, *43*, 953–959.

Veling, W., Susser, E., van Os, J., Mackenbach, J. P., Selten, J. P., & Hoek, H. W. (2008b). Ethnic density of neighborhoods and incidence of psychotic disorders among immigrants. *American Journal of Psychiatry*, *165*(1), 66–73.

Veling, W., Hoek, H. W., Wiersma, D., & Mackenbach, J. P. (2009). Ethnic identity and the risk of schizophrenia in ethnic minorities: a case-control study. *Schizophrenia Bulletin*, *36*, 1149–1156.

Veling, W., Hoek, H. W., Selten, J. P., & Susser, E. (2011). Age at migration and future risk of psychotic disorders among immigrants in the Netherlands: a 7-year incidence study. *American Journal of Psychiatry*, *168*(12), 1278–1285.

CHAPTER

10

The Global Epidemiology and Disease Burden of Cannabis Use and Dependence

L. Degenhardt[*], *A.J. Ferrari*[**,†,‡], *W.D. Hall*[§]

[*]National Drug and Alcohol Research Centre, University of New South Wales, Sydney, NSW, Australia
[**]School of Public Health, University of Queensland, Herston, QLD, Australia
[†]Institute for Health Metrics and Evaluation, University of Washington, Seattle, WA, United States
[‡]Queensland Centre for Mental Health Research, Wacol, QLD, Australia
[§]Centre for Youth Substance Abuse Research, University of Queensland, Herston, QLD, Australia

SUMMARY POINTS

- This chapter discusses the estimated prevalence of cannabis dependence, and its contribution to the global burden of disease.
- The Global Burden of Disease Study 2010 estimated 13.1 million cannabis dependent people globally in 2010 (point prevalence 0.19%)
- Prevalence peaked between 20 and 24 years, was higher in males than females, and in high income regions.
- Cannabis dependence accounted for 2 million disability-adjusted life years (DALYs) globally in 2010; a 22% increase in crude DALYs since 1990, largely due to population growth.
- Countries with statistically higher age-standardized DALY rates included the United States, Canada, Australia, New Zealand, and Western European countries; those with lower rates were from Sub Saharan Africa West, and Latin America.
- Regular cannabis use as a risk factor for schizophrenia accounted for an estimated 7000 DALYs globally.
- Cannabis dependence is a disorder primarily experienced by young adults, especially in high income countries.
- Our estimates suggest that cannabis use as a risk factor for schizophrenia is not a major contributor to population-level disease burden.

KEY FACTS OF CANNABIS DEPENDENCE

- Cannabis is a generic term for preparations (eg, marijuana, hashish, and hash oil) derived from the *Cannabis sativa* plant.
- Cannabis dependence is a substance use disorder primarily experienced by young adults, from high income countries.
- Cannabis dependence causes disability and burden across the globe.
- There were an estimated 13.1 million cannabis dependent people globally in 2010.
- Although it is an established risk factor for schizophrenia, it was not a major contributor to the overall burden attributed to schizophrenia in our study.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00011-9
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

CRA	Comparative risk assessment
DALYs	Disability-adjusted life years
DSM	*Diagnostic and Statistical Manual of Mental Disorders*
GBD	Global burden of disease
GBD 2010	Global Burden of Disease Study 2010
ICD	*International Classification of Diseases*
OR	Odds ratio
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
THC	Δ^9-Tetrahydrocannabinol
UNODC	United Nations Office on Drugs and Crime
WHO	World Health Organization
YLLs	Years lost due to premature mortality
YLDs	Years lived with disability

INTRODUCTION

Cannabis is a generic term for preparations (eg, marijuana, hashish, and hash oil) derived from the *Cannabis sativa* plant (Hall & Degenhardt, 2009). The cannabinoid primarily responsible for the psychoactive effects that cannabis users typically seek is Δ^9-tetrahydrocannabinol or THC (Gaoni & Mechoulam, 1964; Martin & Cone, 1999; Iversen, 2000), found in a resin on the flowering tops and upper leaves of the female plant. THC acts upon a specific cannabinoid receptor (CB_1) in the brain (Hall, Degenhardt et al., 2001).

Cannabis is widely used in developed and developing countries (Hall, Johnston et al., 1999; Hall & Degenhardt, 2007). Global patterns of cannabis use have been estimated by the United Nations Office on Drugs and Crime (UNODC) (UNODC, 2008), but there has not been a systematic review of global, regional and country-level patterns of cannabis dependence and disease burden.

The global burden of disease (GBD) framework uses information on mortality and disability associated with a given disease to estimate the years lost due to premature mortality (YLLs), and the years lived with disability (YLDs). YLLs and YLDs can be summed into disability-adjusted life years (DALYs), an overall summary measure of disease burden.

The Global Burden of Disease Study 2010 (GBD 2010) updated the burden estimation methodology, and estimated the burden of 291 diseases and 67 risk factors, by age, sex, 187 countries, and 21 world regions, for 1990, 2005, and 2010. GBD 2010 estimated both the direct burden attributable to cannabis dependence, and the additional burden arising from cannabis dependence as a risk factor for other health outcomes. As part of GBD 2010, we conducted systematic reviews of the literature to capture all the available data since 1990 on the prevalence and consequences of cannabis dependence. Other methodological improvements included: the use of a Bayesian meta-regression approach to model the epidemiological data and propagate uncertainty around final burden estimates; the quantification of disability for a more comprehensive list of health states using survey data from a more representative sample; and adjusting burden estimates for the effects of comorbid disorders (Murray, Vos et al., 2012; Vos, Flaxman et al., 2012).

This chapter draws heavily on a paper we recently published (Degenhardt, Ferrari et al., 2013) that summarized the GBD 2010 methodology and burden estimates for cannabis use and dependence. It built on our systematic reviews of the epidemiology of cannabis use and dependence (Calabria, Degenhardt et al., 2010a,b,c; Degenhardt, Bucello et al,. 2011), and the relationship between cannabis use and schizophrenia (Degenhardt, Hall et al., 2009; Calabria et al., 2010c; Degenhardt & Hall, 2012), and expanded on previous analyses of the contribution of illicit drug use to the global burden of disease (Lim, Vos et al., 2012; Lozano, Naghavi et al., 2012; Murray et al., 2012; Salomon, Vos et al., 2012; Vos et al., 2012).

DETAILS OF THE BURDEN OF DISEASE STUDY METHODS

Case Definition

The case definition of cannabis dependence was based on the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) (American Psychiatric Association, 2000) and *International Classification of Diseases (ICD)* (World Health Organization, 1993) diagnostic criteria for cannabis dependence (DSM:304.30; ICD:F12.2).

Systematic Reviews

Systematic searches were conducted for studies published since 1990 to identify data sources for the prevalence, incidence, remission, and all-cause excess mortality attributable to cannabis use and dependence. Full detail of these searches has been published elsewhere (Calabria, Degenhardt et al., 2008; Calabria et al., 2010a,b,c; Degenhardt et al., 2011).

All extraction and quality assurance procedures were as recommended by the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (von Elm, Altman et al., 2007). Studies were excluded if they did not contain primary data (eg, review articles) or they contained data collected before 1990. We extracted estimates of annual incidence, current, and period (past year, past-month) prevalence. Remission from cannabis dependence was defined as no longer

fulfilling the diagnostic criteria for the disorder. For remission studies, we included prospective studies that reported on a follow-up of at least 3 years (Calabria et al., 2010a). For excess-mortality studies, we included case-control or cohort studies reporting relative-risk estimates or standardized mortality ratios.

An additional literature search identified any evidence of drug use within other regions such as reports of use (UNODC, 2008), sample estimates, drug-related treatments, drug seizures, drug-related arrests, or other qualitative information related to the use of illicit drugs. Sources included UNODC reports, government reports, surveys, news reports, and journal articles. We located evidence of cannabis use or dependence for almost all of the world's population aged 15–64 years (201 countries/territories; 99.8% of the world's population aged 15–64 years). There were estimates of the prevalence of cannabis use in 108 countries. We identified 13 studies (60 data points) from four GBD regions that reported on the prevalence of cannabis dependence. We found an additional 7 studies (57 data points) from 5 GBD regions reporting on weekly cannabis use and 80 studies (1313 data points) from 17 GBD regions reporting on past year cannabis use. We found only three general population cohort studies that reported on the incidence of cannabis use or dependence. We found no epidemiological evidence of elevated mortality risk attributable to cannabis dependence (for full details see Calabria et al., 2008; Calabria et al., 2010b; Degenhardt et al., 2011)

DisMod-MR Modelling

We pooled these epidemiological estimates in a disease model that was judged to have face validity by the GBD 2010 Expert Group and additional experts in cannabis use epidemiology. For these estimates, we used DisMod-MR (a Bayesian meta-regression tool) (Vos et al., 2012; Flaxman, Vos et al., 2013) to ensure internal consistency between separate estimates of prevalence, incidence, remission, and excess-mortality. DisMod-MR was also used to predict epidemiological estimates for regions with no available data. We preferred high quality, direct epidemiological estimates. In their absence, we used predicted estimates, rather than exclude regions with no epidemiological data from GBD 2010 estimates (Vos et al., 2012; Flaxman et al., 2013).

Disability Weights

To estimate disability weights, a lay person description of cannabis dependence was formulated by the Expert Group as one of 222 lay descriptions that reflected the 291 diseases and their sequelae in GBD 2010. Full details of this process have been reported elsewhere (Salomon, 2010; Salomon et al., 2012). The survey was completed by community samples in five countries (Bangladesh, Indonesia, Peru, the United Republic of Tanzania, and the United States of America), and by respondents to an open-access internet survey. In both, a pair-wise comparison method, asking respondents to nominate the healthier of a pair of health states, was used to arrive at an estimate of the level of disability for each GBD health state.

Full details of the process have been reported elsewhere (Murray et al., 2012; Vos et al., 2012). After adjusting the disability weight for an estimated 51% (47–54%) of cases who had no disability after accounting for comorbidity, the average disability weight for cannabis dependence was 0.162 (0.109–0.224).

Comorbidity Adjustments

In order to correct YLDs for comorbidity, separate microsimulation methods were used to generate hypothetical populations by age, sex, year, and country. Details are reported in full elsewhere (Murray et al., 2012; Vos et al., 2012).

Calculation of YLDs, YLLs, and DALYs

GBD 2010 estimated "prevalent" YLDs by multiplying prevalence estimates from DisMod-MR by the disability weight. DisMod-MR prevalence and burden estimates were stratified by sex, age, country, and 21 GBD regions (see http://www.globalburden.com.au/docs/Regions.pdf for country groupings), and for the years 1990, 2005, and 2010.

We explored time trends in burden by breaking down the change in DALYs between 1990 and 2010 into changes that were attributable to: population growth, population ageing and sex structure, and changes in the epidemiology of cannabis dependence. As we found no evidence of excess deaths (Calabria et al., 2010b) (and therefore YLLs) directly attributable to cannabis dependence, the YLDs comprised all of DALY estimates (Vos et al., 2012).

COMPARATIVE RISK ASSESSMENT (CRA): CANNABIS USE AS A RISK FACTOR FOR SCHIZOPHRENIA

We reviewed existing literature on cannabis use as a risk factor for other health outcomes (Degenhardt et al., 2009; Calabria et al., 2010c; Lim et al., 2012). We concluded that there was sufficient evidence to consider cannabis use as a risk factor for schizophrenia. We decided that cannabis and schizophrenia were most plausibly causally linked as follows: (1) there was greater disorder severity among those using cannabis regularly who have already developed the disorder; and (2) the association reflected earlier onset of schizophrenia among those

who would have developed the disorder (Degenhardt et al., 2009).

Two systematic literature reviews were conducted on the global epidemiology of regular (weekly) cannabis use (Calabria et al., 2010c; Degenhardt et al., 2011), and schizophrenia (Saha, Chant et al., 2005; Ferrari, Saha et al., 2012), respectively. Details are provided below. More information on the methodology is provided elsewhere (Lim et al., 2012).

Data on Regular (Weekly or More Frequent) Cannabis Use in the Past Year

We defined the exposure as weekly or more frequent cannabis use in the previous year because regular use is most consistently associated with this outcome. We found 7 studies reporting prevalence of weekly or more frequent cannabis use in the past year, from 15 countries and 5 GBD world regions; and 80 studies on the prevalence of past-year cannabis use, from 82 countries and 17 GBD world regions (Calabria et al., 2010c; Degenhardt et al., 2011).

The epidemiological data for regular cannabis use were modelled using DisMod-MR to estimate prevalence by country, region, age, sex, and year, using a similar method to that used to model cannabis dependence.

Modelling Earlier Age of Onset of Schizophrenia

The effect of cannabis use on schizophrenia was modelled via two pathways: the first by bringing forward the average age of onset in persons with no cannabis use, and the second by increasing the severity of schizophrenia. To account for the effects of the first pathway, we calculated the counterfactual average duration of schizophrenia across all ages, under a scenario of no cannabis use, and compared this to the observed duration. To determine the counterfactual average duration of schizophrenia, we brought forward incident cases of schizophrenia who used cannabis by 2.70 (95% CIs: 1.96–3.43) years, based on a metaanalysis (Large, Sharma et al., 2011).

We used the estimates of cannabis use by age (in single years), sex, country, and year described above, assuming that the prevalence of regular cannabis use was the same among individuals with and without schizophrenia. Estimates of the number of incident cases and the corresponding duration of schizophrenia by age, sex, country, and year were based on the DisMod-MR model for schizophrenia.

Modelling Increased Severity of Schizophrenia

To calculate the burden associated with the second pathway of shifting severity, we used the odds ratio (OR) from Foti, Kotov et al. (2010) of psychotic symptoms of 1.64 (95% confidence intervals: 1.12–2.34) in people with schizophrenia who regularly use cannabis, compared to those who do not. The percent of time spent in acute psychosis was 63% (38–82%), based on a metaanalysis of six studies covering five GBD world regions (Ferrari et al., 2012).

We used the estimates of cannabis use by age (in single years), sex, country, and year described above, and assumed that the prevalence of regular cannabis use was the same among individuals with and without schizophrenia. Estimates of the number of incident cases and the corresponding duration of schizophrenia by age, sex, country, and year were based on the DisMod-MR model. We used the linear relationship between the estimated change in disability weight (based on the proportion of time spent in a psychotic state) and the prevalence of regular cannabis use to calculate the percent of schizophrenia disability attributable to regular cannabis use. Where we report comparisons of prevalence and DALYs by country or region we use age-standardized values, using direct standardization to the global standard population proposed by the World Health Organization (WHO) in 2001 (http://www.who.int/healthinfo/paper31.pdf).

PREVALENCE OF CANNABIS DEPENDENCE

There were an estimated 11 million cases of cannabis dependence globally in 1990, and 13 million cases in 2010. These translated into pooled point prevalence estimates of 0.20% (95% uncertainty interval: 0.17–0.22%) in 1990, and 0.19% (0.17–0.21%) in 2010. These prevalence estimates were pooled across all regions and standardized by the 2010 global population age and sex. Fig. 10.1 plots the point prevalence in 2010 by region, age, and sex.

Prevalence was higher in males [0.23% (0.2–0.27%)] than females [0.14% (0.12–0.16%)], producing a sex ratio of 1.8 (1.7–1.9). Prevalence peaked in the 20–24 years age group between 0.4% (0.3–0.6%) and 3.4% (2.8–4.2%) in males across regions, and between 0.2% (0.16–0.4%) and 1.9% (1.5–2.4%) in females. It decreased steadily with age, thereafter.

The regional variation in prevalence is summarized in Fig. 10.2 and Table 10.1. Prevalence in high income regions was much higher than that in low to middle income regions, and the global average. Cannabis dependence in Australasia (the region with the highest prevalence, 0.68%) was about 8 times higher than prevalence in Sub-Saharan Africa West (the region with the lowest prevalence, 0.08%). The wide and overlapping 95% uncertainty intervals around some country and regional prevalence estimates indicate that not all variations in prevalence were statistically significant.

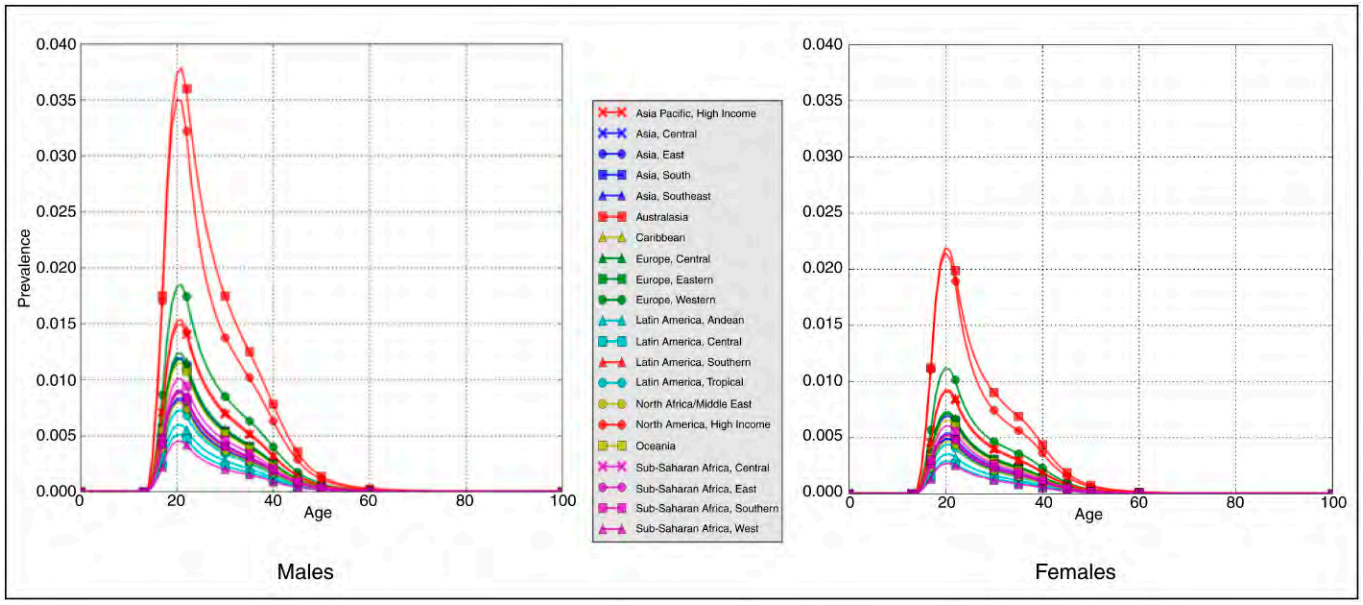


FIGURE 10.1 Estimated prevalence (proportion) of cannabis dependence by age, sex and region, 2010. Shows the prevalence of cannabis dependence (as a proportion of the general population) separately by age, sex, and region for the year 2010.

BURDEN OF CANNABIS DEPENDENCE

Cannabis dependence accounted for 0.07% (0.05–0.1%) of global DALYs in 1990, and 0.08% (0.05–0.12%) in 2010. Although there was no change in the estimated contribution of cannabis dependence to global DALYs, there was an increase in crude DALYs across this period that was attributable to a 25% increase in population, a 6% decrease due to population aging, and a 1% increase in the prevalence rate of cannabis dependence. The net effect was a 22% increase in absolute DALYs attributable to cannabis dependence between 1990 and 2010.

The remainder of the results focuses on 2010 data. There were 2 million DALYs attributable to cannabis dependence in 2010, all YLDs. This accounted for 12.5% of YLDs attributable to illicit drug use, and 0.27% (0.17–0.4%) of global all-cause YLDs. The majority of cannabis dependence YLDs occurred between 20 and 24 years (33.5% of cannabis dependence YLDs). In males, this age group accounted for 64.3% of cannabis dependence YLDs. Fig. 10.3 presents YLDs by age and sex.

Table 10.2 presents the regional estimates of DALYs attributable to cannabis dependence; and Fig. 10.4 presents age standardized DALY rates by country. There was substantial variation in DALYs between regions (Table 10.2) and between countries (Fig. 10.4 (Plot 1)), but most of this variation occurred within wide and often overlapping bounds of uncertainty (Fig. 10.4 (Plot 2)). There was considerable uncertainty around estimated rates for some countries and regions. The countries with statistically higher DALY rates than the global average were all from North America, high income, Australasia, and Europe Western regions. The countries with lower DALY rates than the global average were from Sub-Saharan Africa West, and Latin America Central and Andean.

Fig. 10.5 compares the burden of cannabis dependence to other substance use disorders in GBD 2010. Cannabis dependence was the only substance for which there were no attributable deaths and, hence, zero YLLs. Globally, it accounted for more DALYs than cocaine dependence, but fewer than the other drug use disorders.

In high income regions, such as Australasia, North America, high income, and Europe Western where we had better quality epidemiological data available, the DALY rate (per 100,000) of cannabis dependence surpassed that attributable to amphetamine dependence 93.2 (61.7–134.2) versus 58.5 (31.2–98.1) in Australasia; 43.03 (27.0–62.3) versus 34.5 (19.6–55.2) in Europe Western; and 81.5 (53.6–116.6) versus 33.4 (18.4–52.8) in North America, high income).

ADDITIONAL BURDEN ATTRIBUTABLE TO CANNABIS USE AS A RISK FACTOR FOR SCHIZOPHRENIA

Regular cannabis use as a risk factor for schizophrenia was estimated to account for 7,000 DALYs (3,000–13,000) or 0.04% (0.03–0.06%) of schizophrenia DALYs globally (Table 10.3). Overall, North America high income was the region with highest number of attributable DALYs

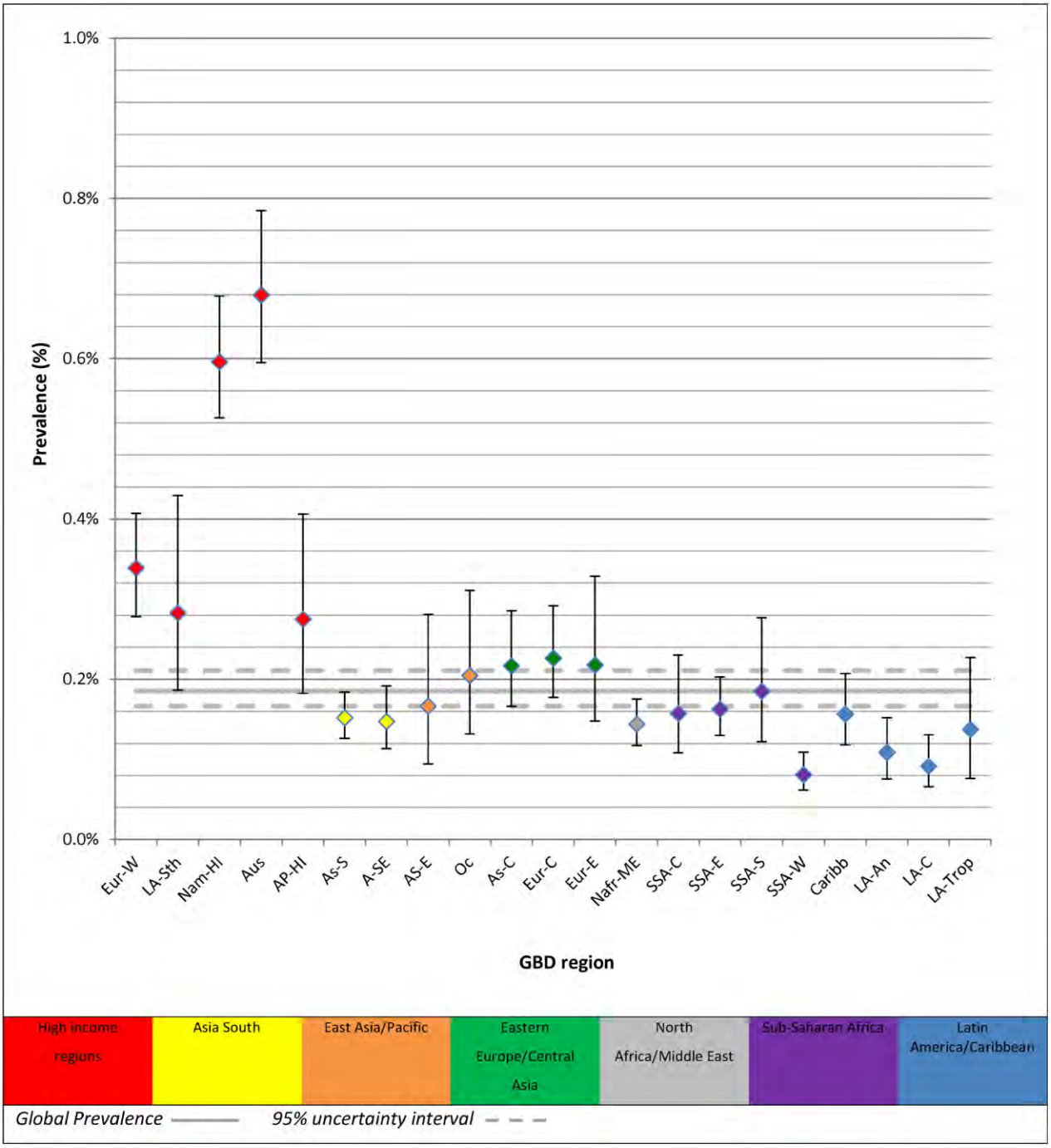


FIGURE 10.2 **Pooled regional prevalence of cannabis dependence, 2010.** Shows the overall prevalence of cannabis dependence by region for the year 2010. Note: Prevalence estimates were standardized by population age and sex; *AP-HI*, Asia Pacific, High Income; *As-C*, Asia Central; *AS-E*, Asia East; *AS-S*, Asia South; *A-SE*, Asia Southeast; *Aus*, Australasia; *Caribb*, Caribbean; *Eur-C*, Europe Central; *Eur-E*, Europe Eastern; *Eur-W*, Europe Western; *LA-An*, Latin America, Andean; *LA-C*, Latin America, Central; *LA-Sth*, Latin America, Southern; *LA-Trop*, Latin America, Tropical; *Nafr-ME*, North Africa/Middle East; *Nam-HI*, North America, High Income; *Oc*, Oceania; *SSA-C*, Sub-Saharan Africa, Central; *SSA-E*, Sub-Saharan Africa, East; *SSA-S*, Sub-Saharan Africa Southern; *SSA-W*, Sub-Saharan Africa, West.

TABLE 10.1 Estimated Age-Standardised Prevalence and Number of Cases of Cannabis Dependence in 2010, by Sex And Region

	Females			Males			Total		
	N	%	95% CI	*N*	%	95% CI	*N*	%	95% CI
Asia, Central	73,000	0.16	(0.11–0.23)	125,000	0.28	(0.19–0.39)	198,000	0.22	(0.17–0.29)
Asia, East	853,000	0.12	(0.05–0.25)	1,547,000	0.21	(0.09–0.4)	2,400,000	0.17	(0.09–0.28)
Asia Pacific, High Income	141,000	0.2	(0.11–0.35)	248,000	0.34	(0.19–0.58)	389,000	0.28	(0.18–0.41)
Asia, South	935,000	0.11	(0.09–0.14)	1,718,000	0.19	(0.15–0.24)	2,653,000	0.15	(0.13–0.18)
Asia, Southeast	359,000	0.11	(0.08–0.16)	616,000	0.19	(0.13–0.26)	975,000	0.15	(0.11–0.19)
Australasia	54,000	0.48	(0.4–0.58)	100,000	0.87	(0.72–1.04)	154,000	0.68	(0.6–0.79)
Caribbean	26,000	0.12	(0.08–0.17)	44,000	0.2	(0.14–0.29)	69,000	0.16	(0.12–0.21)
Europe, Central	89,000	0.16	(0.12–0.23)	161,000	0.29	(0.21–0.4)	250,000	0.23	(0.18–0.29)
Europe, Eastern	163,000	0.16	(0.09–0.28)	273,000	0.27	(0.16–0.46)	436,000	0.22	(0.15–0.33)
Europe, Western	410,000	0.25	(0.19–0.33)	733,000	0.43	(0.33–0.56)	1,143,000	0.34	(0.28–0.41)
Latin America, Andean	23,000	0.08	(0.05–0.13)	39,000	0.14	(0.08–0.22)	62,000	0.11	(0.08–0.15)
Latin America, Central	83,000	0.07	(0.04–0.11)	140,000	0.12	(0.07–0.18)	223,000	0.09	(0.07–0.13)
Latin America, Southern	61,000	0.21	(0.12–0.35)	108,000	0.36	(0.2–0.61)	169,000	0.28	(0.19–0.43)
Latin America, Tropical	108,000	0.1	(0.04–0.21)	186,000	0.17	(0.08–0.33)	294,000	0.14	(0.08–0.23)
North Africa/Middle East	259,000	0.1	(0.08–0.14)	478,000	0.18	(0.14–0.24)	736,000	0.14	(0.12–0.18)
North America, High Income	629,000	0.44	(0.36–0.52)	1,127,000	0.75	(0.63–0.9)	1,755,000	0.6	(0.53–0.68)
Oceania	8,000	0.15	(0.08–0.27)	13,000	0.25	(0.15–0.45)	21,000	0.2	(0.13–0.31)
Sub-Saharan Africa, Central	56,000	0.12	(0.07–0.19)	93,000	0.2	(0.11–0.34)	149,000	0.16	(0.11–0.23)
Sub-Saharan Africa, East	217,000	0.12	(0.09–0.16)	373,000	0.21	(0.15–0.28)	590,000	0.16	(0.13–0.2)
Sub-Saharan Africa, Southern	54,000	0.14	(0.08–0.23)	94,000	0.23	(0.13–0.38)	149,000	0.18	(0.12–0.28)
Sub-Saharan Africa, West	103,000	0.06	(0.04–0.09)	173,000	0.1	(0.07–0.15)	276,000	0.08	(0.06–0.11)
Global	4,704,000	0.14	(0.12–0.16)	8,389,000	0.23	(0.20–0.27)	13,091,000	0.19	(0.17–0.21)

Summarizes the prevalence and number of cases of cannabis dependence by sex and region, for the year 2010.

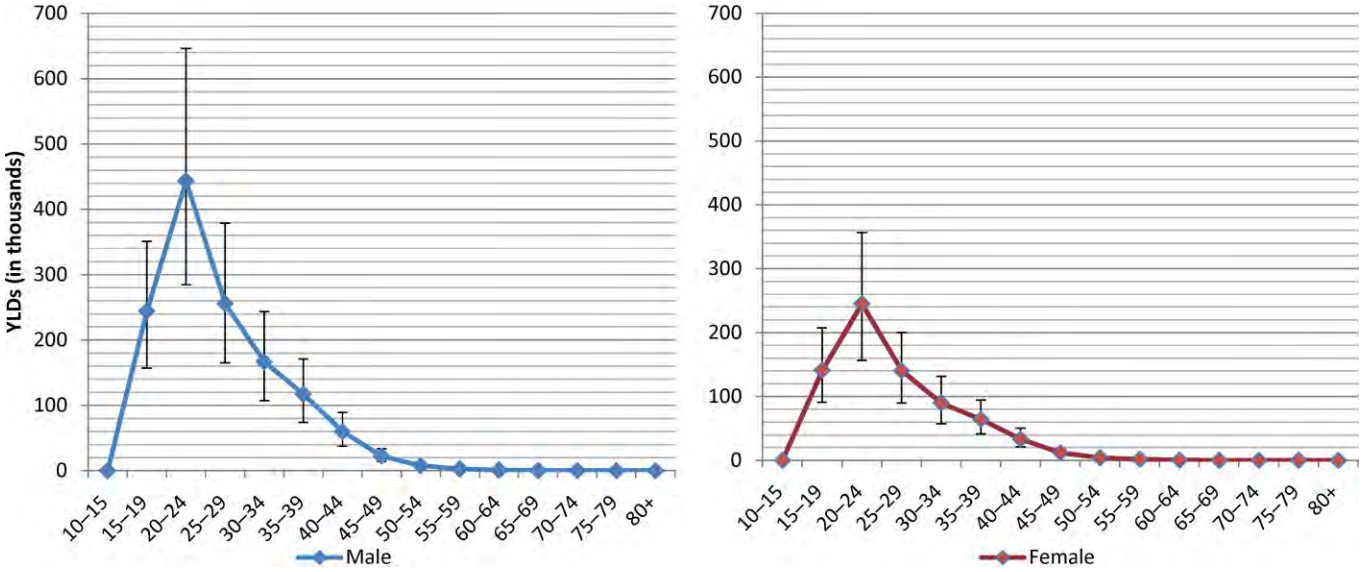


FIGURE 10.3 **Global cannabis dependence DALYs (all YLDs) by age and sex, in thousands, 2010.** Shows the burden of cannabis dependence as disability-adjusted life years (DALYs) by age and sex for the year 2010. Note that DALYs were presented in thousands, and were estimated based on years lived with disability (YLDs) due to cannabis dependence.

TABLE 10.2 Estimated DALYs for Cannabis, by Sex and Region, 2010

	Females			Males			Persons		
	N	Rates per 100,000	95% CI	*N*	Rates per 100,000	95% CI	*N*	Rates per 100,000	95% CI
Asia Pacific, high income	22,000	24.4	(11.8–46.2)	39,000	45.1	(21.5–82.5)	62,000	34.5	(19.2–57.2)
Asia Central	11,000	27.7	(15.7–43.8)	20,000	50.2	(29.2–81.0)	31,000	38.7	(24.3–59.2)
Asia East	135,000	20.1	(7.9–45.2)	247,000	34.1	(14.3–71.0)	382,000	27.3	(13.4–50.5)
Asia South	145,000	18.6	(11.4–28.4)	270,000	32.6	(19.7–48.7)	415,000	25.8	(16.2–37.5)
Asia South East	56,000	18.3	(10.4–29.8)	97,000	32.0	(18.2–51.2)	153,000	25.1	(15.8–37.7)
Australasia	8000	64.2	(41.5–93.7)	16,000	122.5	(79.0–179.6)	24,000	93.2	(61.7–134.2)
Caribbean	4000	18.1	(10.2–29.4)	7,000	31.4	(17.6–49.5)	11,000	24.7	(14.8–36.8)
Europe Central	14,000	22.8	(13.6–35.8)	25,000	43.9	(26.1–70.9)	39,000	33.0	(20.5–50.1)
Europe Eastern	25,000	22.9	(11.0–42.2)	43,000	45.0	(22.3–78.6)	68,000	33.1	(18.5–55.7)
Europe Western	64,000	30.0	(18.4–44.6)	115,000	56.5	(34.4–85.8)	179,000	43.0	(27.0–62.3)
Latin America, Andean	4000	13.4	(6.3–23.0)	6,000	23.0	(11.9–41.0)	10,000	18.2	(10.2–29.8)
Latin America, Central	13,000	11.2	(6.0–19.1)	22,000	19.5	(10.5–32.7)	35,000	15.3	(9.1–23.7)
Latin America, Southern	10,000	31.0	(15.0–59.4)	17,000	57.7	(28.6–106.7)	26,000	44.1	(24.9–75.9)
Latin America, Tropical	17,000	16.5	(6.5–34.5)	29,000	29.4	(11.8–59.9)	46,000	22.9	(11.4–41.0
North Africa/ Middle East	40,000	18.4	(11.4–28.6)	75,000	33.0	(19.9–50.7)	115,000	25.9	(16.2–37.7)
North America, high income	98,000	57.1	(37.0–82.1)	178,000	106.4	(66.6–156.6)	276,000	81.5	(53.6–116.6)
Oceania	1000	24.4	(11.1–47.0)	2,000	41.4	(20.3–81.9)	3,000	33.1	(17.4–57.6)
Sub-Saharan Africa Central	9000	17.7	(8.3–32.7)	14,000	30.1	(14.5–57.0)	23,000	23.9	(12.8–39.7)
Sub-Saharan Africa East	34,000	18.8	(11.1–30.0)	58,000	33.0	(19.9–51.7)	92,000	25.9	(16.5–38.5)
Sub-Saharan Africa South	8000	23.6	(11.4–43.5)	15,000	42.3	(20.9–78.8)	23,000	32.8	(18.0–56.4)
Sub-Saharan Africa West	16,000	9.6	(5.3–15.8)	27,000	16.0	(9.2–27.0)	43,000	12.8	(8.0–20.4)
Global	734,000	21.5	(14.1–31.4)	1,323,000	38.1	(24.4–55.4)	2,057,000	29.9	(19.5–43.1)

Summarizes the burden of cannabis dependence as disability-adjusted life years (DALYs) by sex and region for the year 2010.

(2,000 DALYs). Males were responsible for over twice as many attributable schizophrenia DALYs (5,000 DALYs; 2,000–10,000) than females (2,000; 1,000–4,000); and attributable DALYs peaked between 25 and 30 years, and decreased thereafter. When the additional burden attributable to cannabis use as a risk factor for schizophrenia was added to that of cannabis dependence, cannabis use still accounted for 0.08% of global DALYs in 2010.

WHAT ARE THE IMPLICATIONS OF THESE FINDINGS?

An estimated 13 million people were cannabis dependent in 2010, an age and sex-standardized prevalence of 0.2% (0.17–0.22%). Prevalence was not estimated to have changed significantly from 1990, although increased population size produced an increase in the

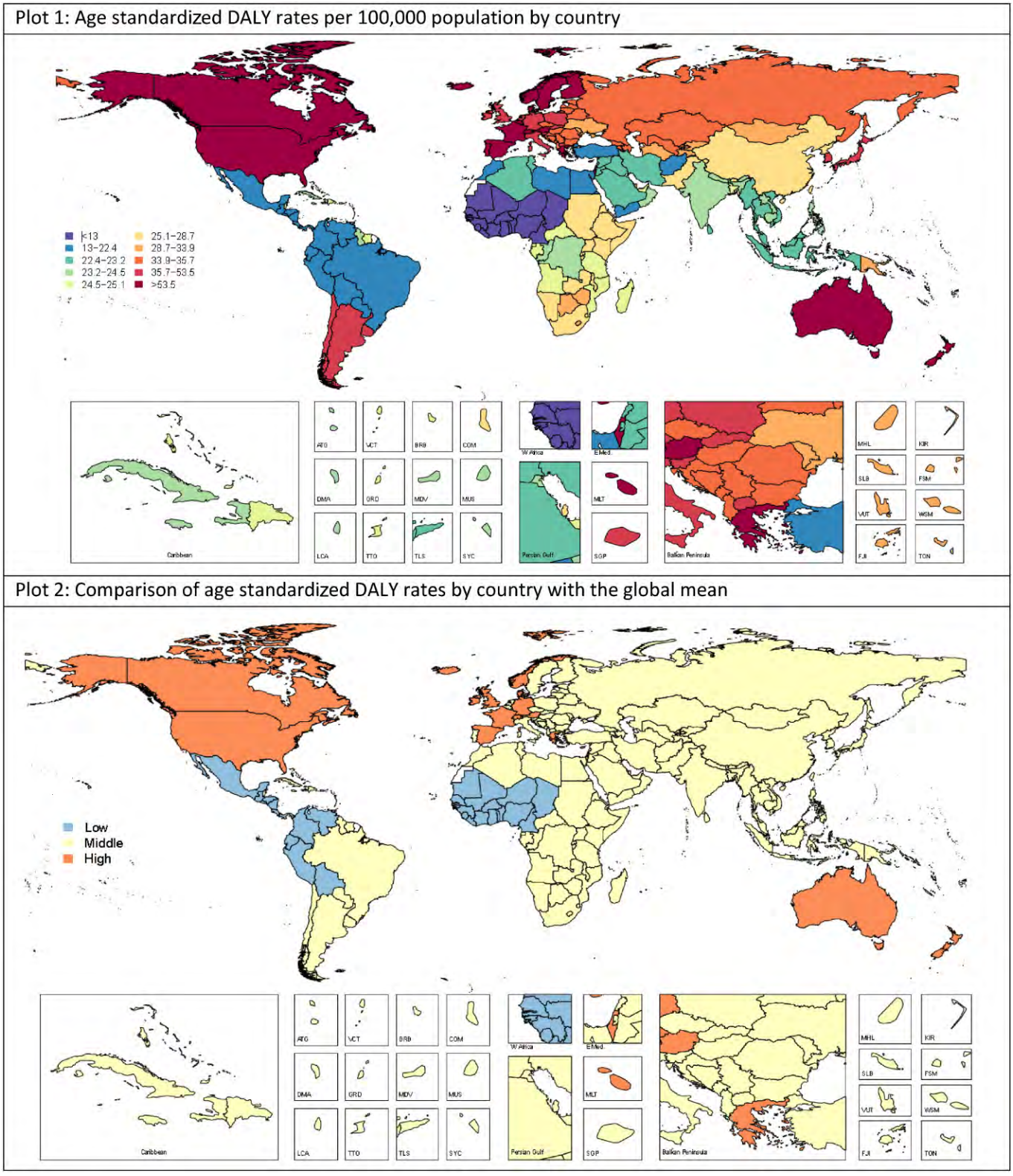


FIGURE 10.4 **Country-level DALYs per 100,000 population for cannabis dependence, age-standardized, for 2010.** Shows the burden of cannabis dependence as disability-adjusted life years (DALYs) by country, for the year 2010. Note that DALYs are age standardized, and presented as a rate per 100,000. Note: *Low*, shows countries with statistically lower DALY rates than global mean; *Middle*, shows countries with DALY rates that are not statistically different to global mean; *High*, shows countries with statistically higher DALY rates than global mean.

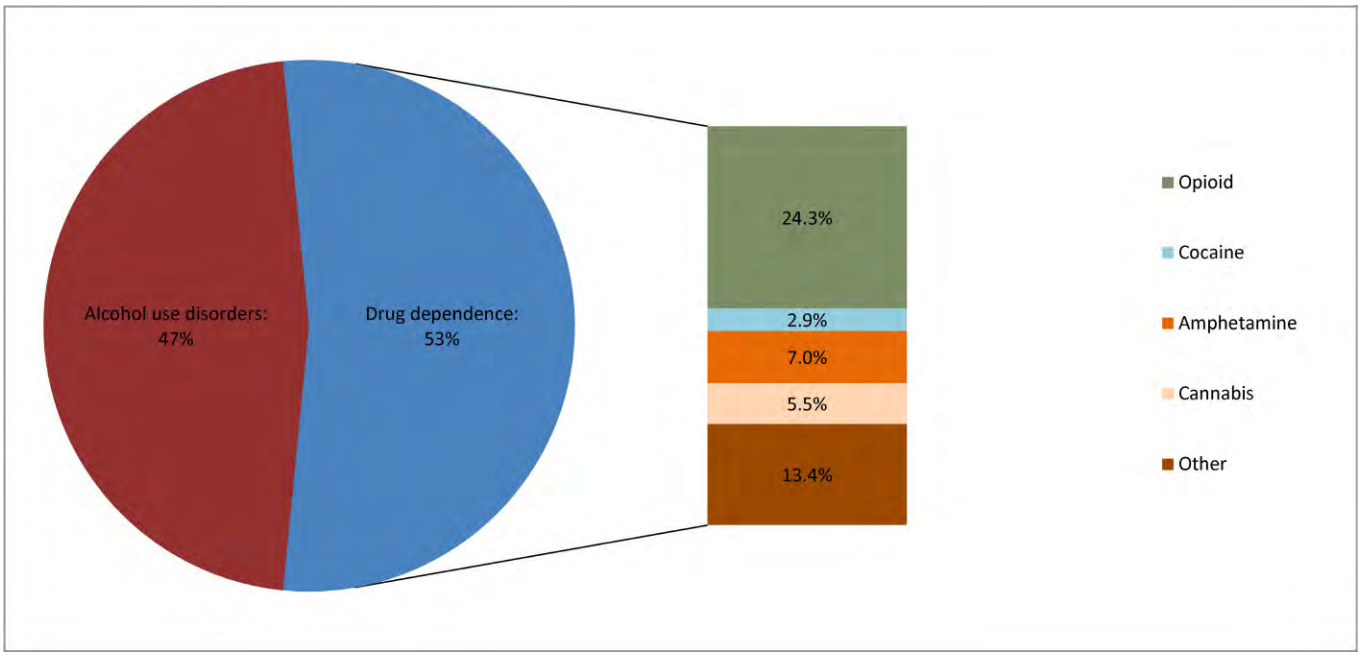


FIGURE 10.5 **The proportion of DALYs due to cannabis dependence relative to other substance use disorders in GBD 2010.** Shows the proportion of burden of cannabis dependence as disability-adjusted life years (DALYs) relative to the proportion of burden due to other substance use disorders. Note: Alcohol use disorders included alcohol dependence and foetal alcohol syndrome; other drugs, burden attributable to illicit drug types other than cannabis, opioid, amphetamine, and cocaine dependence were estimated under residual categories in GBD 2010.

number of cases of cannabis dependence over the period. Levels of cannabis dependence were significantly higher in a number of high income countries including Australia, New Zealand, the United States, Canada, and a number of Western European countries including the United Kingdom. Cannabis dependence caused 2 million DALYs in 2010. DALY rates also varied considerably geographically, with the highest rates again in North America high income, Australasia, and Western Europe.

There is clearly scope to reduce the burden associated with cannabis dependence. Although cannabis dependence was estimated to be a smaller contributor to the overall disease burden than alcohol or opioids dependence, nonetheless some 2 million YLDs were attributed to the drug. Behavioral interventions are effective in the treatment of cannabis dependence (Denis, Lavie et al., 2006; Knapp, Soares et al., 2007), with cognitive behavioral therapy and contingency management showing the greatest promise. Public health campaigns may also be necessary to advise young people of the risks of developing dependence on cannabis because this risk may be underappreciated by many users.

Based on the best available evidence, and models making reasonable assumptions, we found that only 0.04% of the DALYs attributed to schizophrenia were linked to regular (weekly or more frequent) cannabis use. Although epidemiological studies make a consistent case that early and/or heavy cannabis use is linked to a significantly increased risk of schizophrenia (Degenhardt et al., 2009), the modest increase in risk, and the low prevalence of schizophrenia, mean that regular cannabis use accounts for only a very small proportion of the disability associated with schizophrenia.

TABLE 10.3 Estimated DALYs Attributable to Regular Cannabis use as a Risk Factor for Schizophrenia, 2010

	Persons			Males			Females		
	Lower CI	**Mean**	**Upper CI**	**Lower CI**	**Mean**	**Upper CI**	**Lower CI**	**Mean**	**Upper CI**
Attributable YLLs	—	—	—	—	—	—	—	—	—
Attributable YLDs	3,000	7,000	13,000	2,000	5,000	10,000	1,000	2,000	4,000
Attributable DALYs	3,000	7,000	13,000	2,000	5,000	10,000	1,000	2,000	4,000

Summarizes the burden as disability-adjusted life years (DALYs) that can be assigned to cannabis use as a risk factor for schizophrenia, for the year 2010.

LIMITATIONS

We generated estimates based on the best available epidemiological data, and used sophisticated modelling methodology to adjust for known sources of uncertainty in the data. However, our reviews identified clear gaps in existing epidemiological data on cannabis dependence. A significant amount of research is needed to document even the most basic epidemiological parameters for cannabis dependence in most countries. Until such work is done, considerable uncertainty will remain around the exact size of global burden attributable to cannabis and other illicit drugs. This is particularly the case for low income countries, where there is typically limited information on use, less on levels of use, and usually no data on the prevalence of dependence. It is imperative to better assess levels of dependent drug use in these countries, whose populations may be experiencing higher levels of burden than were estimated here.

Further, a range of potential health outcomes of cannabis use were not included in our estimates. We considered including suicide, cancer, and accidental injuries but the evidence for a causal relationship for these outcomes was considered to too weak to generate defensible global estimates (Calabria et al., 2010c).

We also note that we assumed that levels of cannabis use among people with schizophrenia were the same as the general population. This was a conservative assumption. There are data from clinical studies that very high levels of cannabis use are found among patients with schizophrenia (Green, Young et al., 2005). However, a systematic review found that available data on the extent of this elevation were limited (Green et al., 2005) to high income countries. It is also useful to be mindful of the impact of this assumption on the magnitude of the burden we are considering. This particular aspect is only relevant for the "increased severity" analysis. The overall global attributable burden was only 7000 DALYs. Even if levels of cannabis were several times higher among people living with schizophrenia, the estimate of attributable burden would remain very small.

It is also important to acknowledge that, although improved, GBD 2010 disability weights (Salomon et al., 2012), derived from surveys of the general population, have their limitations. As discussed elsewhere (Salomon et al., 2012), it is unclear whether brief lay descriptions can accurately capture the complexity of disability due to drug use disorders. There is also the possibility that considerations other than health status may have influenced respondents' views of "which state was healthier," because it was hard to describe the disability due to cannabis use without mentioning it. Nonetheless, this study has made significant improvements in methods, and in the transparency with which burden estimates have been made.

CONCLUSION

Cannabis dependence causes disability across the globe. It is a disorder primarily experienced by young adults, and our estimates suggest that burden is higher in high income countries. It has not been shown to increase mortality as opioid and other forms of illicit drug dependence do. Nonetheless, in some countries, cannabis dependence produces more years lived with disability than drugs like amphetamines and cocaine, largely because rates of cannabis use are higher than for the stimulant drugs. Our estimates suggest that, although cannabis use is a risk factor for schizophrenia, it is not a major contributor to population level disease burden for that disorder.

MINI-DICTIONARY

Comparative risk assessment (CRA) CRA methods are used in GBD 2010's risk factor analysis to investigate the proportion of burden that could be averted, given a hypothetical absence of a risk factor in the population.

Disability-adjusted life year (DALY) DALYs were the main metric used in GBD 2010 to measure the burden associated to different diseases and injuries. A DALY is equivalent to one healthy year of life lost to a disease or injury, and combines in the one metric both fatal and nonfatal burden.

Global Burden of Disease Study 2010 (GBD 2010) GBD 2010 is the latest iteration of burden of disease studies, published in late 2012. The aim of the study was to quantify and systematically compare the proportion of health loss (or disease burden) associated with 291 diseases and injuries, and 67 risk factors.

Years lived with disability (YLD) YLDs make up the nonfatal component of DALYs, and are estimated by multiplying disease prevalence by the general public's evaluation of the severity of health loss associated to the disease.

Years lost to premature mortality (YLL) YLLs make up the fatal component of DALYs, and are estimated by multiplying deaths due to a disease by the standard life expectancy at the age death occurred.

References

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders (fourth edition - technical revision)*. Washington, DC: American Psychiatric Association.

Calabria, B., Degenhardt, L., et al. (2010a). Systematic reviews of prospective studies investigating "remission" from amphetamine, cannabis, cocaine and opioid dependence. *Addictive Behaviors, 35*, 741–749.

Calabria, B., Degenhardt, L., et al. (2008). Cannabis related mortality. Discussion Paper No. 3. *GBD mental and illicit drug use disorders expert group: illicit drugs discussion papers*. Sydney, Australia: National Drug and Alcohol Research Centre, University of NSW.

Calabria, B., Degenhardt, L., et al. (2010b). Does cannabis use increase the risk of death? Systematic review of epidemiological evidence on adverse effects of cannabis use. *Drug and Alcohol Review, 29*, 318–330.

Calabria, B., Degenhardt, L., et al. (2010c). What do we know about the extent of cannabis use and dependence? Results of a global systematic review. *NDARC technical report No. 307*. Sydney, Australia: National Drug and Alcohol Research Centre, University of NSW.

Degenhardt, L., Bucello, C., et al. (2011). What data are available on the extent of illicit drug use and dependence globally? Results of four systematic reviews. *Drug and Alcohol Dependence*, *117*, 85–101.

Degenhardt, L., Ferrari, A. J., et al. (2013). The global epidemiology and contribution of cannabis use and dependence to the global burden of disease: results from the GBD 2010 Study. *PLoS One*, *8*(10), e76635.

Degenhardt, L., & Hall, W. (2012). Extent of illicit drug use and dependence, and their contribution to the global burden of disease. *Lancet*, *379*(9810), 55–70.

Degenhardt, L., Hall, W., et al. (2009). Should we make burden of disease estimates for cannabis use as a risk factor for psychosis? *PLoS Medicine*, *6*(9), e1000133.

Denis, C., Lavie, E., et al. (2006). Psychotherapeutic interventions for cannabis abuse and/or dependence in outpatient settings. *Cochrane Database of Systematic Reviews*, *3*, CD005336.

Ferrari, A. J., Saha, S., et al. (2012). Health states for schizophrenia and bipolar disorder within the Global Burden of Disease 2010 Study. *Population Health Metrics*, *10*(1), 16.

Flaxman, A. D., Vos, T., et al. (2013). *An integrative metaregression framework for descriptive epidemiology*. Seattle, WA: University of Washington Press.

Foti, D. J., Kotov, R., et al. (2010). Cannabis use and the course of schizophrenia: 10-year follow-up after first hospitalization. *American Journal of Psychiatry*, *167*(8), 987–993.

Gaoni, Y., & Mechoulam, R. (1964). Isolation, structure, and partial synthesis of an active constituent of hashish. *Journal of the American Chemical Society*, *86*(8), 1646–1647.

Green, B., Young, R., et al. (2005). Cannabis use and misuse prevalence among people with psychosis. *British Journal of Psychiatry*, *187*(4), 306–313.

Hall, W., & Degenhardt, L. (2007). Prevalence and correlates of cannabis use in developed and developing countries. *Current Opinion in Psychiatry*, *20*, 393–397.

Hall, W., & Degenhardt, L. (2009). Adverse health effects of non-medical cannabis use. *Lancet*, *374*(9698), 1383–1391.

Hall, W., Degenhardt, L., et al. (2001). *The health and psychological consequences of cannabis use*. Canberra, ACT: Australian Publishing Service.

Hall, W., Johnston, L., et al. (1999). Epidemiology of cannabis use and its consequences. In H. Kalant, W. Corrigall, W. Hall, & R. Smart (Eds.), *The health effects of cannabis* (pp. 71–125). Toronto, ON: Centre for Addiction and Mental Health.

Iversen, L. L. (2000). *The science of marijuana*. Oxford, UK: Oxford University Press.

Knapp, W., Soares, B., et al. (2007). Psychosocial interventions for cocaine and psychostimulant amphetamines related disorders. *Cochrane Database of Systematic Reviews*, *3*, CD003023.

Large, M., Sharma, S., et al. (2011). Cannabis use and earlier onset of psychosis: a systematic meta-analysis. *Archives of General Psychiatry*, *68*(6), 555–561.

Lim, S. S., Vos, T., et al. (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, *380*(9859), 2224–2260.

Lozano, R., Naghavi, M., et al. (2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, *380*(9859), 2095–2128.

Martin, B., & Cone, E. (1999). Chemistry and pharmacology of cannabis. In H. Kalant, W. Corrigal, W. Hall, & R. Smart (Eds.), *The health effects of cannabis* (pp. 19–68). Toronto, ON: Addiction Research Foundation.

Murray, C. J. L., Vos, T., et al. (2012). Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, *380*(9859), 2197–2223.

Saha, S., Chant, D., et al. (2005). A systematic review of the prevalence of schizophrenia. *PLoS Medicine*, *2*(5), e141.

Salomon, J. A. (2010). New disability weights for the global burden of disease. *Bulletin of the World Health Organization*, *88*(12), 879–1879.

Salomon, J. A., Vos, T., et al. (2012). Common values in assessing health outcomes from disease and injury: disability weights measurement study for the Global Burden of Disease Study 2010. *Lancet*, *380*(9859), 2129–2143.

United Nations Office of Drugs and Crime (UNODC). (2008). *World drug report 2008*. New York, NY: United Nations.

von Elm, E., Altman, D. G., et al. (2007). The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet*, *370*, 1453–1457.

Vos, T., Flaxman, A. D., et al. (2012). Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, *380*(9859), 2163–2196.

World Health Organization. (1993). *The ICD-10 classification of mental and behavioural disorders - diagnostic criteria for research*. Geneva, Switzerland: World Health Organization.

CHAPTER

11

International Aspects of Cannabis Use and Misuse: the Australian Perspective

*D.J. Allsop**, *W.D. Hall***

***Psychopharmacology Laboratory, School of Psychology, University of Sydney, Sydney, NSW, Australia**
****Centre for Youth Substance Abuse Research, The University of Queensland, Herston, QLD, Australia**

SUMMARY POINTS

- This chapter gives an overview of patterns of cannabis use, their consequences, and policies relating to cannabis use in Australia.
- Australian cannabis use is placed in its cultural and historical context, and the evolution of cannabis policy is reviewed.
- Cannabis use is common in Australia, and there is tension between the federal government and states on the penalties for cannabis use and possession.
- Australia has well-developed illicit drug monitoring systems that enable drug trends, including availability, purity, and price to be tracked.
- Cannabis accounts for the most treatment episodes of all of the illicit drugs in Australian Drug and Alcohol treatment centers, and is second only to alcohol.
- Australasian cohort studies have reported associations between regular cannabis use, poor educational achievement, and psychotic symptomology.

KEY FACTS OF CANNABIS LAW IN AUSTRALIA

- Australia is a signatory to the United Nations Single Convention on Narcotic Drugs (1961) that requires the government to prohibit and criminalize cannabis use.
- Australia's Federal political system allows individual states and territories to set and enforce their own criminal laws.
- Cannabis use is illegal in all Australian states and territories, but the penalties vary across the country.
- Minor cannabis offences are decriminalized in: the ACT, South Australia, and the Northern Territory. In all other states, criminal penalties apply, but, in reality, police have discretion under some circumstances to divert people into treatment.

KEY FACTS OF CANNABIS AVAILABILITY, PRICE, AND PURITY

- A gram of hydroponic or bush cannabis typically costs AUD$20 in Australia, although an ounce of bush is cheaper ($240) than an ounce of hydroponic ($300).
- In 2013 cannabis in the state of NSW was quantified as containing 15% THC and 0.14% CBD.
- The majority of Australian cannabis growers surveyed in 2014 grew outdoors, and for personal use.

LIST OF ABBREVIATIONS

ACT	Australian Capital Territory
AIC	Australian Institute of Criminology
AIDS	Acquired immunodeficiency syndrome
AIHW	Australian Institute of Health and Welfare
ATSI	Aboriginal and Torres Strait Islanders
CBC	Cannabichromene

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00012-0
Copyright © 2017 Elsevier Inc. All rights reserved.

CBD	Cannabidiol
CBG	Cannabigerol
CBN	Cannabinol
CEN	Cannabis Expiation Notice
CRT	Cannabinoid replacement therapy
GHB	Gamma-hydroxybutyric acid
IDRS	Illicit Drug Reporting System
LSD	Lysergic acid diethylamide
MDMA	3,4-methylenedioxy-*N*-methylamphetamine
MS	Multiple sclerosis
NCADA	National Campaign against Drug Abuse
NCP	National Cannabis Policy
NCPIC	National Cannabis Prevention and Information Centre
NDARC	National Drug and Alcohol Research Centre
NDC	National Drugs Campaign
NDSH	National Drug Strategy Household Survey
NSW	New South Wales
PWID	People who inject drugs
TGA	Therapeutic Goods Administration
THC	Tetrahydrocannabinol
THC-V	Tetrahydrocannabivarian
UNODC	United Nations Office on Drugs and Crime

INTRODUCTION

Cannabis has been cultivated in Australia ever since Sir Joseph Banks sent hemp seeds with the first fleet. Cannabis used as a recreational drug gained its foothold during the Vietnam War, when US soldiers brought cannabis and heroin use to Sydney and Melbourne (Manderson, 1993). Cannabis use spread to Sydney's suburbs throughout the 1960s and 1970s, as part of the surfing culture (McCoy, 1980), extending beyond the criminal milieu, to represent rebellion against established authority, particularly among young people (Penington, 1999).

Cannabis use steadily rose among young adults over the next two decades (Donnelly & Hall, 1994a; Hall & Pacula, 2003). National surveys indicated that less than 20% of young Australians had tried cannabis in the early 1970s; by 1993, 60% had done so (Donnelly & Hall, 1994a). Increased cannabis use among successive birth cohorts normalized its use, increased availability, and reduced the age of first trying cannabis (Degenhardt, Lynskey, & Hall, 2000; Donnelly & Hall, 1994a). A survey of Australian attitudes toward cannabis in 1994 revealed that most interviewees (75%) advocated no criminal penalties for personal use (Bowman & Sanson-Fisher, 1994), and just over half also thought that there should be no criminal penalties for cultivating cannabis for personal use. Strong support remained for criminalizing the commercial production and sale of cannabis (Bowman & Sanson-Fisher, 1994).

National media outlets have also played a role in influencing cultural attitudes toward cannabis in Australia. The media characterization has polarized between two policy choices: legalization or decriminalization, supported by the argument that cannabis use causes few if any harms; and on the other hand, the claim that cannabis use should remain illegal because it causes significant harms to individuals and public health (Hall, 1997).

Since the mid-1990s, cannabis has been the most commonly used illicit drug in Australia. Australasia has one of the highest prevalence rates in the world, with over one-third of the adult population having used, and one in ten have done so in the past year (AIHW, 2011). Cannabis use is most common in young men in their 20s, in Australia (AIHW, 2011). A substantial minority of 14–19 year olds have smoked cannabis in the previous 12 months, according to the 2010 National Drug Strategy Household Survey (NDSHS) (Fig. 11.1), and about one in five Australians reported that they had the opportunity to use cannabis in the previous 12 months.

The tide of public opinion has now swung in favor of removing criminal penalties. A recent discrete choice experiment suggested that the majority of people preferred civil penalties or legalization, compared to cannabis cautioning (current policy) or criminalizing possession and use (Shanahan, Gerard, & Ritter, 2014).

Patterns of Cannabis Use in Relation to Other Substance Misuse

The rise of cannabis use in Australia paralleled a general increase in the use of other psychoactive substances, including amphetamines, heroin, and hallucinogens. In more recent years, party drugs, particularly ecstasy, have increased in prevalence in Australia as cannabis (and heroin) use have declined (Roxburgh, Ritter, Grech, Slade, & Burns, 2011).

Cannabis is frequently used in combination with other psychoactive substances. A 2014 Australian study followed a group of regular nontreatment-seeking cannabis users through 2 weeks of monitored abstinence in the community. It found robust substitution effects, with alcohol (Fig. 11.2) and cigarette (Fig. 11.3) consumption increasing during the 2-week cannabis abstinence, and only returning to baseline levels after resumption of cannabis use (Allsop et al., 2014b).

Cannabis has also been associated with increased nicotine dependence, in a so-called reverse gateway effect. Up to three-quarters of dependent cannabis smokers in Australia mix their cannabis with tobacco, thereby increasing their tobacco exposure (Patton, Coffey, Carlin, Sawyer, & Lynskey, 2005; van Beurden, Zask, Passey, & Kia, 2008).

Cannabis use in Australia is higher amongst those who regularly use other illicit drugs, than in the general

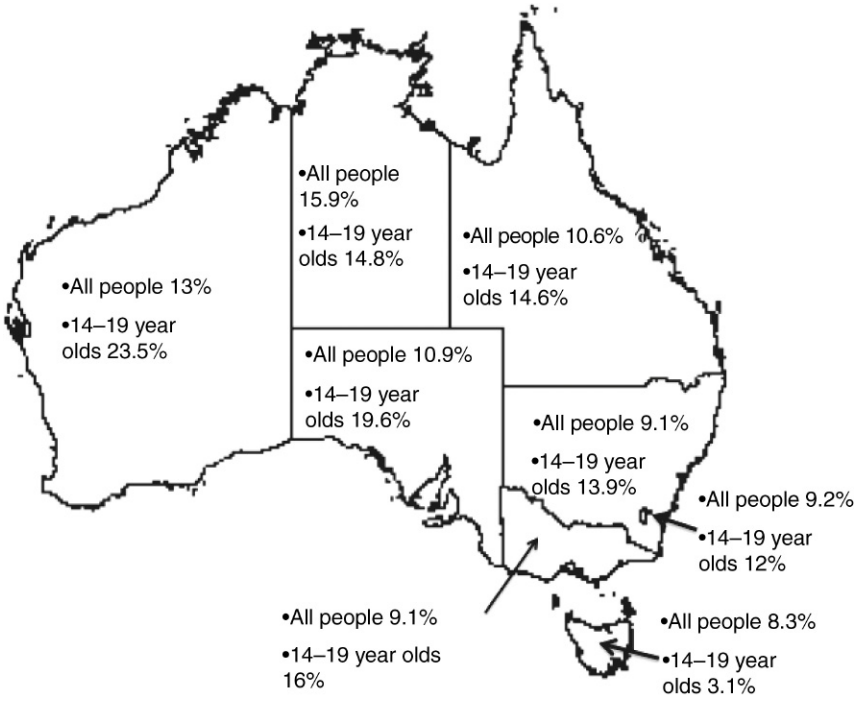


FIGURE 11.1 **Cannabis use prevalence in the Australian States and Territories.** The proportion of each state's population who have smoked cannabis in the previous 12 months split by (1) the average across all age groups (all people); and (2) the 14–19 year age group. *Source: Data from the 2010 National Drug Strategy Household Survey, Australia.*

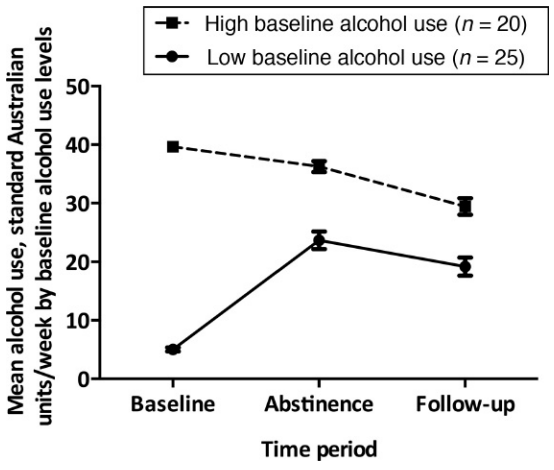


FIGURE 11.2 **Alcohol use increases during cannabis abstinence for those with low baseline alcohol consumption, and decreases with resumption of cannabis use.** The interaction of baseline alcohol use group with time is significant at $F_{2,87.11} = 13.49$, $P < 0.0001$. *Source: Adapted from Allsop et al. (2014b). Changes in cigarette and alcohol use during cannabis abstinence.* Drug and Alcohol Dependence, 138, *54–60.*

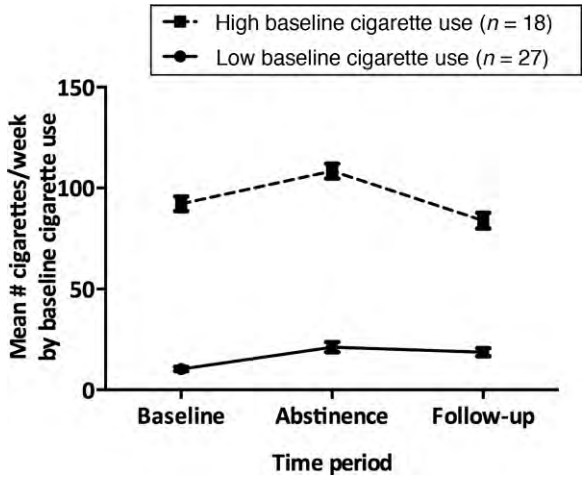


FIGURE 11.3 **Cigarette use increases during cannabis abstinence, and decreases with resumption of cannabis use.** The interaction of baseline cigarette use group with time is significant at $F_{2,89.06} = 4.58$, $P = 0.01$. *Source: Adapted from Allsop et al. (2014b). Changes in cigarette and alcohol use during cannabis abstinence.* Drug and Alcohol Dependence, 138, *54–60.*

population (McLaren & Mattick, 2007). Whilst the majority of cannabis users will not go on to use other illicit drugs, the majority of people who use other illicit drugs (eg, heroin, amphetamines) used cannabis first, and are more likely to be regular cannabis users (McLaren & Mattick, 2007).

Most regular Australian cannabis users are polydrug users. The majority (~86%) of recent cannabis users report using alcohol, 28% report using amphetamines, and 24% report using ecstasy at the same time as cannabis (McLaren & Mattick, 2007). Approximately 20% of Australian dependent cannabis users were also dependent upon opioids, stimulants, or sedatives, compared to only 1% of noncannabis users (Swift, Hall, & Teesson, 1999). Importantly, heavy cannabis users are

more likely to be polydrug users, especially of alcohol, tobacco and stimulants (Swift, Hall, & Copeland, 1997).

Types of Cannabis Products: Availability, Price, and Purity

Australia has well-developed drug use monitoring systems providing useful information about the availability and price of street drugs. The Illicit Drug Reporting System (IDRS) is a strategic early warning system for identifying emerging trends of local and national concern in illicit drug use. IDRS data are gathered from interviews with people who inject drugs (PWID), interviews with key experts, and analysis of indicator data sources related to illicit drug use. In the 2013 IDRS report, hydroponic cannabis was rated as very easy to get (53%) or easy to get (40%), and its potency was rated as high (58%), or medium (30%). Bush cannabis was rated as easy (47%) or very easy (27%) to get, with 25% rating it as difficult to get. The potency of Australian bush cannabis was rated as medium by 54% of PWID interviewed. The price of Australian cannabis was AUD$20 per gram nationally, for hydroponic cannabis, and the same for bush grown cannabis [although bush cannabis (AUD$240) was cheaper than hydroponic ($300), when purchasing an ounce of plant matter].

Claims have been made since the 1990s that the THC content of cannabis produced in Australia has greatly increased, but there has been no data to evaluate this claim (Hall & Swift, 2000). The first systematic analysis of cannabis potency in Australia was conducted in 2013, analyzing the cannabinoid content of 206 different cannabis samples confiscated by police from recreational users, under the New South Wales "cannabis cautioning scheme" (Swift, Wong, Li, Arnold, & McGregor, 2013). The study found high THC content (~15%; Fig. 11.4) and low CBD content (~0.14%; Fig. 11.5). The study also found that there were no significant differences in potency (THC or CBD levels) between indoor (typically hydroponic) or outdoor grown varieties of cannabis (Swift et al., 2013).

Australia has a well-established tradition of domestic cannabis cultivation. Herbal cannabis is most frequently consumed, and there is negligible use of hash (resin), compared to other countries (Potter et al., 2015). A 2014 survey of 491 Australian cannabis growers shows that the majority (41%) cultivated for personal use, and grew outdoor (Potter et al., 2015).

The most visible incorporation of cannabis into Australian culture is seen in the state of New South Wales (NSW), which nominally retains criminal penalties. The North of NSW (known as the Northern Rivers) has a long history of alternative cultural expression, dating back to the early 1970s. The area has long been associated with high rates of cannabis use, normalizing cannabis in the community (Reilly, Didcott, Swift, & Hall, 1998). An annual "Mardi Grass" festival is held in the Northern Rivers in peaceful protest against cannabis prohibition. The primary political organization for cannabis legalization is the Hemp Party (Help End Marijuana Prohibition), founded in 1993. The region also has the highest rates of convictions for cannabis cultivation, use, and possession in the state (Helliwell, Reilly, & Rippingale, 1992; Reilly et al., 1998; van Beurden et al., 2008).

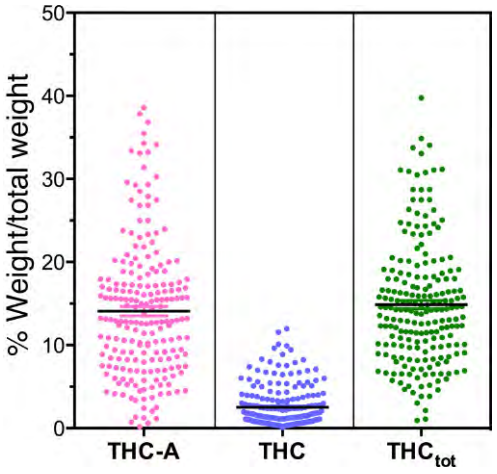


FIGURE 11.4 The levels of THC(A), THC, and THC$_{tot}$ measured in n = 206 Cannabis Cautioning seizures from NSW. Levels of cannabinoids are expressed as a percentage of total weight of sample. THC$_{tot}$ is obtained from adding free THC in the cannabis to the nonpsychoactive form of THC(A), adjusting for their differing molecular weights. *Source: From Swift et al. (2013). Analysis of cannabis seizures in NSW, Australia: cannabis potency and cannabinoid profile.* PLoS One, 8, *e70052.*

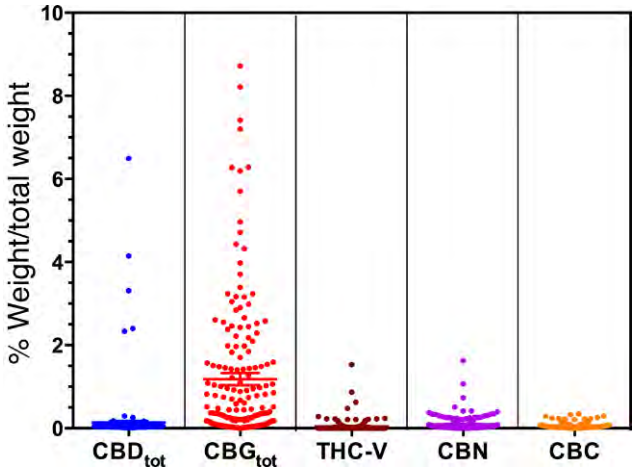


FIGURE 11.5 The levels of CBD$_{tot}$, CBG$_{tot}$, THC-V, CBN, and CBC measured in n = 206 Cannabis Cautioning seizures from NSW. Levels of cannabinoids are expressed as percentage of the total weight of the sample. *Source: From Swift et al. (2013) Analysis of cannabis seizures in NSW, Australia: cannabis potency and cannabinoid profile.* PLoS One, 8, *e70052.*

Indigenous Cannabis Use

Aboriginal and Torres Strait Islanders (ATSI) use cannabis at higher rates than the general population: with 50 and 27% reporting lifetime and past year use of cannabis, respectively, in 2011 (Clough et al., 2006). Cannabis smoking is often associated with tobacco smoking (van Beurden et al., 2008), the primary cause of chronic disease among ATSIs (Ministerial Council on Drug Strategy, 2011). Cannabis use occurs at higher rates in ATSIs than in the general population (AIHW, 2008; Clough et al., 2004; Forero, Bauman, Chen, & Flaherty, 1999; Perkins et al., 1994).

One longitudinal study has demonstrated that the practice of petrol sniffing in remote Aboriginal communities was a strong predictor of later heavy cannabis use (Lee et al., 2009a). This is part of a broader picture of poorer health and well-being, stemming from the experience of alienation and dispossession. Many of the social determinants of harmful substance use are disproportionately common in ATSI communities. There has been very little research on cannabis dependence in this population, until relatively recent studies of the epidemiology of use and harms (Bohanna & Clough, 2012; Lee, Conigrave, Patton, & Clough, 2009), psychiatric consequences (Lee, Clough, Jaragba, Conigrave, & Patton, 2008), and the indigenous experience of cannabis withdrawal (Rogerson & Clough, 2013).

A BRIEF HISTORY OF CANNABIS LAW IN AUSTRALIA

Australia has a Federal political system that arose when the six separate colonies formed a Federal system of government at the beginning of the 20th century (Singleton, Aitken, Jinks, & Warhurst, 2000). Under the constitution, the states have the powers to set and enforce criminal laws, including those relating to drug use. The Commonwealth Government has the responsibility for ensuring compliance with international treaties.

International drug control treaties, such as the Single Convention on Narcotic Drugs (UN, 1961), require signatory states, which include Australia, to prohibit and criminalize cannabis use by adults. Since the late 1970s, rising rates of cannabis use have prompted policy debates in Australia about the appropriateness of these criminal penalties (Hall, 2001). Australian cannabis policy is interesting because it claims to be evidence-based (Fitzgerald & Sewards, 2002), whereas, as in other countries, research is often used to support policies adopted for other reasons (Hall, 2008).

Since cannabis use was prohibited in Australia in the early decades of the 20th century (Manderson, 1993; McCoy, 1980), the initial response to rising rates of recreational cannabis use in the 1960s was to increase enforcement of prohibition. Criminal prosecutions for cannabis offences increased by nearly 1000% between 1966 and 1969. Criminal penalties were also raised, and police were given more funding to disrupt the illicit cannabis market (Manderson, 1993). These efforts had minimal effects on rates of cannabis use which rose steadily between 1970 and the mid-1990s (Hall & Pacula, 2003).

Cannabis Law Reform in Australia

More liberal cannabis policies were first advocated in the late 1970s, when cannabis prosecutions increased among middle class youth (Manderson, 1993). The argument was that having a criminal record caused more harm than cannabis use [Royal Commission into the Non-medical use of Drugs, 1979; Senate Standing Committee on Social Welfare (Baume Committee), 1977], and that a more appropriate penalty for personal possession and use was a civil penalty, such as a fine [Senate Standing Committee on Social Welfare (Baume Committee), 1977]. The Baume Committee also argued that Australia needed to develop a national policy to: reduce cannabis use; discourage cannabis users from driving while intoxicated; and reduce cannabis users' contact with the suppliers of other illicit drugs. State governments generally ignored recommendations for more liberal cannabis policies (Manderson, 1993).

The first Australian state to decriminalize cannabis use was South Australia, in 1987. Under the new law, persons found to possess and use cannabis, or to cultivate up to 10 cannabis plants for personal use, received only a civil fine. There was no criminal conviction recorded, if the fine was paid (the law was termed the Cannabis Expiation Notice System Act, or CEN). Following an inquiry into illicit drug use by the Australian Capital Territory (ACT) in 1991, similar changes in penalties were introduced there (ACT Legislative Assembly Select Committee on HIV, Illegal Drugs and Prostitution, 1991).

The National Task Force on Cannabis

A National Campaign against Drug Abuse (NCADA) was established by Federal and State Ministers in 1985, in response to rising heroin use (Hall, 2004). Cannabis decriminalization was off the agenda (Manderson, 1993). NCADA did, however, establish "harm minimization" as a central principle of Australia's national drug policy. It also initiated regular surveys of drug use in the Australian population that documented the steady rise in cannabis use over subsequent decades.

In 1993 the Federal Labor government established a National Task Force on Cannabis to encourage the states to develop a nationally consistent policy. The Task Force comprised State and Federal health and law enforcement

officials. It commissioned reviews of: patterns of cannabis use (Donnelly & Hall, 1994b), the health and psychological effects of cannabis (Hall, Solowij, & Lemon, 1994), legal options for dealing with cannabis (McDonald, Moore, Norberry, Wardlaw, & Ballenden, 1994), and public attitudes towards these options (Bowman & Sanson-Fisher, 1994).

The Task Force's report recommended that the "possession, unsanctioned cultivation, sale, and nontherapeutic use of cannabis in any quantity remain illegal" (p. xii) but that state governments "consider discontinuing … criminal penalties for the simple personal use or possession of cannabis" (Ali & Christie, 1994, p. xii). It also recommended that the Ministerial Council on Drug Strategy should develop a "comprehensive national policy on cannabis, based on the Task Force research and recommendations" (Ali & Christie, 1994, p. xi). State governments did not accept these recommendations.

In the decade after the National Task Force reported, several states and territories had debates about whether they should change penalties for cannabis use. In 1995 the Northern Territory passed legislation modeled on South Australia's, imposing civil penalties for personal use, possession, and cultivation for personal use. Attempts to legislate for lower cannabis use penalties were unsuccessful in Victoria and New South Wales (Hall, 2008). In 2004 the Western Australian Government decriminalized cannabis use, citing local research that cannabis users prosecuted under that state's laws suffered greater social harm than cannabis users who had been dealt with under the CEN system in South Australia (Lenton, 2004). This law was repealed, and criminal penalties reinstated in 2008, when a conservative government was elected.

The National Cannabis Policy

In Nov. 2004 the Ministerial Council on Drug Strategy commissioned an expert group to develop a National Cannabis Policy (NCP). The issue of penalties for cannabis use was excluded from the brief. The NCP had four aims: to educate the community (and young people in particular) about the adverse health effects of cannabis, and the penalties for using cannabis; to prevent the uptake of cannabis among young people by reducing its availability, and addressing the social vulnerabilities predisposing some young people to its use; to reduce problems related to cannabis use among young people by encouraging users to desist, and reducing the number of users who became regular users; and to respond more effectively to the increasing numbers of problem users seeking help to stop using cannabis. The NCP also advocated more research on cannabis use among young people, and more rigorous evaluations of the effects of different penalties for cannabis use in different Australian jurisdictions on rates of use and harm.

Medicinal Cannabis in Australia

Medicinal use of cannabis was advocated in the early 1990s in Australia, in the first decade of the AIDS epidemic. It was considered by the National Task Force which recommended funding clinical trials. In August 1999, the NSW Premier appointed a Working Party to advise: (1) whether cannabis and cannabinoid drugs had any medical uses, and (2) if they did, how cannabis or cannabinoids could be made available for medical use, without decriminalizing recreational cannabis use. The Working Party concluded that THC was useful in treating HIV- and cancer-related wasting and nausea caused by cancer chemotherapy (Hall, Degenhardt, & Currow, 2001; NSW Working Party, 2000). It also noted suggestive evidence that THC may relieve painful muscle spasms in neurological disorders, and pain that has not responded to conventional analgesics. It recommended further research on the therapeutic use of cannabis and cannabinoid drugs in all of these conditions.

The Working Party also concluded that smoked cannabis would not meet criteria for registration as a therapeutic good (a precondition for any medical use in Australia). It suggested that the best prospects for medical use lay in developing new synthetic cannabinoids, or ways of delivering THC that did not involve smoking cannabis. In the meantime, it recommended that patients be given compassionate access to cannabis by means of an exemption from criminal prosecution for those who were medically certified as having: HIV- or cancer-related wasting; nausea caused by cancer chemotherapy; muscle spasm in neurological disorders or spinal cord injury; and pain unrelieved by conventional analgesia. The government appointed a committee to advise on implementation of the recommendations, but no legislation was introduced.

The medical cannabis debate changed with the advent of Sativex, a botanical extract of *Cannabis sativa* comprising 50% THC and 50% CBD. Prior to May 2009, Sativex was classed as a Schedule 9 drug in Australia, which are prohibited substances for research use only. In May 2010 the Therapeutic Goods Administration (TGA) rescheduled Sativex to Schedule 8 (eg, like methadone), and made it available for use under the TGA's special access scheme. In Nov. 2012 the TGA registered Sativex for use in the treatment of severe spasticity in MS. However, changes are required to the Poisons Act to make prescribing Sativex a practical reality in Australia.

Australian scientists have explored the possibility of using Sativex as a potential Cannabinoid Replacement Therapy (CRT) for treating cannabis withdrawal in dependent cannabis users (Allsop et al., 2014a). This work demonstrated that high-dose Sativex suppressed more of the most clinically relevant symptoms of cannabis withdrawal than placebo (Fig. 11.6). The lack of intoxication suggests that this may be a viable route for future

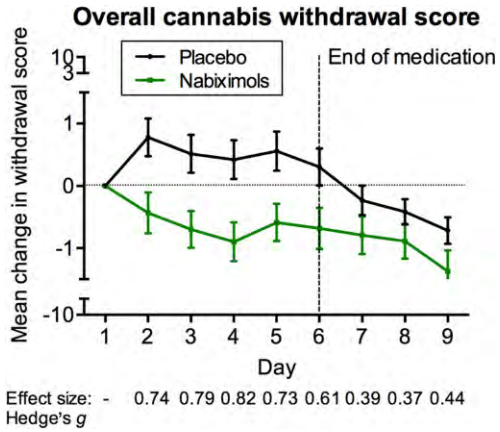


FIGURE 11.6 **Cannabis withdrawal symptoms were suppressed during inpatient cannabis detoxification, when medicated with Sativex (Nabiximols), compared to placebo.** Withdrawal scores are shown relative to baseline (day 1) scores. The interaction of treatment group with time is significant at $F_{8,325.1} = 2.83$, $P = 0.01$. *Source: Adapted from Allsop et al. (2014a). Nabiximols as an agonist replacement therapy during cannabis withdrawal: a randomized clinical trial.* JAMA Psychiatry, *71, 281–291.*

treatment of cannabis dependence. However, there remains much clinical work and policy translation before such an approach can be implemented as standard clinical practice.

A 2013 NSW medicinal cannabis enquiry recommended that medically prescribed cannabinoids (not raw cannabis) be made available for terminally ill patients, as well as allowing terminally ill patients to possess up to 15 g of cannabis. Both patient and carer would need a certificate from a specialist, need to be registered with the Department of Health, and would need photo identification. Recent media articles suggest that the Commonwealth Government has rejected the recommendations due to insufficient evidence of efficacy for cannabis.

EFFECTS OF CANNABIS USE ON AUSTRALIAN COMMUNITIES

Regular cannabis use is associated with a range of social harms. Perhaps the most prevalent and easily recognizable harm in the Australian community is the increased prevalence of adults seeking treatment for cannabis dependence. Examination of the NSW Minimum dataset, a collection of data cataloguing medical treatment episodes in NSW, shows that cannabis is the second most prevalent primary drug of concern (19%) after alcohol (49%), with heroin (17%) and amphetamines (10%) also problematical (Copeland & Allsop, 2014). Cannabis was also the most prevalent "other" or secondary drug of concern (25% of treatment episodes), followed by nicotine (17.6%), amphetamines (8.6%), alcohol (6.2%), benzodiazepines (4%), heroin (3.2%), and ecstasy (2%) (Copeland & Allsop, 2014). These trends are mirrored in other Australian jurisdictions (AIHW, 2013).

Poorer educational outcomes have also been reported in Australasian cannabis smokers who commenced cannabis use at younger ages (Horwood et al., 2010). The worst rates of high school completion, university enrolments, and degree attainment were found to be amongst those who commenced cannabis use prior to age 15, even after controlling for a range of possible confounders, such as socioeconomic status and gender (Horwood et al., 2010). Heavy users who persist in using throughout their 20s report lower life satisfaction, and poorer social outcomes, than those who do not use or who do not persist in using.

Regular cannabis use may impair memory and attention in ways that adversely affect academic performance. Indeed, a longitudinal prospective cohort study from New Zealand, following over a thousand people from birth to age 38, found that persistent cannabis use was associated with IQ decline, after controlling for years of education and other confounding variables (Meier et al., 2012). The largest declines in IQ were found in those who commenced cannabis use during adolescence, and continued to use daily throughout young adulthood.

Regular cannabis use has also been associated with increased prevalence and severity of psychotic illness in the Australian population (McGrath et al., 2010). A younger age at initiation predicts a younger age of onset for psychotic symptoms (Castle, 2013; Large, Sharma, Compton, Slade, & Nielssen, 2011; Stefanis et al., 2013).

STRATEGIES FOR REDUCING MISUSE

Supply control efforts include attempts to detect illicit cultivation, and local policing initiatives, such as the use of sniffer dogs (Dunn & Degenhardt, 2009). Border protection is less of an issue in Australia because most cannabis is domestically cultivated (McLaren & Mattick, 2007; UNODC, 2013).

Cannabis use offences remain the most common drug offences. A significant proportion of people entering the criminal penalty system in Australia for cannabis offences are problematic cannabis users (AIC, 2008). Many of these offenders are diverted into counseling and treatment.

Cannabis Public Information Initiatives

The National Cannabis Policy funded a National Cannabis Prevention and Information Centre (NCPIC) that began in 2008. The NCPIC was established in response to growing community concerns about cannabis use, with the mission of preventing cannabis use, and providing

the community with evidence-based information and interventions. Its key services included: a website providing evidence-based cannabis information; a free cannabis telephone information and help line; regular reports on the latest cannabis research findings; and national training in interventions for cannabis-related problems.

Preventative drug education, including material on cannabis, is included in secondary school health curriculums in all Australian states and territories. An educational resource, *Cannabis and Consequences*, was developed as part of the National School Drug Education Strategy to educate students from an early age about the potential harms of cannabis use, to prevent the uptake of cannabis use, and to develop resilience (Midford, Lenton, & Hancock, 2000). More recently, an interactive computer-based intervention for school children has been developed, CLIMATE Schools: Alcohol and Cannabis course (Newton, Teesson, Vogl, & Andrews, 2010). The program has been tested in a cluster randomized controlled trial, and found to significantly improve knowledge of cannabis (and alcohol) 12 months after the program was delivered, and to reduce alcohol and cannabis use (Newton et al., 2010).

The Australian government has also conducted national media campaigns on cannabis, as part of the National Drugs Campaign (NDC). The most recent campaign, in 2010, focused on ecstasy, marijuana, and methamphetamine, using the Internet, print, and in-venues advertising, a dedicated website, and an information telephone line. Some state governments have also funded cannabis-based public health initiatives.

MINI-DICTIONARY

Bush cannabis Australian vernacular for outdoor grown cannabis. Sometimes can be used to represent cannabis grown indoors that is cultivated in soil, as opposed to hydroponically grown cannabis.
Cannabidiol (CBD) Cannabidiol is a nonpsychotropic phytocannabinoid. CBD has antipsychotic and anxiolytic effects, and may dampen the psychotomimetic actions of THC.
Cannabinoid replacement therapy Using cannabinoid medications (phytocannabinoids or synthetic cannabinoids) to occupy cannabinoid receptor sites in the brain and body, in place of illicit cannabis, or potentially to replace dysfunctional endocannabinoid signaling.
Cannabinoids A diverse group of receptor proteins that activate the endogenous cannabinoid system. They include endogenous substances (eg, anandamide) and synthetic or plant based cannabinoids (THC).
Cannabis decriminalization The abolition of criminal penalties for the use and possession of small amounts of cannabis.
Cohort study An observational study used in medicine, social sciences, and ecology that gathers data on a defined group of people, over a long period of time, in order to elucidate relationships between risk factors (eg, cannabis use) and particular medical conditions or diseases.
Diversion A policy lever for channeling cannabis users away from criminal sanctions and into treatment.
Harm minimization The concept of reducing the harmful effects of alcohol and other drugs using policy levers and educational resources, on the basis of research evidence.
Tetrahydrocannabinol (THC) The main psychoactive component of the cannabis plant that is a partial agonist of the Cannabinoid Type-1 (CB1) receptors in the brain and central nervous system.

References

ACT Legislative Assembly Select Committee on HIV, Illegal Drugs, and Prostitution. (1991). *Marijuana and other illegal drugs: Third interim report*. Canberra, ACT: Australian Capital Territory Chief Minister's Department, Public Affairs Branch.

AIC. (2008). *Police drug diversion: A study of criminal offending outcomes*. Canberra, ACT: Australian Institute of Criminology.

AIHW. (2008). *2007 National Drug Strategy Household Survey: Detailed findings*. Canberra, ACT: Australian Institute of Health and Welfare, Drug statistics series no. 22 Cat. no. PHE 107.

AIHW. (2011). *2010 National Drug Strategy Household Survey report*. Canberra, ACT: Australian Institute of Health and Welfare.

AIHW. (2013). *Alcohol and other drug treatment services in Australia 2011–12*. Canberra, ACT: Australian Institute of Health and Welfare, Drug treatment series no. 21. Cat. no. HSE 139.

Ali, R., & Christie, P. (1994). *Report of the National Task Force on Cannabis*. Canberra, ACT: Australian Government Printing Service.

Allsop, D. J., Copeland, J., Lintzeris, N., Dunlop, A. J., Montebello, M., Sadler, C., … , McGregor, I. S. (2014a) Nabiximols as an agonist replacement therapy during cannabis withdrawal: a randomized clinical trial. *JAMA Psychiatry*, *71*, 281–291.

Allsop, D. J., Dunlop, A. J., Sadler, C., Rivas, G. R., McGregor, I. S., & Copeland, J. (2014b). Changes in cigarette and alcohol use during cannabis abstinence. *Drug and Alcohol Dependence*, *138*, 54–60.

Bohanna, I., & Clough, A. R. (2012). Cannabis use in Cape York Indigenous communities: high prevalence, mental health impacts and the desire to quit. *Drug and Alcohol Review*, *31*, 580–584.

Bowman, J., & Sanson-Fisher, R. W. (1994). *Public perceptions of cannabis legalisation*. Canberra, ACT: Australian Government Publishing Service.

Castle, D. J. (2013). Cannabis and psychosis: what causes what? *F1000 Medicine Reports*, *5*, 1.

Clough, A. R., d'Abbs, P., Cairney, S., Gray, D., Maruff, P., Parker, R., & O'Reilly, B. (2004). Emerging patterns of cannabis and other substance use in Aboriginal communities in Arnhem Land, Northern Territory: a study of two communities. *Drug and Alcohol Review*, *23*, 381–390.

Clough, A. R., Lee, K. S., Cairney, S., Maruff, P., O'Reilly, B., d'Abbs, P., & Conigrave, K. M. (2006). Changes in cannabis use and its consequences over 3 years in a remote indigenous population in northern Australia. *Addiction*, *101*, 696–705.

Copeland, J., & Allsop, D. J. (2014). Dedicated 'cannabis only' treatment clinics in New South Wales, Australia: client and treatment characteristics and associations with first-time treatment seeking. *Drug and Alcohol Review*, *33*, 565–571.

Degenhardt, L., Lynskey, M., & Hall, W. D. (2000). Cohort trends in the age of initiation of drug use in Australia. *Australian and New Zealand Journal of Public Health*, *24*, 421–426.

Donnelly, N., & Hall, W. D. (1994a). *Patterns of cannabis use in Australia*. Canberra, ACT: Australian Government Publishing Service.

Donnelly, N., & Hall, W. D. (1994b). *Patterns of cannabis use in Australia* (p. 27). Canberra, ACT: Australian Government Publishing Service.

Dunn, M., & Degenhardt, L. (2009). The use of drug detection dogs in Sydney, Australia. *Drug and Alcohol Review*, *28*, 658–662.

Fitzgerald, J. L., & Sewards, T. (2002). *Drug policy: the Australian approach*. Canberra, ACT: Australian National Council on Drugs.

Forero, R., Bauman, A., Chen, J. X., & Flaherty, B. (1999). Substance use and socio-demographic factors among Aboriginal and Torres Strait Islander school students in New South Wales. *Australian and New Zealand Journal of Public Health*, *23*, 295–300.

Hall, W. D. (1997). The recent Australian debate about the prohibition on cannabis use. *Addiction*, *92*, 1109–1115.
Hall, W. D. (2001). Reducing the harms caused by cannabis use: the policy debate in Australia. *Drug and Alcohol Dependence*, *62*, 163–174.
Hall, W. D. (2004). The contribution of research to Australian policy responses to heroin dependence 1990–2001: a personal retrospection. *Addiction*, *99*, 560–569.
Hall, W. D. (2008). The contribution of research to the development of a national cannabis policy in Australia. *Addiction*, *103*, 712–720.
Hall, W. D., & Pacula, R. (2003). *Cannabis use and dependence: Public health and public policy*. Cambridge, UK: Cambridge University Press.
Hall, W. D., & Swift, W. (2000). The THC content of cannabis in Australia: evidence and implications. *Australian and New Zealand Journal of Public Health*, *24*, 503–508.
Hall, W. D., Solowij, N., & Lemon, J. (1994). *The health and psychological consequences of cannabis use*. Canberra, ACT: Australian Government Publishing Service, p. 25.
Hall, W. D., Degenhardt, L., & Currow, D. (2001). Allowing the medical use of cannabis. *Medical Journal of Australia*, *175*, 39–40.
Helliwell, D., Reilly, D., & Rippingale, C. (1992). Establishing a drug and alcohol service in an Australian rural area. *Drug and Alcohol Review*, *11*, 371–378.
Horwood, L. J., Fergusson, D. M., Hayatbakhsh, M. R., Najman, J. M., Coffey, C., Patton, G. C., … , Hutchinson, D. M. (2010) Cannabis use and educational achievement: findings from three Australasian cohort studies. *Drug and Alcohol Dependence*, *110*, 247–253.
Large, M., Sharma, S., Compton, M. T., Slade, T., & Nielssen, O. (2011). Cannabis use and earlier onset of psychosis: a systematic meta-analysis. *Archives of General Psychiatry*, *68*, 555–561.
Lee, K. S., Clough, A. R., Jaragba, M. J., Conigrave, K. M., & Patton, G. C. (2008). Heavy cannabis use and depressive symptoms in three Aboriginal communities in Arnhem Land, Northern Territory. *Medical Journal of Australia*, *188*, 605–608.
Lee, K. S., Conigrave, K. M., Clough, A. R., Dobbins, T. A., Jaragba, M. J., & Patton, G. C. (2009a). Five-year longitudinal study of cannabis users in three remote Aboriginal communities in Arnhem Land, Northern Territory, Australia. *Drug and Alcohol Review*, *28*, 623–630.
Lee, K. S., Conigrave, K. M., Patton, G. C., & Clough, A. R. (2009b). Cannabis use in remote Indigenous communities in Australia: endemic yet neglected. *Medical Journal of Australia*, *190*, 228–229.
Lenton, S. (2004). Pot, politics and the press: reflections on cannabis law reform in Western Australia. *Drug and Alcohol Review*, *23*, 223–233.
Manderson, D. (1993). *From Mr Sin to Mr Big: A history of Australian drug laws*. Melbourne, VIC: Oxford University Press.
McCoy, A. W. (1980). *Drug traffic: narcotics and organized crime in Australia*. Sydney, NSW: Harper and Row.
McDonald, D., Moore, R., Norberry, J., Wardlaw, G., & Ballenden, N. (1994). *Legislative options for cannabis in Australia*. Canberra, ACT: Australian Government Publishing Service, p. 26.
McGrath, J., Welham, J., Scott, J., Varghese, D., Degenhardt, L., Hayatbakhsh, M. R., … , Najman, J. M. (2010) Association between cannabis use and psychosis-related outcomes using sibling pair analysis in a cohort of young adults. *Archives of General Psychiatry*, *67*, 440–447.
McLaren, J., & Mattick, R. P. 2007. Cannabis in Australia: use, supply, harms, and responses: Drug Strategy Branch, Australian Government Department of Health and Ageing. Monograph series No. 57.
Meier, M.H., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R.S., … Moffitt, T.E. (2012) Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, E2657–E2664.
Midford, R., Lenton, S., & Hancock, L. (2000). A critical review and analysis: Cannabis education in schools. Report prepared by the National Drug Research Institute, Curtin University. Sydney, NSW: Department of Education and Training.
Ministerial Council on Drug Strategy. (2011). *The National Drug Strategy 2010–2015: a framework for action on alcohol, tobacco and other drugs*. Canberra, ACT: Commonwealth of Australia.
Newton, N. C., Teesson, M., Vogl, L. E., & Andrews, G. (2010). Internet-based prevention for alcohol and cannabis use: final results of the Climate Schools course. *Addiction*, *105*, 749–759.
NSW Working Party. (2000). *Report of the Working Party on the use of cannabis for medical purposes*. Sydney, NSW: National Drug and Alcohol Research Centre, University of New South Wales.
Patton, G. C., Coffey, C., Carlin, J. B., Sawyer, S. M., & Lynskey, M. (2005). Reverse gateways? Frequent cannabis use as a predictor of tobacco initiation and nicotine dependence. *Addiction*, *100*, 1518–1525.
Penington, D. (1999). An international perspective on drug use in Australia. In K. van den Boogert, & N. Davidoff (Eds.), *Heroin crisis: Key commentators discuss the issues and debate solutions to heroin abuse in Australia*. Melbourne, VIC: Bookman Press.
Perkins, J. J., Sanson-Fisher, R. W., Blunden, S., Lunnay, D., Redman, S., & Hensley, M. J. (1994). The prevalence of drug use in urban aboriginal communities. *Addiction*, *89*, 1319–1331.
Potter, G., Barratt, M. J., Malm, A., Bouchard, M., Blok, T., Christensen, A., … , Wouters, M. (2015) Global patterns of domestic cannabis cultivation: sample characteristics and patterns of growing across eleven countries. *International Journal of Drug Policy*, *26*(3), 226–237.
Reilly, D., Didcott, P., Swift, W., & Hall, W. D. (1998). Long-term cannabis use: characteristics of users in an Australian rural area. *Addiction*, *93*, 837–846.
Rogerson, B., & Clough, A. R. (2013). How do Indigenous Australians experience cannabis withdrawal? *Australian and New Zealand Journal of Public Health*, *37*, 186–187.
Roxburgh, A., Ritter, A., Grech, K., Slade, T., & Burns, L. A. (2011). *Trends in drug use and related harms in Australia, 2001 to 2011*. Sydney, NSW: Drug Policy Modelling Program, National Drug and Alcohol Research Centre, University of New South Wales.
Royal Commission into the Non-medical use of Drugs. 1979. Final report. Adelaide: Royal Commission into the Non-medical use of Drugs, South Australian Government.
Senate Standing Committee on Social Welfare [Baume Committee]. (1977). *Drug problems in Australia: an intoxicated society?* Canberra, ACT: Australian Government Publishing Service.
Shanahan, M., Gerard, K., & Ritter, A. (2014). Preferences for policy options for cannabis in an Australian general population: a discrete choice experiment. *International Journal of Drug Policy*, *25*(4), .
Singleton, G., Aitken, D., Jinks, B., & Warhurst, J. (2000). *Australian political institutions*. Sydney, NSW: Longman.
Stefanis, N. C., Dragovic, M., Power, B. D., Jablensky, A., Castle, D., & Morgan, V. A. (2013). Age at initiation of cannabis use predicts age at onset of psychosis: the 7- to 8-year trend. *Schizophrenia Bulletin*, *39*, 251–254.
Swift, W., Hall, W. D., & Copeland, J. (1997). *Cannabis dependence among long-term users in Sydney, Australia*. Sydney, NSW: National Drug and Alcohol Research Centre, University of New South Wales, NDARC Technical Report 47.
Swift, W., Hall, W. D., & Teesson, M. (1999). *Cannabis use disorders among Australian adults: findings from the National Survey of Mental Health and Well-being*. Sydney, NSW: National Drug and Alcohol Research Centre, University of New South Wales, NDARC Technical Report 78.
Swift, W., Wong, A., Li, K., Arnold, J., & McGregor, I. (2013). Analysis of cannabis seizures in NSW. *PLoS One*, *8*, e70052.
UN. (1961). *Single convention on narcotic drugs, 1961 [as amended by the 1972 protocol]*. New York, NY: United Nations.
UNODC. (2013). *World drug report 2013*. New York, NY: United Nations.
van Beurden, E. K., Zask, A., Passey, M., & Kia, A. M. (2008). The Mull Hypothesis: is cannabis use contributing to high tobacco use prevalence among young North Coast males? *NSW Public Health Bulletin*, *19*, 72–74.

CHAPTER

12

International Aspects of Cannabis Use and Misuse: Egypt

*O.M.E. Abdel-Salam**, *A.F. Galal**, *S.A. ElShebiney**, *A.E.D.M. Gaafar***

Department of Toxicology and Narcotics, National Research Centre, Dokki, Greater Cairo, Egypt

***Department of Photochemistry, Chemical Industries Division, National Research Centre, Cairo, Egypt**

SUMMARY POINTS

- This chapter provides a concise review on the history, as well as the present status, of cannabis use and/or abuse in Egypt.
- Cannabis was neither cultivated, nor it was known in Ancient Egypt.
- From the 12th to the 19th century, Egypt was affected by large scale cannabis abuse.
- At the beginning of the 20th century, Egypt called for international restrictions on the cultivation and commerce in cannabis.
- Hashish and herbal cannabis (bango) are the most commonly abused drugs nowadays.
- Hashish is smuggled through the eastern and western borders of Egypt, while the plant is increasingly widely cultivated in Egypt, especially in the Sinai Peninsula and in Upper Egypt.
- Since 1998, seizures of hashish and cannabis herb went up, possibly reflecting increased market demand and/or successful efforts of Egyptian law enforcement officials.

KEY FACTS OF THE DRUG CONTROL AND TREATMENT OF ADDICTION FUND

- Belongs to the Ministry of Insurance and Social Affairs.
- Works in collaboration with the National Council for Addiction Treatment and Prevention.
- Its targets are the prevention and treatment of addiction.
- Performs a range of training programs to educate young people about the issue of drugs, and problems arising from them.
- Establishes clinics and treatment centers for drug addicts.
- Coordinates between the various parties concerned with the problem of drugs of abuse, in cooperation with the authorities and other relevant ministries.
- Works to develop a database on the problem of drug addiction in Egypt.

KEY FACTS OF UNITED NATIONS OFFICE ON DRUGS AND CRIME (UNODC)

- A United Nations agency (in Vienna).
- Was established in 1997 as the Office for Drug Control and Crime Prevention.
- Was renamed the United Nations Office on Drugs and Crime in 2002.
- Deals with the problems of illicit drugs, crime, corruption, terrorism, HIV, and AIDS.
- Works with member states to combat these problems.

LIST OF ABBREVIATIONS

DCTAF	Drug control and treatment of addiction fund
NCATP	National Council for Addiction Treatment and Prevention
SYPE	Survey of young people in Egypt
UNODC	United Nations Office on Drugs and Crime
WHO	World Health Organization

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00013-2

Copyright © 2017 Elsevier Inc. All rights reserved.

INTRODUCTION AND HISTORICAL PERSPECTIVES

Ancient Egypt

The history of herbal medicine can be traced back to ancient Egypt. The Ebers medical papyrus dates to ~ 1550 BC, and lists many herbal prescriptions for treating numerous medical conditions. The Edwin Smith papyrus, the oldest known surgical textbook (~ 1600 BC) describes 48 typical cases, from injuries of the head to the lower spine (Leake, 1952). There is no convincing evidence that the cannabis plant was known to the ancient Egyptians. Wall paintings, tomb reliefs, plant remains in tombs, or papyri constitute our source of information concerning the plants of ancient Egypt. Tomb paintings of private gardens show sycamore fig, *doum* palm, date palm, pomegranates, olives, willows, and *persea*. Flower gardens included cornflowers, water lilies, poppies, mandrakes, hollyhocks, and mallows (Bigelow, 2000). In the ninth year of her reign, Queen Hatshepsut (1473–1458 BC), daughter of Thutmose I, commissioned an expedition to the land of Punt, and which returned laden very heavily with heaps of myrrh-resin, of fresh myrrh-trees, with ebony and pure ivory, and with cinnamon-wood, among many other things. Thutmose III followed queen Hatshepsut and, during his reign, Egypt stretched to Palestine and Syria. From there, plants and trees were brought to Egypt (Breasted, 1945). *Cannabis sativa* was not mentioned in any hieroglyphic texts or paintings, and no cloth made of hemp was found in ancient Egyptian tombs (Peters & Nahas, 1999) (Fig. 12.1).

In 1992, however, the presence of cannabis (tetrahydrocannabinol), nicotine (the main alkaloid in tobacco), and cocaine have been reported in samples from soft tissues, hair, and bone from Egyptian mummies dating back to 1070 BC–395 AD (Balabanova, Parsche, & Pirsig, 1992). In an Egyptian mummy dating to ~ 950 BC, Parsche and Nerlich (1995) detected amounts of the above drugs in internal organs, as well as in hair, bone, skin, muscle, and tendons. Since cannabis, tobacco, and coca plants were not native to Egypt or Africa, their finding in mummies was surprising, and suggested a transoceanic pre-Columbian contact between the Americas and the rest of the world. It might also be that these plants have been available in limited supply for the more prosperous elite of Egyptian society, whose bodies were mummified (Wills, 1998).

Cannabis in Arabic Medicine

Only in the ninth century did Arab scholars become familiar with the medicinal properties of cannabis, after translating the Greek texts of Dioscorides and Galen (Nahas, 1982). Cannabis seemed to have been introduced into Arab culture from India, through Persia; hence it was known as Indian hemp (Hamarneh, 1972). Medicinal uses for oil obtained from hemp seeds first appeared in Arabic literature by al-Razi (857 AD), who proposed its use for the ear, dissolving flatulence, and to cure epilepsy. Hashish is the Arabic name for the cannabis extract used in the Middle East. The word also means "grass." Another Arab physician, al-Badri (1251 AD), recommended hashish to stimulate the appetite and produce a craving for sweets (Peters & Nahas, 1999).

Cannabis in Medieval Arab World

The word "hashshashins," meaning hashish consumers, refers to followers of an 11th century Persian leader, who committed secret assassinations of some of the regional leaders (Nahas, 1982). Hence the word "hashshashins" might have formed the root for the derivation of the English word "assassin" (Raman, 1998; Roffman, Schwartz, & Stephens, 2006). Sufis (a mystical movement of ascetics) in the 12th century had possibly encouraged the use of hashish for seeking spiritual insights, and spread its consumption to Syria and Egypt (Rosenthal, 1971). The Spanish botanist, Ibn Baitar, on his trip to Egypt in 1413, noted that cannabis use in Egypt was public (Iversen, 2000). Egypt was particularly affected by large-scale cannabis abuse since the 13th century (Nahas, 1982, 1985; UNODC, 2008).

Hashish during 18th–20th Centuries

It seems that fear of the influence of hashish on French soldiers in Napoleon's army led, in 1798, to the issuing of a decree that prohibited the preparation and use of hashish as a beverage, as well as the smoking of its seeds throughout Egypt. Nevertheless, it is said that samples of the material, as well as many colorful tales of hashish and its intoxicating effects, were carried back to France by French soldiers (Kalant, 2001; Iversen, 2000).

Hashish use also seemed to have been widespread in Egypt during the 19th century. In 1893, Peterson, a Professor of Nervous Diseases at the University of Vermont, wrote on the toxic effects resulting from the excessive use of "hasheesh" which he observed in 60 men and 4 or 5 women, and described the resulting "insanity." He described the presence of several hasheesh joints in Cairo, and also his visit to one of them: "there were two or three rooms full of men enjoying their pipes and chibouques" (Peterson, 1893).

It is not surprising, therefore, that in 1925, in the Geneva Opium Convention, the Egyptian delegate, M. El Guindy, took a leading role to add international trade in cannabis into the agenda. In his speech, El Guindy claimed that "the illicit use of hashish accounted for 30–60% of the total number of cases of insanity occurring in Egypt" (Zimmer, 1997; UNODC, 2008).

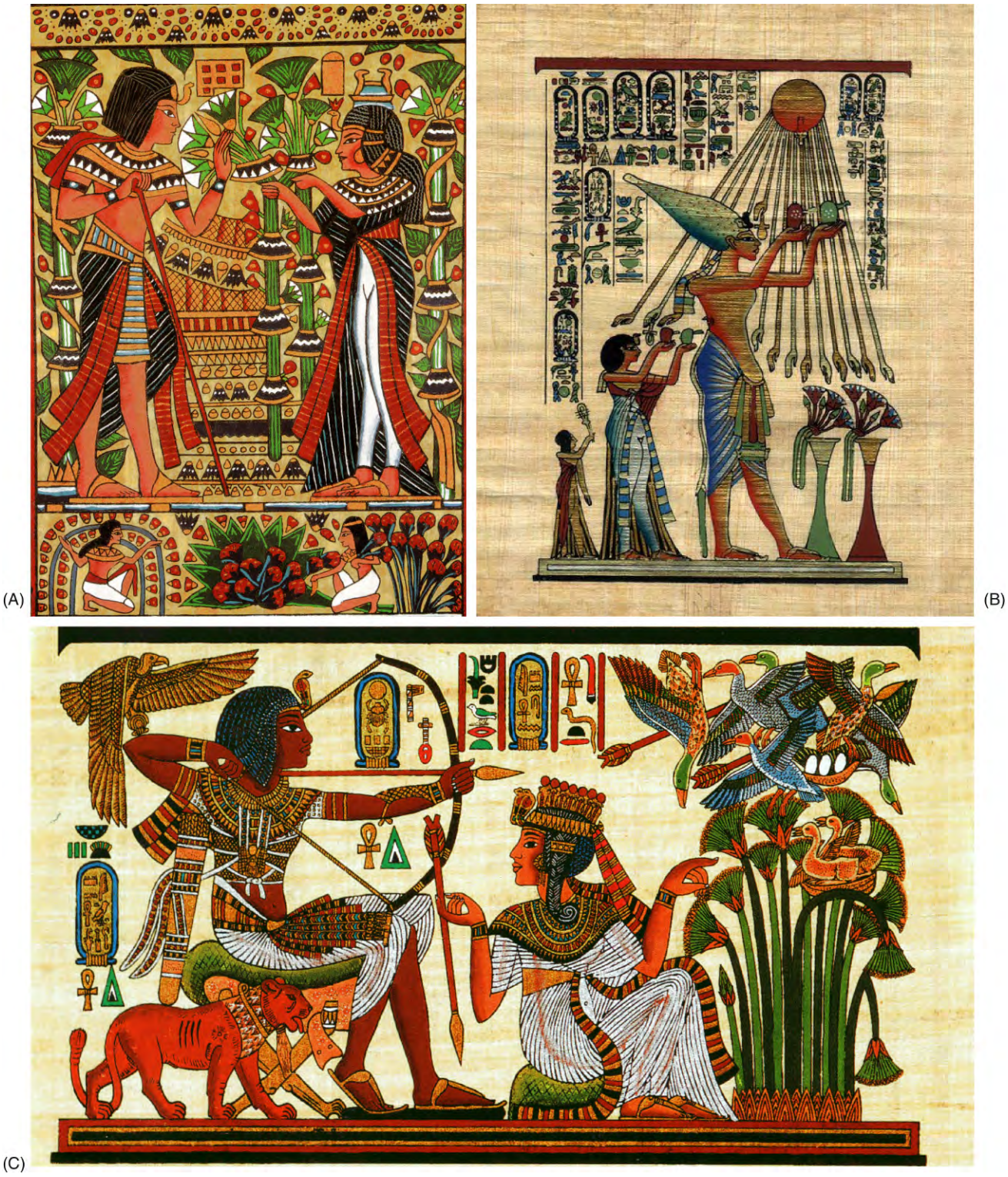


FIGURE 12.1 (A) Papyrus of Tutankhamun and his wife Ankhesenamun offering him bouquets of lotus and papyrus flowers decorated with mandrakes in a garden (Egyptian Museum, Cairo); (B) papyrus depicting Akhenaton, Nefertiti, and their daughter Meritaton; (C) papyrus showing the young Pharaoh and his wife;

(D)

FIGURE 12.1 (*cont.*) (D) agriculture in ancient Egypt. Cannabis was neither cultivated nor known in Ancient Egypt. Tomb paintings and reliefs depicted only papyrus flowers, water lilies or lotus flowers, mandrakes, and date palm.

CURRENT STATUS OF CANNABIS USE IN EGYPT

Egypt is a country with a population of about 80.72 million. The proportion of population under the age of 15 years is 34%. The country is a lower middle income group country (World Bank, 2012). Illegal drugs have made their entry into the Egyptian society, and the most common form of drug abuse is that of cannabis. There are no reliable statistics on the extent of drug abuse, but it is clear that there is a general increase in it, especially in young age (15–25 years) (WHO, 2005).The cannabis products being used in Egypt include "hashish" and marijuana, or "bango." Hashish is usually smoked in a water pipe, known as "goza" or "shisha," while bango is usually smoked in the form of a cigarette, or added to a cigarette. Cannabis is of relatively low price, can be easily obtained, and cultivated illegally in many areas in Egypt (Okasha, 2004). In 2005, the average price of herbal cannabis in Egypt was ~ 2.6 US$ per gram, compared with 5 and 15 US$ in the United Kingdom and the United States, respectively. Meanwhile, the price of hashish was 2.6 US$ per gram, compared with 3.7 US$ in the United Kingdom (UNODC, 2007).

SOURCE OF CANNABIS

Cannabis is one of the most widespread drugs on the illicit trafficking market. There are no registered cases of local production of hashish. This appears to be due to the tight security measures that prevent such activity. Thus, the hashish market depends mainly on importation from producer countries. The main source of hashish smuggled into Egypt is West Africa. Morocco was regarded as the biggest source country. There are also some attempts to smuggle hashish from Pakistan and Afghanistan, through the eastern borders of Egypt (UNODC, 2013). Cannabis herb is widely cultivated year-round in Egypt, especially in Sinai Peninsula and Nile islands, in Upper Egypt. Preventing the cultivation of cannabis presents a challenge, since this requires very costly human, material, and technical capabilities. In the year 2002, Egyptian law enforcement officials eradicated 162.8 hectares of cannabis, and in 2011 control efforts resulted in the eradication of a surface of 65.25 feddans (UNODC, 2012, 2013). Egypt accounted for 90% or more of the annual total cannabis seizures for Africa each year, from 2000 to 2009. In general, seizures of both cannabis resin ("hashish") and herbal cannabis increased

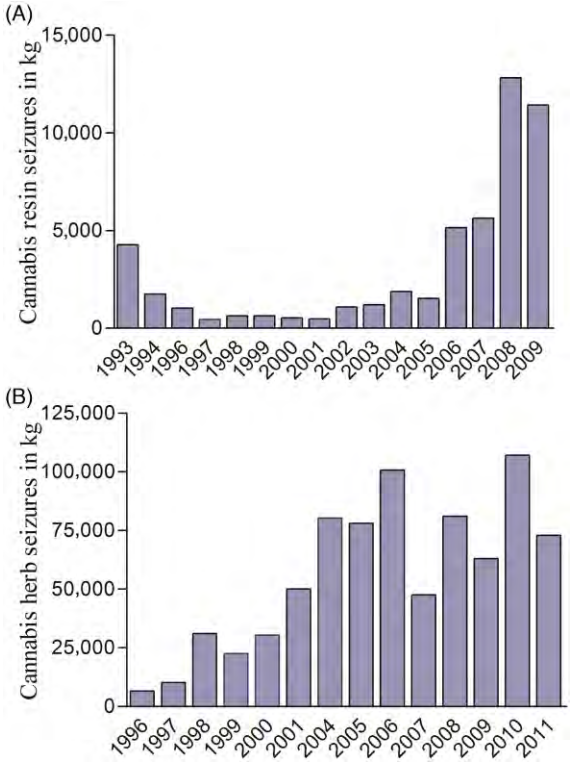


FIGURE 12.2 (A) Cannabis resin (hashish) seizures in Egypt. The columns represent the amount seized in kg during 1998-2009. (B) Cannabis herb (marijuana or bango) seizures in Egypt. The columns represent the amount seized in kg during 1998–2011. *Source: UNODC, 1999, 2000, 2006, 2007, 2008, 2009, 2011; UNODC, 2006, 2007, 2008, 2009, 2011, 2012, 2013.*

markedly since 1998. This might indicate a growing market demand. The rise in seizures in North African countries in 2006, despite the decline in cannabis production in Morocco, might also reflects growing shipments of cannabis resin from Morocco toward Egypt and other countries in North Africa (UNODC, 2008) (Fig. 12.2). Abdel Aal, Abdel Maaboud, and Aly, (2011) found that the percentage of seizures increased in Upper Egypt governorates during the period from 2003 to 2009. Cannabis, as a plant or hashish, was the most widely seized drug. The quantity of seized cannabis plants exceeded that of hashish. In this context, it should be noted that it is generally agreed that drug seizures represent a fifth of drug use in society (Okasha, 2004).

PREVALENCE OF FACTORS ASSOCIATED WITH, AND AFFECTING, CANNABIS ABUSE

In the 1980s, estimates of substance addiction showed that the most commonly abused drug was cannabis. This was followed by opium, solid and liquid hypnosedatives, heroin, and finally cocaine (Okasha, 1996). In 1982, hashish constituted 91% of the mass of drug dealing in Egypt, while opium and its derivatives did not exceed 7% (Darweesh, 1982). Soueif, Yunis, & Taha (1986) indicated that, apart from alcohol and tobacco, cannabis remained the most widely abused psychoactive substance. This was based on a series of large scale field surveys conducted since 1957. The authors reported that 30% of male industrial workers, and 20% of male students, had used drugs at some point in their lifetimes, and that approximately 25% became chronic users. In 1985, Okasha reported that 34% of University students in Egypt, who had succeeded in their studies, and 42% of those who had failed, used drugs. In the 1990s, there was an exponential increase in synthetic psychoactive drug use, these becoming the third most commonly available drug following cannabis and alcoholic beverages (Okasha, 1996, 2004). One study found that 21.4% of experimenters in secondary schools admitted continuing to take synthetic drugs (Soueif et al., 1990). But toward the second half of the same decade, marijuana (bango) became the most prevalent form of cannabis abused (Okasha, 1996, 2004). Studying drug abuse as a risk factor affecting juvenile delinquency showed that 12.7% had cannabinoids on urine testing (Moustafa, El-Defrawi, Kheder, & Hagras, 2001).

In a recent study on the lifetime prevalence of alcohol and substance use in Egypt, cannabis was the most misused drug, while alcohol was a distant second. The sample comprised 44,000 subjects from eight governorates. The lifetime prevalence of any substance abuse varied between 7.25 and 14.5%. Substance abuse was more prevalent in males (13.2%) compared with females (1.1%). Youth (15–19 years), males of Bedouin origin, seaside governorates, low levels of education, and certain occupations experienced far more substance abuse (Hamdi et al., 2013).

In a study on 457 patients with drug dependence (from the neuropsychiatry department in Tanta University Hospital), the majority of addicts were males (70.0%), aged 20–40 years (81.4%), from urban areas (75.7%), current smokers (95.6%), not married (single, divorced, and widowed) (70.0%), and of low educational level (El-Sawy, Abdel Hay, & Badawy, 2010). In a study on hired hospital cleaners, substance abuse, particularly tramadol and marijuana (bango), was significantly higher in temporary compared to permanent cleaners. Urine-based screening tests were positive for tramadol (39.7% vs 20.6%), amphetamine (1.2% vs 0.96%), marijuana (bango) (35.9% vs 24.4%), morphine (8.7% vs 4.3%), and benzodiazepines (17.8% vs 14.8%). The study also suggested that being unmarried, of male sex, with family history of mental disorder, aged ≥40 years, smoking, and with length of service of ≥8 years, were the factors associated with substance abuse (Abbas, Hammam, El-Gohary, Sabik, & Hunter, 2013).

In a survey on drug use in young people (15–29 years), 1.5% had tried drugs, representing 2.7% of all male

respondents, and 0.1% of all female respondents. These figures were probably underreported because more respondents reported that their family members had tried drugs (2.9% overall, 3.6% male, and 2.1% female), and even more reported that their friends had tried drugs (5.3% overall, 9.6% male, and 0.8% female) The most commonly used drug was hashish, which 89.2% tried, followed by marijuana (bango) (23.1%), pills (10.3%), stimulants (3.1%), and sedatives (3.2%) (SYPE, 2011).

CANNABIS ABUSE IN SELECTED POPULATION GROUPS

Cannabis Use in Patients with Drug Overdose

Studies among patients admitted to the Mansoura emergency hospital (Eastern Nile Delta) showed cannabis to be the most commonly abused drug. In a study conducted on 150 patients presenting with drug overdose over the period of 2002–2004, AbouEleinen, Mostafa, Ghanem, Elnaggar, and Elbayomy (2008) reported the presence of cannabis in urine samples of 68% of cases (followed by opiates, and barbiturates, in 42.7% and 26.7% of samples, respectively). The diagnosis of polysubstance abuse was more prevalent than single substance abuse. In 2010, Ghanem, Abdel Rahman, Mandour, and Attia, (2010) detected the presence of substance abuse in 65% and 43.8% of urine samples from 399 patients with drug overdosage. Fifty-six percent of these patients were aged between 20 to 30 years. Cannabis accounted for 16.1%, benzodiazepines for 10.2%, opiates for 8.5%, and barbiturates for 9% of samples, respectively. Amr, El-Gilany, El-Mogy, and Fathi (2014) examined 1317 patients attending the same hospital, and found cannabis to be the most common drug of abuse (3.6% of patients); being of young age (<30 years), male and single, with unsatisfactory income, or having a psychiatric illness (mainly anxiety disorders in 60.7%) were predictors of drug abuse. Tramadol and polysubstance abuse followed with a prevalence of 1.8 and 1.9%, respectively. Substance abuse was present in 63.8% of those with low educational level (below secondary school) versus 31.7% in those with a higher level of education.

Cannabis Use in School and University Students

Cannabis abuse is a problem among school and University students. Alcoholic beverages were used by 18.4% of high school students, hashish and opium by 36%, and psychiatric drugs (tranquilizers 51%) by 5.2%. Hashish abuse accounted for 81.4% of natural drug abusers (Darweesh, 1982). One study that included 14,656 male secondary school students showed that users tended to see drug consumption as either harmless or even useful, and that significantly more urban than rural students smoked tobacco, and tried cannabis and alcoholic beverages. Arts students were more likely to smoke tobacco, ever use prescription psychotropic substances, narcotics, and alcohol, compared with their science and mathematics studying counterparts (Soueif et al., 1990). In another survey, 5.1% of male technical secondary schools students have used natural narcotics at some time in their lives. The respondents ranked cannabis as the most abused (84.3%) or coabused with opium (3.4%). Age onset was 16 years for all natural narcotics. Regarding continuing or ceasing abuse, 20.8% admitted to continue abuse, while 74% stopped after the first trial for different reasons; the most important factor was a general consensus that these substances had an overall damaging effect (51.1%). Other factors were religious (11.8%), financial (9.4%), and social (6%) factors (Soueif et al., 2000).

In a study among students of secondary schools in Assuit province (south of Cairo), 11.6% tested positive for cannabis in urine; being a male, higher income, large families, and parental problems were among risk factors in this group. Cannabis addiction was reflected in decreased school activities, and increased aggressive behavior among students. The same study reported cannabis abuse in 12% of a sample of drivers, especially the younger aged (21–41 years). In students and drivers, cigarette smoking played an important role as a gateway for cannabis abuse; those who abused cannabis were also shisha smokers (Yassa, Dawood, Shehata, Abdel-Hady, & Abdel-Aal, 2009).

Studies among male University students showed that cannabis remained the most widely abused psychoactive substance, where 9.05% of students had tried it at least once. Abusers started experimentation at 18–20 years of age. The first use of cannabis most often occurred during social occasions, or in the company of friends. In all cases of "ever using" cannabis, those who stopped the practice (81.42%) were mainly motivated by fear of expected physical as well as psychological harm (38.61%). Another factor was religious prohibition of these substances, while fear of legalization was almost an ineffective reason. Approximately 30% continued cannabis consumption for pleasure and euphoria, and only 9.3% continued abuse to cope with social stress, while 5.23% continued as a result of addiction (Soueif, 1995).

Another study by Refaat (2004) found that 6.8% of University students had tried marijuana, and 2.5% were current users of marijuana. ElShair & Shafik (2012) found that one-quarter of student hostels in Cairo University were smokers who showed significantly higher use of shisha, cannabis (bango), and alcohol, compared with their nonsmoker counterparts. AbouSeif, AbouElezz, Elgaafary, Ibrahem, and El Sotohy (2011) reported drug addiction in 7.9% of 419 students from Ain Shams University (Cairo). Smoking and alcohol intake were present in 20.9% and 11.6%, respectively.

TABLE 12.1 Prevalence of Drug Abuse in Different Studies

Category	Prevalence (%)	Study
Industrial workers	30	Soueif et al. (1986)
Students	20	Soueif et al. (1986)
University students	42	Okasha (1985)
University students	2.5–6.8	Refaat (2004)
Experimenters in secondary schools	21.4	Soueif et al. (1990)
Juvenile delinquents	12.7	Moustafa et al. (2001)
General population	7.25–14.5	Hamdi et al. (2013)
High school students	36	Darweesh (1982)
Secondary school students	5.1	Soueif et al. (1990)
Secondary school students	11.6	Yassa et al. (2009)
Drivers	12	Yassa et al. (2009)
University students	9.05	Soueif (1995)
University students	7.9	ElShair & Shafik, (2012)
Psychiatric patients	26	Asaad et al. (2003)
Patients with organic erectile dysfunction	16.8	El-Bendary et al. (2009)

The table shows the proportion of those who have abused drugs reported by different studies. The drugs abused were mostly cannabis.

Cannabis Use in Psychiatric Patients

In a study on 100 schizophrenic outpatients (Ain Shams University Psychiatric Institute, Cairo), drug abuse was prevalent in 26% of patients; antiparkinsonian drugs ranked first, followed by cannabis, opioids, and benzodiazepines. The authors found no significant association between drug abuse and schizophrenia subtype or neuroleptic drug dosage. In contrast, male gender, high level of depression, smoking, and premorbid personality were strong risk factors (Asaad, Okasha, El-Khouly, & Abdel Azim, 2003). Another study on 120 inpatients of the addiction center and outpatients of the psychiatric clinic (Menoufia University Hospitals) found tramadol the most prevalent drug (20%). Other important drugs were cannabis (10%), sedative tablets (10%), and alcohol (10%), while polysubstance abuse accounted for 38.3% (Mohamed et al., 2013). Psychiatrically ill patients who abused cannabis exhibited worsened symptoms of schizophrenia, major depressive disorder, and generalized anxiety disorder, as well as decreased quality of life, compared with those patients not abusing cannabis (Sehlo, 2008).

Cannabis Use in Patients with Other Medical Conditions

The use of recreational drugs was reported in 16.8% of Egyptian patients (<40 years) complaining of organic erectile dysfunction; marijuana (bango) being the most common (8.8%), followed by hashish (4.1%), street drugs (codeine containing antitussives, and narcotic analgesics, eg, tramadol). In this sample, smoking and the use of recreational drugs were the most significant risk factors for the erectile problem (El-Bendary, El-Gamal, & Salem, 2009). Among 109 drug addicts at Abbasia chest hospital (Cairo), Saeed, Riad, & Kamel (2011) found cannabis the most commonly abused drug (67% of abused substances). Cigarette smoking was frequent in all patients (Tables 12.1 and 12.2).

GENDER AND DEMOGRAPHIC DIFFERENCES IN CANNABIS ABUSE

In a survey made in 2000, hashish abusers constituted 61.5% and 80.5% of the total male drug users in Lower and Upper Egypt, respectively. Meanwhile, 19.3% and 3.7% tended to use marijuana (bango) in Lower and Upper Egypt, respectively (together with other substances, such as opium or heroin), indicating the high prevalence and preference for hashish. Females constituted 17% and 35.6% of hashish users in Lower and Upper Egypt, respectively. In Lower Egypt, most drug abusers were males (83%), the majority of male abusers (64.3%) were 18–40 years old, while 26.6% fell in the age group 40–55 years. On the other hand, 82.1% of female abusers in Lower Egypt were in the age group of 18–40 (Abdel-Gawad, 2000). In Upper Egypt, there were less female abusers reported, where the culture plays a role in starting or experimenting with abuse. Low prevalence

TABLE 12.2 Factors Associated With Cannabis Abuse

Category	Factor	Study
Students	Unhealthy family environment	Soueif et al. (1990)
Students	High monthly income	Soueif et al. (1990)
Students	High daily funds	Yassa et al. (2009)
Students	Family problems (eg, divorce)	Yassa et al. (2009)
Students	Large families	Yassa et al. (2009)
University students	Coping with psychological or physical discomfort, or stress-triggering situations	Soueif et al. (1986)
Students	Entertainment on happy social occasions	Soueif (1994)
Workers	Difficult working conditions	Soueif (1994)
Subjects attending outpatient neuropsychiatry clinic	Coping with anxiety disorders or depressive disorders.	El-Sawy et al. (2010)

Studies point to social and economic factors as important factors associated with cannabis abuse.

of drugs among females is mainly attributed to society's values, together with higher exposure of males to drug during marriage celebrations, which constitute a major circumstantial cause of engagement of men in drug abuse (Hegazy, 1994).

El-Sawy, Abdel Hay, and Badawy, (2010) found that males started drug abuse at an earlier age than females, with a longer duration of addiction, and that single males were more vulnerable to abuse than females. Gad, El-Dod, Eissa, El-Sawi, and El-Fadaly (2004) found that female marijuana addicts were similar to males in causes of first use, negative consequences, and mood disorders, except that cognitive decline was higher in males than females. Demographics play such an important role in the discrepancy of drug abuse trends because of the kind of schools, financial level, and clubs availability are important factors in limiting the spread of drugs in the countryside among the young (Albaghdady, 2002).

Soueif and Abdel-Monem (2004) found that the percentage of males who abused cannabis was higher in urban (55.3%) than in rural areas (44.4%). The percentage increased in married persons, rather than in single ones. The authors suggested that marriage involves some kind of hardship. It involves new events and responsibilities that might make married couples more prone to abuse. Skillful workers and commercial workers constituted the highest percentage of abusers (45.3%), whereas the number of agricultural workers who abuse cannabis was significantly lower (23.8%). Finally, cigarette smoking appeared to increase the likelihood of drug abuse, especially at early ages, as 79.7% of abusers were intensive cigarette smokers (of which 18.4% smoke 40 cigarettes or more), compared to 49% of nonabusers. Smokers were thus more vulnerable to drug culture than nonsmokers (Fig. 12.3)

INITIATION OF DRUG/CANNABIS ABUSE

Marijuana use typically starts in adolescence. The mean age of first use in the 1993 survey done among the Egyptian population was 16 years, among them 11.2% reported using cannabis only, while 12.4% used alcohol, and 32.7% turned to psychiatric drugs, where 26% of them preferred to use sedatives and tranquilizers. In the survey performed in 2000 on a representative sample of general population, the mean age of starting abuse was 19.8 ± 6.9 years for the male population in Lower Egypt, and 21.6 ± 6.7 years for males in Upper Egypt. Meanwhile, the females in Lower Egypt usually started drug abuse at the age of 22.3 ± 6.8 years (Soueif et al., 1990).

The drug experience usually begins out of curiosity, in addition to peer-pressure, which plays a major role. Obtaining the information about drugs and their effects through schoolmates, friends, and relatives increases the urge to undergo the experience. Furthermore, giving the sons a high monthly stipend, besides the prevalence of smoking as a socially accepted behavior, encourages the experimentation of drugs, especially cannabis, in the form of smoked marijuana (bango) joints commonly found between teenagers (Soueif et al., 1990). Social occasions or the company of friends mostly accounted for the first use of cannabis, but coping with psychological or physical discomfort, or stress-triggering situations were the reasons for initiating psychotropic substance use (Soueif et al., 1986). Entertainment on happy social occasions was the main reason for drug use (mostly hashish) among secondary school students. Sedatives and hypnotics were next to hashish, but used to overcome physical exhaustion, fatigue, and to cope with psychosocial problems, the stress of studying and examinations, or difficult working conditions (Soueif, 1994).

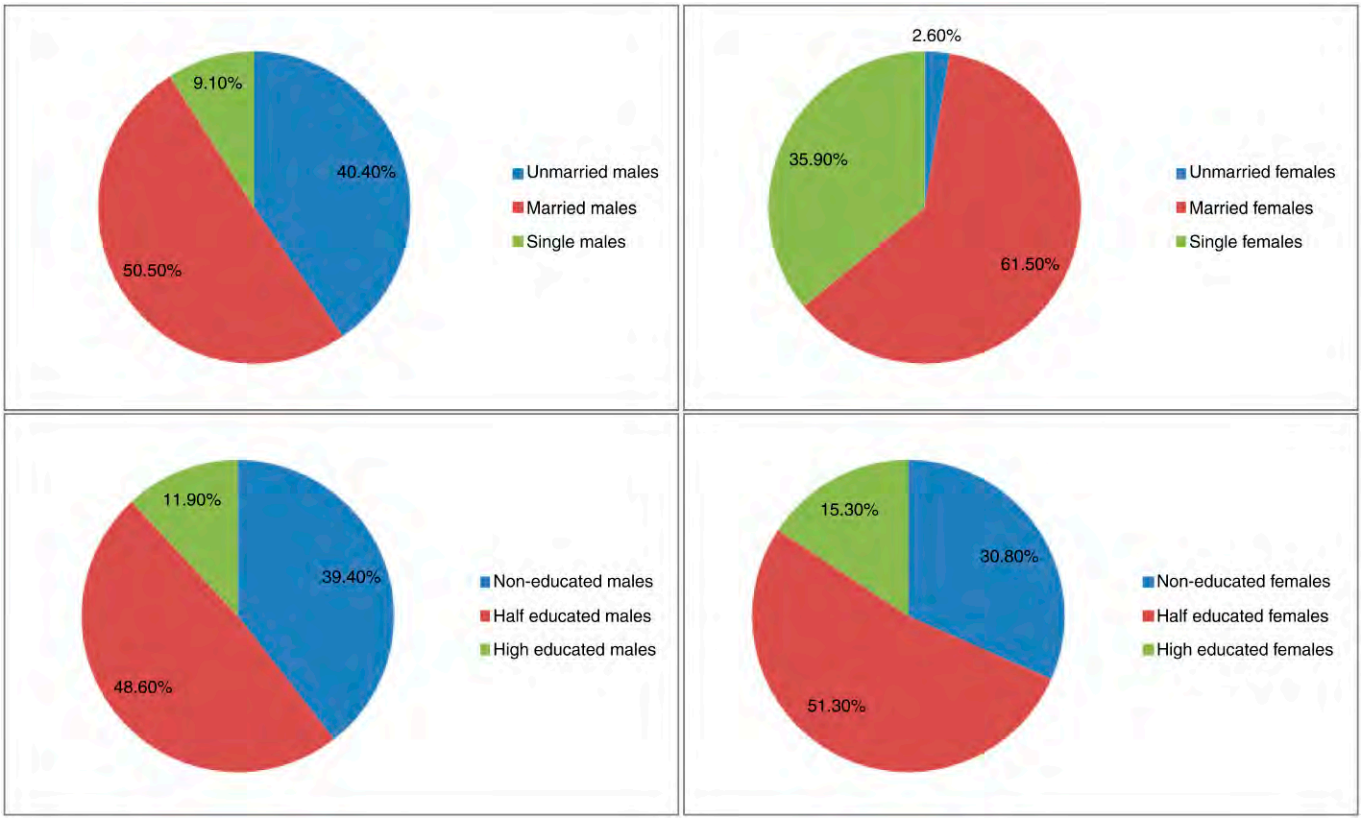


FIGURE 12.3 Relevance of cannabis use to educational level and marital status in Lower Egypt. Cannabis use increases in married and in medium educated subjects. *Source: Data from Hegazy (1994) and Albaghdady (2002).*

Among technical secondary schools students, the first experimentation with narcotics was during social occasions (51.6%) or friends' gatherings (23.6%). Other reasons were satisfying certain motives (experience, imitating, and outlook), and facing psychological stress (Soueif et al., 2000). Among university students, pleasure and euphoria, but also in order to cope with social stress, were the reasons for continuing drug abuse (Soueif, 1995). Other workers found that peer pressure was the most common motivating factor for drug abuse in males, in contrast to family troubles and sexual abuse in females. Other motivations, such as anxiety disorders, were more common in males while depressive disorders were more common in females (El-Sawy et al., 2010). There was also a strong association between exposure to drug culture and drug taking (Soueif et al., 1990) (Table 12.3).

CIGARETTE SMOKING AND CANNABIS

The rate of cigarette smoking among high school students reached 11.4% (16% in urban areas and 8% in rural areas), and 18.4% of technical high school students. The rate of cigarette smoking seemed relatively constant since 1987, which had a ratio of 10.77, and reached 10.8% in 1993 (Soueif, Taha, Gomaa, Makarem, & Amer, 2000). This established fact explains how teenagers and high school students could be easily drawn to cannabis use having the acquaintance of cigarette smoking. Tobacco cigarette smoking was the main entrance to drug abuse, where studies have shown that the percentage of drug addicts among cigarette smokers is higher than that of nonsmokers (Darweesh, 1982).

SOCIAL AND ECONOMIC FACTORS

The social and economic disruptions that took place in the 1990s in Egypt, where certain social categories rose up, together with the deterioration of the economic status and decreased standard of living of other categories, have resulted in alterations of the prevailing values in society, where the moral inherent values were replaced by luxury consumptive values (Helal, 2002). This disruption evoked psychological and social conflicts that encouraged pathological behavioral patterns, such as addiction, and ultimately contributed to increased mobility of the drug market among young people in Egypt (Albaghdady, 2002).

Social studies have also suggested that the economic crisis and the growing unemployment among youth in general, and the well-educated ones in particular, caused negative feelings and frustration that stimulated the

TABLE 12.3 Drug Abuse Pattern Among Different Categories in Egypt

Category	Hashish (%)	Bango (%)	Opiates (%)	Tramadol (%)	Heroin (%)	Stimulants (%)	Sedatives (%)	Alcohol (%)	Study
University students	34.4		7.9	10.8	5.5	11.5	9.6	18.4	Soueif (1995)
High school students	36						5.6	18.4	Darweesh (1982)
Secondary school students		11.6							Yassa et al. (2009)
Cleaning workers (temporary/permanent)	35.9/20.6		8.7/4.3	39.7/20.6	—	1.2/0.96	17.8/14.8	—	Abbas et al. (2013)
Farmers	17.1		2.2	1.7	—	—	—	11.5	Oda, El Boghdady, El Husseiny, El Sayed, & Fathallah (2011)
Addicts		10		20			10	10	Mohamed et al. (2013)
Slums	19.4	93.8	6.6	42.7	2.8	4.3	22.3	19.4	DCTAF (2002)
Juvenile delinquents	35.6		12.5	60.1	6	3.9	8.5	—	NCATP (2002)
Patients with drug overdose	68		42.7				26.7		AbouEleinen et al. (2008)
Patients with drug overdose	16.1		8.5				10.2		Ghanem et al. (2010)
Patients with drug overdose	3.6			1.8					Amr et al. (2014)

Hashish and bango (marijuana) account for high proportion of abused drugs, though tramadol abuse is increasingly growing.

resort to another world, where dreams come true, and where they seek the absent happiness (Helal, 2002).

Soueif (1995) identified unhealthy family environment as a major factor for hashish addiction. There was also a positive correlation between high monthly income (and consequently high student pocket money) and abuse. High income encouraged families to respond to all financial demands of their children, encouraging them to break ethical standards. Studies among drug abusers and addicts in prisons and hospitals revealed a correlation between drug abuse and some family problems, such as the mother's work, and failure of the father to satisfy the family's needs, thus thinking of drugs as a means of escape (Soueif et al., 1990). Studies have suggested, in addition, an important role for the media in spreading drugs, with drug dealers and takers are portrayed as glamorous and living in luxury while negative effects are ignored (Hegazy, 1994).

MINI-DICTIONARY

Chibouque A Turkish tobacco pipe with a mouthpiece often made of amber
Feddan Land unit that equals about 4200 square meters.
Goza A water pipe where the tobacco smoke passes through water prior to inhalation.
Hashish or hasheesh The dried resinous exudate of the tops of the cannabis plant.
Lower Egypt Northern Egypt, from Cairo and Giza to the Mediterranean Sea.
Marijuana or bango The dried leaves and flowering tops of the cannabis plant.
Shisha A waterpipe that differs slightly in design from goza, with a longer tube.
Upper Egypt South of Cairo and Giza to the border with Sudan.

References

Abbas, R. A., Hammam, R. A., El-Gohary, S. S., Sabik, L. M., & Hunter, M. S. (2013). Screening for common mental disorders and substance abuse among temporary hired cleaners in Egyptian Governmental Hospitals, Zagazig City, and Sharqia Governorate. *International Journal of Occupational and Environmental Medicine*, *4*, 3–26.

Abdel Aal, K. M., Abdel Maaboud, R. M., & Aly, N. S. (2011). Illicit drugs seizures as an indicator of drug abuse pattern in Upper Egypt from 2003 to 2009. *Egyptian Journal of Natural Toxins*, *8*, 58–80.

Abdel-Gawad, E. (2000). Comprehensive survey of the phenomenon of abuse and drug addiction. The first phase exploratory study of prison inmates in the Greater Cairo. National center for social & criminology research, Cairo. *National Criminal magazine*, *43*, 127–165.

AbouEleinen, R., Mostafa, M., Ghanem, A., Elnaggar, E., & Elbayomy, A. (2008). *Psychoactive Drug Use in Toxicology Unit Patients, Mansoura Emergency Hospital, Egypt*. Bethesda, MD: NIDA international program.

AbouSeif, H., AbouElezz, N. F., Elgaafary, M., Ibrahem, J. M., & El Sotohy, M. (2011). Health risk behaviors among university students, Cairo. *Egyptian Journal of Community Medicine*, *29*, 1–11.

Albaghdady, N. (2002). The social perspective studies of the urge to narcotics. In N. Fawwal (Ed.), *Narcotics phenomenon in Egypt, documentary and analytical study of the social researches and studies. 2nd report* (pp. 41–55). Cairo, Egypt: National Center for Social and Criminology Research.

Amr, M., El-Gilany, A. -H., El-Mogy, A., & Fathi, W. (2014). Substance abuse and dependence among patients attending an emergency hospital in eastern Nile delta, Egypt. *African Journal of Psychiatry*, *17*, 532–537.

Asaad, T. A. A., Okasha, T. A., El-Khouly, G. A., & Abdel Azim, K. A. A. (2003). Substance abuse in a sample of Egyptian schizophrenic patients. *Addictive Disorders and their Treatment*, *2*, 147–150.

Badr, K. (1999). Psychoactive substances among male high school students. In M. Soueif (Ed.), *Substance abuse affecting the nerves among general high school students: field studies in the Egyptian reality. 8th volume*. Cairo, Egypt: The permanent program for drug abuse research.

Balabanova, S., Parsche, F., & Pirsig, W. (1992). First identification of drugs in Egyptian mummies. *Naturwissenschaften*, *79*, 358.

Bigelow, J. M. H. (2000). Ancient Egyptian gardens. *Ostracon*, *11*, 1–11.

Breasted, J. H. (1945). *A history of Egypt* (2nd ed. pp. 276–277). New York: Charles Scribner's Sons.

Darweesh, Z. A. (1982). Scientific efforts of the National Center for Social and Criminological Research on the problem of drug abuse in Egypt. In *Conference training seminar for teachers about the problems of drug use among students and educational methods to address them, February 13, 1982* (pp. 26). Cairo, Egypt: National Center for Educational Research.

Drug control and treatment of addiction fund. (2002). *Drug abuse in slums: field study of the area of Sharabeyya* (1st ed.). Cairo, Egypt: Drug control and treatment of addiction fund, 85–130.

El-Bendary, M. A., El-Gamal, O. M., & Salem, K. A. (2009). Analysis of risk factors for organic erectile dysfunction in Egyptian patients under the age of 40 years. *Journal of Andrology*, *30*, 520–524.

El-Sawy, H., Abdel Hay, M., & Badawy, A. (2010). Gender differences in risks and pattern of drug abuse in Egypt. *Egyptian Journal of Neurology, Psychiatry and Neurosurgery*, *47*, 413–418.

ElShair, I. H., & Shafik, S. A. (2012). Health instructions to control tobacco use among Cairo University students hostels. *Journal of American Science*, *8*, 360–370.

Gad, E-S. A., El-Dod, A. E., Eissa, M. A., El-Sawi, H. E-D. F., & El-Fadaly, N. H. (2004). Gender difference in Bhang dependence in Egypt. *Egyptian Journal of Neurology, Psychiattry and Neurosurgery*, *41*, 171–182.

Ghanem, A. A., Abdel Rahman, R. H., Mandour, R. A., & Attia, A. M. (2010). Detection of some substances of abuse during daily practice in emergency hospital, Mansoura University. *Mansoura Journal of Forensic Medicine and Clinical Toxicology*, *XVIII*, 65–79.

Hamarneh, S. (1972). Pharmacy in Medieval Islam and the history of drug addiction. *Medical History*, *16*, 226–237.

Hamdi, E., Gawad, T., Khoweiled, A., Sidrak, A. E., Amer, D., Mamdouh, R., Fathi, H., & Loza, N. (2013). Lifetime prevalence of alcohol and substance use in Egypt: a community survey. *Substance Abuse*, *34*, 97–104.

Hegazy, A. M. (1994). *Drugs in contemporary Egyptian reality*. Cairo, Egypt: Culture house for publications.

Helal, A. (2002). Psychological studies of the urge to drug abuse. In N. Fawwal (Ed.), *Narcotics phenomenon in Egypt, documentary and analytical study of the social researches and studies. 2nd report* (pp. 59–75). Cairo, Egypt: National Center for Social and Criminology Research.

Iversen, L. L. (2000). *The science of marijuana* (pp. 22–23). New York, NY: Oxford University Press.

Kalant, H. (2001). Medicinal use of cannabis: history and current status. *Pain Research and Management*, *6*, 80–91.

Leake, C. D. (1952). *The old Egyptian medical papyri, Logan Clendening lectures on the history and philosophy of medicine. Second series*. Lawrence, KA: University of Kansas Press.

Mohamed, N. R., Hammad, S. A., El Hamrawy, L. G., Rajab, A. Z., El Bahy, M. S., & Soltan, M. R. (2013). Dual diagnosis and psychosocial correlates in substance abuse in Menoufia, Egypt. *Menoufia Medical Journal*, *26*, 114–121.

Moustafa, N. M., El-Defrawi, M. H., Kheder, S., & Hagras, A. (2001). Risk factors related to juvenile delinquency in Egypt during the year 1998-1999. *Suez Canal University Medical Journal*, *4*, 75–85.

Nahas, G. G. (1982). Hashish and Islam: 9th to 18th century. *Bulletin of the New York Academy of Medicine*, *58*, 814–831.

Nahas, G. G. (1985). Hashish and drug abuse in Egypt during 14th-20th century. *Bulletin of the New York Academy of Medicine*, *61*, 428–444.

National Council for Addiction Treatment and Prevention. (2002). *Study of juvenile abusers in juvenile crime establishments* (pp. 12–84). Cairo, Egypt: National Council for Addiction Treatment and Prevention.

Oda, M., El Boghdady, N., El Husseiny, R., El Sayed, Y., & Fathallah, A. (2011). *Drug abuse in Egyptian rurals: a field study in some villages*. Cairo, Egypt: National Council for Addiction Treatment and Prevention, 83-L 110.

Okasha, A. (1985). Young people and the struggle against drug abuse in the Arab countries. *Bulletin on Narcotics*, *37*, 67–73.

Okasha, A. (1996). Substance use is a major public health hazard. In *Proceedings of the first Egyptian international conference on addiction and drug abuse*. Cairo: Ministry of Health.

Okasha, A. (2004). Focus on psychiatry in Egypt. *British Journal of Psychiatry*, *185*, 266–272.

Rosenthal, F. (1971). *The herb: hashish versus medieval Muslim society*. Leiden, the Netherlands: E.J. Brill.

Parsche, F., & Nerlich, A. (1995). Presence of drugs in different tissues of an Egyptian mummy. *Fresenius' Journal of Analytical Chemistry*, *352*, 380–384.

Peters, H., & Nahas, G. G. (1999). A brief history of four millennia (BC 2000–AD 1974). In G. G. Nahas et al.,(Ed.), *Marihuana and medicine* (pp. 3–7). Totowa, NJ: Humana Press.

Peterson, F. (1893). *Medical notes in Egypt*. New York, NY: M.J. Rooney.

Raman, A. (1998). The Cannabis Plant: Botany, Cultivation, and Processing for Use. In D. T. Brown (Ed.), *Cannabis, the genus Cannabis* (pp. 29–54). Amsterdam, the Netherlands: Harwood Academic Publishers.

Refaat, A. (2004). Practice and awareness of health risk behaviour among Egyptian university students. *Eastern Mediterranean Health Journal*, *10*, 2–81.

Roffman, R. A., Schwartz, S., & Stephens, R. S. (2006). Themes in the history of cannabis dependence. In R. A. Roffman, & R. S. Stephens (Eds.), *Cannabis dependence: its nature, consequences, and treatment* (pp. 3–20). New York, NY: Cambridge University Press.

Saeed, A., Riad, N., & Kamel, M. (2011). Study of respiratory problems of addiction among patients at Abbasia chest hospital. *Egyptian Journal of Bronchology*, *5*, 131–141.

Sehlo, M. (2008). Evaluation of cannabis abuse effect in a sample of Egyptian psychiatric ill patients. *Current Psychiatry*, *15*, 95.

Soueif, M. I. (1994). *Extent and patterns of drug use among students and working class mMen in Egypt*. Cairo, Egypt: National Centre for Social and Criminological Research.

Soueif, M. (1995). Natural narcotics abuse among male students: an overview. In *Psychoactive substances abuse among male Universities students: a field study in Egyptian society* (pp. 63–109). Cairo, Egypt: National Centre for Criminal and Social Research.

Soueif S M., & Abdel-Monem, E. (2004). Psychoactive drugs abuse: an overview. In *Psychoactive substances abuse among countryside Egyptians: an exploratory study* (pp. 21–27). Cairo, Egypt: National Centre for Criminal and Social Research.

Soueif, M. I., Yunis, F. A., & Taha, H. S. (1986). Extent and patterns of drug abuse and its associated factors in Egypt. *Bulletin on Narcotics*, *38*, 113–120.

Soueif, M. I., Taha, H., Gomaa, M., Makarem, F. A., & Amer, A. (2000). *Substance abuse affecting the nerves between students of general high schools and students of high technical schools. Comparative studies on field basis. 10th volume*. Cairo, Egypt: The permanent program for drug abuse research.

Soueif, M. I., Youssuf, G. S., Taha, H. S., Moneim, H. A., Sree, O. A., Badr, K. A., Salakawi, M., & Yunis, F. A. (1990). Use of psychoactive substances among male secondary school pupils in Egypt: a study on a nationwide representative sample. *Drug and Alcohol Dependence*, *26*, 63–79.

Population council, West Asia and North Africa office. (2011). *Survey of young people in Egypt. Final report* (pp. 30–32). Cairo, Egypt: The Population Council.

UNODC. (1999). *World Drug Report*. Vienna, Austria: United Nations Publications, Sales No. E.99.XI.16.

UNODC. (2000). *World Drug Report*. Vienna, Austria: United Nations Publications.

UNODC. (2006). *World Drug Report*. Vienna, Austria: United Nations Publications, Sales No. E.06.XI.10.

UNODC. (2007). *World Drug Report*. Vienna, Austria: United Nations Publication, Sales No. E. 07.XI.5.

UNODC. (2008). *World Drug Report*. Vienna, Austria: United Nations Publications, Sales No. E.08.XI.1.

UNODC. (2009). *World Drug Report*. Vienna, Austria: United Nations Publications, Sales No. E.09.XI.12.

UNODC. (2011). *World Drug Report*. Vienna, Austria: United Nations Publications, Sales No. E.11.XI.10.

UNODC. (2012). *World Drug Report*. Vienna, Austria: United Nations Publications, Sales No. E.12.XI.1.

UNODC. (2013). *World Drug Report*. United Nations Publication: Vienna, Austria, Sales No. E.13.XI.6.

Wills, S. (1998). Cannabis use and abuse by man: an historical perspective. In D. T. Brown (Ed.), *Cannabis: the genus Cannabis* (pp. 1–28). Amsterdam: Harwood Academic Publishers.

World Bank. (2012). *World Bank Report*. Washington, DC: The World Bank Group.

World Health Organization. (2005). *Mental health atlas*. Geneva, Switzerland: WHO, 176.

Yassa, H. A., Dawood, Ael-W., Shehata, M. M., Abdel-Hady, R. H., & Abdel-Aal, K. M. (2009). Risk factors for bango abuse in Upper Egypt. *Environmental Toxicology and Pharmacology*, *28*, 397–402.

Zimmer, L. (1997). Chapter one: the history of cannabis prohibition. In L. Böllinger (Ed.), *Cannabis science: from prohibition to human right* (pp. 15–34). New York: Peter Lang.

CHAPTER

13

Cannabis Body Packing: A Caribbean Perspective

S.O. Cawich, D. Dan, V. Naraynsingh

Department of Clinical Surgical Sciences, University of the West Indies, St Augustine Campus, St Augustine, Trinidad and Tobago

SUMMARY POINTS

- This chapter gives an overview of cannabis body packing, including its consequences and complications.
- There has been a change in the demographics of the body packer in the Caribbean, with the majority of packers being young males.
- The practice brings significant potential earnings, with earnings of up to US$10,000 per successful trip.
- There are several potential complications recorded, including bowel obstruction, intestinal perforation, and potential mortality, although no deaths from acute toxicity have been reported.
- There is an urgent need for legislation to be developed to protect healthcare workers in these environments when treating body packers.

KEY FACTS OF CANNABIS BODY PACKING IN THE ANGLOPHONE CARIBBEAN

- The Anglophone Caribbean consists of 17 English-speaking nations in the Caribbean basin, with a cumulative estimated population of 6,426,914 persons.
- All Anglophone Caribbean nations adhere to the United Nations Convention on Narcotic Drugs (1961) that requires the government to criminalize cannabis.
- To meet the demand for the drug in developing countries smuggling is commonplace.
- Body packing is a common method of smuggling cannabis across international borders, and is epidemic in the Caribbean.
- The average body packer transports 1 kg of a drug, and can earn up to US$10,000 for each successful trip.
- The practice is accompanied by several potential complications, including intestinal obstruction, and colonic perforation.

LIST OF ABBREVIATION

Δ^9-THC Δ^9-Tetrahydrocannabinol

INTRODUCTION

Cannabis consumption is commonplace across the globe. It has been estimated that up to 40% of persons in the Caribbean have used some form of cannabis, with usage transcending all gender, social, economic, and educational groups (Jefferson, Harding, Cawich, & Jackson-Gibson, 2013; Boekhout van Solinge, 1996). It is commonly used to induce euphoria (Iversen, 2003; Chait & Pierri, 1992), promote meditation (Jefferson et al., 2013), heighten perception and cognition (Hollister, 1986), increase visual acuity (Jefferson et al., 2013), enhance physical performance (Hollister, 1986), temper anxiety (Jefferson et al., 2013), and relieve chronic pain (Hohmann & Suplita, 2006; Mackie, 2008).

Although there has been a thrust to legalize cannabis over the past few decades, it is still considered illegal

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00014-4
Copyright © 2017 Elsevier Inc. All rights reserved.

in many nations across the globe. The high demand for cannabis and simultaneous prohibition of its distribution by most legal systems fuels the illegal drug trade, and increases the opportunities for smuggling (Cawich, Williams, Simpson, Evans, & Johnson, 2008; Traub, Hoffman, & Nelson, 2003; East, 2005).

BODY PACKING AS A MEANS OF SMUGGLING

A common method of drug transportation is for smugglers to ingest packages containing contraband drugs in order to avoid detection by authorities (Cawich et al., 2008; Traub et al., 2003). Most ingest multiple packages, usually averaging about one kilogram net weight (Traub et al., 2003). In the early years, crude packaging methods were encountered, including plastic wrap (Traub et al., 2003; East, 2005), aluminum foil (Cawich et al., 2008), and condoms (East, 2005). But as the trade matured, we have encountered more sophisticated packaging in our practice in the Caribbean (Fig. 13.1).

Several names have been ascribed to this practice including "body stuffing," "body packing," or internal concealment. The smugglers are commonly called "body packers," "swallowers," or "drug mules." The packers transport their cargo to the delivery points, often using commercial transportation (Cawich et al., 2008). Upon arrival at their destination, laxatives, cathartics and/or enemas are used to expedite delivery of the cargo (Cawich et al., 2008; Traub et al., 2003; East, 2005).

DEMOGRAPHICS OF THE DRUG TRADE

The tropical climate in the Caribbean and Latin America is conducive to outdoor growth of cannabis. Although it is consumed in these developing nations, cannabis has significantly greater resale value in developed countries. Therefore, there is a steady stream of cannabis and other illegal drugs flowing toward developed countries in North America and Europe (Cawich et al., 2008). The developing countries in the Caribbean and Latin America make ideal transshipment points (East, 2005; Simon, 1990; De La Haye, 2004a; Cawich, Valentine, Evans, Harding, & Crandon, 2009; De La Haye, 2004b) along this drug stream because they offer opportunities for: (1) restocking/refueling when traversing long distances, (2) convenient redistribution of product, (3) increased availability of packers, and (4) reduced detection if packers are seemingly originating from "safer" destinations.

Although they are encountered across the globe, most packers originate from underdeveloped countries with high poverty rates (East, 2005). The practice has reached epidemic proportions in the Caribbean, as evidenced by the British High Commission's report that there are approximately 30 body packers traveling on every flight from Kingston to London (Cawich et al., 2008), and the

FIGURE 13.1 **Multiple types of packaging from pellets extracted from drug packers.** The figure demonstrates the range of packaging used by body packers.

United States Drug Enforcement Agency's report that Jamaica alone is responsible for 30% of cocaine smuggled into the United States (Cawich et al., 2009a).

Several factors contribute to the popularity of body packing in the Caribbean. Many of the territories are classified as low- or middle-income countries, and they do not have sufficient resources to devote to continuous drug surveillance. Many are underpopulated, or have their populations concentrated in clusters, leaving abundant isolated land space for unimpeded drug-related activities. Additionally, Latin American and Caribbean countries are notorious for administrative corruption (Collier, 2002) and gang activity, making the atmosphere ripe for drug-related activities.

These countries have a large population of impoverished persons, with high levels of unemployment (Cawich et al., 2008). The drug trade organizations target these individuals using the lure of enormous financial rewards (Cawich et al., 2008; Traub et al., 2003; East, 2005; Simon, 1990; De La Haye, 2004a). For obvious reasons, accurate figures are not available, but it has been reported that body packers can earn between US$2,000 and US$10,000 for one successful trip (Cawich et al., 2008; De La Haye, 2004a; Cawich et al., 2009a; De La Haye, 2004b). The lure of rapid, large earnings is sufficient for impoverished individuals to volunteer as packers, despite the knowledge of legal consequences if caught (Cawich et al., 2009a).

DEMOGRAPHICS OF THE BODY PACKER

The typical demographics of body packers have been extensively researched. The information is commonly used by antidrug task forces to facilitate passenger profiling in order to select suspicious individuals at airports and borders, for detailed searches (Cawich et al., 2009a).

In the latter 20th century, the majority of the detained body packers were single females from underprivileged backgrounds, usually with multiple dependents (Traub et al., 2003; East, 2005; De La Haye, 2004a). Once drug traffickers appreciated that authorities were utilizing passenger profiling as one means of detecting body packers, they responded by hiring packers who did not fit these profiles. We then saw a change in packer demographics in the Caribbean, with more young men (mean age 34 years) becoming body packers (Cawich et al., 2009a). Male offenders have now become significantly more prevalent, with a 4:1 male to female ratio (Cawich et al., 2009a).

LEGAL CONSIDERATIONS

Body packing is an illegal practice. Even the body packers are aware of the potential of legal consequences, including incarceration (Cawich et al., 2008). However, they may choose to take the risk because they view the likelihood of being detected lower that the potential for large earnings after they deliver the drug. Antidrug task forces are fully aware of this practice, and maintain surveillance programs at many points of emigration, in an attempt to stem trafficking (Lancashire, Legg, Lowe, Davidson, & Ellis, 1988). They heavily publicize all antidrug trafficking efforts, in an attempt to make the population aware of their activity, and of the likelihood of detection (Cawich et al., 2009a). This resulted in a consistent decline in the number of body packers detected at ports of origin in Caribbean countries, as well as their destination in countries at the turn of the 21st century (Cawich et al., 2009a).

On the other hand, many packers are aware that the impact of cannabis possession and trafficking may not be taken as seriously as other contraband drugs. To illustrate the point, Wikipedia reported that in several countries possession is "illegal but tolerated" (Wikipedia Reports, 2014). To further illustrate the point, many Caribbean countries have laws that are so antiquated that it may actually be worth the risk even if a packer is caught. Currently, any person convicted of "exporting or taking steps preparing to export ganja" before a Circuit Court in Jamaica is liable to "a fine not less than $500.00" (US$5.65 equivalent) per ounce of marijuana, "or imprisonment for a term not exceeding 35 years," Laws of Jamaica; Section 7A;1(b):1994(30)S3b (Government of Jamaica, 2014). Considering that the average drug mule ingests 1 kg (35.27 ounces) of marijuana, the monetary penalty for a convicted marijuana drug mule will only be US$199.28.

CLINICAL CONSIDERATIONS: SYMPTOMATIC PATIENTS

Apart from being illegal, body packing may be dangerous from a clinical perspective. Several complications may occur, including mechanical bowel obstruction from impacted pellets, intestinal perforation, or acute drug toxicity from ruptured packaging. These are all potentially fatal conditions.

Body packing is not unique to cannabis. In fact, reports of cannabis body packing are much less common (Cawich et al., 2010) than with heroin (Jill & Graham, 2002; Hutchins et al., 2000; Sribanditmongkol, Supasingsirpreecha, Thampitak, & Junkuy, 2006; Soriano-Perez, Serrano-Carrillo, Marin-Montin, & Cruz-Caballero, 2009) and cocaine (Traub et al., 2003; East, 2005; Khan, 2005; Simon, 1990; Cawich et al., 2009a). The reason is simple economics. The potential financial return from the same quantity of drug is significantly greater with cocaine and heroin, than it would for cannabis (Cawich et al., 2009a). Consequently, more reports of complications from heroin and cocaine body packing exist.

Acute Toxicity

Although it has become less common with the use of second-generation packaging (Cawich, Williams, Evans, & Johnson, 2009; Pidoto et al., 2002), there is still a risk of acute drug toxicity if the ingested packets rupture, exposing the drug to the large absorptive mucosal surface of the gut. Rupture is potentially fatal when the packages contain cocaine (Jill & Graham, 2002; Koehler et al., 2005; Norfolk, 2007) or heroine (Hutchins et al., 2000; Sribanditmongkol et al., 2006), but we could find no reports of deaths from acute toxicity with cannabis packet rupture, in medical literature. Even in medical literature, many consider cannabis a "soft drug" (Nahas & Latour, 1992) because of the absence of "life-threatening effects no more damaging than coffee or tobacco" (Hollister, 1986).

Package rupture will expose the packer to the psychoactive constituent of cannabis, Δ-tetrahydrocannabinol (THC). This compound produces many central nervous system effects: altered behavior, cognition, perception, and performance (Stadmauer, Beyer, Bardina, & Sicherer, 2003), heightened senses resulting in euphoria with distorted time–space perception (Boekhout van Solinge, 1996), impaired memory, complex problem solving, and motor function (Hollister, 1986; Stadmauer et al., 2003). There are also cardiovascular effects including transient sinus tachycardia and orthostatic hypotension (Jones, 2002; Wu, Tashkin, Djahed, & Rose, 1988). The effect of THC on an individual is unpredictable (Jefferson et al., 2013; Hollister, 1986; Aggarwal et al., 2009). Many factors determine the individual user's response, including the user's baseline personality, current psychological state, previous cannabis use, and the quantity of THC ingested (Hollister, 1986), but there has never been a reported case of fatality that can be directly attributed to THC overdose.

Bowel Obstruction

Bowel obstruction is the commonest complication encountered with cannabis body packing. This occurs when large indigestible pellets become impacted at areas of luminal narrowing, especially at the pylorus and the ileocecal valve (East, 2005). These patients present with cardinal features of mechanical bowel obstruction: colicky abdominal pain, abdominal distention, vomiting, constipation, and/or obstipation.

The diagnosis may be suspected when patients divulge the history of "packing," but it is well recognized that these patients are unreliable and deceptive historians (Cawich et al., 2008). Many cases, therefore, are only detected when obturated pellets are seen on imaging. The pellets are easily detected on plain radiographs in 80–90% of cases (Traub et al., 2003; Hergan, Kofler, & Oser, 2004; Beerman, Nunez, & Wetli, 1986), as demonstrated in Fig. 13.2. Contrast studies and computed tomographic scans (Hierholzer et al., 1995; Marc et al., 1990) are reserved only for cases with diagnostic uncertainty.

FIGURE 13.2 **Plain abdominal radiograph of the abdomen of a confirmed cannabis body packer.** This X-ray shows the classic findings seen in body packing: multiple elongated sausage shaped radioopacities with regular, smooth, rounded edges.

Mechanical obstruction from pellet obturation should be treated operatively. The pellets are extracted through enterotomies by a laparoscopic or open approach. Many authorities frown on the prospect of endoscopic retrieval (East, 2005; Mandava, Chang, & Wang, 2011) since there is a greater risk of package rupture when they are handled with endoscopic instruments. While there are no documented cases of deaths from THC toxicity, there is no way to tell what the pellets contain until they are retrieved and forensically examined. And, with "packers" recognized to be deceptive historians (Cawich et al., 2008), surgeons should be wary of the packer's account of the content.

Intestinal Perforation

Impacted pellets may place mechanical pressure on the intestinal wall leading to perforation and intraabdominal sepsis. This is the rationale behind emergent surgical management in cases of mechanical obstruction. Intraabdominal sepsis is not uncommon because many "packers" present to hospital late. These patients require emergent laparotomy to achieve peritoneal toilet, control contamination, and seal the perforation. Supplemental antibiotics and supportive management are also required.

Medical literature contains several reports of mortality from intestinal perforation when body packers attempted to transport heroin (Hutchins et al., 2000; Sribanditmongkol et al., 2006) and cocaine (Jill & Graham, 2002; Koehler et al., 2005; Norfolk, 2007), but there have only been two reported deaths from colonic perforation due to cannabis body packing (Cawich et al., 2010; Barnett & Codd, 2002). Although most authorities suggest that colonic pellets are less dangerous than small bowel and gastric pellets (East, 2005), it is interesting that both reported deaths from intestinal perforation with cannabis body packing were due to colonic perforations (Cawich et al., 2010; Barnett & Codd, 2002).

ETHICAL CONSIDERATIONS

Cannabis body packers may present to clinicians in one of three ways (Cawich et al., 2008):

- symptomatic packers with one of the complications detailed previously;
- asymptomatic packers detained by law enforcement agents;
- asymptomatic packers seeking medical attention on their own.

Persons who engage in body packing are usually fully aware of the potential medical and legal consequences, and take a calculated risk to engage in this practice (Cawich et al., 2008). The symptomatic packers are usually very cooperative with medical personnel because they are fearful of the potential mortality that they believe the medical team may be able to prevent. Admittedly, mortality from acute overdose has never been reported from THC, but multiple deaths from acute overdose with cocaine have been reported (Cawich et al., 2008; East, 2005; Marc et al., 1990; Ambre et al., 1988; Gay, 1982) and >50% of the patients with free rupture dying before reaching hospital (East, 2005; Cawich et al., 2009b; Schaper, Hofmann, Ebbecke, Desel, & Langer, 2003). The patients are not aware of the difference, and are only cognizant of the fact that complications during packing can cause death. Patients who seek medical attention on their own tend to be cooperative as well because they have presented to hospital with a genuine desire for medical assistance.

On the other hand, packers who are asymptomatic when detained tend to be uncooperative with medical teams (Cawich et al., 2008) because they are cognizant of the legal consequences, and wish to conceal the evidence as long as possible. Ironically, these are the easiest patients to manage from an ethical perspective because it is clear that they have already been identified, detained, and will face legal consequences. This removes any potential for blame from being ascribed to the physician teams. In this case, the physician's responsibility is to confirm the diagnosis with imaging (Cawich et al., 2008; Traub et al., 2003; Hergan et al., 2004; Beerman et al., 1986; Hierholzer et al., 1995; Marc et al., 1990), and to be vigilant in the event that a complication occurs (Cawich et al., 2008; East, 2005). Body packers have the right to refuse any examination or investigation (Cawich et al., 2008), and when this occurs they are treated expectantly until the pellets spontaneously pass. Laxatives, high fiber diets, clear fluid diets and suppositories can be administered, but their benefit remains unproven (Das, Baha, & Mackway-Jones, 2003).

While the detained patients are straightforward to manage, it is the asymptomatic body packers that pose a serious ethical dilemma from two points of view: reporting and drug disposal. Most countries do not have legislation in place to mandate reporting body packers to the authorities. The decision is many times left up to the attending physicians (Cawich et al., 2008), who often have mixed views on reporting. Many persons believe that there is insufficient reason to override patient–physician confidentiality solely on the basis of the crime committed, or the amount of illicit drugs involved (Traub et al., 2003; Khan, 2005; East, 2005), unless there has been a credible threat of violence (Cawich et al., 2008).

In 2003 the United States Department of Health and Human Services implemented the Health Insurance Portability and Accountability Act, Public Law 104-191 (Kreismann, Gang, & Goldfrank, 2006). It states that "healthcare providers are permitted, but not required

to, disclose protected health information without the patient's permission" to the authorities, in order to "undertake an enforcement action." This includes data regarding "federally regulated products or activities," and "to inform law enforcement about the commission, nature, location, victims, and perpetrator of any crime" (Kreismann et al., 2006). This is still subject to interpretation.

There are no comparable regulations in Caribbean countries where body packing is rampant (Cawich et al., 2009b). The decision to disclose is made on an individualized basis, depending on the physician's ethical viewpoint, and guided by hospital policies. This places the physician in a precarious position because, in small communities, as would exist in Caribbean countries, it is known that reporting is not mandatory (Cawich et al., 2009b). The drug lords are usually aware of this fact, and they may view sporadic reporting as a hostile action deserving violent protest (Cawich et al., 2009b). Motive is easy to appreciate, since the healthcare team's actions separate the drug lord from their valuable product. This type of intimidation puts enormous pressure on the healthcare team, who are usually unprepared (Cawich et al., 2009b). The importance of this is highlighted by recent reports that some Caribbean countries rank among the top ten countries worldwide for homicides, 50% of which were reprisal and drug related murders (Lemard & Hemenway, 2006).

Another problem arises with unreported cases when it comes to handling the extracted drugs. Many hospitals have policies to address the issue that usually involve hospital security officers confiscating the drug, and their disposal by a pharmacist (East, 2005; Cawich et al., 2009b). But the "unauthorized" disposal of this expensive product may again be viewed as antagonistic, especially in small developing countries where drug lords can monitor these actions (Cawich et al., 2009b).

SECURITY CONSIDERATIONS

Incarcerated offenders pose the least security concerns as they are already guarded by police officers. Asymptomatic patients who have not been detected by law enforcement, however, pose a security concern. Although hospital security officers guard these patients in the absence of law enforcement agents, they are not equipped for this. Often they cannot prevent the packers from leaving the hospital, or protect hospital staff from intimidation by drug lords (Cawich et al., 2009b).

There have been instances of healthcare worker intimidation from drug lords and their affiliates. Obviously, this has great impact on the doctors' management decisions, as well as any decision to report to the authorities, and even handling of the extracted drugs. The medical teams can find themselves in a moral dilemma between being coerced to comply with drug lords' instructions (Cawich et al., 2008), and taking the decision to follow the law at the risk of their personal security. This is put into perspective when we consider that some Caribbean territories are among the world's top five countries for homicide rates, 50% of which were reprisal or drug-related killings (Lemard & Hemenway, 2006).

MINI-DICTIONARY

Body packing The practice in which a smuggler ingests drug-filled packages in order to transport the drug without detection by authorities.
Body stuffing The practice in which a smuggler ingests drug-filled packages in order to transport the drug without detection by authorities.
Drug toxicity Rapid exposure to the median lethal dose of a drug.
Internal concealment The practice in which a smuggler ingests drug-filled packages in order to transport the drug without detection by authorities.
Intestinal perforation Rupture of the wall of the intestine resulting in contamination, intraabdominal sepsis, and potential mortality.
Mechanical bowel obstruction The absence of aboral flow of intestinal contents due to the presence of a mechanical blockade.
Smuggling Transportation of illegal substances across an international border, in violation of regulations.
Tetrahydrocannabinol (THC) The main psychoactive component of the cannabis plant.

References

Aggarwal, S. K., Carter, G. T., Sullivan, M. D., ZumBrunnen, C., Morrill, R., & Mayer, J. D. (2009). Medicinal use of cannabis in the United States: historical perspectives, current trends, and future directions. *Journal of Opioid Management*, *5*(3), 153–168.

Ambre, J. J., Belknap, S. M., Nelson, J., Ruo, T. I., Shin, S. G., & Atkinson, A. J. (1988). Acute tolerance to cocaine in humans. *Clinical Pharmacology and Therapeutics*, *44*(1), 1–8.

Barnett, J., & Codd, G. (2002). Sudden, unexpected death of a cannabis bodypacker, due to perforation of the rectum. *Journal of Clinical and Forensic Medicine*, *9*(2), 82–84.

Beerman, R., Nunez, D., Jr, & Wetli, C. V. (1986). Radiographic evaluation of the cocaine smuggler. *Gastrointestinal Radiology*, *11*(4), 351–354.

Boekhout van Solinge, T. (1996). Ganja in Jamaica. *Amsterdams Drug Tijdschrift*, *2*, 11–14.

Cawich, S. O., Downes, R., Martin, A. C., Evans, N. R., Mitchell, D. I., & Williams, E. W. (2010). Colonic perforation: a lethal consequence of cannabis body packing. *Journal of Forensic and Legal Medicine*, *17*(5), 269–271.

Cawich, S. O., Valentine, C., Evans, N. R., Harding, H. E., & Crandon, I. W. (2009a). The changing demographics of body packers in Jamaica. *International Journal of Forensic Science*, *3*, 2.

Cawich, S. O., Williams, E. W., Evans, N. R., & Johnson, P. B. (2009b). Short report. Occupational hazard: treating cocaine body packers in Caribbean countries. *International Journal of Drug Policy*, *20*(4), 377–380.

Cawich, S. O., Williams, E. W., Simpson, L. K., Evans, N. R., & Johnson, P. (2008). Treating cocaine body packers: the unspoken personal risks. *Journal of Forensic and Legal Medicine*, *15*(4), 231–234.

Chait, L. D., & Pierri, J. (1992). Effects of smoked marijuana on human performance: a critical review. In: L. Murphy, A. Bartke (Eds.), *Marijuana/Cannabinoids: Neurobiology and neurophysiology* (2nd ed.). Boca Raton, FL: CRC Press.

Collier, M.W. (2002) The effects of political corruption on Caribbean development. Caribbean Studies. Available from http://www10.iadb.org/intal/intalcdi/PE/2009/02799.pdf.

Das, S., Baha, A., & Mackway-Jones, K. (2003). Conservative management of asymptomatic cocaine body packers. *Emergency Medicine Journal*, *20*, 172–174.

De La Haye, W. G. (2004a). Exposure opportunity to substances of abuse. *West Indian Medical Journal*, *53*(3), 141–142.

De La Haye, W. G. (2004b). Community-based prevention of substance abuse. *West Indian Medical Journal*, *53*(6), 420–423.

East, J. M. (2005). Surgical complications of cocaine body-packing: a survey of Jamaican hospitals. *West Indian Medical Journal*, *54*(1), 38–41.

Gay, G. R. (1982). Clinical management of acute and chronic cocaine poisoning. *Annals of Emergency Medicine*, *11*(10), 562–572.

Government of Jamaica. (2014). Dangerous Drug Act. 1987; 17(S6): Sec 7A-D. Available from www.moj.gov.jm/laws/statutes/The%20Dangerous%20Drugs%20Act.pdf

Hergan, K., Kofler, K., & Oser, W. (2004). Drug smuggling by body packing: what radiologists should know about it. *European Radiology*, *14*, 736–742.

Hierholzer, J., Cordes, M., Tantow, H., Keske, U., Maurer, J., & Felix, R. (1995). Drug smuggling by ingested cocaine-filled packages: conventional x-ray and ultrasound. *Abdominal Imaging*, *20*, 333–338.

Hohman, A. G., & Suplita, R. L. (2006). Endocannabinoid mechanisms of pain modulation. *AAPS Journal*, *8*(4), 79.

Hollister, L. E. (1986). Health aspects of cannabis. *Pharmacological Reviews*, *38*, 1–32.

Hutchins, K. D., Pierre-Louis, P. J., Zaretski, L., Williams, A. W., Lin, R. L., & Natarajan, G. A. (2000). Heroin body packing: three fatal cases of intestinal perforation. *Journal of Forensic Science*, *45*(1), 42–47.

Iversen, L. (2003). Cannabis and the brain. *Brain*, *126*(6), 1252–1270.

Jefferson, D. A., Harding, H. E., Cawich, S. O., & Jackson-Gibson, A. (2013). Postoperative analgesia in the Jamaican cannabis user. *Journal of Psychoactive Drugs*, *45*(3), 227–232.

Jill, J. R., & Graham, S. M. (2002). Ten years of "body packers" in New York City: 50 deaths. *Journal of Forensic Science*, *47*(4), 843–846.

Jones, R. T. (2002). Cardiovascular system effects of marijuana. *Journal of Clinical Pharmacology*, *42*(11S), 58–63.

Khan, F. Y. (2005). The cocaine 'body packer' syndrome. Diagnosis and treatment. *Indian Journal of Medical Sciences*, *59*(10), 457–458.

Koehler, S., Ladham, S., Rozin, L., Shakir, A., Omalu, B., Dominick, J., & Wecht, C. (2005). The risk of body packing: a case of a fatal cocaine overdose. *Forensic Science International*, *30*(15), 81–84.

Kreismann, E., Gang, M., & Goldfrank, L. R. (2006). The interface: ethical decision making, medical toxicology, and emergency medicine. *Emergency Medicine Clinics North America*, *24*(3), 769–784.

Lancashire, M. J., Legg, P. K., Lowe, M., Davidson, S. M., & Ellis, B. W. (1988). Surgical aspects of international drug smuggling. *British Medical Journal*, *296*, 1035–1037.

Lemard, G., & Hemenway, D. (2006). Violence in Jamaica: an analysis of homicides 1998 to 2002. *Injury Prevention*, *12*, 15–18.

Mackie, K. (2008). Cannabinoid receptors: where they are and what they do. *Journal of Neuroendocrinology*, *20*(S1), 10–14.

Mandava, N., Chang, R. S., & Wang, J. H. (2011). Establishment of a definitive protocol for the diagnosis and management of body packers (drug mules). *Emergency Medicine Journal*, *28*, 98–101.

Marc, B., Baud, F. J., Aelion, M. J., Gherardi, R., Diamant-Berger, O., & Blery, M. (1990). The cocaine body-packer syndrome: evaluation of a method of contrast study of the bowel. *Journal of Forensic Science*, *35*(2), 345–355.

Nahas, G., & Latour, C. (1992). The human toxicity of marijuana. *Medical Journal of Australia*, *156*(7), 495–497.

Norfolk, G. A. (2007). The fatal case of a cocaine body-stuffer and a literature review—towards evidence based management. *Journal of Forensic and Legal Medicine*, *14*(1), 49–52.

Pidoto, R. R., Agliata, A. M., Bertolini, R., Mainini, A., Rossi, G., & Giani, G. (2002). A new method of packaging cocaine for international traffic and implications for the management of cocaine body packers. *Journal of Emergency Medicine*, *23*(2), 149–153.

Schaper, A., Hofmann, R., Ebbecke, M., Desel, H., & Langer, C. (2003). Cocaine body packing: infrequent indication for laparotomy. *Der Chirurg*, *74*, 626–631.

Simon, L. C. (1990). The cocaine body packer syndrome. *West Indian Medical Journal*, *39*, 250–255.

Soriano-Perez, M. J., Serrano-Carrillo, J. L., Marin-Montin, I., & Cruz-Caballero, A. (2009). Hashish body packing: a case report. *Case Reports in Medicine*, *712573*, 1–3.

Sribanditmongkol, P., Supasingsirpreecha, W., Thampitak, S., & Junkuy, A. (2006). Fatal heroin intoxication in body packers in northern Thailand during the last decade: two case reports. *Journal of the Medical Association of Thailand*, *89*(1), 106–110.

Stadmauer, G., Beyer, L., Bardina, L., & Sicherer, S. H. (2003). Anaphylaxis to ingestion of hempseed (*Cannabis sativa*). *Journal of Allergy and Clinical Immunology*, *112*, 216–217.

Traub, S., Hoffman, R. S., & Nelson, L. S. (2003). Body packing—the internal concealment of illicit drugs. *New England Journal of Medicine*, *26*(349), 2519–2526.

Wikipedia Reports. (2014). Legality of cannabis by country. Available from http://en.wikipedia.org/wiki/Legality_of_cannabis_by_country

Wu, T. C., Tashkin, D. P., Djahed, B., & Rose, J. E. (1988). Pulmonary hazards of smoking marijuana as compared with tobacco. *New England Journal of Medicine*, *318*, 347–351.

CHAPTER

e1

Tetrahydrocannabinol Concentration and Genetic Characterization of Cannabis

F. Cascini, I. Boschi

Public Health Institute, Department of Forensic Medicine, Università Cattolica del Sacro Cuore, Rome, Italy

SUMMARY POINTS

- The species *C. sativa* L. includes varieties suitable for recreational and therapeutic purposes (commonly named marijuana or simply cannabis), as well as varieties appropriate for industrial use only (usually named hemp).
- Published studies show the total content of THC or CBD found in the plant material generally corresponds to the sum of its free form, and its respective acidic precursor.
- According to total THC and total CBD contents, three main chemotypes (chemical phenotypes) of cannabis have been recognized.
- The absolute THC content (quantitative chemotype) reflects polygenic genetic mechanisms showing Gaussian distributions in progenies and populations being strongly subject to environmental factors.
- The CBD/THC ratio (qualitative chemotype) is controlled by simple mendelian genetic mechanisms.
- Some authors found the upregulation of cannabinoid pathway genes, and the exclusive presence of functional THCA-synthase in the genome and transcriptome of high-THC plant strains/varieties.
- The correlation between the quantity of the THCA synthase gene and the production of THC appeared to be random and variable, in high and low potency varieties of *Cannabis*.
- To date, a great part of genetic research on *Cannabis* has been focused on molecular techniques and markers to distinguish high-THC (drug-type) from low/absent-THC (fiber-type) varieties.
- The function of genetic markers is threesome: evaluation and distinction of cannabis varieties, application in order to facilitate the use of *Cannabis* for therapeutic purposes and, concurrently, an effective monitoring tool of the illegal market.
- Advances in molecular genomics are providing genetic maps that could permit marker-assisted breeding.

KEY FACTS OF THC CONCENTRATION IN CANNABIS

- Different destinations and uses of *Cannabis* plants are related to the biological capacity of each variety or strain to synthesize cannabinoids, hence dividing drug-type from fiber-type varieties/strains.
- The amount of the main active cannabinoid Δ^9-tetrahydrocannabinol in the plant naturally depends on the influence that factors such as genotype, plant age, and environment have on the biosynthesis of cannabinoids.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00003-X
Copyright © 2017 Elsevier Inc. All rights reserved.

- Cannabinoids have been found in all tissues of the plant, but the amount in which they are present differs considerably among the tissues: they are most abundant in flowers (female plant), and especially in the glands.
- The storage cavity of glandular trichomes of the cannabis plant would not only be the site for the accumulation of cannabinoids, but also the site for THCA biosynthesis.
- Flowering tops and leaves of female plants belonging to drug type strains are traditionally known to have a THC level rarely exceeding 5%, while significantly higher THC concentrations in cannabis have been recently documented in the literature.
- The availability of a more THC-concentrated form of cannabis could cause an increase in the total amount of THC consumed, with foreseeable consequences for public health.
- Scientific research on *Cannabis* is trying to explain, through chemical and genetic investigations, the biological processes behind the differences in cannabinoids levels among varieties.

LIST OF ABBREVIATIONS

AFLP	Amplified Fragment Length Polymorphism
CBC	Cannabichromene
CBCA	Cannabichromene acid
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBG	Cannabigerol
CBGA	Cannabigerolic acid
ISSR	Inter-Simple Sequence Repeat
ITS	Internal Transcribe Spacer
RAPD	Random Amplified Polymorphic DNA
STR	Short Tandem Repeats
THC	Tetrahydrocannabinol
THCA	Tetrahydrocannabinolic acid

INTRODUCTION

Cannabis sativa L. (commonly called *Cannabis*) is a herbaceous plant belonging to the *Cannabis* genus, family of Cannabaceae. It is normally a wind-pollinated, dioecious herb, which means that male and female reproductive structures are on separate plants, although monoecious plants can occur in some populations (Divashuk, Alexandrov, Razumova, Kirov, & Karlov, 2014).

The *Cannabis* genus includes wild and cultivated forms that are morphologically variable, resulting in confusion and controversy over the taxonomic organization: some authors have proposed a monotypic genus, *C. sativa*, while others have argued that *Cannabis* is composed of two species, *C. sativa* and *Cannabis indica*, and some have included a third species, *Cannabis ruderalis*, in the genus (Hillig, 2005).

Beside this taxonomic uncertainty, the species *C. sativa* L. includes varieties suitable for recreational and therapeutic purposes (commonly named marijuana, or simply cannabis), as well as varieties appropriate for industrial use only (usually named hemp). This feature depends on the constitution of each *Cannabis* variety or strain to synthesize and store chemicals known as cannabinoids. They represent a group of more than 100 natural products (Elsohly & Slade, 2005; Mehmedic et al., 2010) within which tetrahydrocannabinolic acid (THCA) is the main (psycho)active compound.

CANNABINOIDS BIOSYNTHESIS

Tethraydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA) are from the same precursor cannabigerolic acid (CBGA). THCA-synthase enzyme or CBDA-synthase enzyme are responsible for the synthesis of THCA or CBDA, respectively (Fellermeier & Zenk, 1998; Fellermeier, Eisenreich, Bacher, & Zenk, 2001; Raharjo et al., 2004a; Taura, Morimoto, & Shoyama, 1995, 1996; Thakur, Duclos, & Makriyannis, 2005).

Cannabinoids are actually biosynthesized in a carboxylated form in plant tissues. Then, they are progressively decarboxylated to THC and CBD by drying, and especially under intense heating, such as when cannabis is smoked (Dussy, Hamberg, Luginbühl, Schwerzmann, & Briellmann, 2005) while, in vivo, this conversion appears to be limited (Jung et al., 2009).

Almost no neutral cannabinoid can be found in fresh plant material. The carboxyl group is not very stable, and easily lost under the influence of heat or light, resulting in the corresponding neutral cannabinoids. However, the total content of THC or CBD found in the plant material generally corresponds, in published studies, to the sum of the free cannabinoid and its respective acidic precursor.

Cannabinoids have been found in all tissues of the plant, but the amount in which they are present differs considerably among the tissues (Fetterman, Doorenbos, Keith, & Quimby, 1971): these chemicals are, in fact, most abundant in flowers (female plant), especially in the glands. This raises the question of whether biosynthesis of cannabinoids occurs in all tissues but in different quantities, or only in one tissue, implying the following transfer to other tissues. In both scenarios, the expression level of the genes involved in the cannabinoids biosynthesis would be different among the tissues. On this topic, Raharjo et al. (2004b) identified that some proteins in *Cannabis* are expressed more in flowers than in the leaves.

FIGURE e1.1 Resin drops and glandular trichomes of resin-type cannabis. The drops of resin containing THC are visible in the picture, and appear at the stage of maturity of the plant. The secretory cells of glandular trichomes are believed to secrete cannabinoids that are then accumulated into the storage cavity of these glands.

However, in the comparison of the proteome of leaves and flowers they did not find proteins involved in cannabinoid biosynthesis. They moved to a more specific part of the flower, the glands, where the levels of cannabinoids are the highest. They also found that THCA synthase, CBDA synthase, and CBCA synthase enzymes, involved in the cannabinoid biosynthesis, are expressed at very low levels even in the glands. Biochemical processes concerning cannabinoids biosynthesis and storage in selective tissues then remained uncertain.

On the other hand, Sirikantaramas et al. (2005) suggested a sorting pathway for THCA synthase from secretory cells into the storage cavity, meaning that the glandular trichome (Fig. e1.1), believed to secrete small molecules such as resinous compounds or volatile oils, can also secrete biosynthetic enzyme. Therefore, the storage cavity of glandular trichomes of the *Cannabis* plant would not only be the site for the accumulation of cannabinoids, but also the site for THCA biosynthesis. This hypothesis has not been further investigated in *Cannabis*.

However, a different tissue-specific DNA banding could also be possible. It has been actually identified in plants (Donini, Elias, Bougourd, & Koebner, 1997), and this is not overly surprising, due to the selective amplification or mutation of genes during tissue development.

The studies previously cited (Fellermeier et al., 2001; Raharjo et al., 2004a; Thakur et al., 2005) demonstrate that significant efforts have been made by the scientific community to understand the cannabinoids biosynthetic pathways. This topic raises more interest and curiosities, if we consider that the cannabis market is changing worldwide toward an increasing level of drug potency. A literature review (with metaanalysis by year), based on 21 studies containing 75 total mean THC observations from 1979 to 2009 (Cascini, Aiello, & Di Tanna, 2012), have shown a significant correlation between year and mean THC in cannabis, revealing a temporal trend of increasing potency (5% above the mean THC value in the Poisson regression analysis), although this increase is not constant, and does not exceed 5% globally (Figs. e1.2 and e1.3). However, it would be right to consider a 200% increase of potency over the years, possibly due to the rising market of intensively grown indoor cannabis.

Amounts and ratios of cannabinoids determine the chemical phenotype of the plant and its unique pharmacological properties, including analgesic, antiemetic, and

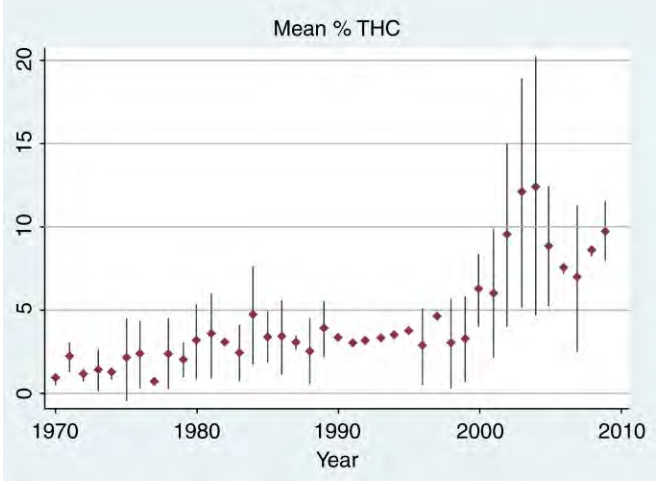


FIGURE e1.2 Per year metaanalysis graph showing the mean THC value with 95% CI. Metaanalysis was performed by year on 75 total mean THC observations from 1970 to 2009, using the random effect model. Metaregression was performed on the same dataset, using the year as the covariate. The results revealed great variability within studies (I2 = 99.91%) and a significant association (from the metaregression) (p value < 0.0001) between the mean THC in herbal cannabis and year (coef. 0.214; 95% CI = 0.163–0.266). *Source: From Cascini et al. (2012a).*

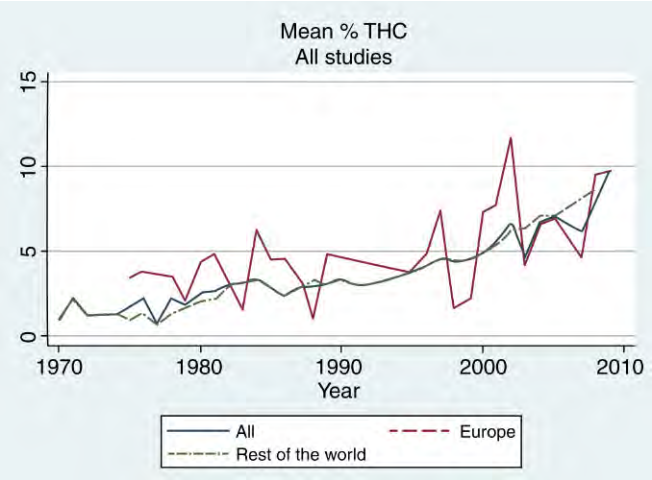


FIGURE e1.3 Time trend of the mean THC value for all samples and disaggregated by zone. The combined data from the 21 studies included in the metaanalysis indicated an increasing temporal trend in potency; the Poisson regression suggested an average increase in the mean THC value of 4.694% (95% CI 3.129–6.283). This rise in mean THC seems to have been more rapid in the last decade. Analysis of the trend, according to geographical zone, indicated a smaller increase in Europe (2.799%, 95% CI 1.157–4.467; significant at p = 0.001) compared to the rest of the world, almost totally represented by the United States (5.279%, 95% CI 3.590–6.996), but this may reflect the fact that the data from Europe started with a higher mean THC. *Source: From Cascini et al. (2012a).*

appetite-stimulating effects. The chemical phenotype naturally depends on the influence that factors such as genotype, age of the plant, and environment exercise on the biosynthesis of cannabinoids.

CANNABIS GENOME

Cannabis has a diploid genome ($2n = 20$) with a karyotype composed of nine autosomes and a pair of sex chromosomes; female plants are homogametic (XX), and males heterogametic (XY), with sex determination controlled by an X-to-autosome balance system (Ming, Bendahmane, & Renner, 2011). The genome size ranges from 0.84 to 0.91 pg: the estimated size of the haploid genome is 818 Mb for female plants, and 843 Mb for male plants, owing to the larger size of the Y chromosome (Sakamoto, Akiyama, Fukui, Kamada, & Satoh, 1998). Divashuk et al. (2014), using modern cytogenetic methods, characterized the karyotype of male and female *C. sativa*, allowing to identify it in early developmental stages of the plant. Furthermore, karyotype analysis of chromosomes suggest that the X chromosome is submetacentric, and the Y chromosome is subtelocentric, with a satellite at the terminus of its short arm.

Other lines of genetic research on *Cannabis* have also been evaluated over time to establish genetic relationships, as well as to highlight genetic differences among plant varieties (with different chemical phenotypes and different psychoactive effects).

The most investigated genes of *Cannabis* to date are related to cannabinoids biosynthesis, and concern several key enzymes, including a candidate polyketide synthase and two oxidocyclases but, in particular, THCA synthase (THCAS) and cannabidiolic acid synthase (CBDAS) (Figs. e1.4 and e1.5). (Sirikantaramas et al., 2004; Taura et al., 2007, 2009).

The gene for the enzyme THCAS responsible for the production of THC from the CBG precursor has been identified (Taura et al., 1995; Sirikantaramas et al., 2004). Several functional and nonfunctional sequence variants of this gene have been published, and sequence polymorphisms have been employed for marker application.

Kojoma, Seki, Yoshida, & Muranaka (2006) published 13 different strains of *Cannabis* plants,. distinguishing, by implementing a specific PCR marker, high-THC (drug-type) from low/absent-THC (fiber-type) varieties.

Rotherham and Harbison (2010) developed a single nucleotide polymorphism (SNP) assay, based on 4 polymorphisms of THCA synthase gene for the differentiation of drug and nondrug cannabis plants.

Other authors (Staginnus, Zörntlein, & de Meijer, 2014) have recognized three main, discrete chemotypes (chemical phenotypes) of *Cannabis* according to total THC and total CBD contents (Fig. e1.6):

1. drug-type plants (chemotype I, also known as THC predominant type) show a low CBD/THC ratio, usually 0.00–0.05 (or, alternatively, some authors prefer to consider a high THC/CBD ratio $\gg 1$)
2. intermediate-type plants (chemotype II) show an intermediate ratio, usually a CBD/THC ratio 0.5–3
3. fiber-type plants (chemotype III, also known as CBD predominant type) exhibit a high CBD/THC ratio, usually 15–25 (or, alternatively, a low THC/CBD ratio $\ll 1.0$).

Chemotype III is tipical of plants called hemp, refers to varieties that have low THC concentrations (lower than 0.2%, according to the EU legislation CE 73/2009 for Agriculture), but contain CBD as a major compound. (Hillig & Mahlberg, 2004; Pacifico, Miselli, Carboni, Moschella, & Mandolino, 2008; Staginnus et al., 2014).

The absolute THC content (quantitative chemotype) reflects polygenic genetic mechanisms showing Gaussian distributions in progenies and populations that are strongly subject to environmental factors. Total cannabinoid content of a plant is therefore a complex feature, while the relative CBD/THC ratio is fairly constant throughout the plant's life, and relatively unaffected by external factors (De Backer, Maebe, Verstraete, & Charlier, 2012; de Meijer, Van der Kamp, & Van Eeuwijk, 1992). The CBD/THC ratio (qualitative chemotype) is controlled

FIGURE e1.4 DNA sequencing electropherogram of a portion of THCA-synthase gene. The picture shows the linear nucleotide order of a segment of THCA synthase gene of a fiber-type plant (variety "Tygra").

by simple mendelian genetic mechanisms. This means that genotypes of offspring that inherit from one parent the same alleles (or a combination of alleles for the same gene or group of genes, ie, haplotype) will have the same cannabinoid ratios (CBD/THC ratio) while having different absolute THC content (quantitative chemotype). This also applies to clones of the same plant.

In particular, *Cannabis* expresses three discrete chemical phenotypes (qualitative chemotypes) depending on polymorphisms within the THCA synthase sequence. The CBD/THC ratio is determined by a single locus (B) of the THCA synthase gene having two codominant alleles (B_T and B_D). The homozygous B_T/B_T genotype underlies the THC-predominant phenotype, and B_D/B_D is CBD-predominant.

The intermediate phenotype is induced by the heterozygous state (B_T/B_D). (de Meijer et al., 2003). Expanding this line of research, Staginnus et al. (2014) tested specific polymorphisms within the THCA synthase sequence for forensic applicability have shown to be absolutely linked to either the THC-predominant (B_T/B_T) or the THC-intermediate (B_T/B_D) chemotype. The marker system they found (D589) proved to be a reliable tool to detect a B_T/B_T or B_T/B_D genotype that works independently from the developmental stage or the tissues examined.

Some *C. Sativa* L. individuals that lack the ability to form cannabinoids are known. This is generally due to a knockout factor that inhibits the metabolic pathway upstream of THCA synthase (Pacifico et al., 2006). In these plants, neither CBD nor THC can be produced, due to the absence of upstream precursors in the pathway (de Meijer, Hammond, & Sutton, 2009).

The presence of a THCA synthase gene in these individuals cannot be ruled out: the o/o genotype underlying the cannabinoid-free chemotype has been found in individuals of fiber hemp cultivars. They belonged to the B_D/B_D group, and did not show a marker signal. All this data then confirms that cannabinoid content and composition is highly variable among *Cannabis* plants, explaining distinctions between chemotypes I, II, and III.

Working on this topic, some authors (Bakel et al., 2011) found the upregulation of cannabinoid pathway genes,

FIGURE e1.5 DNA sequencing electropherogram of a portion of CBDA-synthase gene. The picture shows the linear nucleotide order of a segment of CBDA synthase gene of a resin-type plant (variety "Shiatsu Kush").

and the exclusive presence of functional THCA-synthase in the genome and transcriptome of high-THC plant strains/varieties (they studied the Purple Kush). These authors have presented a draft genome and transcriptome of cannabis, and compared the genomes and flower transcriptomes of high-THC and low-THC producing strains: Purple Kush as high, Finola as low, and USO-31 as low to absent. THCA synthase, the gene encoding the oxidocyclase enzyme that forms the THC, is found in the genome and transcriptome of Purple Kush, whereas CBDA synthase dominates in the "Finola" hemp cultivar. Moreover, they found that most of the cannabinoid biosynthetic pathway enzymes are highly expressed in flower tissues containing glandular trichomes, and that the expression of the cannabinoid biosynthetic enzymes is elevated in the high-THC strain (Purple Kush).

It has been also investigated (Cascini, Passerotti, & Martello, 2012) whether or not the THCA synthase gene, which codes the enzyme, influences the production and storage of THC in a dose-dependent manner. Quantification of the THCA synthase gene using the real-time PCR technique revealed a gene copy number that is independent of the chemical phenotype. This means that the correlation between the quantity of the THCA synthase gene and the production of THC appeared to be random and variable in high and low potency varieties of *Cannabis*. Furthermore, the same samples did not show a correlation between the THCA synthase gene expression and the THC storage in leaves and inflorescence (Cascini, Passerotti, & Boschi, 2013).

Concerning investigations on other genes involved in the cannabinoid biosynthesis, the sequence of CBDA synthase resulted to be very similar to the one of THCA synthase gene (homology 87.9%)(Yoshikai, Taura, Morimoto, & Shoyama, 2001). A total of 37 major amino-acid substitutions were detected in the alignment of the sequences from high-THC type and low/absent-THC type strains. It appeared that the amino acid substitutions induced a decrease in THCA synthase activity in the fiber-type cannabis plants.

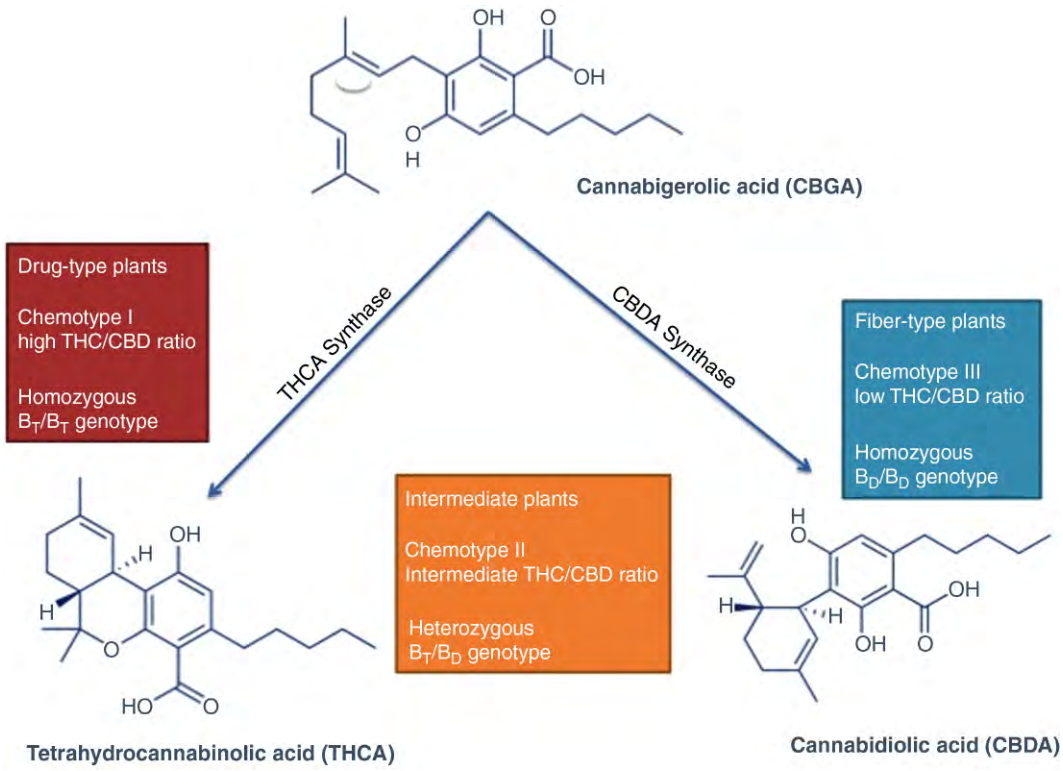


FIGURE e1.6 **Genotypes and chemical phenotypes of cannabis.** The picture shows the genetic base of cannabinoids biosynthesis. According to total THC and total CBD contents, it is possible to distinguish: (1) drug-type plants (chemotype I also known as THC predominant type); (2) intermediate-type plants (chemotype II showing an intermediate ratio); and (3) fiber-type plants (chemotype III also known as CBD predominant type). These three discrete chemical phenotypes (qualitative chemotypes) depend on polymorphisms within the THCA synthase sequence. The CBD/THC ratio is determined by a single locus (B) of the THCA synthase gene having two codominant alleles (B_T and B_D). The homozygous B_T/B_T genotype underlies the THC-predominant phenotype, and B_D/B_D is CBD-predominant. The intermediate phenotype is induced by the heterozygous state (B_T/B_D).

GENETIC MARKERS FOR FORENSIC IDENTIFICATION

To this date, beside investigations concerning biosynthesis processes, the greater part of genetic research on *Cannabis* has focused on molecular markers for forensic investigations to fight the illegal market.

Various approaches have been suggested, even if definitive conclusions haven't been reached in regard to genetic markers characterized by high levels of reproducibility and reliability.

Cannabis strains have been classified based on genomic DNA markers that revealed informative genetic diversity, such as random amplified polymorphic DNA (RAPD) (Gillan, Cole, Linacre, Thorpe, & Watson, 1995; Jagadish, Robertson, & Gibbs, 1996; de Meijer et al., 2003), amplified fragment length polymorphisms (AFLP) (Miller et al., 2003), and inter simple sequence repeat amplification (ISSR) (Kojoma, Iida, Makino, Sekita, & Satake, 2002).

Short tandem repeat (STR, microsatellite) markers have been mostly developed and widely tested (Hsieh et al., 2003; Gilmore et al., 2003; Alghanim & Almirall, 2003). Some of these STRs have been optimized for being analyzed in multiplex PCRs (Howard, Gilmore, Robertson, & Peakall, 2008; Mendoza et al., 2009; Köhnemann, Nedele, Schwotzer, Morzfeld, & Pfeiffer, 2012), but they are still scarcely used (Gilmore, Peakall, & Robertson, 2003; Howard et al., 2008; Gilmore, Robertson, & Peakall, 2009; Allgeier, Hemenway, Shirley, LaNier, & Coyle, 2011) because they are not standardized, making their results unreliable.

Valverde et al. (2014a) have recently sequenced and characterized several alleles of 15 of the most common *Cannabis* STRs, and proposed a nomenclature following forensic international guidelines.

The characterization of all these alleles has allowed the establishment of a nomenclature to be applied for standardizing the results and making comparisons between laboratories. The sequencing of every STR locus has revealed several nucleotide variations inside the repeated structure and/or in the flanking regions. This has permitted the construction of an initial SNPSTR database of all the haplotypes found, also including the previously available sequence raw data for these STR loci.

Valverde et al. (2014b) published also a new *Cannabis* STRs nomenclature proposal based on only 7 STRs and their SNPSTR data, to facilitate standardization, performance, and reliability of these analyses, thus facilitating the implementation of routine forensic investigations on *Cannabis*. Nonetheless, additional investigations concerning published STR loci in different populations and in related plants would be useful for the purpose. This would set the basis for establishing frequency databases and mutation rates for identification of varieties and strains.

In addition, because microsatellite markers are seldom located in functional genomic regions, mutations in them tend to be evolutionarily neutral. Thus, they are unaffected by selective pressure. This permits the rapid (in terms of centuries) accumulation of variations, permitting varietal (but usually not clonal) differentiation.

Among other methods to genetically characterize *Cannabis*, analysis of the organelle DNA of plants, particularly using chloroplast DNA (cpDNA) is well established as a tool to study evolutionary and population processes (Palmer, 1990; Olmstead & Palmer, 1994). It has superior attributes to nuclear DNA in discerning evolutionary trends, due to its low mutation rate and the absence of genetic recombination, which is typical of nuclear DNA. Chloroplasts and mitochondria are generally uniparentally (maternally) inherited in angiosperms, and hence have the potential to provide complementary evolutionary and biogeographic insights to nuclear DNA markers such as STRs. Numerous studies in plants have used cpDNA restriction site and sequence data to elucidate species and higher level phylogenetic relationships (Borsch et al., 2003; Soltis & Soltis, 2000). PCR amplification of cpDNA, followed by restriction fragment length polymorphism analysis of the amplicons (PCR-RFLP), has provided a rapid method of assaying information among populations, for some plant groups (Petit et al., 2002). PCR-RFLP of cpDNA has been facilitated by publication of large numbers of universal PCR primer sequences, producing amplicons that cover large tracts of the chloroplast genome (Taberlet, Gielly, Pautou, & Bouvet, 1991; Dumolin-Lapegue, Pemonge, & Petit, 1997).

APPLICATIONS OF GENOTYPING

Many researchers around the world are undergoing a mutual effort to define the genetics of *C. sativa* L. Advances in molecular genomics are providing genetic maps showing the location of interesting genes, and possibly adjacent polymorphic markers that could permit marker-assisted breeding, where the adjacent sites facilitate recognition of the desired genes.

Most of the properties associated with commercial success of cannabis are quantitative (ie, THC concentration), and depend on the accumulative action of multiple genes (maybe located on separate chromosomes), although nowadays a quantitative prediction of the final THC content in a *Cannabis* plant still cannot be made.

Genetic markers can, thus, represent a valuable tool for therapeutic and forensic purposes.

Clear indicators of gene expression are highly desirable, especially if suitable for the identification of important genetic traits at an early stage of plant development. Concerning, for example, the selection of varieties/strains susceptible to obtain a high THC chemotype, desirable for therapeutic purposes, genomic features related to chemical factors can be greatly useful in the early selection of suitable offspring. Embryos or seedlings not possessing the desirable gene(s) can be discarded early, saving time, effort, and space. The procedure also streamlines traditional breeding programs, by clearly identifying plants likely to possess the desired traits for subsequent crossing.

Also regarding forensic applications, the advantages that the genetic individualization of *Cannabis* plants could provide in the illegal trade investigations are enormous, since it offers the possibility of establishing relations between different criminal cases, and assessing their belonging to a trade network.

However, due to the illegal nature of the *Cannabis* plant in many countries, insufficient data on its genetic features has been accumulated. Then genotyping has not been yet adopted as routine beside toxicological analyses.

MINI-DICTIONARY

Allele One of different, alternative forms of a gene locus.
Autosome A chromosome that is not a sex-determining chromosome.
Genetic characterization Identification and description of some genetic structures related to certain phenotypic features.
Genotype Genetic structure of an individual/organism. It can be related to single or multiple traits.
Haplotype A group of tightly linked genes in a chromosome that are likely to be inherited together.
Heterogametic Having different gametes, sex chromosomes (X and Y).
Homogametic Having a matching pair of sex chromosomes (X and X).
Karyotype Number and appearance of chromosomes.
Phenotype The entire complex of the external (morphological, physiological, developmental) features.
Proteome The entire set of proteins expressed by a genome.
Strain Descendant/offspring produced from an ancestor (sharing uniform genotype and phenotype).
Variety One of the possible forms of a botanical taxon (subspecies/species) differing from the other forms in minor characteristics.

References

Alghanim, H. J., & Almirall, J. R. (2003). Development of micro satellite markers in *Cannabis sativa* for DNA typing and genetic relatedness analyses. *Analytical and Bioanalytical Chemistry*, *376*, 1225–1233.

Allgeier, L., Hemenway, J., Shirley, N., LaNier, T., & Coyle, H. M. (2011). Field testing of collection cards for *Cannabis sativa* samples with a single hexanucleotide DNA marker. *Journal of Forensic Sciences*, *56*, 1245–1249.

Bakel, H., Stout, J., Cote, A., Tallon, C., Sharpe, A., Hughes, T. R., & Page, J. E. (2011). The draft genome and transcriptome of *Cannabis sativa*. *Genome Biology*, *12*, R102.

Borsch, T., Hilu, K. W., Quandt, D., Wilde, V., Neinhuis, C., & Barthlott, W. (2003). Noncoding plastid trnT-trnF sequences reveal a well resolved phylogeny of basal angiosperms. *Journal of Evolutionary Biology*, *16*, 558–576.

Cascini, F., Aiello, C., & Di Tanna, G. (2012a). Increasing delta-9-tetrahydrocannabinol (Δ-9-THC) content in herbal cannabis over time: systematic review and meta-analysis. *Current Drug Abuse Review*, *5*, 32–40.

Cascini, F., Passerotti, S., & Martello, S. (2012b). A real-time PCR assay for the relative quantification of the tetrahydrocannabinolic acid (THCA) synthase gene in herbal Cannabis samples. *Forensic Science International*, *217*, 134–138.

Cascini, F., Passerotti, S., & Boschi, I. (2013). Analysis of THCA synthase gene expression in cannabis: a preliminary study by real-time quantitative PCR. *Forensic Science International*, *231*, 208–212.

De Backer, B., Maebe, K., Verstraete, A., & Charlier, C. (2012). Evolution of the content of THC and other major cannabinoids in drug-type cannabis cuttings and seedlings during growth of plants. *Journal of Forensic Sciences*, *57*, 918–922.

de Meijer, E., Van der Kamp, H., & Van Eeuwijk, F. (1992). Characterization of Cannabis accessions with regard to cannabinoid content in relation to other plant characters. *Euphytica*, *62*, 187–200.

de Meijer, E., Bagatta, M., Carboni, A., Crucitti, P., Moliterni, V., Ranalli, P., & Mandolino, G. (2003). The inheritance of chemical phenotype in *Cannabis sativa* L. *Genetics*, *163*, 335–346.

de Meijer, E., Hammond, K., & Sutton, A. (2009). The inheritance of chemical phenotype in *Cannabis sativa* L. (IV): cannabinoid-free plants. *Euphytica*, *168*, 95–112.

Divashuk, M. G., Alexandrov, O. S., Razumova, O. V., Kirov, I. V., & Karlov, G. I. (2014). Molecular cytogenetic characterization of the dioecious *Cannabis sativa* with an XY chromosome sex determination system. *PLoS One*, *21*(9), e85118.

Donini, P., Elias, M. L., Bougourd, S. M., & Koebner, R. M. D. (1997). AFLP fingerprinting reveals pattern differences between template DNA extracted from different plant organs. *Genome*, *40*, 521–526.

Dumolin-Lapegue, S., Pemonge, M. H., & Petit, R. J. (1997). An enlarged set of consensus primers for the study of organelle DNA in plants. *Molecular Ecology*, *6*, 393–397.

Dussy, F. E., Hamberg, C., Luginbühl, M., Schwerzmann, T., & Briellmann, T. A. (2005). Isolation of Delta9-THCA-A from hemp and analytical aspects concerning the determination of Delta9-THC in cannabis products. *Forensic Science International*, *149*, 3–10.

Elsohly, M. A., & Slade, D. (2005). Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life Science*, *78*, 539–548.

Fellermeier, M., & Zenk, M. H. (1998). Phenylation of olivetolate by a hemp transferase yields cannabigerolic acid,the precursor of tetrahydrocannabinol. *FEBS Letters*, *427*, 283–285.

Fellermeier, M., Eisenreich, W., Bacher, A., & Zenk, M. H. (2001). Biosynthesis of cannabinoids. Incorporation experiments with C^{13}-labeled glucoses. *European Journal Biochemistry*, *268*, 1596–1604.

Fetterman, P. S., Doorenbos, N. J., Keith, E. S., & Quimby, M. W. (1971). A simple gas liquid chromatography procedure for determination of cannabinoidic acids in *Cannabis sativa* L. *Experientia*, *27*, 988–990.

Gillan, R., Cole, M. D., Linacre, A., Thorpe, J. W., & Watson, N. D. (1995). Comparison of *Cannabis sativa* by random amplification of polymorphic DNA (RAPD) and HPLC of cannabinoid: a preliminary study. *Science and Justice*, *35*, 169–177.

Gilmore, S., Peakall, R., & Robertson, J. (2003). Short tandem repeat (STR) DNA markers are hypervariable and informative in *Cannabis sativa*: implications for forensic investigations. *Forensic Science International*, *131*, 65–74.

Gilmore, S., Robertson, J., & Peakall, R. (2009). A *Cannabis sativa* STR genotype database for Australian seizures: forensic applications and limitations. *Journal of Forensic Sciences*, *54*, 556–563.

Hsieh, H., Hou, R., Tsai, L., Wei, C., Liu, S., Huang, L., Kuo, Y., Linacre, A., & Lee, J. (2003). A highly polymorphic STR locus in *Cannabis sativa*. *Forensic Science International*, *131*, 53–58.

Hillig, K. W., & Mahlberg, P. G. (2004). A chemotaxonomic analysis of cannabinoid variation in Cannabis (Cannabaceae). *American Journal of Botany*, *91*, 966–975.

Hillig, K. (2005). Genetic evidence for speciation in *Cannabis* (Cannabaceae). *Genetic Resources and Crop Evolution*, *52*, 161–180.

Howard, C., Gilmore, S., Robertson, J., & Peakall, R. (2008). Developmental validation of a *Cannabis sativa* STR multiplex system for forensic analysis. *Journal of Forensic Sciences*, *53*, 1061–1067.

Jagadish, V., Robertson, J., & Gibbs, A. (1996). RAPD analysis distinguished *Cannabis sativa* samples from different sources. *Forensic Science International*, *79*, 113–121.

Jung, J., Meyer, M. R., Maurer, H. H., Neusüss, C., Weinmann, W., & Auwärter, V. (2009). Studies on the metabolism of the Delta-9-tetrahydrocannabinol precursor delta-9-tetrahydrocannabinolic acid A (Delta9-THCA-A) in rat using LC-MS/MS, LC-QTOF MS and GC-MS techniques. *Journal of Mass Spectrometry*, *44*, 1423–1433.

Köhnemann, S., Nedele, J., Schwotzer, D., Morzfeld, J., & Pfeiffer, H. (2012). The validation of a 15 STR multiplex PCR for Cannabis species. *International Journal of Legal Medicine*, *126*, 601–606.

Kojoma, M., Iida, O., Makino, Y., Sekita, S., & Satake, M. (2002). DNA fingerprinting of *Cannabis sativa* using inter-simple sequence repeat (ISSR) amplification. *Planta Medica*, *68*, 60–63.

Kojoma, M., Seki, H., Yoshida, S., & Muranaka, T. (2006). DNA polymorphisms in the tetrahydrocannabinolic acid (THCA) synthase gene in "drug type" and "fibre type" *Cannabis sativa* L. *Forensic Science International*, *159*, 132–140.

Mehmedic, Z., Chandra, S., Slade, D., Denham, H., Foster, S., Patel, A. S., Ross, S. A., Khan, I. A., & ElSohly, M. A. (2010). Potency trends of Δ^9-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. *Journal of Forensic Sciences*, *55*, 1209–1710.

Mendoza, M. A., Mills, D. K., Lata, H., Chandra, S., ElSohly, M. A., & Almirall, J. R. (2009). Genetic individualization of *Cannabis sativa* by a short tandem repeat multiplex system. *Analytical and Bioanalytical Chemistry*, *393*, 719–726.

Miller, C. H., Shutler, G., Abrams, S., Hanniman, J., Neylon, S., Ladd, C., Palmbach, T., & Lee, H. C. (2003). A simple DNA extraction method for marijuana samples used in amplified fragment length polymorphism (AFLP) analysis. *Journal of Forensic Sciences*, *48*, 343–347.

Ming, R., Bendahmane, A., & Renner, S. S. (2011). Sex chromosomes in land plants. *Annual Review of Plant Biology*, *62*, 485–514.

Olmstead, R. G., & Palmer, J. D. (1994). Chloroplast DNA systematics: a review of methods and data analysis. *American Journal of Botany*, *81*, 1205–1224.

Pacifico, D., Miselli, F., Micheler, M., Carboni, A., Ranalli, P., & Mandolino, G. (2006). Genetics and marker-assisted selection of the chemotype in *Cannabis sativa* L. *Molecular Breeding*, *17*, 257–268.

Pacifico, D., Miselli, F., Carboni, A., Moschella, A., & Mandolino, G. (2008). Time course of cannabinoid accumulation and chemotype development during the growth of *Cannabis sativa* L. *Euphytica*, *160*, 231–240.

Palmer, J. D. (1990). Contrasting modes and tempos of genome evolution in land plant organelles. *Trends in Genetics*, *6*, 115–120.

Petit, R. J., Csaikl, U. M., Bordacs, S., Burg, K., Coart, E., Cottrell, J., van Dam, B., Deans, J. D., Dumolin-Lapegue, S., Fineschi, S., Finkeldey, R., Gillies, A., Glaz, I., Goicoechea, P. G., Jensen, J. S., Konig, A. O., Lowe, A. J., Madsen, S. F., Matyas, G., Munro, R. C., Olalde, M., Pemonge, M. H., Popescu, F., Slade, D., Tabbener, H., Taurchini, D., de Vries, S. G. M., Zeigenhagen, B., & Kremer, A. (2002). Chloroplast DNA variation in European white oaks. Phylogeography and patterns of diversity based on data from over 2600 populations. *Forest Ecology and Management*, *156*, 5–26.

Raharjo, T. J., Chang, W. T., Verberne, M. C., Peltenburg-Looman, A. M., Linthorst, H. J., & Verpoorte, R. (2004a). Cloning and over-expression of a cDNA encoding a polyketide synthase from *Cannabis Sativa*. *Plant Physiology and Biochemistry*, *42*, 291–297.

Raharjo, T. J., Widjaja, I., Roytrakul, S., & Verpoorte, R. (2004b). Comparative proteomics of *Cannabis sativa* plant tissues. *Journal of Biomolecular Techniques*, *15*, 97–106.

Rotherham, D., & Harbison, S. (2010). Differentiation of drug and non-drug Cannabis using a single nucleotide polymorphism (SNP) assay. *Forensic Science International*, *207*, 1–3.

Sakamoto, K., Akiyama, Y., Fukui, K., Kamada, H., & Satoh, S. (1998). Characterization; genome sizes and morphology of sex chromosomes in hemp (*Cannabis sativa* L.). *Cytologia*, *63*, 459–464.

Sirikantaramas, S., Morimoto, S., Shoyama, Y., Ishikawa, Y., Wada, Y., Shoyama, Y., & Taura, F. (2004). The gene controlling marijuana psychoactivity: molecular cloning and heterologous expression of Delta1-tetrahydrocannabinolic acid synthase from *Cannabis sativa* L. *The Journal of Biological Chemistry*, *279*, 39767–39774.

Sirikantaramas, S., Taura, F., Tanaka, Y., Ishikawa, Y., Morimoto, S., & Shoyama, Y. (2005). Tetrahydrocannabinolic acid synthase, the enzyme controlling marijuana psychoactivity, is secreted into the storage cavity of the glandular trichomes. *Plant Cell Physiology*, *46*, 1578–1582.

Soltis, E. D., & Soltis, P. S. (2000). Contributions of plant molecular systematics to studies of molecular evolution. *Plant Molecular Biology*, *42*, 45–75.

Staginnus, C., Zörntlein, S., & de Meijer, E. (2014). A PCR marker linked to a THCA synthase polymorphism is a reliable tool to discriminate potentially THC-rich plants of *Cannabis sativa* L. *Journal of Forensic Sciences*, *59*, 919–926.

Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, *17*, 1105–1109.

Taura, S., Morimoto, S., & Shoyama, Y. (1995). First direct evidence for the mechanism of D-1-tetrahydrocannabinolic acid biosynthesis. *Journal of the American Chemical Society*, *117*, 9766–9767.

Taura, F., Morimoto, S., & Shoyama, Y. (1996). Purification and characterization of cannabidiolic-acid synthase from *Cannabis sativa* L. *Journal of Biological Chemistry*, *271*, 17411–17416.

Taura, F., Sirikantaramas, S., Shoyama, Y., Yoshikai, K., Shoyama, Y., & Morimoto, S. (2007). Cannabidiolic-acid synthase, the chemotype-determining enzyme in the fiber-type *Cannabis sativa*. *FEBS Letters*, *581*, 2929–2934.

Taura, F., Tanaka, S., Taguchi, C., Fukamizu, T., Tanaka, H., Shoyama, Y., & Morimoto, S. (2009). Characterization of olivetol synthase, a polyketide synthase putatively involved in cannabinoid biosynthetic pathway. *FEBS Letters*, *583*, 2061–2066.

Thakur, G. A., Duclos, R. I., & Makriyannis (2005). A. Natural cannabinoids: templates for drug discovery. *Life Sciences*, *78*, 454–466.

Valverde, L., Lischka, C., Scheiper, S., Nedele, J., Challis, R., de Pancorbo, M. M., Pfeiffer, H., & Kohnemann, S. (2014a). Characterization of 15 STR Cannabis loci: nomenclature proposal and SNPSTR haplotypes. *Forensic Science International: Genetics*, *9*, 61–65.

Valverde, L., Lischka, C., Erlemann, S., de Meijer, E., de Pancorbo, M. M., Pfeiffer, H., & Kohnemann, S. (2014b). Nomenclature proposal and SNPSTR haplotypes for 7 new *Cannabis sativa* L. STR loci. *Forensic Science International: Genetics*, *13*, 185–186.

Yoshikai, K., Taura, T., Morimoto, S., Shoyama, Y. DNA encoding cannabidiolate synthase, Patent in Japan JP 2000-78979A, 2001, DDBJ/EMBL/GenBank database accession numbers E55107.

PART II

PERSONAL, SOCIAL AND COMMUNITY ASPECTS OF CANNABIS USE

14 *Gender differences in cannabis use disorders* 131
15 *The role of age in the onset and further development of cannabis use disorders* 138
16 *Effects of cannabis use on neurocognition in adolescents and emerging adults* 151
17 *Correlates and consequences of prenatal cannabis exposure (PCE): Identifying and characterizing vulnerable maternal populations and determining outcomes for exposed offspring* 160
18 *Cannabis and clubbing: Relevance of cannabis and polydrug use in the clubbing culture today* 171
19 *Cannabis and sexual behavior* 180
20 *Friendships and cannabis use* 188
21 *Students' knowledge of cannabis* 198
22 *Childhood trauma and cannabis: Risk factors in severe mental disorders?* 208
23 *Parent's influence on children's cannabis use* 215
24 *Cannabis users and premorbid intellectual quotient* 223
25 *Cannabis and traffic accidents* 234

Page left intentionally blank

CHAPTER

14

Gender Differences in Cannabis Use Disorders

R. Secades-Villa, S. Fernández-Artamendi

Addictive Behaviors Research Group, Department of Psychology, University of Oviedo, Oviedo, Spain

KEY FACTS

- Rates of cannabis use and CUD used to be greater among men than women.
- Males are more likely to transition from cannabis use to CUD.
- Women exhibit a telescoping effect.
- Neurocognitive effects of cannabis might be different for males and females.
- Males clearly outnumber females in treatment services for cannabis use disorders.
- Treatment demand is equally low for both genders.
- Triggers of treatment seeking, change in cannabis use, or cessation, show different patterns for males and females.
- Further research is needed to analyze gender differences in CUD.

LIST OF ABBREVIATIONS

A-CRA	Adolescent-community reinforcement approach
CBT	Cognitive behavioral therapy
CUD	Cannabis use disorders
MET	Motivational enhancement therapy

INTRODUCTION

Despite the fact that cannabis is the most widely used and misused illicit drug in the world (United Nations Office on Drugs and Crime, 2012) and is the drug with the highest rate of abuse or dependence (Compton, Grant, Colliver, Glantz, & Stinson, 2004; Stinson, Ruan, Pickering, & Grant, 2006), the gender differences among individuals with cannabis use disorders (CUD) remain poorly characterized. Nevertheless, recent research has brought to attention the important gender differences in this disorder that can lead to clinical consequences in the management of patients, and in the development of prevention strategies. The goal of this chapter is to review the main findings concerning gender differences related to development, clinical features, and treatment trends in CUD.

PREVALENCE OF CANNABIS USE AND CUD

Rates of cannabis use and CUD used to be greater among men than women. Surveys conducted in Europe indicate a cannabis use level of 4.4% for females versus 9.1% for males, among the population aged 15–64 (United Nations Office on Drugs and Crime, 2012). In all European countries, rates of cannabis use are higher for males than for females, with an increase in male-female differences from younger to older groups (Fig. 14.1). Among students aged 15–16 years old, the male to female ratio of lifetime cannabis use is 1.0–1.8, whereas it rises to 1.25–4.0 among adults (European Monitoring Centre for Drugs and Drug Addiction, 2005). These differences are even higher when the focus is switched from lifetime use toward more recent use.

These figures are very similar to those in the United States (Substance Abuse and Mental Health Services Administration, 2013). Rates of marijuana use among adolescents aged 12–17 are 9.0% for males versus 6.7% for

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00015-6
Copyright © 2017 Elsevier Inc. All rights reserved.

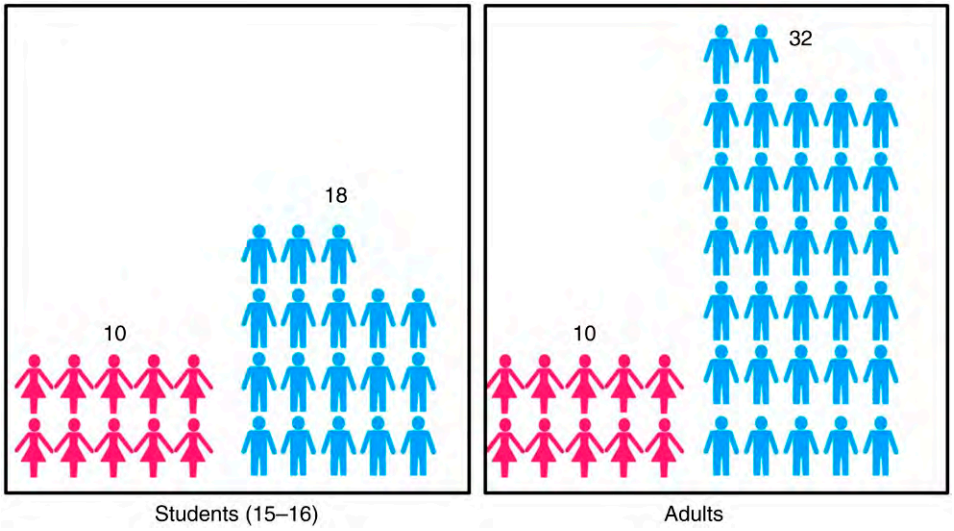


FIGURE 14.1 **Male to female ratio of lifetime cannabis use.**

females, with the difference increasing to 9.3% and 4.9% for the general population (SAMHSA, 2013).

In a nationally representative epidemiologic study of the US population, the prevalence of any 12-month and lifetime CUD was twice for men (2.2% and 11.8%) than for women (0.8% and 5.4%). The odds of both lifetime cannabis abuse and dependence were significantly greater for men than for women (Stinson et al., 2006). A nationally representative report in Australia found that the prevalence of CUD was comparable with that in the United States, and that both cannabis abuse and cannabis dependence were more common in males (Teesson et al., 2012).

In Western countries, gender differences in the rates of cannabis use seem to appear during late adolescence (Goncy & Mrug, 2013), with an increasing gender gap indicating higher consumption in males (ter Bogt et al., 2014). Recent population-based reports with young adults in the United States (Haberstick et al., 2014) have also found that rates of cannabis abuse and dependence were greater among males (5.6% and 10.9%) than among females (2.4% and 6.1%). As in the adult population, the odds for meeting diagnostic criteria for lifetime cannabis abuse and dependence were also greater among males.

Despite the higher prevalence of CUD among men than women, the incorporation of women to cannabis use has grown in recent cohorts, giving rise to gender convergence, and to the fact that the gender gap in the prevalence of CUD has been narrowing (Colell, Sánchez-Niubò, & Domingo-Salvany, 2013; Johnson & Gerstein, 2000).

An interesting issue is that even though males are more likely than females to transition from cannabis use to CUD (Looby & Earleywine, 2007), evidence also

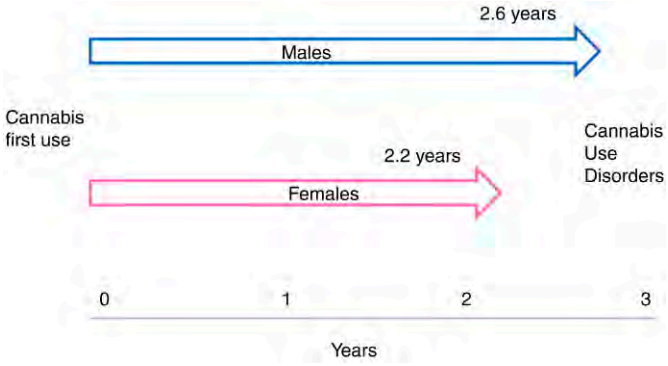


FIGURE 14.2 **Transition from cannabis first use to cannabis use disorder.** *Source: Khan et al. (2013).*

suggests the existence of a "telescoping effect" in the progression from substance use to substance use disorders in women. That is, women would experience an accelerated progression from the first stages of use toward diagnosis of a CUD. The occurrence of this phenomenon has been confirmed in the case of cannabis use by different studies (Khan et al., 2013). Women show shorter intervals between the age of first cannabis use and the onset of a CUD (Fig. 14.2). The factors that put women at higher risk of developing a CUD are not well understood, but it has been suggested that it could be a consequence of associated factors such as patterns of use, or related comorbidities.

CLINICAL FEATURES OF CUD

Results from clinical and population-based studies have shown several gender differences in the clinical features of CUD. In one clinical study with adolescent

patients, the likelihood to meet the criteria for cannabis abuse without cannabis dependence was higher for women than for men (Crowley, Macdonald, Whitmore, & Mikulich, 1998). In another clinical study with adolescents entering outpatient treatment primarily for cannabis abuse or dependence, females were more likely to be diagnosed with more severe CUD (namely dependence) than their male counterparts. The author attributed this result to the higher comorbidity of female participants. Similar results were found by Hernandez-Avila, Rounsaville, & Kranzler (2004) in a clinical study with 271 substance-dependent patients. Despite women experiencing fewer years of regular use of cannabis, they reported more severe psychiatric, medical, and employment complications. Results of a controlled laboratory study indicate that when matched for cannabis use, women are more sensitive to the subjective effects related to cannabis abuse liability, relative to men, fact that may contribute to the enhanced vulnerability to developing CUD (Cooper & Haney, 2014).

Research on the neurocognitive effects of cannabis is still scarce, but is growing rapidly. Results from a nontreatment-seeking community sample of young adults showed that cannabis use was more consistently associated with poorer episodic memory performance in females than males, and, conversely, with poorer decision-making performance for males, but not females (Crane, Schuster, Fusar-Poli, & Gonzalez, 2013). Although still nascent, the current evidence points to sex differences in the impact of cannabis on neurocognitive functioning, but more so for longer term cannabis use than acute effects (Crane et al., 2013).

Studies in community samples suggest that younger individuals and men may be more likely to use cannabis heavily and, consequently, to develop cannabis dependence (Chen, Kandel, & Davies, 1997; von Sydow et al., 2001). Fergusson et al. reported that men were 4.2 and 19.2 times more likely to be arrested and convicted, respectively, on a cannabis-related offense, even after accounting for gender differences in prevalence of CUD (Fergusson, Swain-Campbell, & Horwood, 2003). Men and women also have differences in endorsement rates for most cannabis abuse and dependence symptoms. Results from another community sample showed that the largest discrepancy occurs for hazardous use, endorsed by 53% of the men and 35% of the women, which was attributable to increased drug-taking among men as compared to women (Grant et al., 2006). An interesting result of this study was the highest endorsement of cannabis withdrawal among women in some of the subtypes of cannabis problems analyzed. Other clinical (Levin et al., 2010) and community studies (Agrawal & Lynskey, 2007) also suggest that women may be more likely to report cannabis withdrawal, although some studies find no age or sex differences (Copersino et al., 2010; Swift, Hall, & Copeland, 2000; Wiesbeck et al., 1996). Nevertheless, these findings came from samples of men and women who were not matched according to prequit cannabis use. Assessing cannabis withdrawal and relapse under controlled laboratory conditions would provide critical data to determine the clinical significance of sex in cannabis use disorders (Cooper & Haney, 2014).

Agrawal and Lynskey (2007) found that certain CUD criteria may be more potent markers for cannabis problems in one gender versus another. Specifically, two dependence criteria (quit and problems) indicated a greater level of severity for women, whereas the abuse criteria, legal and hazard, were more difficult for men. Results from a nationally representative sample of the US population (Khan et al., 2013) showed that the severity of CUD was higher in men. Men met more criteria for cannabis abuse, smoked a greater number of joints per day, had longer episodes of CUD, and were older at remission. Despite the fact that severity of CUD was higher in men, there were no significant gender differences in the age at first use of cannabis, the age at heavy use, and the age at onset of the disorder. Nevertheless, these gender differences in mean dose of cannabis smoked daily (joints per day) were not found by another previous study (Lev-Ran et al., 2012). In this study, CUD were associated with significantly poorer mental health and quality of life, and this relationship was particularly pronounced in females with CUD (Lev-Ran et al., 2012). As no significant differences existed in the cannabis smoked daily, the gender differences in mental quality of life were not attributed to a dose-response effect (Table 14.1).

While little work has focused directly on gender differences in psychiatric comorbidity, some studies have found important differences between males and females with CUD. Particularly, previous work has shown women with CUD have higher rates of comorbid mood and anxiety disorders (Grant, 1995; Khan et al., 2013), whereas men with CUD are more likely to have externalizing disorders (Khan et al., 2013). The comorbidity of internalizing disorders among women with CUD may simply reflect the higher rates of mood and anxiety disorders that occur among women in the general population, but a novel finding shows that CUD increase the risk of externalizing disorders among women, compared to

TABLE 14.1 Gender Differences in Severity Markers

Higher severity or prevalence among	
Males	**Females**
Legal and hazard DSM-IV criteria	Difficulty with quitting
Greater number of joints per day	DSM-IV criteria
Longer episodes of CUD	Associated problems
Older at remission	DSM-IV criteria
Comorbid externalizing disorders	Comorbid mood and anxiety disorders

TABLE 14.2 Clinical Features of CUD: Summary Table

- Findings about gender differences in clinical features of CUD are mixed
- Although most of the studies indicate that the severity of CUD is higher among males than among females, some of them found greater severity of cannabis use in women
- Future research is needed to extend these results, and to examine whether gender differences may be due to the inclusion of different samples (clinical vs community samples)
- These gender differences may have implications for gender-specific treatment approach

men (Khan et al., 2013). One of the most comprehensive studies analyzing the effects of cooccurring problems in 5150 adolescents receiving Adolescent-Community Reinforcement Approach (A-CRA) showed that females were overrepresented in the group with internalizing problems (40.8%) and the group with mixed problems (ie, both internalizing and externalizing; 39,5%), compared to externalizing (16.4%) and noncomorbidity groups (16.1%) (Godley et al., 2014). Although this study included users of all drugs, the majority of adolescents presented with cannabis use as the primary substance. Nevertheless, these findings should be verified in future studies (Table 14.2).

TREATMENT SERVICES

Given the higher prevalence of cannabis use and CUD among males, it is not surprising that these figures also replicate among cannabis users in treatment. In these services, males are consistently predominant. Similarly low rates of females have been reported in several studies with various populations, and from different countries. As an example, rates of males in treatment for cannabis as primary substance are above 80% among young adolescents (Battjes, Gordon, O'Grady, Kinlock, & Carswell, 2003; Tims et al., 2002; Fernández-Artamendi, Fernández-Hermida, Godley, & Secades-Villa, 2014), and above 70% for adult cannabis users (Connor et al., 2013; Copeland, Swift, & Rees, 2001; Hathaway, MacDonald, & Erickson, 2008; Hoch et al., 2014; Jungerman & Laranjeira, 2008; Mariani et al., 2011). Even though these rates are consistent with the figures of cannabis users in the general population, Hathaway et al. detected that the rate of males among users in treatment (70%) was significantly higher that among users not in treatment (62%) (Hathaway et al., 2008). Nevertheless, other studies have failed to detect such significant differences (average = 68.5%; Gates, Taplin, Copeland, Swift, & Martin, 2009).

The low presence of females in these services does not seem to be specific of CUD treatment services. Urbanosky et al. compared the characteristics of a big sample of Canadian treatment seekers (Urbanoski, Strike, & Rush, 2005), and found that the rate of males presenting with cannabis as the primary drug of abuse was not significantly different to that of cocaine, alcohol, or opiate users. Accordingly, Mariani et al. (2011) found that male/female ratios are alike among cannabis users and users with other drugs of referral. One factor that influenced the ratio of males was the use of additional drugs. In the study by Arendt and Munk-Jorgensen (2004), 78.4% of all treatment seekers with cannabis use as primary substance were males; this rate rose up to 83.9% among cannabis users who also reported using hard drugs.

What holds true for cannabis treatment services is that treatment demand is very low among both male (14.20%) and female (12.43%) cannabis users; but with no significant differences between them (Khan et al., 2013). Nevertheless, some differences have been ascertained in the sources of these treatment referrals, as well as on the motives stated for moving towards a change in their cannabis use. In the study by Urbanoski et al. (2005), men and women in treatment for their CUD, who were older than 20, were more likely to be self-referred to these services, whereas those under this age indicated family and friends as primary sources of referral. Gender differences arouse in referrals from the legal or justice system since, regardless of age, men were twice as likely to be referred to treatment by these sources. On the other hand, and also irrespective of age, women were more likely to be referred to treatment by physicians or psychiatrists. It would seem that different consequences of cannabis use are triggering treatment demand for males and females.

Among young adolescents, some studies have failed to detect significant gender differences in the factors that encourage the motivation to change (Battjes et al., 2003). However, some other longitudinal studies following this population have found differences in the factors determinant for cessation (Chen & Kandel, 1998). Among women, first-time pregnancy and parenthood were the most important social roles leading to stop cannabis use, and these events were significantly more important than for men. Although the effect of getting married for the first time did not seem to have a differential weight in males than in females, it resulted a significant predictor of cessation for boys, but not for girls. According to the aforementioned studies, external pressures or changes would be exerting greater influence on males' motivation to change and seek treatment, compared to factors, such as, health concerns or motherhood for women. Similar conclusions have been noted in previous studies with alcohol users (Blomqvist & Cristophs, 2005), but more studies are still needed before confirming such hypothesis with cannabis smokers (Fig. 14.3).

Gender differences have been found also in the attitude reported toward one's own cannabis use. In the study by Fernández-Artamendi, Fernández-Hermida, García-Fernández, Secades-Villa, and García-Rodriguez (2013) with adolescent regular cannabis users, boys were

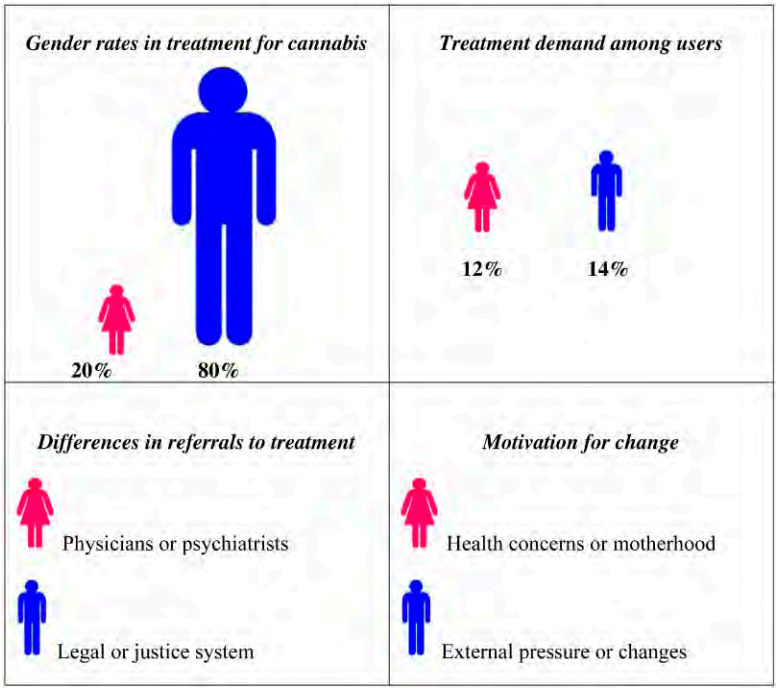


FIGURE 14.3 **Gender differences in treatment services.**

significantly more likely to express no intention to change their cannabis use, whereas girls were more likely to assert attempting a reduction on their own. In fact, Khan et al. (2013) found that rates of remission of CUD in the general population showed to be slightly higher among females (82.1%) than among males (77.8%). The age at remission was also lower for women (23.08 years old vs 25.32) than for men. In this same direction, the longitudinal study by Chen & Kandel (1998) found that, at the age of 34, 76.2% of male cannabis users had stopped using, compared to 82.3% of females. The "telescoping effect" shows that women have faster progression toward CUD. On the other hand, progression to remission, by means of treatment services or on their own, seems to happen faster and earlier, too. Some data support the idea that the telescoping effect would cause women to show a more rapid progression to treatment entry (Hernandez-Avila, Rounsaville, & Kranzler, 2004), which would also facilitate eventual remission. Once cannabis users have decided to quit, no significant differences arise in the barriers deterring males and females from attending treatment (Khan et al., 2013) (Table 14.3).

TABLE 14.3 Treatment Services: Summary Table

- Rates of males in treatment for cannabis use disorders are usually higher than those of women
- Treatment demand for cannabis use disorders is very low among both male and female
- First-time pregnancy and parenthood used to be the most important social roles leading to stop cannabis use among women

TREATMENT EFFECTIVENESS

Some gender differences in the consequences associated to cannabis use, as well as on factors influencing progress toward remission, have been found. Additionally, women seem to be more likely to speak about their cannabis use, and although they are more likely to remain abstinent after treatment, they seem more vulnerable to interpersonal problems and negative feelings that might spark relapse easier than in males (Fattore, 2013).

It remains unanswered, however, whether these differences somehow influence treatment effectiveness. To our knowledge, current research does not provide many specific studies on the possible effects of gender differences on the clinical outcomes and effectiveness of treatments for CUD. The Marijuana Treatment Research Project (2004), one of the most comprehensive clinical trials comparing Motivational Enhancement Therapy (MET) and Cognitive Behavioral Therapy (CBT), with 450 adults, found that clinical results were not influenced by gender. Pretreatment dropout rates were also similar between males and females in this study (Vendetti, McRee, Christiansen, & Herrell, 2002), and, in another brief intervention for adults, completers and noncompleters did not differ by gender either (Copeland et al., 2001). Accordingly, gender did not result to be a moderator of treatment effects in an intervention for adolescents (Hendriks, van der Schee, & Blanken, 2012).

An in-depth analysis of 1819 adolescents receiving A-CRA for their drug use (mostly cannabis use) did not

find significant gender differences in initiation, engagement, or retention (Godley, Hedges, & Hunter, 2011). Although male adolescents reported greater intake severity, rates of change after treatment were equivalent to those of females. Males also reported higher satisfaction with treatment, apparently due to their greater recognition that the staff had significantly helped them. Treatment services are often characterized by a culturally sensitive approach that deals with gender differences during treatment, and could be, therefore, preventing significant differences in clinical outcomes.

In conclusion, greater prevalence of cannabis use and CUD among males translates to greater rates of males in treatment services for these disorders. Some differences have been found in the factors that trigger treatment seeking and motivation to change, but it seems that treatment outcomes do not significantly differ between genders. Research studies on these areas are, however, relatively scarce, and more investigation is needed to determine how gender affects treatment.

MINI-DICTIONARY

Cannabis use disorders (CUD) Cannabis use and/or cannabis dependence according to DSM-IV criteria.
Cannabis withdrawal Abstinence symptoms and signs during withdrawal from chronic cannabis use.
Externalizing disorders Liability to experience substance use disorders, conduct disorder, and antisocial personality disorder.
Internalizing disorders Liability to experience mood and anxiety disorders, such as, major depressive disorder, generalized anxiety disorder, panic disorder, and social anxiety disorder.
Prevalence of CUD Percentage of the population reporting cannabis use or cannabis dependence.
Psychiatric comorbidity Cooccurrence of cannabis abuse or cannabis dependence with other psychiatric disorder.
Telescoping effect Faster progression from substance use to substance abuse or substance dependence.

References

Agrawal, A., & Lynskey, M. T. (2007). Does gender contribute to heterogeneity in criteria for cannabis abuse and dependence? Results from the national epidemiological survey on alcohol and related conditions. *Drug and Alcohol Dependence*, *88*(2–3), 300–307.

Arendt, M., & Munk-Jorgensen, P. (2004). Heavy cannabis users seeking treatment- prevalence of psychiatric disorders. *Social Psychiatry and Psychiatric Epidemiology*, *39*(2), 97–105.

Battjes, R. J., Gordon, M. S., O'Grady, K. E., Kinlock, T. W., & Carswell, M. A. (2003). Factors that predict adolescent motivation for substance abuse treatment. *Journal of Substance Abuse Treatment*, *24*(3), 221–232.

Blomqvist, J. & Cristophs, I. (2005). Vagen till varden: kvinnors och mans skal att soka hjalp respektive borja behandling for alkoholproblem *FoU-rapport* (p. 9). Stockholm: Socialtjanstforvaltningen, Forsknings- och utvecklingsenheten.

Colell, E., Sánchez-Niubò, A., & Domingo-Salvany, A. (2013). Sex differences in the cumulative incidence of substance use by birth cohort. *International Journal of Drug Policy*, *24*(4), 319–325.

Compton, W. M., Grant, B. F., Colliver, J. D., Glantz, M. D., & Stinson, S. (2004). Prevalence of marijuana use disorders in the United States: 1991-1992 and 2001-2002. *Journal of the American Medical Association*, *291*(17), 2114–2121.

Connor, J. P., Gullo, M. J., Chan, G., Young, R. M., Hall, W. D., & Feeney, G. F. (2013). Polysubstance use in cannabis users referred for treatment: drug use profiles, psychiatric comorbidity and cannabis-related beliefs. *Frontiers in Psychiatry*, *4*, 79.

Cooper, Z. D., & Haney, M. (2014). Investigation of sex-dependent effects of cannabis in daily cannabis smokers. *Drug and Alcohol Dependence*, *136*, 85–91.

Copeland, J., Swift, W., & Rees, V. (2001). Clinical profile of participants in a brief intervention program for cannabis use disorder. *Journal of Substance Abuse Treatment*, *20*(1), 45–52.

Copersino, M.L., Boyd, S.J., Tashkin, D.P., Huestis, M.A., Heishman, S.J., Dermand, J.C., ..., Gorelick, D.A. (2010). Sociodemographic characteristics of cannabis smokers and the experience of cannabis withdrawal. *Am J Drug Alcohol Abuse*, *36*(6), 311–319.

Crane, N. A., Schuster, R. M., Fusar-Poli, P., & Gonzalez, R. (2013). Effects of cannabis on neurocognitive functioning: recent advances, neurodevelopmental influences, and sex differences. *Neuropsychology Reviews*, *23*(2), 117–137.

Crowley, T. J., Macdonald, M. J., Whitmore, E. A., & Mikulich, S. K. (1998). Cannabis dependence, withdrawal, and reinforcing effects among adolescents with conduct symptoms and substance use disorders. *Drug and Alcohol Dependence*, *50*(1), 27–37.

Chen, K., Kandel, D. B., & Davies, M. (1997). Relationships between frequency and quantity of marijuana use and last year proxy dependence among adolescents and adults in the United States. *Drug and Alcohol Dependence*, *46*(1-2), 53–67.

Chen, K., & Kandel, D. B. (1998). Predictors of cessation of marijuana use: an event history analysis. *Drug and Alcohol Dependence*, *50*(2), 109–121.

European Monitoring Centre for Drugs and Drug Addiction. (2005). Differences in Patterns of Drug Use between Women and Men. *European drug situation. Technical data sheet*. Lisbon, Portugal: EMCDDA.

Fattore, L. (2013). Considering gender in cannabinoid research: a step towards personalized treatment of marijuana addicts. *Drug Testing and Analysis*, *5*(1), 57–61.

Fergusson, D. M., Swain-Campbell, N. R., & Horwood, L. J. (2003). Arrests and convictions for cannabis related offences in a New Zealand birth cohort. *Drug and Alcohol Dependence*, *70*(1), 53–63.

Fernández-Artamendi, S., Fernández-Hermida, J. R., García-Fernández, G., Secades-Villa, R., & García-Rodriguez, O. (2013). Motivation for change and barriers to treatment among young cannabis users. *European Addiction Research*, *19*(1), 29–41.

Fernández-Artamendi, S., Fernández-Hermida, J. R., Godley, M. D., & Secades-Villa, R. (2014). Evidence-based treatments for adolescents with cannabis use disorders in the Spanish Public Health System. *International Journal of Clinical and Health Psychology*, *14*(3), 186–194.

Gates, P., Taplin, S., Copeland, J., Swift, W., & Martin, G. (2009). *Barriers and facilitators to cannabis treatment*. Randwick, NSW: National Cannabis Prevention and Information Centre, NCPIC Techical Report No.1:..

Godley, S. H., Hedges, K., & Hunter, B. (2011). Gender and racial differences in treatment process and outcome among participants in the adolescent community reinforcement approach. *Psychology of Addictive Behaviors*, *25*(1), 143–154.

Godley, S. H., Hunter, B. D., Fernández-Artamendi, S., Smith, J. E., Meyers, R. J., & Godley, M. D. (2014). A comparison of treatment outcomes for Adolescent Community Reinforcement Approach participants with and without co-occurring disorders. *Journal of Substance Abuse Treatment*, *46*(4), 463–471.

Goncy, E. A., & Mrug, S. (2013). Where and when adolescents use tobacco, alcohol, and marijuana: comparisons by age, gender, and race. *Journal of Studies on Alcohol and Drugs*, *74*(2), 288–300.

Grant, B. F. (1995). Comorbidity between DSM-IV drug use disorders and major depression: results of a national survey of adults. *Journal of Substance Abuse*, *7*(4), 481–497.

Grant, J. D., Scherrer, J. F., Neuman, R. J., Todorov, A. A., Price, R. K., & Bucholz, K. K. (2006). A comparison of the latent class structure of cannabis problems among adult men and women who have used cannabis repeatedly. *Addiction*, *101*(8), 1133–1142.

Haberstick, B. C., Young, S. E., Zeiger, J. S., Lessem, J. M., Hewitt, J. K., & Hopfer, C. J. (2014). Prevalence and correlates of alcohol and cannabis use disorders in the United States: results from the national longitudinal study of adolescent health. *Drug and Alcohol Dependence*, *136*, 158–161.

Hathaway, A. D., MacDonald, S., & Erickson, P. G. (2008). Reprioritizing dependence and abuse: a comparison of cannabis clients in treatment with a nontreatment sample of users. *Addiction Research and Theory*, *16*(5), 495–502.

Hendriks, V., van der Schee, E., & Blanken, P. (2012). Matching adolescents with a cannabis use disorder to multidimensional family therapy or cognitive behavioral therapy: treatment effect moderators in a randomized controlled trial. *Drug and Alcohol Dependence*, *125*(1–2), 119–126.

Hernandez-Avila, C. A., Rounsaville, B. J., & Kranzler, H. R. (2004). Opioid-, cannabis- and alcohol-dependent women show more rapid progression to substance abuse treatment. *Drug and Alcohol Dependence*, *74*(3), 265–272.

Hoch, E., Buhringer, G., Pixa, A., Dittmer, K., Henker, J., Seifert, A., & Wittchen, H. U. (2014). CANDIS treatment program for cannabis use disorders: findings from a randomized multi-site translational trial. *Drug and Alcohol Dependence*, *134*, 185–193.

Johnson, R. A., & Gerstein, D. R. (2000). Age, period, and cohort effects in marijuana and alcohol incidence: United States females and males, 1961-1990. *Substance Use and Misuse*, *35*(6–8), 925–948.

Jungerman, F. S., & Laranjeira, R. (2008). Characteristics of cannabis users seeking treatment in Sao Paulo, Brazil. *Revista Panamericana de Salud Publica – Pan American Journal of Public Health*, *23*(6), 384–393.

Khan, S. S., Secades-Villa, R., Okuda, M., Wang, S., Perez-Fuentes, G., Kerridge, B. T., & Blanco, C. (2013). Gender differences in cannabis use disorders: results from the National Epidemiologic Survey of Alcohol and Related Conditions. *Drug and Alcohol Dependence*, *130*(1-3), 101–108.

Lev-Ran, S., Imtiaz, S., Taylor, B. J., Shield, K. D., Rehm, J., & Le Foll, B. (2012). Gender differences in health-related quality of life among cannabis users: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Drug and Alcohol Dependence*, *123*(1–3), 190–200.

Levin, K. H., Copersino, M. L., Heishman, S. J., Liu, F., Kelly, D. L., Boggs, D. L., & Gorelick, D. A. (2010). Cannabis withdrawal symptoms in non-treatment-seeking adult cannabis smokers. *Drug and Alcohol Dependence*, *111*(1–2), 120–127.

Looby, A., & Earleywine, M. (2007). Negative consequences associated with dependence in daily cannabis users. *Substance Abuse Treatment, Prevention and Policy*, *2*(3), 1–7.

Mariani, J. J., Cheng, W. Y., Bisaga, A., Sullivan, M., Carpenter, K., Nunes, E. V., & Levin, F. R. (2011). Comparison of clinical trial recruitment populations: treatment-seeking characteristics of opioid-, cocaine-, and cannabis-using participants. *Journal of Substance Abuse Treatment*, *40*(4), 426–430.

Stinson, F. S., Ruan, W. J., Pickering, R., & Grant, B. F. (2006). Cannabis use disorders in the USA: prevalence, correlates and co-morbidity. *Psychological Medicine*, *36*(10), 1447–1460.

Substance Abuse and Mental Health Services Administration. (2013). *Results from the 2012 National Survey on Drug Use and Health: summary of national findings*. Rockville, MD: SAMHSA, NSDUH Series H-46.

Swift, W., Hall, W., & Copeland, J. (2000). One year follow-up of cannabis dependence among long-term users in Sydney, Australia. *Drug and Alcohol Dependence*, *59*(3), 309–318.

Teesson, M., Slade, T., Swift, W., Mills, K., Memedovic, S., Mewton, L., Hall, W., et al. (2012). Prevalence, correlates and comorbidity of DSM-IV Cannabis use and Cannabis Use Disorders in Australia. *Australian and New Zealand Journal of Psychiatry*, *46*(12), 1182–1192.

ter Bogt, T. F. M., de Looze, M., Molcho, M., Godeau, E., Hublet, A., Kokkevi, A., Pickett, W., et al. (2014). Do societal wealth, family affluence and gender account for trends in adolescent cannabis use? A 30 country cross-national study. *Addiction*, *109*(2), 273–283.

The Marijuana Treatment Project Research Group. (2004). Brief treatments for cannabis dependence: findings from a randomized multisite trial. *Journal of Consulting and Clinical Psychology*, *72*(3), 455–466.

Tims, F. M., Dennis, M. L., Hamilton, N., Buchan, J. B, Diamond, G., Funk, R., & Brantley, L. B. (2002). Characteristics and problems of 600 adolescent cannabis abusers in outpatient treatment. *Addiction*, *97*, 46–57.

United Nations Office on Drugs and Crime. (2012). *World drug report*. New York, NY: UNODC.

Urbanoski, K. A., Strike, C. J., & Rush, B. R. (2005). Individuals seeking treatment for cannabis-related problems in Ontario: demographic and treatment profile. *European Addiction Research*, *11*(3), 115–123.

Vendetti, J., McRee, B., Christiansen, K., & Herrell, J. (2002). Correlates of pre-treatment drop-out among persons with marijuana dependence. *Addiction*, *97*, 125.

von Sydow, K., Lieb, R., Pfister, H., Höfler, M., Sonntag, H., & Wittchen, H. U. (2001). The natural course of cannabis use, abuse and dependence over four years: a longitudinal community study of adolescents and young adults. *Drug and Alcohol Dependence*, *64*(3), 347–361.

Wiesbeck, G. A., Schuckit, M. A., Kalmijn, J. A., Tipp, J. E., Bucholz, K. K., & Smith, T. L. (1996). An evaluation of the history of a marijuana withdrawal syndrome in a large population. *Addiction*, *91*(10), 1469–1478.

CHAPTER

15

The Role of Age in the Onset and Further Development of Cannabis Use Disorders

S. Behrendt

Institute of Clinical Psychology and Psychotherapy, Technische Universitaet Dresden, Dresden, Germany

SUMMARY POINTS

- The main incidence phases for cannabis use (CU) and disorders (CUD) are in adolescence and early adulthood.
- A younger age at first CU is associated with a higher risk of CUD.
- This association might be explained by features of adolescence, susceptibility to cannabis effects, impairment of executive functions, a vulnerability for disinhibitory psychopathology, psychosocial derailment, and problematic use motivation.
- A heuristic model on the role of earlier CU onset in CUD risk integrates nonnormative risk, age-related maturational processes, and coping abilities.
- Relatively little is known about the role of earlier CU onset in the course of CUD.

KEY FACTS OF CANNABIS USE AND DISORDERS

- Cannabis is a widely used, often illegal, substance.
- Cannabis abuse refers to a CUD characterized by repeated negative psychosocial consequences of CU such as neglect of role obligations, or interpersonal problems.
- Cannabis dependence refers to a CUD characterized by clinical symptoms such as tolerance, or loss of control over consumption.
- Only a relatively small proportion of subjects who try cannabis develop a CUD.
- A substantial proportion of transitions from first use to CUD occur within a few years after first use.

LIST OF ABBREVIATIONS

CU	Cannabis use
CUD	Cannabis use disorder
EDSP	Early Developmental Stages of Psychopathology Study
NESARC	National Epidemiologic Survey on Alcohol and Related Conditions
NHSDA	National Household Survey on Drug Abuse
NSDUH	National Survey on Drug Use and Health

INTRODUCTION

Cannabis is a widely used psychotropic substance in many western countries (European Monitoring Centre for Drugs and Drug Addiction, 2012; United Nations Office on Drugs and Crime, 2012). Still, (conditional[a]) substance use disorder rates are usually lower for cannabis than for alcohol and nicotine (Compton, Grant, Colliver, Glantz, & Stinson, 2004; Grant, Hasin, Chou, Stinson, & Dawson, 2004; Grant et al., 2004b; Wagner & Anthony, 2002; Wittchen et al., 2008). Given relatively low cannabis use disorder rates (DSM-IV abuse and dependence; CUD) it is important for targeted preventive efforts to specifically identify those cannabis users who are at elevated risk of CUD. A robust predictor for substance use disorders is a younger age at onset of substance use. This has been shown for nicotine, cannabis and alcohol (Behrendt, Wittchen, Höfler, Lieb, & Beesdo, 2009; Chen, O'Brien, & Anthony, 2005; DeWit, Adlaf, Offord, & Ogborne, 2000).

This chapter focuses on the role of age in the risk of CUD. It provides an overview of high-risk age periods for incident cannabis use (CU) and CUD, and of

[a]Conditional disorder rates: rates of disorder among those who tried the substance at least once.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00016-8
Copyright © 2017 Elsevier Inc. All rights reserved.

empirical evidence on the association between early first CU and CUD risk. Putative explanatory mechanisms are presented and integrated in a heuristic model of early CU in CUD risk.

HIGH-RISK AGE PERIODS FOR CU AND CUD INCIDENCE

There exist several epidemiological studies—predominantly from Western countries—on age of onset distributions of CU. Contrasting differences in prevalence rates, high-risk incidence periods are relatively consistent across countries, clearly revealing adolescence and early adulthood as high-risk periods for first CU (Vega, Aguilar-Gaxiola, Andrade, Bijl, & Borges, 2002). For example, studies with samples aged between 14 and 15 years find lifetime CU rates up to about 30% (Kokkevi, Gabhainn, Spyropoulou, & Risk Behaviour Focus Group of the HBSC, 2006), and peaks of incidence rates at about the age of 14 (Monshouwer, Smit, de Graaf, van Os, & Vollebergh, 2005). These findings from young samples could, however, be influenced by right censoring.

Still, in a German representative community sample (EDSP-Study) followed until the ages of 21–34, the main incidence phase for CU were ages 14–19 (Behrendt et al., 2009). In a Swiss national survey including subjects aged 15–49 years, about 93% of CU onsets occurred up to the age of 24 (Müller & Gmel, 2002). Comparable results were shown in national epidemiological samples from the United States and Australia, including middle-aged and older adults (Lenton, Liang, & Chikritzhs, 2011; Wagner & Anthony, 2002, 2007). In contrast, late onset of CU appears rare. In an epidemiological survey (baseline age 18–99 years), only 1.9% reported first CU at a 3-year follow-up. The majority of these subjects were young adults (Agrawal & Lynskey, 2009).

Less information is available on high-risk periods for CUD onset. In the EDSP-Study, the main incidence phase for DSM-IV cannabis abuse and dependence was at the ages of 15–19 years (Behrendt et al., 2009). In the National Comorbidity Survey, the high-risk phase for DSM-III-R cannabis dependence was in adolescence and early adulthood (Wagner & Anthony, 2002). A study with a baseline age-range of 18–64 years (de Graaf, ten Have, Tuithof, & van Dorsselaer, 2013) showed 3-year follow-up CUD incidence rates below 0.4%, possibly indicating that CUD incidence in middle and later adulthood is rare.

In summary, research clearly identifies the age periods of adolescence and early adulthood as high-risk phases for incident CU and CUD. Moreover, a substantial proportion of DSM-IV CUD cases occur in the first few years after first CU (Behrendt et al., 2009; Lopez-Quintero et al., 2011), leaving little time for secondary preventive efforts.

YOUNG AGE AT CU ONSET AND ITS ASSOCIATION WITH CUD RISK

Empirical Evidence

As outlined previously, age is related to CU and CUD onset in terms of specific high-risk periods in life. Beyond this, the question arises whether the timing of first CU (a younger age at CU onset compared to an older age) is a risk factor for CUD.

Several epidemiological studies address the topic, all of which report higher CUD risk in those with earlier onset. Winters and Lee (2008) report a higher risk of DSM-IV CUD by recent CU initiation in subjects aged between 13 and 18 years, compared to subjects aged 22–26. NHSDA data revealed a higher risk of DSM-IV cannabis dependence for early CU initiation defined as, respectively, 11–13, 14–15, and 16–17 years versus initiation after the age of 20 (Chen et al., 2005). In the NESARC, CU onset before the age of 14 was associated with a higher risk of DSM-IV cannabis dependence (Lopez-Quintero et al., 2011). In the EDSP-Study, early first CU was defined by taking into account the high-incidence phase of CU in the sample. For each year of the high-incidence phase, CU onset at a later age was compared to earlier onset. Later CU onset was consistently associated with a lower risk of DSM-IV cannabis abuse and dependence (Behrendt et al., 2009).

Of note, in all studies except one, these associations remained significant after adjustment for potentially influential covariates as alcohol use, nicotine use, alcohol use disorders, and nicotine dependence prior to CUD onset (Behrendt et al., 2009), externalizing disorders, and paternal and maternal alcohol dependence (in another EDSP-analysis[b]; Behrendt et al., 2012), socio-economic status, and drug use history (Chen et al., 2005; Winters & Lee, 2008). However, the association observed in the NESARC was nonsignificant, after thorough adjustment for mental disorders and socio-economic variables (Lopez-Quintero et al., 2011).

Methodological Considerations

While these results may serve as compelling support for earlier CU onset as a risk factor for CUD, several methodological factors have to be considered:

1. A commonly agreed definition of early CU onset is lacking. Applied definitions differ by study, making comparisons difficult. It has been suggested to define early onset in consideration of the main incidence

[b]With age at onset of CU as a continuous measure.

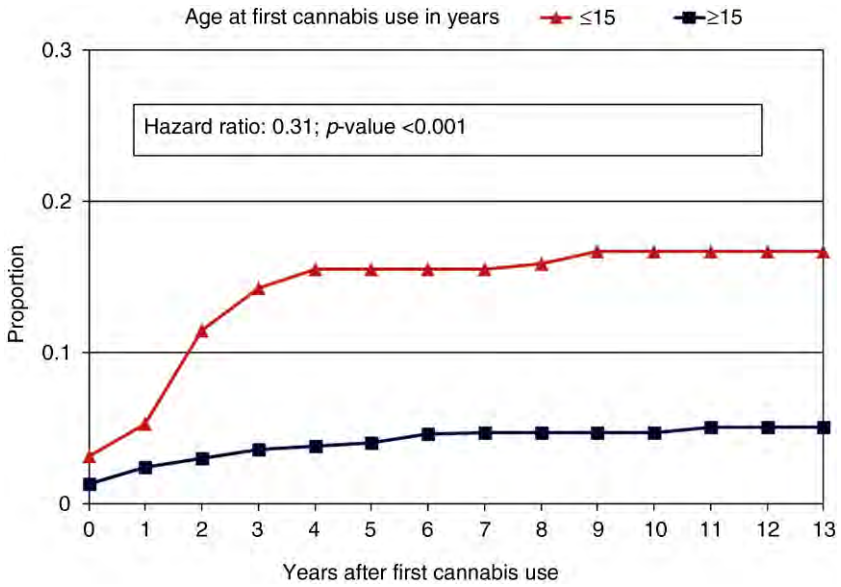


FIGURE 15.1 **Risk of DSM-IV cannabis dependence by age at first CU.** A higher age at first CU was associated with a lower risk of cannabis dependence in the EDSP-Study. *Source: Behrendt et al. (2009); unpublished figure.*

period for CU in the respective sample (Behrendt et al., 2009). This constitutes an alternative to arbitrary definitions or substitute definitions that do not apply for CU, such as, legal drinking age. Comparing several groups with early onset to the group with "latest onset" (Chen et al., 2005; Winters & Lee, 2008) may be problematic, as it can lead to extreme comparisons for those groups with very early onset. Moreover, it may not be helpful in identifying specific risk-phases within the main incidence phase of CU.

2. The association between early onset of substance use and a higher risk of substance use disorders could be an artefact, as subjects with earlier use onset simply have more time to consume the substance and to develop a disorder (Anthony & Petronis, 1995). For cannabis, two well-designed studies address this problem by investigating CUD risk by early CU onset, in subjects with recent CU onset only (Chen et al., 2005; Winters & Lee, 2008). Both find an elevated CUD risk for those with earlier CU initiation. Moreover, Winters and Lee (2008) showed that the proximity of incident CU was not influential in this association.
3. Current CUD are rarer among older individuals (Compton et al., 2004) and CUD are not necessarily stable (Perkonigg et al., 2008). Hence, two factors in combination could lead to an over-report of "late onset CU and no CUD" in older subjects: forward telescoping (the more distal the event of interest, the higher the probability to incorrectly report a higher age of onset) and forgetfulness for transient past CUD episodes. However, two studies on recent CU onset and CUD development—a study design that controls this problem—find associations between early CU and higher CUD risk (Chen et al., 2005; Winters & Lee, 2008) (Fig. 15.1; Tables 15.1 and 15.2).

Possible Explanations for the Association between a Younger Age at First CU and CUD Risk

The findings on the association between early onset CU and an elevated risk of CUD described previously agree with findings on other substances, such as, nicotine (Behrendt et al., 2009; Breslau, Fenn, & Peterson, 1993) and alcohol (DeWit et al., 2000). However, it has to be noted that these studies stem from few Western countries.

Despite this evidence, it is hard to envision how chronological age itself might contribute to CUD risk. Accordingly, Obot, Wagner, & Anthony (2001) speculated that "…early-onset drug use qualifies as a non-causal marker of special vulnerability to later drug problems…". The question arises as to which factors could underlie the association between early onset of CU and CUD risk. In the following, the role of early CU in CUD risk is discussed in the context of existing models on substance use disorder development, and related empirical evidence. For the latter, the focus will be on studies that compare early to later CU onset, instead of accepting the broader comparison category "later or no CU" when considering the effect of earlier CU on putative mediating factors in CUD risk (eg, psychosocial derailment following early CU). Studies using the broader category do not permit conclusions on specifically early versus later first CU. Evidence exists for differential results for comparisons with later onset users and nonusers (Gruber, Sagar, Dahlgren, Racine, & Lukas, 2012).

TABLE 15.1 Epidemiological Studies on Earlier Onset of CU in CUD Risk

Study	Sample	Definition of early CU onset	Result on the risk of CUD by early onset of CU	Consideration of covariates	Methodological advantages[a]	Methodological limitations[a]
NHSDA (Chen et al., 2005)	*N* = 3352 recent onset cannabis users from a representative sample from the United States (*N* = 114,241) Age: 12+ Cross-sectional survey	Early onset: 11–13 years, 14–15 years, 16–17 years, 18–20 years Late onset (comparison group): after age of 20	Higher risk of DSM-IV cannabis dependence in individuals with onset between ages of 11 and 17 *These results were also significant after covariate adjustment*	Socio-economic variables Drug use history	The design reduces the risk of • an artefact due to "more time to develop a CUD" in subjects with earlier onset of use • forward telescoping • problems remembering CUD symptoms	Comparison with "latest" onset
NSDUH (Winters & Lee, 2008)	Data from a subsample of the representative NSDUH from the United States Age: 12–26 years Cross-sectional survey	Early onset: recent onset (in past 2 years) in subjects aged 12, 13, 14, 15, 16, 17, or 18 Late onset (comparison group): recent onset in subjects aged 22–26	Higher risk of DSM-IV CUD in individuals with earlier onset (for ages 13–18) *These results were significant after covariate adjustment*	Socio-economic variables Alcohol use prior to CU	The design reduces the risk of • an artefact due to "more time to develop a CUD" in subjects with earlier onset of use • forward telescoping • problems remembering CUD symptoms	Comparison with oldest group among recent onset users Information on age at interview among recent onset users instead of information on age at onset of CU
EDSP (Behrendt et al., 2009)	Representative community sample (*N* = 3021) from metropolitan Munich, Germany Baseline age: 14–24 years; age at last follow-up: 21–34 years Longitudinal study	Early onset (for analysis on cannabis abuse): at age ≤14, ≤15, ≤16, ≤17, ≤18, ≤19 Early onset (for analysis on cannabis dependence): at age ≤14, ≤15, ≤16 Late onset: any onset occurring later	Consistently lower risk of DSM-IV cannabis abuse and DSM-IV cannabis dependence in subjects with later onset *Results were significant after covariate adjustment*	Alcohol and nicotine use before onset of CUD Alcohol use disorder and nicotine dependence before onset of CUD (separate analysis)	Early onset of cannabis is defined in reference to the high-risk phase of first CU in the sample Young sample (reduction of risk of forward telescoping and problems remembering CUD symptoms)	The assessment period covered the high-risk phase of CUD in the sample. This may reduce the risk of an artefact due to "more time to develop CUD" in subjects with earlier onset of use, but this risk cannot be excluded

(*Continued*)

TABLE 15.1 Epidemiological Studies on Earlier Onset of CU in CUD Risk (*cont.*)

Study	Sample	Definition of early CU onset	Result on the risk of CUD by early onset of CU	Consideration of covariates	Methodological advantages[a]	Methodological limitations[a]
Behrendt et al. (2012)		Continuous measure of age at first CU	Higher risk of DSM-IV CUD *Results were significant after covariate adjustment*	Externalizing disorders, paternal and maternal alcohol dependence, parental illegal drug dependence, age at onset of nicotine and alcohol use	A young sample (reduction of risk of forward telescoping and problems remembering CUD symptoms)	No cut-off for early CU applied The temporal sequence between CUD onset and covariates was not established The assessment period covered the high-risk phase of CUD in the sample. This may reduce the risk of an artefact due to "more time to develop CUD" in subjects with earlier onset of use, but this risk cannot be excluded
NESARC (Lopez-Quintero et al., 2011)	Subsample of lifetime cannabis users ($N = 7389$) of the nationally representative NESARC from the United States (participants of wave one and follow-up assessment) Baseline age: 18+	Early onset: at age ≤13 Late onset: any onset occurring later	Higher risk of DSM-IV cannabis dependence *This association was not found after covariate adjustment*	Socio-economic variables Mental disorders		Early onset not explicitely defined in reference to the high-risk phase of first CU in the sample Wide age range: possible risk of forward telescoping for first CU and problems remembering CUD in older subjects

[a]*Specific methodological advantages and disadvantages of studies on early CU onset as a risk factor for CUD as described in Table 15.2.*

Several epidemiological studies find an association between earlier onset of CU and CUD risk. CU, cannabis use; CUD, cannabis use disorder.

TABLE 15.2 Important Methodological Aspects of Studies on Early Onset of Substance Use and Substance Use Disorder Risk

Methodological obstacles	Possible solutions
There is no commonly agreed definition of early substance use onset	Definition of early onset of substance use in reference to the main incidence phase of substance use in the sample
Comparisons with latest onset of use do not permit identifying high-risk phases within the main incidence period of substance use	Consideration of other than extreme comparisons
Subjects with earlier onset of use may have had more time to develop a substance use disorder	Investigation of substance use disorder onset in subjects with first substance use within a defined and limited recent time period
Older subjects may show forward telescoping for first substance use. They may forget more distant past episodes of substance use disorders	Incidence of substance use and disorders mainly occurs in the second and third decade of life. Studies should focus on subjects from age groups for whom this phase of life is not too distant

Specific methodological aspects have to be considered in studies on early onset of substance use and substance use disorder risk.

Before focusing on the etiological models, two important features of earlier CU as a risk factor for CUD are addressed: its association with CU escalation, and its substance-specificity.

ESCALATION OF CU

Increases in CU amount and frequency after initiation are a prerequisite for CUD development. Earlier CU onset has to be linked with CUD risk via an association with a greater risk of intensified CU. Surprisingly, empirical evidence for a higher risk of such CU-escalation in early onset CU is relatively rare. In a large convenience sample aged 12–49, CU onset before the age of 16 was associated with more frequent CU (Baumeister & Tossmann, 2005). In an Australian study (Silins et al., 2013), CU before the age of 16 was associated with continued regular use in early adulthood. Of note, the studies measured CU in the 4 weeks and 12 months, respectively, prior to the assessment of the outcome, thus not reporting problematic CU that might have occurred earlier. They did not address CU duration. In both studies, the observed use patterns may have occurred in individuals who were already dependent. To summarize, there is somewhat restricted documentation of a higher risk of escalating CU in the association between earlier CU onset and CUD risk leaving an important part of the progression from earlier CU to CUD understudied.

HOW SPECIFIC IS EARLIER CU AS A RISK FACTOR FOR CUD?

It is of interest whether the association between earlier CU and CUD risk is cannabis-specific. If it was not cannabis-specific and, for example, mainly explained by a general vulnerability for externalizing behavior (Hicks et al., 2004) that manifested itself in early experimentation with different substances and lack of control over progressing use, these unspecific factors would deserve more weight in explanatory models, than cannabis-specific aspects. In a high-risk sample, earlier onset of alcohol use was associated with CUD risk (Kirisci et al., 2013), mediating the association between a heritable vulnerability for substance use disorders and CUD. In an epidemiological study, early smoking, but not drinking, was associated with CUD, even after adjustment for, for example, externalizing problems (van Leeuwen et al., 2013). Both studies are in support of cannabis-unspecific risk. Another study that considered earlier CU in the analysis (Behrendt et al., 2012) found that earlier CU, externalizing disorders, and paternal alcohol dependence, but not earlier drinking and smoking, were associated with CUD risk. In summary, findings to date indicate that cannabis-specific and nonspecific factors may underlie the association between earlier CU and CUD.

In the following, the role of earlier CU in CUD risk is presented in the context of selected models for substance use disorder development[c] (for an overview, see Tables 15.3 and 15.4).

THE ROLE OF EARLY CU IN CUD RISK IN THE CONTEXT OF ETIOLOGICAL MODELS FOR SUBSTANCE USE DISORDERS

Adolescence as a Phase of Vulnerability for Addiction

Adolescence is a period of transition from childhood into adulthood. Typical behavioral and motivational

[c]The models listed here are influential concepts on substance use disorder development in adolescents. They do not necessarily include early CU as a core factor, but permit a discussion of a role of early CU onset within the model. The core factors and mechanisms in the different models presented here are partially overlapping.

TABLE 15.3 Important Features of Earlier CU as a Risk Factor for CUD

Summary	
Is earlier onset of CU a risk factor for escalating CU?	**Is earlier onset of CU a substance-specific risk factor?**
A problematic increase of CU is a prerequisite for the development of CUD Empirical evidence from longitudinal epidemiological studies on earlier CU onset as a risk factor of problematic use progression is relatively rare This constitutes an important missing link between earlier CU onset and the risk of CUD Research is needed on earlier CU onset as a risk factor for problematic CU patterns that can lead to CUD. The specific features of these use patterns have to be identified	This question is crucial for understanding the role of earlier CU in CUD risk. It is also important for the choice of preventive efforts The association between earlier onset of CU and CUD could be either due to cannabis-specific factors (eg, THC effects) or unspecific factors (eg, impulsivity) Empirical evidence yields mixed results. It is possible that both substance-specific and unspecific factors contribute to the observed association between earlier onset of CU and CUD

It is important to understand whether earlier onset of CU is related to escalating CU and a substance-specific risk factor for CUD. CU, cannabis use; CUD, cannabis use disorder.

features of adolescence are, for example, increased novelty seeking, risk taking, and greater peer influence. These can be understood as functional in the "transition… to independence…" and new roles (Doremus-Fitzwater, Varlinskaya, & Spear, 2010). Adolescence is also a period of brain maturation, with an imbalance between slower development of the prefrontal cortex, which is important for executive functions and faster development of the motivation system (Chambers, Taylor, & Potenza, 2003; Crews, He, & Hodge, 2007; Romer et al., 2011).

It has been postulated that, while generally adaptive, these features may make adolescents vulnerable to the influence of drug using peers and rewarding substance effects, and place them at risk of undercontrolled substance use (Chambers et al., 2003; Crews et al., 2007; Doremus-Fitzwater et al., 2010; Romer et al., 2011). Following this hypothesis, tendencies of undercontrolled behavior typical for adolescence could link earlier CU to CUD.

Vulnerability for THC Effects in Adolescence

Pleasant subjective reactions to CU in adolescence are related to a higher CUD risk (Fergusson, Horwood, Lynskey, & Madden, 2003). Increased positive subjective CU-effects and reduced negative effects earlier in adolescence could influence CU-related reward learning—in terms of reduced experience of negative CU-consequences and increased rewarding effects in earlier CU—and thus lead to CUD. Results on THC-reactivity from animal studies show reduced aversive effects in adolescent, compared to adult rodents (Quinn et al., 2008; Schramm-Sapyta et al., 2007). However, in humans, subjective aversive effects were not associated with reduced CUD risk (Fergusson et al., 2003). Also, a review on animal studies found no evidence for more rewarding effects or self-administration in adolescent versus adult animals (Schramm-Sapyta, Walker, Caster, Levin, & Kuhn, 2009). Because of ethical considerations, however, objective THC-effects in younger versus older human adolescents are difficult to study.

It can be criticized that models on adolescence as a phase of vulnerability do not explain why only a small proportion of adolescents show risky CU (Romer et al., 2011).

Impairment of Executive Functions

Alternatively, it is not common features of adolescence, but individual risk factors, such as impairments in executive functions, that could link early CU with CUD risk. Such impairments and dominance of automatic over controlled cognitive processes are widely regarded as influential in substance use disorder etiology (Bühringer, Wittchen, Gottlebe, Kufeld, & Goschke, 2008; Redish, Jensen, & Johnson, 2008; Wiers, Gladwin, Hofmann, Salemink, & Ridderinkhof, 2013). Impaired cognitive functioning, such as poor regard of long-term consequences, could lead to earlier CU onset, undercontrolled CU, and CUD. It might be hypothesized, that in this process, early CU effects might disrupt maturation of executive control which could add to the risk of undercontrolled CU.

Comparing earlier and later CU, and earlier regular with later regular CU onset, respectively, studies find some indications for reduced executive functioning in earlier CU onset, but results are inconsistent over task conditions and domains of cognitive functioning (Becker, Wagner, Gouzoulis-Mayfrank, Spuentrup, & Daumann, 2010; Gruber et al., 2012; Pope et al., 2003), and two studies (Gruber et al., 2012; Pope et al., 2003) used samples with heavy CU. Importantly, available studies are often cross-sectional, obscuring the temporal sequence of executive function and early CU.

TABLE 15.4 Early CU in CUD Risk in the Context of Existing Models of Substance Use Disorder Etiology

Model	Core assumption	Important features	Important criticism or need of future research	Future research questions
Adolescence as a phase of vulnerability for addiction	Generally adaptive changes in behavior and motivation in adolescence contribute to the risk of substance experimentation, risky use, and substance use disorders An imbalance in brain maturation in adolescence (faster maturation of the motivational system vs the prefrontal cortex) is involved in these risks	Not cannabis-specific	Does not explain why only a small proportion of adolescents develop a CUD	Can the higher risk of CUD in subjects with earlier onset of use (partially) be attributed to the degree of expression of typical behavioral and motivational features and brain maturation in adolescence?
Vulnerability for THC effects in adolescence	Adolescents may be more susceptible to pleasant (rewarding), and less susceptible to unpleasant effects of THC. This could lead to frequent CU	Cannabis-specific	Does not explain why only a small proportion of adolescents develop a CUD Difficulty to obtain objective information on humans with respectively early and late onset	Which objective reactions to THC do younger and older human adolescents show? Of note: this research question is difficult to address in humans due to ethical restraints
Impairment of executive functions	A dominance of automatic over controlled cognitive processes contributes to substance use disorder etiology Lower executive functioning can lead to early first use and progression to CUD Alternatively, earlier substance use can disrupt processes of brain maturation and lead to less executive control over CU	This model considers cannabis-specific (disruption of brain maturation) or unspecific (if preexisting low executive functions lead to CU initiation) factors	Relatively little is known about executive functions in early versus late onset of CU Especially, more longitudinal studies that permit clarification of the temporal sequence of impaired executive function and early CU are needed	For which executive function can impairment predict early CU initiation and progression to CUD?
General heritable vulnerability for disinhibitory psychopathology	Substance use disorders and other mental disorders characterized by undercontrolled and impulsive behaviors share a common and unspecific heritable vulnerability Earlier onset of CU and the risk of CUD are both related to this underlying vulnerability	Not cannabis-specific	Other factors—such as THC effects—may also be of influence in the association between earlier CU onset and CUD	It would be of interest to study the vulnerability for disinhibitory psychopathology in earlier onset of use and CUD risk in relation to other factors, such as, THC effects, cognitive development or coping behavior
Psychosocial derailment	The nonnormative earlier onset of CU leads to adverse psychosocial consequences which lead to increased use and CUD	The model is cannabis-specific as it refers to the nonnormative aspects of earlier CU onset: • Cannabis is an illegal substance in many countries • Among cannabis users, early CU onset can be a deviation from the main time-period of CU initiation	Additional longitudinal-epidemiological studies that investigate possibly derailing psychosocial consequences of earlier versus later CU onset that could act as possible mediators in the link between earlier CU and CUD are needed	It would be of interest to further identify specific consequences of earlier (in contrast to later) onset of CU in the areas of, for example, cognitive, social and behavior development, mental health, and educational achievement
Problematic use motivation in earlier CU onset	Earlier CU is linked with increased CUD risk because of problematic use motives that are associated with early onset of use and increased use after initiation	Not cannabis-specific	Additional epidemiological-longitudinal studies on CU motives that are specifically related to earlier onset of CU are needed	Which CU motives are associated with earlier onset of CU? Are they also associated with the risk of CUD?

Earlier onset of CU is associated with CUD risk—but the underlying mechanisms remain to be identified. CU, cannabis use; CUD, cannabis use disorder.

General Heritable Vulnerability for Disinhibitory Behavior and Psychopathology

In this model, the core underlying factor is a heritable vulnerability that is common to substance use disorders, and other mental disorders characterized by impulsive behaviors. Twin studies including parental information support this model (Hicks, Krueger, Iacono, McGue, & Patrick, 2004; Marmorstein, Iacono, & McGue, 2009). Earlier CU onset could be regarded as an expression of undercontrolled behavior (eg, it violates norms on drug use). The link with CUD risk could then be explained by the underlying vulnerability for disinhibitory behavior that could contribute to escalating CU.

In support of this, epidemiological data showed that children with an alcohol dependent parent initiate CU earlier than controls (Obot et al., 2001). McGue, Iacono, and Krueger (2006) showed that the shared vulnerability for externalizing psychopathology is accounted for by genetic factors, as is the phenotypic link between early behavior problems and externalizing psychopathology. In a high-risk sample, earlier CU onset mediated the association between the degree of familial transmissible SUD liability, covering self-regulation, impulsive and antisocial behavior, and CUD (Kirisci et al., 2013). Moreover, an association between early CU onset and risk of current CUD in the NESARC was attenuated and nonsignificant, after controlling for covariates, including impulsivity. However, here, the outcome of the 12-month CUD may have included recurrent and stable CUD cases (Blanco et al., 2014).

One study found an association between earlier CU onset and CUD, after adjusting for early use of legal substances, externalizing disorders, and parental alcohol dependence (Behrendt et al., 2012). This finding may suggest an additional role of other factors (eg, THC effects).

Psychosocial Derailment and Problem Accumulation

One explanation for the association between earlier CU onset and CUD could be that especially earlier and thus, nonnormative CU leads to adverse psychosocial consequences, which then lead to intensified CU and CUD.

For example, in support of this, in a Latent Class Analysis, Wittchen et al. (2009) found an association between earlier CU onset and the probability of being in a class with elevated probabilities of a range of substance-related mental and social problems. However, no temporal order was established in the analysis.

No significant difference in the risk of subsequent depressive symptomatology was found for CU onset before the age of 18 (compared to age ≥ 18) in the cross-sectional NSDUH (Fairman & Anthony, 2012). To date, there are relatively few epidemiological, prospective-longitudinal studies on derailing psychosocial consequences of earlier versus later CU initiation as possible mediators in the association between earlier CU and CUD.

Problematic Use Motivation in Early Onset CU

Early CU could be linked with CUD risk via problematic use motives that are associated with early use, as well as the risk of intensified consumption and CUD. For alcohol, an epidemiological study found that subjects with adverse life events (eg, physical abuse) were more likely to report coping motives for their drinking (Rothman, Edwards, Heeren, & Hingson, 2008). Of interest, these adverse life events were associated with a higher risk of drinking before the age of 15 (compared to onset after the age of 20). This might imply that early adversity (eg, life events, mental disorders) and subsequently arising problematic use motives (coping and self-medication motives) could contribute to both earlier CU onset and escalation into CUD.

AN INTEGRATIVE MODEL OF THE ROLE OF YOUNGER AGE AT CU ONSET IN CUD RISK

Based on the existing insight discussed previously, an integrative model on putative mechanisms underlying the observed association between earlier CU onset and CUD is presented (see Fig. 15.2). The function of this heuristic model is to integrate different categories of putative underlying factors, and to model their putative relationship with age, CUD, and each other.

The model incorporates aspects of other models of CUD development, for example, the vulnerability-stress-model of mental disorder development, and the CUD development model recently presented by Blanco et al. (2014) as combined effects of different risk factors, and the relative impact of a risk factor depending on its occurrence in certain developmental periods.

A core assumption of the model is that chronological age at first CU is indirectly (not directly) connected with CUD risk, via different factors and pathways. The postulated associations between chronological age and the influential factors may vary in strength between factors and age-periods. Another core assumption of the model is that the underlying factors act on CUD risk via intensified CU, as a prerequisite for CUD. The model integrates three categories of putative influential factors. It is assumed that factors can interact within and between the three categories. These are: (1) the status of expectable

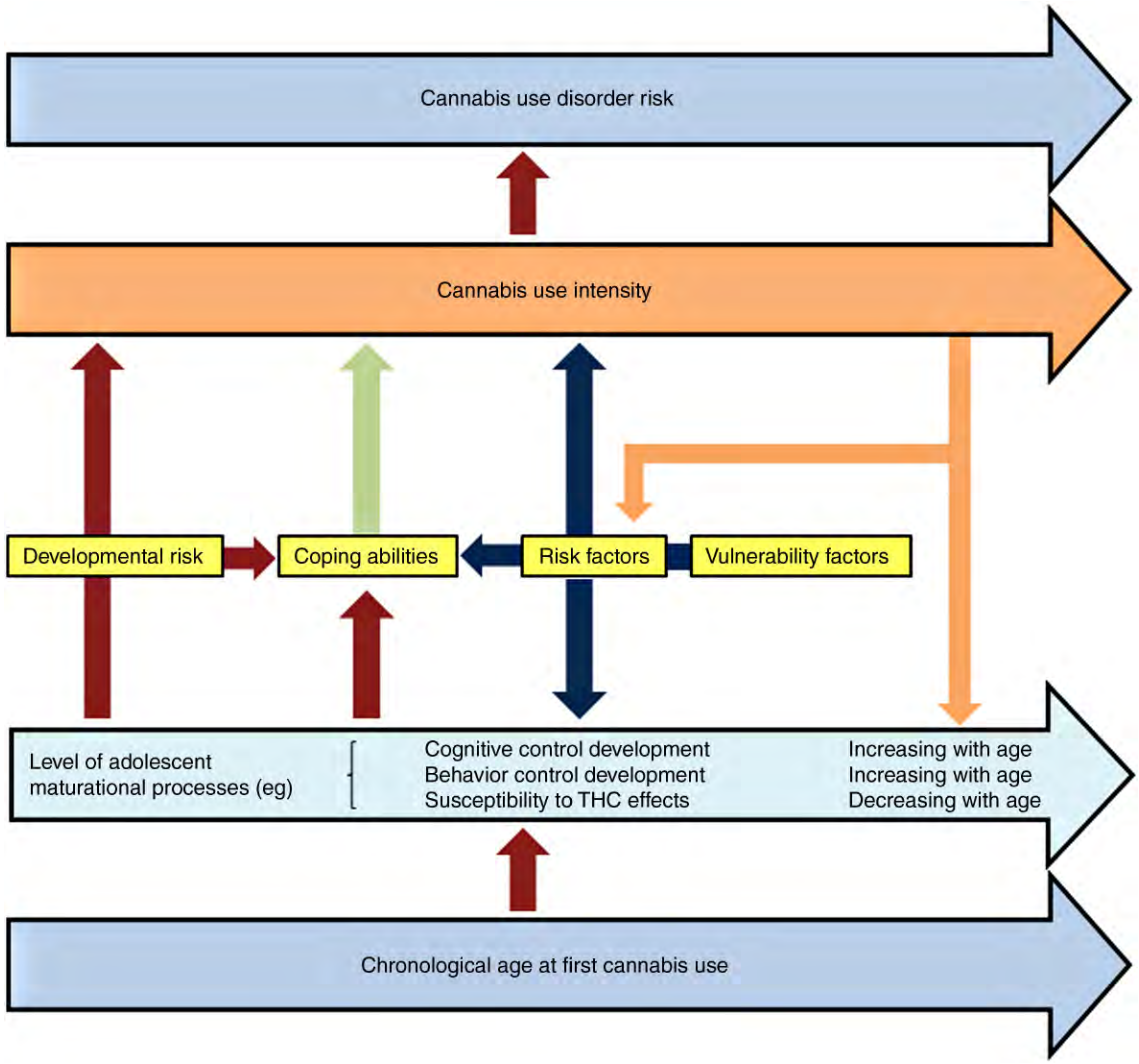


FIGURE 15.2 **What links earlier CU onset with the risk of CUD?** Putative pathways connecting early first CU with CUD risk.

age-related maturational processes, (2) nonnormative individual and social vulnerabilities and risk factors (eg, familial substance use disorders, peer use), and (3) coping abilities.

The category of age-related maturation comprises the developmental status of the individual, regarding factors that are undergoing changes during adolescence (and are thus related to age), such as, the development of cognitive control or of susceptibility to drug effects. This category is distinguished from vulnerabilities and risk factors, as it includes expectable maturational processes. In contrast, the category of vulnerabilities and risk factors comprises factors that are developmentally nonnormative, such as, an inherited vulnerability for low inhibitory control. The third category includes the ability to cope with stressors, and to limit negative consequences in critical situations (eg, ability to resist peer pressure, negative affect regulation). This ability is expected to be related to the maturational level of development. While the model focuses on factors explaining the link between earlier CU and CUD, it is also assumed that some of the factors may be involved in both the risk of earlier CU and the risk of CUD.[d]

How are these categories related to age at CU onset and intensified CU?

The status of *maturational processes* is expected to be related to age. A more immature status of such normative processes is expected in younger subjects, and expected to be linked to the risk of CU progression and CUD. For example, lower cognitive control in young subjects could contribute to a greater disregard of future risks and, thus, to intensified CU. A greater susceptibility

[d]This aspect is not included in Fig. 15.2 in order to enhance clarity of the figure.

for pleasant cannabis effects in younger subjects could also contribute to CU escalation. It is assumed that the maturational level can be directly linked with CUD risk (*direct developmental pathway*). It is also assumed that the maturational status can act via coping abilities. For example, a strong susceptibility to cannabis effects could interact with knowledge about drugs (*indirect developmental pathway*).

Risk factors can link early CU directly with increased CU, for example, via disregard for rules in conduct disorder (*nondevelopmental pathway*). Alternatively, risk factors can act via *coping abilities* or *maturational status* that are both related to age and individual development (*combined developmental and nondevelopmental pathway*). For example, peer CU could act via low social competence. Familial substance dependence could act via self-regulation development. Of note, in this pathway, the risk factor may cooccur with a certain maturational status or coping abilities, but it may also influence these factors (eg, lack of supervision could have an impact on behavior control development).

The model also includes a feedback component, assuming that, in subjects with early CU onset, CU and its escalation[e] can interfere with development, or contribute to risk factor occurrence.

AGE AT FIRST CU AND CUD ONSET AND THE COURSE OF CUD

Given the information on the high-risk incidence phases for CU and CUD and the model of adolescence as a period of vulnerability for addiction, the question arises whether age at CUD onset is related to a more severe course of CUD. Information on CUD course by earlier CU and CUD onset is relatively rare, to date. Interestingly, for alcohol, using cross-sectional data, Hingson, Heeren, & Winter (2006a, 2006b) showed that an earlier age at drinking, and alcohol dependence onset, respectively, was associated with indicators of greater course severity.

SUMMARY AND OUTLOOK

CU and CUD incidence mainly occurs in adolescence and early adulthood. A younger age at first CU is a risk factor for CUD. Putative explanatory factors are, for example, features of adolescence, psychosocial derailment, and an underlying vulnerability for disinhibitory psychopathology. As for outlook, prospective-longitudinal studies on a range of putative explanatory factors are needed.

MINI-DICTIONARY

Cannabis use disorder DSM-IV cannabis abuse or dependence.
Incidence First occurrence of an event.
Psychotropic substance Substance with effects on the central nervous system.
Right censoring When age-specific incidence risk is measured in a population, some subjects have not reached the highest age yet. For these subjects, information on age-periods above their age is unavailable (censored).
Risk factor A factor associated with an elevated risk of a subsequent outcome.

References

Agrawal, A., & Lynskey, M. T. (2009). Correlates of later-onset cannabis use in the National Epidemiological Survey on Alcohol and Related Conditions (NESARC). *Drug and Alcohol Dependence*, *105*(1–2), 71–75.

Anthony, J. C., & Petronis, K. R. (1995). Early-onset drug use and risk of later drug problems. *Drug and Alcohol Dependence*, *40*, 9–15.

Baumeister, S. E., & Tossmann, P. (2005). Association between early onset of cigarette, alcohol and cannabis use and later drug use patterns: an analysis of a survey in European metropolises. *European Addiction Research*, *11*(2), 92–98.

Becker, B., Wagner, D., Gouzoulis-Mayfrank, E., Spuentrup, E., & Daumann, J. (2010). The impact of early-onset cannabis use on functional brain correlates of working memory. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *34*(6), 837–845.

Behrendt, S., Beesdo-Baum, K., Höfler, M., Perkonigg, A., Bühringer, G., Lieb, R., et al. (2012). The relevance of age at first alcohol and nicotine use for initiation of cannabis use and progression to cannabis use disorders. *Drug and Alcohol Dependence*, *123*(1–3), 48–56.

Behrendt, S., Wittchen, H. -U., Höfler, M., Lieb, R., & Beesdo, K. (2009). Transitions from first substance use to substance use disorders in adolescence: is early onset associated with a rapid escalation? *Drug and Alcohol Dependence*, *99*, 68–78.

Blanco, C., Rafful, C., Wall, M. M., Ridenour, T. A., Wang, S., & Kendler, K. S. (2014). Towards a comprehensive developmental model of cannabis use disorders. *Addiction*, *109*(2), 284–294.

Breslau, N., Fenn, N., & Peterson, E. L. (1993). Early smoking initiation and nicotine dependence in a cohort of young adults. *Drug and Alcohol Dependence*, *33*, 129–137.

Bühringer, G., Wittchen, H. -U., Gottlebe, K., Kufeld, C., & Goschke, T. (2008). Why people change? The role of cognitive-control processes in the onset and cessation of substance abuse disorders. *International Journal of Methods in Psychiatric Research*, *17*, 4–15.

Chambers, R. A., Taylor, J. R., & Potenza, M. N. (2003). Developmental neurocircuitry of motivation in adolescence: a critical period of addiction vulnerability. *American Journal of Psychiatry*, *160*(6), 1041–1052.

Chen, C. -Y., O'Brien, M. S., & Anthony, J. C. (2005). Who becomes cannabis dependent soon after onset of use? Epidemiological evidence from the United States: 2000–2001. *Drug and Alcohol Dependence*, *79*, 11–22.

[e]It may be the case that subjects with early onset of CU are especially vulnerable to the effects of escalating CU which may constitute an important contribution to CUD risk in early onset CU. However, as the focus of this chapter is on the role of early use initiation, the putative effects of escalating CU in young subjects are not focused on here.

Compton, W. M., Grant, B. F., Colliver, J. D., Glantz, M. D., & Stinson, F. S. (2004). Prevalence of marihuana use disorders in the United States. 1991–1992 and 2001–2002. *Journal of the American Medical Association*, *291*(17), 2114–2121.

Crews, F., He, J., & Hodge, C. (2007). Adolescent cortical development: a critical period of vulnerability for addiction. *Pharmacology Biochemistry and Behavior*, *86*(2), 189–199.

de Graaf, R., ten Have, M., Tuithof, M., & van Dorsselaer, S. (2013). First-incidence of DSM-IV mood, anxiety and substance use disorders and its determinants: results from The Netherlands Mental Health Survey and Incidence Study-2. *Journal of Affective Disorders*, *149*(1–3), 100–107.

DeWit, D. J., Adlaf, E. M., Offord, D. R., & Ogborne, A. C. (2000). Age at first alcohol use: a risk factor for the development of alcohol disorders. *American Journal of Psychiatry*, *157*, 745–750.

Doremus-Fitzwater, T. L., Varlinskaya, E. I., & Spear, L. P. (2010). Motivational systems in adolescence: possible implications for age differences in substance abuse and other risk-taking behaviors. [Review]. *Brain and Cognition*, *72*(1), 114–123.

European Monitoring Centre for Drugs and Drug Addiction. (2012). *Annual report 2010: The state of the drugs problem in Europe*. Luxembourg: Publications Office of the European Union.

Fairman, B. J., & Anthony, J. C. (2012). Are early-onset cannabis smokers at an increased risk of depression spells? *Journal of Affective Disorders*, *138*(1–2), 54–62.

Fergusson, D. M., Horwood, L. J., Lynskey, M. T., & Madden, P. A. F. (2003). Early reactions to cannabis predict later dependence. *Archives of General Psychiatry*, *60*(10), 1033–1039.

Grant, B. F., Hasin, D. S., Chou, S. P., Stinson, F. S., & Dawson, D. A. (2004a). Nicotine dependence and psychiatric disorders in the United States—results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Archives of General Psychiatry*, *61*(11), 1107–1115.

Grant, B. F., Stinson, F. S., Dawson, D. A., Chou, S. P., Dufour, M. C., Compton, W., et al. (2004b). Prevalence and co-occurrence of substance use disorders and independent mood and anxiety disorders. Results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Archives of General Psychiatry*, *61*, 807–816.

Gruber, S. A., Sagar, K. A., Dahlgren, M. K., Racine, M., & Lukas, S. E. (2012). Age of onset of marijuana use and executive function. *Psychology of Addictive Behaviors*, *26*(3), 496–506.

Hicks, B. M., Krueger, R. F., Iacono, W. G., McGue, M., & Patrick, C. J. (2004). Family transmission and heritability of externalizing disorders—a twin-family study. [Article]. *Archives of General Psychiatry*, *61*(9), 922–928.

Hingson, R. W., Heeren, T., & Winter, M. R. (2006a). Age at drinking onset and alcohol dependence. Age at onset, duration and severity. *Archives of Pediatrics and Adolescent Medicine*, *160*, 739–746.

Hingson, R. W., Heeren, T., & Winter, M. R. (2006b). Age of alcohol-dependence onset: associations with severity of dependence and seeking treatment. *Pediatrics*, *118*(3), 755–763.

Kirisci, L., Tarter, R., Ridenour, T., Zhai, Z. W., Fishbein, D., Reynolds, M., et al. (2013). Age of alcohol and cannabis use onset mediates the association of transmissible risk in childhood and development of alcohol and cannabis disorders: evidence for common liability. *Experimental and Clinical Psychopharmacology*, *21*(1), 38–45.

Kokkevi, A., Gabhainn, S. N., & Spyropoulou, M. Risk Behaviour Focus Group of the HBSC. (2006). Early initiation of cannabis use: a cross-national European perspective. *Journal of Adolescent Health*, *39*, 712–719.

Lenton, S., Liang, W., & Chikritzhs, T. (2011). Evidence of increasing age of onset of cannabis use among younger Australians. *Addiction*, *107*(3), 650–657.

Lopez-Quintero, C., Perez de los Cobos, J., Hasin, D. S., Okuda, M., Wang, S., Grant, B. F., et al. (2011). Probability and predictors of transition from first use to dependence on nicotine, alcohol, cannabis, and cocaine: Results of the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC). *Drug and Alcohol Dependence*, *115*(1–2), 120–130.

Marmorstein, N. R., Iacono, W. G., & McGue, M. (2009). Alcohol and illicit drug dependence among parents: associations with offspring externalizing disorders. [Article]. *Psychological Medicine*, *39*(1), 149–155.

McGue, M., Iacono, W. G., & Krueger, R. (2006). The association of early adolescent problem behavior and adult psychopathology: a multivariate behavioral genetic perspective. *Behavior Genetics*, *36*(4), 591–602.

Monshouwer, K., Smit, F., de Graaf, R., van Os, J., & Vollebergh, W. (2005). First cannabis use: does onset shift to younger ages? Findings from 1988 to 2003 from the Dutch National School Survey on Substance Use. *Addiction*, *100*, 963–970.

Müller, S., & Gmel, G. (2002). Changes in the age of onset in cannabis use: results of the second Swiss Health Survey 1997. [Article]. *Sozial Und Praventivmedizin*, *47*(1), 14–23.

Obot, I. S., Wagner, F. A., & Anthony, J. C. (2001). Early onset and recent drug use among children of parents with alcohol problems: data from a national epidemiologic survey. *Drug and Alcohol Dependence*, *65*, 1–8.

Perkonigg, A., Goodwin, R. D., Fiedler, A., Behrendt, S., Beesdo, K., Lieb, R., et al. (2008). The natural course of cannabis use, abuse and dependence during the first decades of life. *Addiction*, *103*, 439–449.

Pope, H. G., Gruber, A. J., Hudson, J. I., Cohane, G., Huestis, M. A., & Yurgelun-Todd, D. (2003). Early-onset cannabis use and cognitive deficits: what is the nature of the association? *Drug and Alcohol Dependence*, *69*(3), 303–310.

Quinn, H. R., Matsumoto, I., Callaghan, P. D., Long, L. E., Arnold, J. C., Gunasekaran, N., et al. (2008). Adolescent rats find repeated Delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. [Article]. *Neuropsychopharmacology*, *33*(5), 1113–1126.

Redish, A. D., Jensen, S., & Johnson, A. (2008). Addiction as vulnerabilities in the decision process. *Behavioral and Brain Sciences*, *31*, 461–487.

Romer, D., Betancourt, L. M., Brodsky, N. L., Giannetta, J. M., Yang, W., & Hurt, H. (2011). Does adolescent risk taking imply weak executive function? A prospective study of relations between working memory performance, impulsivity, and risk taking in early adolescence. *Developmental Science*, *14*(5), 1119–1133.

Rothman, E. F., Edwards, E. M., Heeren, T., & Hingson, R. W. (2008). Adverse childhood experiences predict earlier age of drinking onset: results from a representative US sample of current or former drinkers. [Article]. *Pediatrics*, *122*(2), E298–E304.

Schramm-Sapyta, N. L., Cha, Y. M., Chaudry, S., Wilson, W. A., Schwartzwelder, H. S., & Kuhn, C. M. (2007). Differential anxiogenic, aversive, and locomotor effects of THC in adolescent and adult rats. *Psychpharmacology*, *191*, 867–877.

Schramm-Sapyta, N. L., Walker, Q. D., Caster, J. M., Levin, E. D., & Kuhn, C. M. (2009). Are adolescents more vulnerable to drug addiction than adults? Evidence from animal models. [Review]. *Psychopharmacology*, *206*(1), 1–21.

Silins, E., Hutchinson, D., Swift, W., Slade, T., Toson, B., & Rodgers, B. (2013). Factors associated with variability and stability of cannabis use in young adulthood. *Drug and Alcohol Dependence*, *133*(2), 452–458.

United Nations Office on Drugs and Crime. (2012). World Drug Report 2012.

van Leeuwen, A. P., Creemers, H. E., Verhulst, F. C., Vollebergh, W. A. M., Ormel, J., van Oort, F., et al. (2013). Legal substance use and the development of a DSM-IV cannabis use disorder during adolescence: the TRAILS study. *Addiction*, *109*(2), 303–311.

Vega, W. A., Aguilar-Gaxiola, S., Andrade, L., Bijl, R., & Borges, G. (2002). Prevalence and age of onset for drug use in seven international sites: results from the international consortium of psychiatric epidemiology. *Drug and Alcohol Dependence*, *68*, 285–297.

Wagner, F. A., & Anthony, J. C. (2002). From first drug use to drug dependence: developmental periods of risk for dependence upon marijuana, cocaine, and alcohol. *Neuropsychopharmacology*, *26*(4), 479–488.

Wagner, F. A., & Anthony, J. C. (2007). Male-female differences in the risk of progression from first use to dependence upon cannabis, cocaine and alcohol. *Drug and Alcohol Dependence*, *86*, 191–198.

Wiers, R. W., Gladwin, T. E., Hofmann, W., Salemink, E., & Ridderinkhof, K. R. (2013). Cognitive bias modification and cognitive control training in addiction and related psychopathology: mechanisms, clinical perspectives, and ways forward. *Clinical Psychological Science*, *1*, 192–212.

Winters, K. C., & Lee, C. -Y. S. (2008). Likelihood of developing an alcohol and-cannabis use-disorder during youth: Association with recent use and age. *Drug and Alcohol Dependence*, *92*(1–3), 239–247.

Wittchen, H. -U., Behrendt, S., Höfler, M., Perkonigg, A., Lieb, R., Bühringer, G., et al. (2008). What are the high risk periods for incident substance use and transitions to abuse and dependence? Implications for early intervention and prevention. *International Journal of Methods in Psychiatric Research*, *17*, 16–29.

Wittchen, H. -U., Behrendt, S., Hoefler, M., Perkonigg, A., Rehm, J., Lieb, R., et al. (2009). A typology of cannabis-related problems among individuals with repeated illegal drug use in the first three decades of life: evidence for heterogeneity and different treatment needs. [Article]. *Drug and Alcohol Dependence*, *102*(1–3), 151–157.

CHAPTER

16

Effects of Cannabis Use on Neurocognition in Adolescents and Emerging Adults

N.E. Wright, K.E. Maple, K.M. Lisdahl

Department of Psychology, University of Wisconsin-Milwaukee, Milwaukee, WI, United States

SUMMARY POINTS

- Significant neuromaturation occurs between the ages of 12 and 25, making adolescent and young adult brains more susceptible to damage from substances, such as, cannabis.
- Cannabis use in adolescence has been related to poorer attention, memory, inhibition, processing speed, IQ, and decision making.
- Adolescent and emerging adult cannabis users have different patterns of gray matter volumes, cortical thickness, and white matter integrity. These findings are often associated with functional deficits in neurocognitive performance.
- Functional magnetic resonance imaging (fMRI) studies reveal deficits in memory, attention, and motor tasks. Increased functional connectivity has also been found across parietal, frontal, temporal, and cerebellar regions.
- Earlier onset of use is related to worse outcomes in neurocognitive, neuroanatomical, and neurological function.
- More frequent and greater quantities of use of cannabis are related to worse outcomes.
- Gender moderates some of the effects of cannabis use.
- Future research should be directed at establishing causal, rather than correlational, relationships.
- Clinicians should be more proactive in assessing substance use in adolescence, so as to begin interventions early.

KEY FACTS OF NEUROCOGNITIVE DIFFERENCES IN ADOLESCENT AND YOUNG ADULT CANNABIS USERS

- Adolescent and young adult cannabis users have slower processing speed, poorer ability to sustain their attention, worse verbal memory, and poorer decision-making, in comparison to controls.
- These neurocognitive problems have real-world implications. Adolescent and young adult cannabis users also reported increased symptoms of inattention, depression, anxiety, poorer sleep, increased school problems, and lower grades.
- They are more likely to engage in impulsive behavior, and have less ability to inhibit inappropriate responses.
- Overall, neurocognitive problems are worse in heavy (daily) users, and in those who began using cannabis regularly (at least weekly) before they are 18 years old.

KEY FACTS OF NEURONAL DIFFERENCES IN ADOLESCENT AND YOUNG ADULT CANNABIS USERS

Structural

- These cognitive problems are related to brain structure abnormalities in areas of the brain, such as, the amygdala, hippocampus, nucleus accumbens, cerebellum, and prefrontal cortex.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00017-X
Copyright © 2017 Elsevier Inc. All rights reserved.

- Most studies point to diminished white matter integrity in cannabis users, compared to controls.
- Other factors may influence the relationship between cannabis use and brain structure, including age of use onset, gender, other substance use, and cannabis content.

Functional

- During attention and inhibition tasks, cannabis users exhibit increased activation in the motor cortex and frontal and parietal regions, but have dispersed anterior cingulate cortex activation during an inhibition task.
- During a faces memory task, greater parahippocampal gyrus activation was related to greater cannabis use; however, while engaging working memory, cannabis users have more activation in the anterior cingulate cortex, dorsolateral prefrontal cortex, and inferior frontal gyrus, compared to controls.
- A spatial working memory task elicited decreased activity in the right parietal cortex, and increased activity in the dorsolateral prefrontal cortex, while a visuospatial memory study found increased activation in the right inferior frontal gyrus, left middle frontal gyrus, and right superior temporal gyrus, in cannabis users versus controls.
- Cannabis users have greater resting state functional connectivity and task-based functional connectivity in several networks.
- Age of use onset, length of abstinence, and gender, in addition to cannabis use, may all impact brain activation patterns.

LIST OF ABBREVIATIONS

Brain Regions

ACC Anterior cingulate cortex
dlPFC Dorsolateral prefrontal cortex
IFOF Inferior fronto-occipital fasciculus
ILF Inferior longitudinal fasciculus
PFC Prefrontal cortex

Brain Imaging Terms

BOLD Blood oxygen level dependent
CBF Cerebral blood flow
DTI Diffusion tensor imaging
FA Fractional anisotropy
fcMRI Functional connectivity magnetic resonance imaging
fMRI Functional magnetic resonance imaging
MD Mean diffusivity
MRI Magnetic resonance imaging
THC Tetrahydrocannabinol

RISING RATES OF USE

The last 20 years have seen a rise in adolescent cannabis use, with 6.5% of 12th graders reporting daily use (Johnston, O'Malley, Bachman, & Schulenberg, 2013). Concurrently, as increasing numbers of US states have legalized medical use and, in two states, recreational use, perception of risk has decreased (Johnston et al., 2013). This increase in use and abuse in this vulnerable population is creating an increasing need to understand the neurocognitive effects of chronic cannabis use. The present review will summarize the neuronal consequences of regular cannabis use in adolescents and young adults (ages 12–25), while paying particular attention to unique effects by gender and other moderators. We will then discuss clinical implications, and address broader considerations for those working with and studying emerging adults who use cannabis.

THE DEVELOPING BRAIN

Neurological developmental changes are abundant in adolescence, including pruning of gray matter and cortical thinning, in conjunction with improved performance in areas associated with executive functioning, such as, the prefrontal cortex (PFC) (Gogtay et al., 2004). Further, the endocannabinoid system itself is going through vast changes that make it particularly vulnerable to exogenous cannabinoids (for review, see Schneider, 2008). These changes may lead to increased vulnerability to risky behavior, and the neurotoxic effects of drug use (for review, see Bava & Tapert, 2010). Some suggest that, due to this malleable time period of brain development, changes later in life may not be able to correct for alterations in brain development during teen years (Blakemore & Choudhury, 2006). As a result of the sensitive nature of this time period, it is important to understand the neurocognitive and neuronal consequences of cannabis abuse.

NEUROCOGNITIVE IMPLICATIONS OF CANNABIS USE

Regular (weekly to daily) cannabis use has been associated with several neurocognitive deficits, including attention (Ehrenreich et al., 1999; Medina et al., 2007a; Tapert, Granholm, Leedy, & Brown, 2002), executive functioning (Gruber, Sagar, Dahlgren, Racine, & Lukas, 2012; Fontes et al., 2011; McHale & Hunt, 2008;

Medina et al., 2007a), impulsive behavior and inhibition (Gruber et al., 2012a; Gruber, Dahlgren, Sagar, Gonenc, & Lukas, 2013; Lisdahl & Price, 2012; Solowij et al., 2012; Becker, Collins, & Luciana, 2014), psychomotor speed (Medina et al., 2007a; Lisdahl & Price, 2012), visuospatial memory (Harvey, Sellman, Porter, & Frampton, 2007; Becker et al., 2014), verbal memory (Gonzalez et al., 2012; Jacobsen, Pugh, Constable, Westerveld, & Mencl, 2007; Solowij et al., 2011; Tait, Mackinnon, & Christensen, 2011; Becker et al., 2014), and overall intelligence (Fried, Watkinson, & Gray, 2005; Meier et al., 2012; see Fig. 16.1).

These effects appear to be moderated by frequency (Fried et al., 2005; Gonzalez et al., 2012; Meier et al., 2012; Medina et al., 2007a; Lisdahl & Price, 2012), age of onset (eg, Gruber, Dahlgren, Sagar, Gönenc, & Killgore, 2012; Ehrenreich et al., 1999), and gender (Lisdahl & Price, 2012). As would be expected, heavier use of cannabis, both in frequency and quantity, has been linked to greater deficits in neurocognitive functioning (Fried et al., 2005; Gonzalez et al., 2012; Lisdahl & Price, 2012; Medina et al., 2007a; Meier et al., 2012). Further, adolescents who begin regularly (at least weekly) using cannabis when they are under 16–18 have more pronounced deficits in inhibition, attention, and executive functioning, compared to those who started using later (Pope et al., 2003; Gruber et al., 2012a; Ehrenreich et al., 1999; Fontes et al., 2011; Meier et al., 2012). When looking at gender differences, emerging adult male cannabis users tend to have more pronounced difficulty with psychomotor speed, compared to female users (Lisdahl & Price, 2012). It should be noted that analysis of interactive gender effects has been limited, though a number of studies that have assessed gender as a moderator have found null results (Tait et al., 2011; Solowij et al., 2011). Therefore, further assessment of potential interactive effects between gender and cannabis use is needed.

Importantly, differences in cognitive performance appear to remain, even after up to a month of monitored abstinence in adolescents (Hanson et al., 2010; Medina et al., 2007a), and longitudinal changes have been observed. For instance, Tapert and colleagues (2002) followed 16 year olds for 8 years, finding that cannabis use over the study period predicted poorer attention at follow-up periods. Similarly, Tait and colleagues (2011) found light and heavy users had a decline in performance across cognitive domains over an 8-year period, while former and nonusers did not exhibit the same pattern. In the largest prospective study conducted to date, Meier et al. (2012) assessed participants prior to cannabis use (7–13 years old), and followed them until they were 38 years old. They found that regular use of cannabis across 3 or more study time points resulted in a 6 point IQ decline, while those who never used cannabis had an almost one point increase. Results were maintained after controlling for childhood IQ, education, recent cannabis use, tobacco, other drug, and alcohol dependence, as well as schizophrenia diagnosis. Impairments were also found across neuropsychological domains, including memory, processing speed, verbal comprehension, and perceptual reasoning.

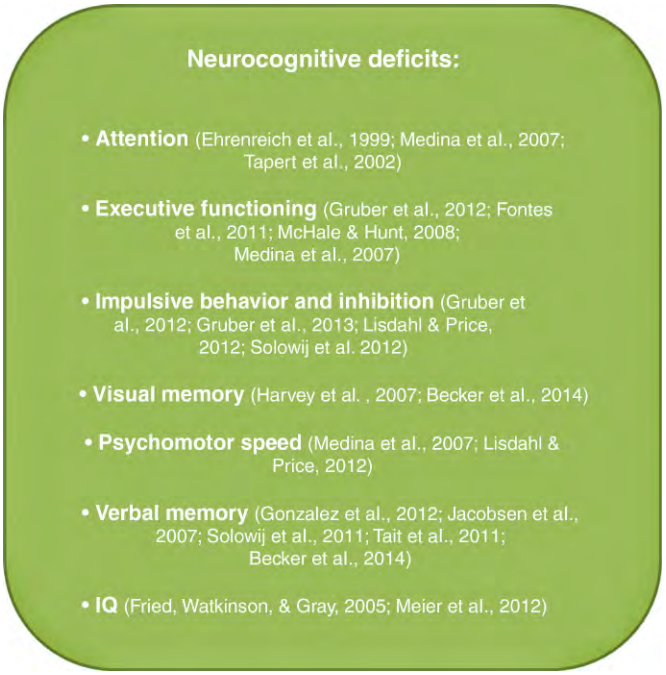


FIGURE 16.1 Neurocognitive deficits in emerging adult cannabis users. Overview of deficits in neurocognitive functioning in adolescent and young adult cannabis users, including citations for studies that have found these differences.

STRUCTURAL IMPLICATIONS OF CANNABIS USE

As function often follows structure (Greicius, Supekar, Menon, & Dougherty, 2009), it is perhaps unsurprising that neuroanatomical differences have been found between cannabis users and nonusers (see Figs. 16.2, 16.3).

Gray Matter. Converging lines of evidence suggest cannabis use in this sensitive time period may be related to differing patterns of gray matter architecture, perhaps due to immature neuronal development. Differences in gray matter volume between users and nonusers are assumed to relate to inefficient pruning of unnecessary synapses or inflammation in the case of larger regions or decreased synaptic density in smaller regions. Due to greater CB1 densities in regions, such as, the amygdala, hippocampus, hypothalamus, basal ganglia, cerebellum, anterior cingulate cortex, and prefrontal cortex (Iversen, 2003), these areas may be particularly susceptible to structural differences between cannabis users and nonusers. Increased volume has been found primarily in adolescent samples, including

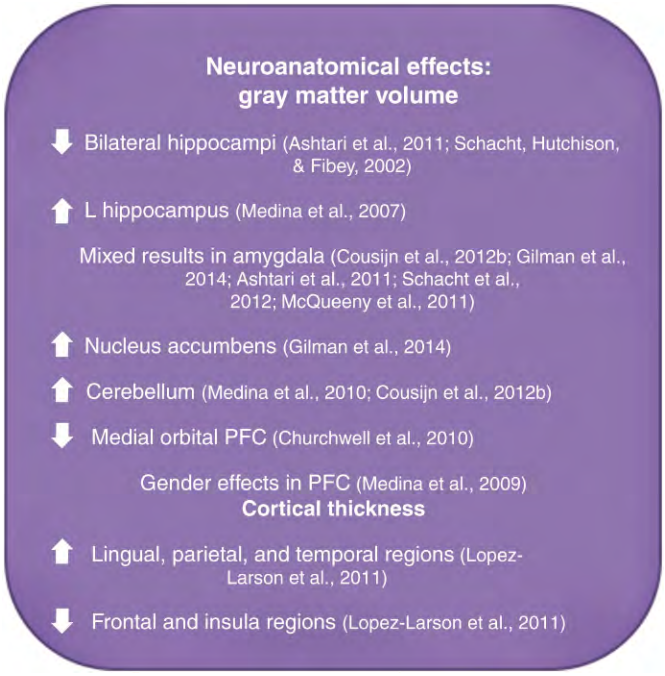


FIGURE 16.2 Differences in gray matter volume in emerging adult cannabis users. Overview of differences in gray matter volume in adolescent and young adult cannabis users, including citations for studies that have found these differences.

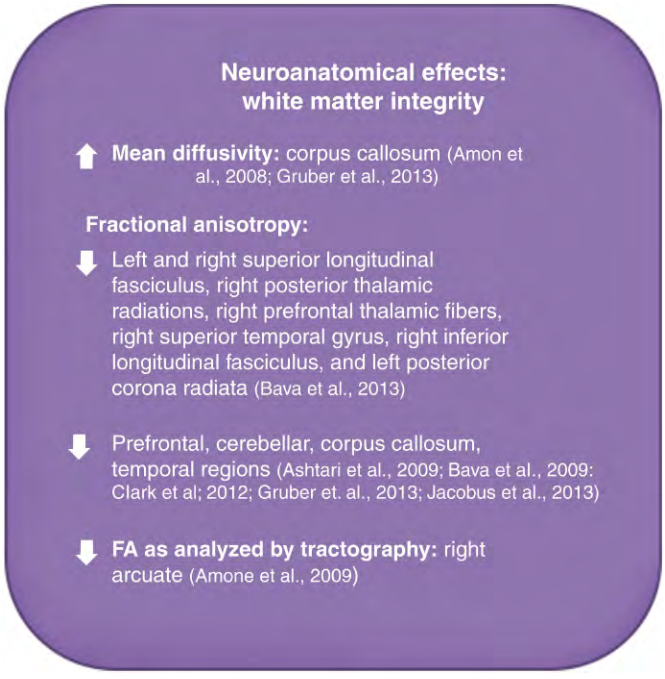


FIGURE 16.3 Differences in white matter integrity in emerging adult cannabis users. Overview of differences in white matter integrity in adolescent and young adult cannabis users, including citations for studies that have found these differences.

the right amygdala in females (McQueeny et al., 2011), left hippocampus (Medina, Schweinsburg, Cohen-Zion, Nagel, & Tapert, 2007b), nucleus accumbens (Gilman et al., 2014), cerebellum (Medina, Nagel, & Tapert, 2010; Cousijn et al., 2012b), and inferior PFC in females (Medina et al., 2009). In young adults, one study found no differences (Ashtari et al., 2011), one found increased (Gilman et al., 2014), and another decreased (Schacht, Hutchison, & Filbey, 2012) amygdala volumes. Cousijn and colleagues (2012b) found larger amygdala volumes predicted increased cannabis use problem severity. Young adult cannabis users have been found to have smaller bilateral hippocampal (Ashtari et al., 2011; Schacht et al., 2012) and medial orbital PFC volumes (Churchwell, Lopez-Larson, & Yurgelun-Todd, 2010). Patterns of cortical thickness have thus far shown decreased thickness in frontal and insula regions and increased thickness in lingual, parietal, and temporal regions (Lopez-Larson et al., 2011). Potency and content of cannabis use may impact structural findings. For example, use of cannabis with higher THC: cannabidiol ratios, two primary components of cannabis, have been found to be predictive of larger bilateral hippocampus volume, indicating that potency of THC also plays a role (Demirakca et al., 2011).

As structural studies have thus far been cross-sectional, these differences may be a result of cannabis use or, alternatively, may predispose young adults to initiate use of cannabis. For example, in a 4-year study of neuroanatomical volume, having smaller orbitofrontal volume at age 12, most specifically the right lateral orbitofrontal cortex, was found to be predictive of onset of cannabis use by age 16 (Cheetham et al., 2012). Whether cannabis is causing or reflecting neuronal differences, abnormalities in gray matter volume in areas, such as, the amygdala, cerebellum, and PFC have been found to be related to greater depressive symptoms, executive dysfunction, or increased cannabis related problems (Cousijn et al., 2012b; McQueeny et al., 2011; Medina et al., 2009, 2010), underscoring the importance of the functional implications related to neuronal differences.

White Matter. Myelinated axons, or white matter, reach within and across brain regions to convey necessary information quickly. Improved white matter integrity results in increased communication efficiency. Studies use diffusion tensor imaging (DTI), assessing mean diffusivity (MD) and fractional anisotropy (FA), or diffusion of water and coherence across white matter tracts, respectively. With few exceptions (DeLisi et al., 2006; Cousijn et al., 2012b), deficits in white matter integrity have also been reported in cannabis users. In young adult samples, findings suggest increased MD in the corpus callosum (Arnone et al., 2008; Gruber et al., 2013), as well as decreased FA in the left and right genu and left internal capsule in early regular onset (before age 16) cannabis use (Gruber et al., 2013). In adolescents, results indicate decreased FA in prefrontal, parietal, cerebellar, corpus callosum, and temporal regions (Ashtari et al., 2009; Bava et al., 2009; Clark, Chung, Thatcher,

Pajtek, & Long, 2012; Jacobus, Squeglia, Infante, Bava, & Tapert, 2013), and decreased FA in the right arcuate fasciculus, as well as increased radial diffusivity in the left and right arcuate fasciculus, as measured by tractography (Ashtari et al., 2009). Longitudinally, Bava, Jacobus, Thayer, and Tapert (2013) found deficits in FA in seven clusters: the left and right superior longitudinal fasciculus, right posterior thalamic radiations, right prefrontal thalamic fibers, right superior temporal gyrus, right inferior longitudinal fasciculus, and the left posterior corona radiata. However, in contrast to the cross-sectional studies that statistically controlled for alcohol, Bava et al. (2013) found that changes over the 18-month follow-up period were predicted by frequency of alcohol use, rather than cannabis. Additional longitudinal studies examining the relative impact of alcohol versus cannabis use on white matter microstructure are needed.

Potential Confounding or Moderating Factors. Other factors besides cannabis exposure may contribute to structural differences. As stated above, increased alcohol consumption has also been linked to worse white matter integrity in adolescents (Bava et al., 2013), although the majority of the aforementioned studies did statistically control for alcohol use. Age of regular use onset has similarly been related to poorer FA (Gruber, Silveri, Dahlgren, & Yurgelun-Todd, 2011; Gruber et al., 2013) and smaller medial orbital PFC volumes (Churchwell et al., 2010). Results regarding interactions between gender and cannabis use on brain structure have been mixed. Some have found no difference (Medina et al., 2007a; Gilman et al., 2014; Cousijn et al., 2012b), while others have found gender to significantly interact with use, especially in adolescent users. Medina and colleagues (2009) found female cannabis users had larger PFC volumes, compared to female controls, while male cannabis users had smaller PFC volumes, compared to male controls. McQueeny and colleagues (2011) found cannabis-using females had larger right amygdala volumes than male users and male and female controls.

FUNCTIONAL IMPLICATIONS OF CANNABIS USE

Various functional MRI (fMRI) tasks have found different activation patterns in cannabis users, in comparison to controls (see Figs. 16.4, 16.5). In general, adolescent and young adult cannabis users appear to compensate for deficits in functioning through overactivation of important brain regions, as well as recruiting additional regions to assist in completing tasks (eg, Abdullaev, Posner, Nunnally, & Dishion, 2010; Tapert et al., 2007). In particular, cannabis-using adolescents and young adults have less efficient functioning in attention and inhibition tasks. In an attention task, greater activation in the right PFC, motor cortex, and bilateral parietal cortex

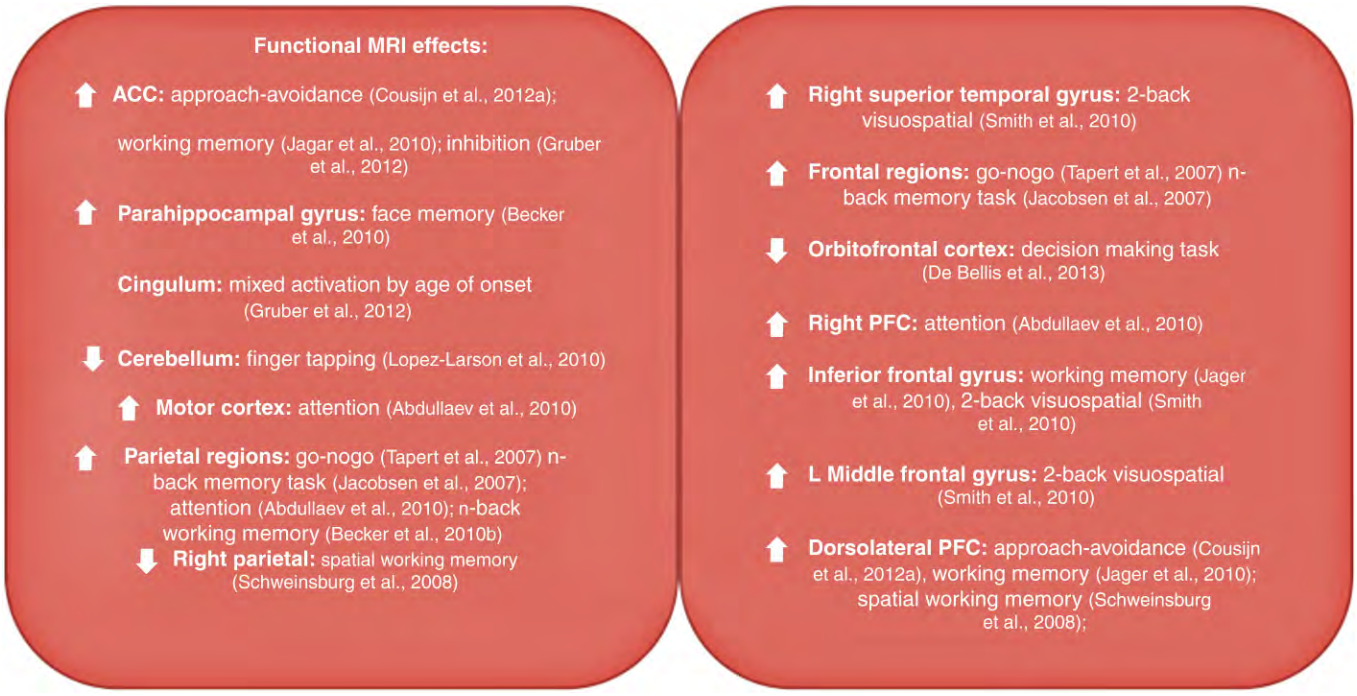


FIGURE 16.4 **Difference in neuronal function as measured by fMRI in emerging adult cannabis users.** Overview of differences in blood oxygen level dependent (BOLD) response by task and brain region in adolescent and young adult cannabis users, including citations for studies that have found these differences.

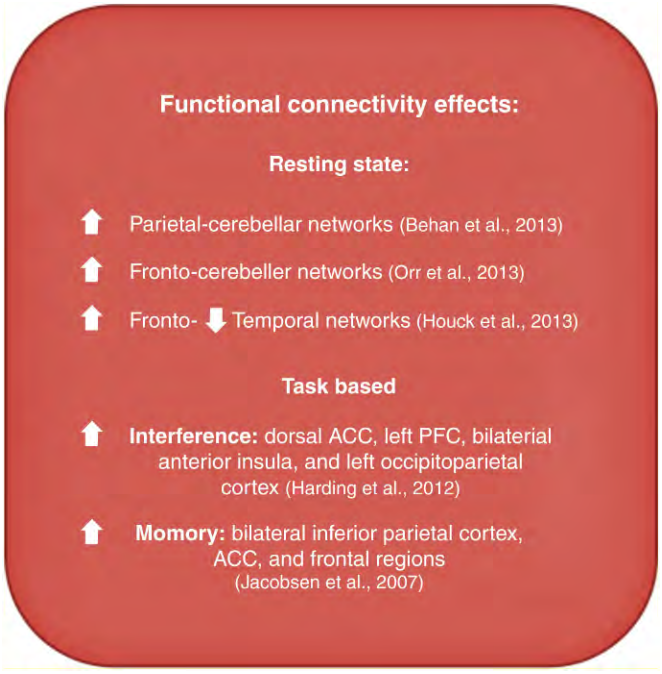


FIGURE 16.5 Differences in functional connectivity in emerging adult cannabis users. Overview of differences in functional connectivity by task and brain region in adolescent and young adult cannabis users, including citations for studies that have found these differences.

have been found and, even with this increased activation, adolescent users had worse performance over all (Abdullaev et al., 2010).

One go-nogo task found no group differences in activation in adolescent users (Behan et al., 2013). However, others (Tapert et al., 2007) have found adolescents to have increased activation in frontal and parietal regions after 28 days of abstinence during similar go-nogo tasks. In an fMRI interference task assessing inhibition, Gruber and colleagues (2012b) found cannabis use to result in broader activation of the ACC. Further, they found that the cingulum was activated differently based on early (before 16 years old) or late onset of cannabis use.

Differences in working memory function are also apparent. In a faces memory task, young adult heavy users, in comparison to occasional users, had more activation in the parahippocampal gyrus (Becker, Wagner, Gouzoulis-Mayfrank, Spuentrup, & Daumann, 2010a). Frequency of use, as well as age of onset, has been assessed in young adults, with early onset found to be predictive of greater left parietal activation during a working memory task (Becker, Wagner, Gouzoulis-Mayfrank, Spuentrup, & Daumann, 2010b). When not controlling for recent cannabis use or binge drinking through required abstinence, greater activation in the right inferior frontal gyrus, left middle frontal gyrus, and right superior temporal gyrus has been found in young adult cannabis users, in comparison to healthy controls, during a visuospatial memory task (Smith, Longo, Fried, Hogan, & Cameron, 2010). Male adolescent cannabis users have demonstrated overactivity in the ACC, dlPFC, and inferior frontal gyrus, during a working memory task (Jager, Block, Luijten, & Ramsey, 2010). Decreased activity in the right parietal cortex, but increased activity in the dlPFC, has also been found during a spatial working memory task in adolescents (Schweinsburg et al., 2008). Further, recent (2–7 days) cannabis users had greater activation in frontal regions than abstinent (27–60 days) adolescent users, but both had significantly greater activation than nonusing controls, again indicating overuse and compensation of frontal regions during spatial working memory tasks (Schweinsburg et al., 2010). Cannabis and nicotine comorbid adolescent users in nicotine withdrawal demonstrated increased activation in frontal and parietal regions, in comparison to controls during a verbal memory task (Jacobsen et al., 2007).

In adolescents, decreased activation, suggesting underutilization of important areas, has also been found in the cerebellum, in a motor skills task using finger tapping (Lopez-Larson et al., 2012), and in the orbitofrontal cortex in a decision making task (De Bellis et al., 2013). In young adults, an approach-avoidance task found no group differences, though dlPFC and ACC activation predicted cannabis problem severity at 6-month follow-up (Cousijn et al., 2012a).

Functional connectivity across and within regions has also been assessed in adolescents. Resting state functional connectivity (fcMRI) analyses have found increased connectivity in parietal-cerebellar networks, fronto-cerebellar, and fronto-temporal networks (Behan et al., 2013; Orr et al., 2013; Houck, Bryan, & Feldstein Ewing, 2013). In looking at functional connectivity in a verbal memory task, comorbid cannabis and nicotine users had increased connectivity between the right and left inferior parietal cortex, the anterior cingulate gyrus, and frontal regions, with decreased connectivity when in nicotine withdrawal (Jacobsen et al., 2007). In adults with early age of onset, greater connectivity was found within the dorsal ACC, the left PFC, the left and right anterior insula, and the left occipitoparietal cortex, during an interference task (Harding et al., 2012). Together, these findings across fMRI and fcMRI studies indicate different patterns related to use that are frequently associated with poorer performance and outcomes.

Age of onset appears to influence differences in BOLD response (Harding et al., 2012; Schweinsburg et al., 2008; Becker et al., 2010b), though this finding is not universal (Becker et al., 2010a). Gender has not often been assessed, but in the few circumstances it has, no difference has been found (Jacobsen et al., 2007). Length of abstinence is also an important issue in assessing differences. Jacobus et al. (2012) found that adolescents had decreased cerebral blood flow in four regions, including the frontal gyrus, and increased CBF in the right precuneus at

baseline; differences disappeared at 4 weeks abstinence. Therefore, there is some preliminary evidence for recovery of function with sustained abstinence.

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

Prevalence of cannabis use and abuse is rising in the United States (Johnston et al., 2013). Rates of use are particularly disconcerting in adolescent and young adult populations, as neuromaturational changes in this time period may make them more susceptible to the neurotoxic effects of cannabis (Schneider, 2008). Adolescents demonstrate more robust and diverse cognitive (eg, working memory, verbal memory, complex attention, inhibition, decision making, processing speed) and brain structural abnormalities, compared to adult users. Other important moderators influence the impact of cannabis use, including extent of use, gender, and age of regular cannabis use onset.

Although there has been replication in cross-sectional studies that are consistent with animal models and longitudinal studies, additional longitudinal data is needed to determine the whether these neurocognitive abnormalities measured in cannabis users are risk factors for use, or directly caused by cannabis exposure. Additional research examining potential gender differences is also necessary, as few studies have adequately addressed this issue. Teasing apart alcohol and cannabis couse is also important, as cannabis users often binge drink, a fact that may be a more robust predictor of neurocognitive deficits (eg, Bava et al., 2013). Finally, while not addressed in this review, due to space limitations, greater understanding is needed regarding the specific effects of specific components of cannabis (eg, THC and cannabidiol), as well as the influence of psychiatric comorbidities on cannabis use and neurocognitive consequences.

These converging lines of evidence regarding the neurocognitive consequences of cannabis use in adolescence and young adulthood highlight the dire need to protect the developing brain through screening, prevention, and treatment. Unfortunately, the majority of physicians may not be screening for alcohol, let alone drug use (Town, Naimi, Mokdad, & Brewer, 2006). As earlier age of regular use onset is related to worse outcomes, prevention of cannabis use initiation should be a top priority. Therefore, in clinical and school settings, early screening for cannabis use is critical. For example, CRAFFT (Knight, Sherritt, Shrier, Harris, & Chang, 2002) is a brief computerized program that asks six substance use questions to provide individualized feedback on risk for developing a substance use disorder, and can be implemented in many clinical settings. Encouraging emerging adults to wait until they are *at least* 18 to begin using any substances (ideally 25) would reduce the likelihood of developing a cannabis use disorder, and reduce risk for developing negative neurocognitive effects (Meier et al., 2012; Substance Abuse and Mental Health Services Administration, 2013).

MINI-DICTIONARY

Executive functioning Executive functioning refers to higher level functioning, such as management, regulation, and goal-directed behavior, that occurs within the prefrontal cortex.
Gray matter Composed of neurons, gray matter is a major component of the cortex. It provides much of the structure for the brain, so reductions in volume, and less complex patterns of folding within gray matter are often related to functional deficits.
Inhibition The ability to prevent inappropriate or incorrect responding.
Processing speed The ability to efficiently and accurately process information.
Sustained attention The ability to maintain focus on a task over an extended period of time, typically measured through long and uninteresting tasks.
Visuospatial memory Memory related to visual stimuli.
White matter White matter refers to axons covered in a fatty sheath called myelin. Such myelinated axons increase the speed of communication within and across brain regions. Therefore, analysis of white matter integrity is used to measure how efficiently different brain regions communicate.
Working memory The ability to hold and manipulate information in your mind for a short period of time.

References

Abdullaev, Y., Posner, M. I., Nunnally, R., & Dishion, T. J. (2010). Functional MRI evidence for inefficient attentional control in adolescent chronic cannabis abuse. *Behavioral Brain Research*, *215*(1), 45–57.

Arnone, D., Barrick, T. R., Chengappa, S., Mackay, C. E., Clark, C. A., & Abou-Saleh, M. T. (2008). Corpus callosum damage in heavy marijuana use: preliminary evidence from diffusion tensor tractography and tract-based spatial statistics. *NeuroImage*, *41*(3), 1067–1074.

Ashtari, M., Cervellione, K., Cottone, J., Ardekani, B. A., Sevy, S., & Kumra, S. (2009). Diffusion abnormalities in adolescents and young adults with a history of heavy cannabis use. *Journal of Psychiatric Research*, *43*(3), 189–204.

Ashtari, M., Avants, B., Cyckowski, L., Cervellione, K. L., Roofeh, D., Cook, P., Gee, J., Sevy, S., & Kumra, S. (2011). Medial temporal structures and memory functions in adolescents with heavy cannabis use. *Journal of Psychiatric Research*, *45*(8), 1055–1066.

Bava, S., & Tapert, S. F. (2010). Adolescent brain development and the risk for alcohol and other drug problems. *Neuropsychology Review*, *20*, 398–413.

Bava, S., Frank, L. R., McQueeny, T., Schweinsburg, B. C., Schweinsburg, A. D., & Tapert, S. F. (2009). Altered white matter microstructure in adolescent substance users. *Psychiatry Research*, *173*(3), 228–237.

Bava, S., Jacobus, J., Thayer, R. E., & Tapert, S. F. (2013). Longitudinal changes in white matter integrity among adolescent substance users. *Alcoholism: Clinical and Experimental Research*, *37*(1), 181–189.

Becker, B., Wagner, D., Gouzoulis-Mayfrank, E., Spuentrup, E., & Daumann, J. (2010a). Altered parahippocampal functioning in cannabis users is related to the frequency of use. *Psychopharmacology*, *209*, 361–374.

Becker, B., Wagner, D., Gouzoulis-Mayfrank, E., Spuentrup, E., & Daumann, J. (2010b). The impact of early-onset cannabis use on functional brain correlates of working memory. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *34*, 837–845.

Becker, M. P., Collins, P. F., & Luciana, M. (2014). Neurocognition in college-aged daily marijuana users. *Journal of Clinical and Experimental Neuropsychology*, *36*(4), 379–398.

Behan, B., Connolly, C. G., Datwani, M. C., Ivanovic, J., Morioka, R., Stone, A., Watts, R., Smyth, B., & Garavan, H. (2013). Response inhibition and elevated parietal-cerebellar correlations in chronic adolescent cannabis users. *Neuropharmacology*, *2908*(13), 241–244.

Blakemore, S. J., & Choudhury, S. (2006). Development of the adolescent brain: implications for executive function and social cognition. *Journal of Child Psychology and Psychiatry*, *47*(3), 296–312.

Cheetham, A., Allen, N. B., Whittle, S., Simmons, J. G., Yücel, M., & Lubman, D. I. (2012). Orbitofrontal volumes in early adolescence predict initiation of cannabis use: a 4-year longitudinal and prospective study. *Biological Psychiatry*, *71*(8), 684–692.

Churchwell, J. C., Lopez-Larson, M., & Yurgelun-Todd, D. A. (2010). Altered frontal cortical volume and decision making in adolescent cannabis users. *Frontiers in Psychology*, *1*, 225.

Clark, D. B., Chung, T., Thatcher, D. L., Pajtek, S., & Long, E. C. (2012). Psychological dysregulation, white matter disorganization and substance use disorders in adolescences. *Addiction*, *7*(1), 206–214.

Cousijn, J., Goudriaan, A. E., Ridderinkhof, R., van den Brink, W., Veltman, D. J., & Wiers, R. W. (2012a). Approach-bias predicts development of cannabis problem severity in heavy cannabis users: results from a prospective fMRI study. *PLOS One*, *7*(9), e42394.

Cousijn, J., Wiers, R. W., Ridderinkhof, K. R., van den Brink, W., Veltman, D. J., & Gourdiaan, A. E. (2012b). Grey matter alterations associated with cannabis use: results of a VBM study in heavy cannabis users and healthy controls. *NeuroImage*, *59*, 3845–3851.

De Bellis, M. D., Wang, L., Bergman, S. R., Yaxley, R. H., Hooper, S. R., & Huetel, S. A. (2013). Neural mechanisms of risky decision-making and reward response in adolescent onset cannabis use disorder. *Drug and Alcohol Dependence*, *133*, 134–145.

DeLisi, L. E., Bertisch, H. C., Szulc, K. U., Majcher, M., Brown, K., Bappal, A., & Ardekani, B. A. (2006). A preliminary DTI study showing no brain structural change associated with adolescent cannabis use. *Harm Reduction Journal*, *3*, 17.

Demirakca, T., Sartorius, A., Ende, G., Meyer, N., Welzel, H., Skopp, G., Mann, K., & Hermann, D. (2011). Diminished gray matter in the hippocampus of cannabis users: possible protective effects of cannabidiol. *Drug and Alcohol Dependence*, *114*(2/3), 242–245.

Ehrenreich, H., Rinn, T., Kunert, H. J., Moeller, M. R., Poser, W., Schilling, L., Gigerenzer, G., & Hoehe, M. R. (1999). Specific attentional dysfunction in adults following early start of marijuana use. *Psychopharmacology*, *142*(3), 295–301.

Fontes, M. A., Bolla, K. I., Cunha, P. J., Almeida, P. P., Jungerman, F., Laranjeira, R. R., Bressan, R. A., & Lacerda, A. L. (2011). Cannabis use before age 15 and subsequent executive functioning. *British Journal of Psychiatry*, *198*(6), 442–447.

Fried, P. A., Watkinson, B., & Gray, R. (2005). Neurocognitive consequences of marihuana- a comparison with pre-drug performance. *Neurotoxicology and Teratology*, *27*(2), 231–239.

Gilman, J. M., Kuster, J. K., Lee, S., Lee, M. J., Kim, B. W., Makris, N., van der Kouwe, A., Blood, A. J., & Breiter, H. C. (2014). Cannabis use is quantitatively associated with nucleus accumbens and amygdala abnormalities in young adult recreational users. *Journal of Neuroscience*, *34*(16), 5529–5538.

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., Nugent, T. F., 3rd, Herman, D. H., Clasen, L. S., Toga, A. W., Rapoport, J. L., & Thompson, P. M. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(21), 8174–8179.

Gonzalez, R., Schuster, R. M., Mermelstein, R. J., Vassileva, J., Martin, E. M., & Diviak, K. R. (2012). Performance of young adult cannabis users on neurocognitive measures of impulsive behavior and their relationship to symptoms of cannabis use disorders. *Journal of Clinical and Experimental Neuropsychology*, *34*(9), 962–976.

Greicus, M. D., Supekar, K., Menon, V., & Dougherty, R. F. (2009). Resting-state functional connectivity reflects structural connectivity in the default mode network. *Cerebral Cortex*, *19*(1), 72–78.

Gruber, S. A., Silveri, M. M., Dahlgren, M. K., & Yurgelun-Todd, D. (2011). Why so impulsive? White matter alterations are associated with impulsivity in chronic marijuana smokers. *Experimental and Clinical Psychopharmacology*, *19*(3), 231–242.

Gruber, S. A., Dahlgren, M. K., Sagar, K. A., Gönenc, A., & Killgore, W. D. (2012a). Age of onset of marijuana use impacts inhibitory processing. *Neuroscience Letters*, *511*(2), 89–94.

Gruber, S. A., Sagar, K. A., Dahlgren, M. K., Racine, M., & Lukas SE (2012b). Age of onset of marijuana use and executive function. *Psychology of Addictive Behaviors*, *26*(3), 486–506.

Gruber, S. A., Dahlgren, M. K., Sagar, K. A., Gonenc, A., & Lukas, S. E. (2013). Worth the wait: effects of age of onset of marijuana use on white matter and impulsivity. *Psychopharmacology*, *231*(8), 1455–1465.

Hanson, K. L., Winward, J. L., Schweinsburg, A. D., Medina, K. L., Brown, S. A., & Tapert, S. F. (2010). Longitudinal study of cognition among adolescent marijuana users over three weeks of abstinence. *Addictive Behaviors*, *35*(11), 970–976.

Harding, I. H., Solowij, N., Harrison, B. J., Tajagi, M., Lorenzetti, V., Lubman, D. I., Seal, M. L., Pantelis, C., & Yucel, M. (2012). Functional connectivity in brain networks underlying cognitive control in chronic cannabis users. *Neuropsychopharmacology*, *37*, 1923–1933.

Harvey, M. A., Sellman, J. D., Porter, R. J., & Frampton, C. M. (2007). The relationship between non-acute adolescent marijuana use and cognition. *Drug and Alcohol Review*, *26*(3), 309–319.

Houck, J. M., Bryan, A. D., & Feldstein Ewing, S. W. (2013). Functional connectivity and cannabis use in high-risk adolescents. *American Journal of Drug and Alcohol Abuse*, *39*(6), 414–423.

Iversen, L. (2003). Cannabis and the brain. *Brain*, *126*, 1252–1270.

Jacobsen, L. K., Pugh, K. R., Constable, R. T., Westerveld, M., & Mencl, W. E. (2007). Functional correlates of verbal memory deficits emerging during nicotine withdrawal in abstinent adolescent marijuana users. *Biological Psychiatry*, *61*(1), 31–40.

Jacobus, J., Goldenberg, D., Wierenga, C. E., Tolentino, N. J., Liu, T. T., & Tapert, S. F. (2012). Altered cerebral blood flow and neurocognitive correlates in adolescent cannabis users. *Psychopharmacology (Berlin)*, *222*(4), 675–684.

Jacobus, J., Squeglia, L. M., Infante, M. A., Bava, S., & Tapert, S. F. (2013). White matter integrity pre- and post marijuana and alcohol initiation in adolescence. *Brain Sciences*, *3*(1), 396–414.

Jager, G., Block, R. I., Luijten, M., & Ramsey, N. F. (2010). Cannabis use and memory brain function in adolescent boys: a cross-sectional multicenter functional magnetic resonance imaging study. *Journal of the American Academy of Child and Adolescent Psychiatry*, *49*(6), 561–572.

Johnston, L. D., O'Malley, P. M., Bachman, J. G., & Schulenberg, J. E. (2013). *Monitoring the Future national results on adolescent drug use: overview of key findings, 2012*. Ann Arbor, MI: Institute for Social Research, The University of Michigan, 83 pp.

Knight, J. R., Sherritt, L., Shrier, L. A., Harris, S. K., & Chang, G. (2002). Validity of the CRAFFT substance abuse screener test among adolescent clinic patients. *Archives Pediatric and Adolescent Medicine*, *156*, 607–614.

Lisdahl, K. M., & Price, J. S. (2012). Increased marijuana use and gender predict poorer cognitive functioning in healthy emerging adults. *Journal of the International Neuropsychological Society*, *18*(4), 678–688.

Lopez-Larson, M. P., Bogorodzki, P., Rogowska, J., McGlade, E., King, J. B., Terry, J., & Yurgelun-Todd, D. (2011). Altered prefrontal and

insular cortical thickness in adolescent marijuana users. *Behavioral Brain Research*, *220*(1), 164–172.

Lopez-Larson, M. P., Rogowska, J., Bogorodzki, P., Bueler, C. E., McGlade, E. C., & Yurgelun-Todd, D. A. (2012). Cortico-cerebellar abnormalities in adolescents with heavy marijuana use. *Psychiatry Research*, *202*(3), 224–232.

McHale, S., & Hunt, N. (2008). Executive function deficits in short-term abstinent cannabis users. *Human Psychopharmacology*, *23*(5), 409–415.

McQueeny, T. M., Padula, C., Price, J., Medina, K. L., Logan, P., & Tapert, S. F. (2011). Gender effects on amygdala morphometry in adolescent marijuana users. *Behavioral Brain Research*, *224*(1), 128–134.

Medina, K. L., Hanson, K., Schweinsburg, A. D., Cohen-Zion, M., Nagel, B. J., & Tapert, S. F. (2007a). Neuropsychological functioning in adolescent marijuana users: subtle deficits detectable after 30 days of abstinence. *Journal of the International Neuropsychological Society*, *13*(5), 807–820.

Medina, K. L., Schweinsburg, A. D., Cohen-Zion, M., Nagel, B. J., & Tapert, S. F. (2007b). Effects of alcohol and combined marijuana and alcohol use during adolescence on hippocampal asymmetry. *Neurotoxicology and Teratology*, *29*, 141–152.

Medina, K. L., McQueeny, T., Nagel, B. J., Hanson, K. L., Yang, T., & Tapert, S. F. (2009). Prefrontal morphometry in abstinent adolescent marijuana users: subtle gender effects. *Addiction Biology*, *14*(4), 457–468.

Medina, K. L., Nagel, B. J., & Tapert, S. F. (2010). Cerebellar vermis abnormality in adolescent marijuana users. *Psychiatry Research: Neuroimaging*, *182*(2), 152–159.

Meier, M. H., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R. S., McDonald, K., Ward, A., Poulton, R., & Moffitt, T. E. (2012). Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(40), 2657–2664.

Orr, C., Morioka, R., Behan, B., Datwani, S., Doucet, M., Ivanovic, J., Kelly, C., Weierstall, K., Watts, R., Smyth, B., & Garavan, H. (2013). Altered resting-state connectivity in adolescent cannabis users. *American Journal of Drug and Alcohol Abuse*, *39*(6), 372–381.

Pope, H., Jr., Gruber, S. A., Hudson, J., Cohane, G., Huestis, M., & Yurgelun-Todd, D. (2003). Early-onset cannabis use and cognitive deficits: what is the nature of the association? *Drug and Alcohol Dependence*, *69*(3), 303.

Substance Abuse and Mental Health Services Administration. (2013). *The TEDS Report: marijuana admissions aged 18 to 30. Early vs. adult initiation*. Rockville, MD: SAMHSA, Center for Behavioral Health Statistics and Quality.

Schacht, J. P., Hutchinson, K. E., & Flibey, F. M. (2012). Associations between cannabinoid receptor-1 (CNR1) variation and hippocampus and amygdala volumes in heavy cannabis users. *Neuropsychopharmacology*, *37*(11), 2368–2376.

Schneider, M. (2008). Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. *Addiction Biology*, *13*, 253–263.

Schweinsburg, A. D., Nagel, B. J., Schweinsburg, B. C., Park, A., Theilmann, R. J., & Tapert, S. F. (2008). Abstinent adolescent marijuana users show altered fMRI response during spatial working memory. *Psychiatry Research*, *163*(1), 40–51.

Schweinsburg, A. D., Schweinsburg, B. C., Medina, K. L., McQueeny, T., Brown, S. A., & Tapert, S. F. (2010). The influence of recency of use on fMRI response during spatial working memory in adolescent marijuana users. *Journal of Psychoactive Drugs*, *42*(3), 401–412.

Smith, A. M., Longo, C. A., Fried, P. A., Hogan, M. J., & Cameron, I. (2010). Effects of marijuana on visuospatial working memory: an fMRI study in young adults. *Psychopharmacology (Berlin)*, *210*(3), 429–438.

Solowij, N., Jones, K. A., Rozman, M. E., Davis, S. M., Ciarrochi, J., Heaven, P. C., Lubman, D. I., & Yücel, M. (2011). Verbal learning and memory in adolescent cannabis users, alcohol users and non-users. *Psychopharmacology (Berlin)*, *216*(1), 131–144.

Solowij, N., Jones, K. A., Rozman, M. E., Davis, S. M., Ciarrochi, J., Heaven, P. C., Pesa, N., Lubman, D. I., & Yücel, M. (2012). Reflection impulsivity in adolescent cannabis users: a comparison with alcohol-using and non-substance-using adolescents. *Psychopharmacology (Berlin)*, *219*(2), 575–586.

Tait, R. J., Mackinnon, A., & Christensen, H. (2011). Cannabis use and cognitive function: 8-year trajectory in a young adult cohort. *Addiction*, *106*(12), 2195–2203.

Tapert, S. F., Granholm, E., Leedy, N. G., & Brown, S. A. (2002). Substance use and withdrawal: neuropsychological functioning over 8 years in youth. *Journal of the International Neuropsychological Society*, *8*(7), 873–883.

Tapert, S. F., Schweinsburg, A. D., Drummond, S. P., Paulus, M. P., Brown, S. A., Yang, T. T., & Frank, L. R. (2007). Functional MRI of inhibitory processing in abstinent adolescent marijuana users. *Psychopharmacology (Berlin)*, *194*, 173–183.

Town, M., Naimi, T. S., Mokdad, A. H., & Brewer, R. D. (2006). Health care access among US adults who drink alcohol excessively: missed opportunities for prevention. *Preventing Chronic Disease*, *3*(2), epub A53.

CHAPTER

17

Correlates and Consequences of Prenatal Cannabis Exposure (PCE): Identifying and Characterizing Vulnerable Maternal Populations and Determining Outcomes for Exposed Offspring

L.K. Brents

Brain Imaging Research Center, Psychiatric Research Institute, University of Arkansas for Medical Sciences, Little Rock, AR, United States

SUMMARY POINTS

- Between 5% and 15% of pregnant women abuse illicit drugs.
- Of these women, 65–75% use cannabis during pregnancy, meaning that an estimated 5–10% of neonates are exposed to cannabis in utero.
- Prevalence estimates rely on a combination of self-report and analysis of biological samples (ie, maternal hair, urine, oral fluids, and neonatal meconium), which indicate that a small minority (<35%) of women are forthcoming about their cannabis use during pregnancy.
- Women who use cannabis during pregnancy are more likely to be younger, unmarried, of lower socioeconomic status (ie, lower education and income), and to have had unplanned pregnancies, compared to pregnant women who don't use cannabis.
- Effects of PCE are dose-dependent, with more severe negative effects occurring with heavier prenatal exposure.
- PCE is associated with lower birth weight, shorter birth length, lifelong smaller head circumference, reduced length of gestation, neonatal neurological disturbances, reduced function in specific cognitive domains (memory, verbal, and abstract/visual reasoning), but not overall intelligence, during grade school, increased impulsivity and hyperactivity, depression at age 10, poor school achievement and delinquency in adolescence, increased use of tobacco and marijuana in adolescence, and altered neural function for visuospatial working memory and motor impulse control, in early adulthood.

KEY FACTS OF CANNABIS EXPOSURE

- *Cannabis* is a plant that contains over 400 chemicals.
- The psychoactive effects of cannabis are caused by the cannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC).
- The effects of Δ^9-THC and other pharmacologically active cannabinoids are mediated by the endocannabinoid system (ECS); specifically, Δ^9-THC activates at least two G-protein coupled receptors: the Cannabinoid 1 Receptor (CB1R) and the Cannabinoid 2 Receptor (CB2R).

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00018-1
Copyright © 2017 Elsevier Inc. All rights reserved.

- The ECS is integrally involved in a variety of biological processes that are vulnerable to disruption by exogenous cannabinoid exposure: mood, cognition, appetite, energy storage and metabolism, bone formation, reproduction, and embryonic and fetal neural development.
- Acute exposure to cannabis distorts spatial and temporal perception, elevates mood, increases appetite, impairs memory and cognition, and has a suspected link to adverse cardiovascular episodes.
- Cannabis with high Δ^9-THC content can cause anxiety and/or lead to psychosis and schizophrenia onset in susceptible individuals.
- Chronic cannabinoid exposure can lead to cognition and memory problems, disrupt the function of the male and female reproductive systems, and lead to cannabis use disorder, which is characterized by dependence and withdrawal symptoms upon cessation.
- Younger individuals (adolescents) are more susceptible than adults to the adverse effects of marijuana, especially dependence and psychosis.

LIST OF ABBREVIATIONS

CB1R, CB2R	Cannabinoid Receptors 1 and 2
Δ^9-THC	Δ^9-Tetrahydrocannabinol
ECS	Endocannabinoid system
fMRI	Functional magnetic resonance imaging
MHPCD	Maternal Health Practices and Child Development
OPPS	Ottawa Prenatal Prospective Study
PCE	Prenatal cannabis exposure
SAMHSA	Substance Abuse and Mental Health Services Administration
SIDS	Sudden Infant Death Syndrome
UDS	Urine drug screening

INTRODUCTION

Cannabis is the most abused illicit drug worldwide (WHO, 2014) and has been used for millennia across diverse cultures for recreational, medicinal, religious, and industrial purposes (Zuardi, 2006). Because cannabis has never been documented as the primary cause of even a single death (Sidney, 2003), its use is regarded as safe by many in the public, leading to several strong movements throughout the world to legalize marijuana use for medicinal and recreational purposes (Johnson, 2014). Considering the widespread use of cannabis in the general population, it is not surprising that cannabis is used by women immediately before, during, and after pregnancy. Despite the perceived safety of cannabis throughout the general public, unnecessary use of pharmacologically active substances during pregnancy is widely discouraged, especially when their effects on the mother–fetus dyad are unknown, or suspected to potentially cause harm. The issue of marijuana's harm versus benefit in the general population is a controversial subject; similarly, reports on many of the suspected effects of prenatal cannabis exposure (PCE) are conflicted, or nuanced, and require careful consideration. This chapter aims to explore the correlates and consequences of PCE by examining the characteristics of women who use cannabis during pregnancy, and summarizing the scientific literature that describes the lifelong consequences of PCE on offspring.

PREVALENCE OF PCE AND BEHAVIORAL PATTERNS OF CANNABIS USE ACROSS PREGNANCY

With few exceptions, the possession, sale, and transport of cannabis is illegal throughout the world. Nevertheless, cannabis use is highly prevalent. In a 2013 Pew Research Center poll in the United States, 48% of respondents reported ever using marijuana, with 12% of respondents reporting use in the past year (Desilver, 2013). An estimated 17.4% of past-year cannabis users were also daily or near-daily users (SAMHSA, 2014). Consistent with these data, 51.4% of young adults in an Australian sample reported ever using cannabis; importantly, more than 21% of individuals in this sample were diagnosed as ever having a cannabis use disorder by age 21 (Hayatbakhsh, Najman, Bor, O'Callaghan, & Williams, 2009).

To determine the prevalence and impact of PCE, one should focus on drug use by women. According to the US. Substance Abuse and Mental Health Services Administration (SAMHSA), 11.4% of all women and 5.4% of pregnant women between ages of 15 and 44 were current illicit drug users in 2012–13, with rates as high as 14.6% for pregnant teenagers aged 15–17 (SAMHSA, 2014). In close agreement with the US SAMHSA data, a large Australian study (n = 25,049) from 2000 to 2006 determined that 9.3% of nonpregnant women of childbearing age self-reported regular cannabis use (Hayatbakhsh et al., 2011), suggesting that a significant proportion of women use drugs during the periconceptual period. Investigators in a London study screened 807 consecutive positive urine pregnancy tests for licit and illicit drugs, and determined that 14.5% tested positive for cannabis, confirming that there is a high prevalence of early PCE (Sherwood, Keating, Kavvadia, Greenough, & Peters, 1999).

Most women abstain from drug use during pregnancy. Upon recognition of the pregnancy, at least half of women who use illicit drugs remit at some point in their pregnancy (SAMHSA, 2014). Such women are able to progressively reduce drug consumption, and even achieve complete abstinence by the third trimester (Ebrahim & Gfroerer, 2003; Gray et al., 2010). Remission of drug use during pregnancy is associated with baseline severity of drug use, psychosocial stressors, and initiation of prenatal care (Corse & Smith, 1998). Postpartum relapse is highly prevalent, with cannabis consumption returning to prepregnancy levels within a year of birth (Fried, Barnes, & Drake, 1985).

Marijuana is, by far, the most commonly abused illicit drug during pregnancy. Of pregnant and postpartum women who use illicit drugs, an overwhelming proportion—between 64% and 75%—use marijuana (Ebrahim & Gfroerer, 2003; Falcon et al., 2012). Furthermore, prenatal marijuana consumption is reduced much less than prenatal alcohol and nicotine consumption (Fried et al., 1985). Although many women achieve abstinence during pregnancy, a significant proportion of the population is being exposed to cannabis at one or more points during early development, potentially resulting in peripartum complications, and long-term alterations in development.

PCE DETECTION METHODS AND ACCURACY OF PREVALENCE ESTIMATES

Many women are hesitant to report their drug use to health care providers, possibly due to the intense stigma of prenatal drug abuse, and to a fear of losing parental rights to their children. Estimates of prenatal drug use were derived from a combination of self-report and chemical analysis of urine, hair and oral fluids from pregnant and peripartum women, and meconium from neonates (Gray & Huestis, 2007). The prevalence of analysis-confirmed cannabis use in pregnancy can depend on participant sampling, the types of biological tissues that are analyzed, and the temporal relationship of drug use to biological sampling. Analytical results often conflict with self-report, due to underreporting or inadvertent sampling of prepregnancy biological specimens (eg, hair). One small, single site study (n = 107) in Spain detected drugs of abuse in 15.9% of the maternal hair samples obtained, with cannabis being the most prevalent (detected in 10.3% of the total sample), although less than 1% reported cannabis use during pregnancy (Friguls et al., 2012). Another study in Spain detected metabolites of cannabis in 5.3% of meconium samples, although only 1.7% of mothers disclosed cannabis use in the pregnancy (Lozano et al., 2007). These two studies suggest that less than 35% of women who use cannabis during pregnancy will disclose their drug use. The window of detection in meconium samples is restricted to the third trimester of pregnancy; therefore, a cannabis-positive meconium sample is indicative of third trimester PCE. Considering that most women who abuse drugs taper their use during pregnancy (Ebrahim & Gfroerer, 2003), third trimester use indicates that a drug was likely used throughout pregnancy, resulting in a greater overall burden of in utero exposure that occurs across most or all prenatal developmental stages. Detection of drug exposure in fetal and placental tissues is inconsistent with detection in the respective maternal hair. Less than 17% of placental and fetal tissues (gestational age 17–22 weeks) from women with a cannabis-positive hair test also tested positive for cannabis. Possible explanations of this phenomenon are that undetectable amounts of cannabis byproducts accumulate in fetal and placental tissues due to pharmacokinetic activity (ie, reduced fetal distribution, rapid metabolism, and complete excretion of cannabis from the placenta and fetus) and/or hair testing detects previous cannabis use that may have occurred only before pregnancy (Falcon et al., 2012). In contrast to hair, urine drug screening (UDS), which is the most common screening method, has a much shorter window of detection for cannabis. In a large study in the Netherlands (n = 3997), 71 pregnant women had positive cannabis UDS, even though 92 women reported use during pregnancy (El Marroun et al., 2011a). Additionally, several of these positive screens were from women who self-reported only prepregnancy cannabis use, further increasing the disparity between self-reports of drug use and UDS. Despite the advances in analytical screening, it is clear that limitations exist in determining accurate, valid information on PCE. Clinicians must rely on a combination of self-report, family report, and multiple analytical screenings across pregnancy and the postpartum period to accurately confirm maternal drug abuse.

CHARACTERISTICS OF WOMEN WHO USE CANNABIS DURING PREGNANCY

Women who abuse drugs during pregnancy tend to be younger, less educated, unemployed, and unmarried, with a history of childhood trauma, delinquency, and unplanned pregnancy (SAMHSA, 2014; Correia, Oliveria, Almeida, Sing, & Nobrega, 1995; Ebrahim & Gfroerer, 2003; El Marroun et al., 2008). Cannabis use by the biological father of the baby robustly increases the odds of a pregnant woman using cannabis (odds ratio = 38; El Marroun et al., 2008). One subgroup of prenatal drug users are women who decrease alcohol use and abstain from using some recreational drugs, specifically cocaine and ecstasy, but continue to use tobacco and cannabis at prepregnancy levels (Moore et al., 2010),

even though all of these substances are considered detrimental to the offspring. It is possible that this pattern of polydrug use is due to a misconception that tobacco and cannabis use is safer than other drugs, or to the highly rewarding or addictive properties of tobacco and cannabis. While it is presumed that most pregnant women who use cannabis do so for recreational purposes, a subset of this population reported using cannabis medicinally to treat severe morning sickness, and over 92% rated it as effective for this indication (Westfall, Janssen, Lucas, & Capler, 2006). Understanding who uses cannabis during pregnancy and their motivation for doing so is important to identifying such patients, and educating them about the potential harm of their drug use on their offspring.

THE EFFECTS OF PCE ON OFFSPRING

Drug use during pregnancy is associated with complications of childbirth (Saleh Gargari et al., 2012) and negative physical, behavioral, and cognitive effects on the offspring (Steinhausen, Blattmann, & Pfund, 2007; Day, Richardson, Goldschmidt, & Cornelius, 2000; Conradt et al., 2013), but causal relationships between specific prenatal exposures and these negative outcomes can be difficult to establish. Potentially confounding variables that often correlate with PCE, such as, socioeconomic status, age, and coexposures to tobacco products, alcohol, and other illicit drugs complicate the identification of PCE-induced effects. Another challenge is determining the dosage of maternal cannabis use during pregnancy, and the relative in utero exposures of the offspring. To circumvent these issues, three large-scale prospective longitudinal studies that are examining offspring outcomes with an emphasis on PCE have been undertaken: the Ottawa Prenatal Prospective Study (OPPS; Fried, 1995), the Maternal Health Practices and Child Development (MHPCD) study (Day & Richardson, 1991), and the Generation R study (Jaddoe et al., 2006). The populations recruited into each study differ in demographics, and thus help to discern effects that may be due to variables other than cannabis exposure; that is, consistent results across the cohorts strengthens the case that PCE plays a causal role. The OPPS cohort consists of a "low-risk," racially homogenous middle class sample of pregnant women in Ottawa, Ontario in Canada who were recruited between 1978 and 1983. The MHPCD study recruited "high-risk" urban, racially and ethnically heterogeneous pregnant women of lower socioeconomic status in the Pittsburgh, Pennsylvania area in the United States, between May 1983 and Jan. 1986. The Generation R study, which began in 2001, recruited an ethnically diverse sample of slightly higher socioeconomic status in Rotterdam, the Netherlands. Much of what is known about the effects of in utero cannabis exposure comes from these studies.

In utero cannabinoid exposure has a suspected association with minor physical abnormalities, including facial–visual system anomalies, such as, ocular hypertelorism and severe epicanthus (Fried & Smith, 2001), particularly in children who had heavy PCE. Third trimester exposure to marijuana predicted a greater risk of chest and head shape abnormalities, after controlling for covariates in one study of 6-year-olds with prenatal exposure to cocaine and marijuana (Minnes et al., 2006). As shown in Table 17.1, there are also associated cardiovascular abnormalities of the PCE offspring, including isolated simple ventricular septal defect (Williams, Correa, & Rasmussen, 2004), and smaller fetal aortic diameter. Alterations in fetal blood supply that are associated with cannabis exposure include increased pulsatility and resistance indices of the uterine artery, indicating an increase in placental vascular resistance (El Marroun et al., 2010). Interestingly, decreased uterine artery pulsatility and resistance was observed when cannabis exposure was restricted to early pregnancy (El Marroun et al., 2010), suggesting that the developmental stage at which exposure occurs is critical in determining the ultimate effects on the fetal–placental complex.

Reports on how PCE affects offspring growth are conflicted (Table 17.2). While several report no effect on birth weight (English, Hulse, Milne, Holman, & Bower, 1997), a surprisingly positive relationship between PCE and

TABLE 17.1 Cardiovascular Changes Associated With PCE

Age group	Prenatal exposure	Effects	References
All			
	Regular	Increased risk of isolated simple ventricular septal defect	Williams et al. (2004)
Prenatal			
	Regular	Decreased fetal aortic diameter	El Marroun et al. (2010)
	Regular	Increased uterine artery pulsatility and resistance	El Marroun et al. (2010)
	Early pregnancy only	Decreased uterine artery pulsatility and resistance	El Marroun et al. (2010)

PCE offspring has an increased risk of a cardiac defect and greater fetal–placental cardiovascular alterations.

TABLE 17.2 Size Effects Associated With PCE

Age group	Prenatal exposure	Effects	References
All			
	Heavy	Smaller head circumference	Fried et al. (1999), Gray et al. (2010)
Prenatal and neonatal			
	a	Small for gestational age	Hayatbakhsh et al. (2012), Hurd et al. (2005)
	a	Reduced fetal foot length	Hurd et al. (2005)
	a	No effect on birth weight	English et al. (1997), meta-analysis
	Infrequent	Elevated birth weight	Day and Richardson (1991), Fried and O'Connell (1987)
	More closely associated with regular, moderate use	Low birth weight	Hayatbakhsh et al. (2012), Sherwood et al. (1999), El Marroun et al. (2009)
	a	Decreased length	Gray et al. (2010)
Infant			
	a	Decreased body length	Barr et al. (1984)
Toddler			
	Continuous	Increased weight	Fried and O'Connell (1987)
Adolescent			
	a	No effect on height	Fried et al. (1999)

Overall associates of PCE with offspring size are conflicted. Conflicts may be due to differences in prenatal cannabis exposure (ie, low exposures are associated with increased size, and larger exposures with decreased size, resulting in no net effect when data are unstratified by exposure levels). *PCE*, prenatal cannabis exposure.
[a]*Unspecified.*

birth weight was measured in the OPPS and the MHPCD study, with exposed babies being heavier than nonexposed (Day & Richardson, 1991), and remaining heavier up to 24 months of age (Fried & O'Connell, 1987). Heavier, more frequent use of cannabis during pregnancy was associated with low birth weight in several studies (Sherwood et al., 1999; Fried & Smith, 2001), including the Generation R cohort (El Marroun et al., 2009; Huizink, 2014). Conflicting results between the older OPPS and MHPCD study and the more recent Generation R study may be due, in part, to differences in the content of the psychoactive chemical of marijuana, Δ^9-tetrahydrocannabinol (Δ^9-THC), during the two time periods. Over time, marijuana has been selectively bred to produce a higher concentration of Δ^9-THC (Cascini, Aiello, & Di Tanna, 2012), potentially resulting in greater maternal intake and in utero exposure to Δ^9-THC, in more recent studies. The size of cannabis-exposed fetuses was measured for the first time in the Generation R study. This study found that cannabis-exposed 17–22 week fetuses possessed slightly reduced foot length and body weight, with foot length decreasing in a dose-dependent manner (Hurd et al., 2005). In the OPPS cohort, PCE was negatively associated with birth length, but this relationship was not significant at 8 months, 1 year, 6 years or adolescence (Fried, Watkinson, & Gray, 1999; Fried & Smith, 2001). In contrast, prenatally exposed infants in a different cohort were still significantly shorter at 8 months (Barr, Streissguth, Martin, & Herman, 1984). Children with heavy PCE had statistically smaller head circumferences at all ages, from early infancy to early adolescence (Fried et al., 1999). As shown in Table 17.3, gestation was reduced by almost a week in heavy users in the OPPS cohort (Fried, Watkinson, & Willan, 1984), and other studies reported an association of shortened gestation and increased risk of prematurity, as well (Sherwood et al., 1999). Reports on the effects of PCE on neonate size and gestation length are conflicting, but seem to suggest a dose-dependent, negative association between PCE and two pregnancy outcomes: birth size, and length of gestation.

There is an apparent association between neonatal and infant mortality and in utero cannabis exposure (Table 17.3). A large five-site study of stillbirth determined that the presence of cannabis in umbilical cord homogenate was associated with more than a twofold increased odds of stillbirth (Varner et al., 2014), a result that may be partially confounded by concurrent tobacco use. An Australian sample of PCE offspring also had a 2.23-fold increased risk of suffering from Sudden Infant Death Syndrome (SIDS; Scragg et al., 2001); in contrast, another large study (n = 2964) found no association

TABLE 17.3 Associations of PCE With Gestation and Infant Mortality

Age group	Prenatal exposure	Effects	References
Prenatal and neonatal			
	a	Increased preterm labor or birth	Hayatbakhsh et al. (2012), Fried et al. (1984), Sherwood et al. (1999)
	a	No effect on preterm birth	Fried and Smith (2001)
	a	Increased risk of stillbirth	Varner et al. (2014)
	a	Increased NICU admissions	Hayatbakhsh et al. (2012)
Infant			
	a	Increased risk of Sudden Infant Death Syndrome	Scragg et al. (2001)
Toddler			
	Cannabis-positive meconium (third trimester exposure)	No increased risk of death before 2 years of age	Ostrea et al. (1997)

Reports on the association PCE with preterm labor, stillbirth, and infant mortality are conflicted. Disagreements in the literature may be due to methodological differences between studies. *PCE*, prenatal cannabis exposure.
[a]*Unspecified.*

between having cannabis-positive meconium samples and risk of infant death within the first 2 years of life (Ostrea, Ostrea, & Simpson, 1997).

There are apparent long-term effects of PCE on offspring neurological and cognitive development (Table 17.4). Markers of altered neurological function in neonates and infants prenatally exposed to cannabis include increased risk of tremors, an exaggerated and prolonged startle response, altered autonomic arousal, a high-pitched cry, less time in quiet sleep, and poorer habituation to visual, but not auditory, stimuli (Fried & Smith, 2001). There is consistently no effect of PCE on the Bayley Scales of Infant Development, except for in children of exceptionally heavy users (one or more joints per day during the third trimester). These children had reduced mental scores on the Bayley Scales at 9 months, but not at 18 months (Richardson, Day, & Goldschmidt, 1995). After the age of 3, there were distinguishable dose-dependent effects of PCE on specific cognitive domains, including short-term memory, verbal, and abstract/visual reasoning, while composite intelligence scores were not affected (Fried & Smith, 2001; Day et al., 1994; Griffith, Azuma, & Chasnoff, 1994). Although there was no association of PCE with cognitive and receptive language development in 5- and 6-year-olds that were followed by the OPPS (Fried, O'Connell, & Watkinson, 1992a), high-risk 6-year-olds in the MHPCD who were heavily exposed during the first trimester of pregnancy had significantly lower verbal reasoning scores on the Stanford-Binet Intelligence Scale than nonexposed children (Goldschmidt, Richardson, Willford, & Day, 2008). Similarly, prenatally exposed school-aged children (ages 9–12) showed deficits in specific Wechsler Intelligence Scale for Children subtests of visuospatial/visuomotor intelligence and planning, the Block Design and the Picture Completion subtests, but were equal to nonexposed children in global IQ measures (Fried, Watkinson, & Gray, 1998), and there was no effect of PCE on reading or language outcomes in this cohort (Fried, Watkinson, & Siegel, 1997). Prenatally exposed 9–12 year olds also showed poorer impulse control, and impaired hypothesis testing (Fried et al., 1998). In sum, PCE is associated with abnormal behavior in neonates, and deficits in multiple cognitive domains in preschool and school-aged children, including verbal reasoning, perceptual reasoning, memory, planning, and attentional processes, without affecting overall intelligence.

Neuroimaging studies have provided insights on the neural mechanisms underlying cognitive processing (Table 17.4). Visuospatial working memory was examined at age 18–22, in the OPPS cohort, using functional magnetic resonance imaging (fMRI) to measure neural activity while participants performed the two-back working memory task. Although behavioral performance did not differ between the prenatally exposed versus unexposed participants, there was a positive, dose-dependent relationship between PCE and neural activity in the left inferior and middle frontal gyri, left parahippocampal gyrus, left middle occipital gyrus and left cerebellum, and a negative relationship between exposure and activity in the right inferior and middle frontal gyri, during the task (Smith, Fried, Hogan, & Cameron, 2006). Similarly, a task of response inhibition that measures motor impulse, and is a correlate of drug use disorders, was measured in the 18–22 year old OPPS offspring cohort. After controlling for possible confounders, the prenatally exposed group exhibited more commission errors than the unexposed group, but both groups had similar reaction times. Brain

TABLE 17.4 Associations of PCE on Neurocognitive Development

Age group	Prenatal exposure	Effects	References
Prenatal and neonatal			
	a	Increased tremors	Fried and Smith (2001)
	a	Reduced habituation to visual stimuli	Fried and Smith (2001)
	a	Prolonged and exaggerated startle	Fried and Smith (2001)
	a	Sleep disturbances	Fried and Smith (2001)
Toddler			
	Heavy	Reduced mental scores on Bayley Scales of Infant Development (9, but not 18, months)	Richardson et al. (1995)
	Mild-moderate	No effect on Bayley Scales of Infant Development (9 and 18 months)	Richardson et al. (1995)
School-aged			
	a	No effect on cognitive development (5 and 6 years)	Fried et al. (1992a)
	a	No effect on receptive language development	Fried et al. (1992a)
	Heavy first trimester	Reduced verbal reasoning (6 years)	Goldschmidt et al. (2008)
	a	Deficits in WISC Block Design Task (spatial analysis, abstract visual reasoning) (9–12 years)	Fried et al. (1992b)
	a	Deficits in Picture Completion task (alertness to detail, visual discrimination) (9–12 years)	Fried et al. (1992b)
	a	Reduced planning (9–12 years)	Fried et al. (1992b)
	a	Impaired hypothesis testing (9–12 years)	Fried et al. (1992b)
	a	No effect on global and composite intelligence (6, 9–12 years)	Fried et al. (1992b), Goldschmidt et al. (2008)
	a	No effect on verbal fluency (9–12 years)	Fried et al. (1997)
	a	No effect on reading (9–12 years)	Fried et al. (1997)
Young adult			
	a	No effect on two-back performance (visual-spatial working memory task) (18–22 years)	Smith et al. (2006)
	Dose-dependent effect	Altered neural activity during two-back task (18–22 years)	Smith et al. (2006)
	a	Greater errors in response inhibition (18–22 years)	Smith et al. (2004)
	Dose-dependent effect	Altered neural activity during response inhibition task (18–22 years)	Smith et al. (2004)

Offspring with PCE show deficits and alterations in selective measures of neurocognitive development. *PCE*, prenatal cannabis exposure.
[a]*Unspecified.*

activity measured with fMRI during the task showed a positive dose-dependent relationship between PCE and activity in the bilateral prefrontal cortex and right premotor cortex, during response inhibition (Smith, Fried, Hogan, & Cameron, 2004). These functional neuroimaging studies indicate that PCE alters the neural strategy used in completing tasks of cognition.

PCE is also associated with alterations in emotional and behavioral development that are highly consistent between the "low risk" OPPS cohort and the "high risk" MHPCD cohort (Table 17.5). A sex-specific effect of PCE was identified in 18-month old girls in the Generation R cohort, who exhibited notably greater aggressive and inattentive behavior, but this effect was not significant at 36 months (El Marroun et al., 2011b). Depression and anxiety at 10 was predicted by PCE that was specific to exposure during the first and third trimesters (Gray, Day, Leech, & Richardson, 2005). Prenatally exposed 10-year-olds in the MHPCD cohort had higher ratings of hyperactivity, inattention,

TABLE 17.5 Behavioral Alterations in Children Prenatally Exposed to Cannabis

Age group	Prenatal exposure	Effects	References
Toddler			
	a	Aggressive, inattentive behavior (girls only, 18 months, but not 36 months)	El Marroun et al. (2011b), Generation R Study
School-aged			
	a	Reduced impulse control (9–12 years)	Fried et al. (1992b), OPPS
	Dose-dependent effect	Increased externalizing behavior and delinquency (10 and 14 years)	Goldschmidt et al. (2000), Day et al. (2011), MHPCD
	a	Increased hyperactivity, inattention, impulsivity (10 years)	Goldschmidt et al. (2000), MHPCD
	First and third trimester exposure	Increased depression and anxiety (age 10)	Gray et al. (2005), Leech, Larkby, Day, & Day (2006), MHPCD
Adolescent			
	a	Reduced academic achievement (14 years)	Goldschmidt et al. (2012), MHPCD
Young adult			
	a	Increased risk of marijuana and daily cigarette use (16–21 years)	Porath and Fried (2005), MHPCD
	a	Predictive of marijuana use (22 years)	Sonon et al. (2015), OPPS

Offspring with PCE exhibit greater behavioral and academic problems than nonexposed cohorts. *PCE*, prenatal cannabis exposure.
[a]*Unspecified.*

impulsivity, externalizing problems, and delinquency, with inattention mediating the effect between exposure and delinquency (Goldschmidt, Day, & Richardson, 2000). Heavier PCE increased odds of delinquency at the age of 14, mediated by depression at the age of 10 (Day, Leech, & Goldschmidt, 2011). Porath and Fried (2005) reported that adolescents and young adults (16–21 years) with PCE had greater than twofold increased odds of initiating marijuana and daily cigarette use. Similarly, prenatally exposed participants in the MHPCD cohort had higher rates of marijuana use at the age of 22 than nonexposed controls, even after controlling for maternal and offspring sociodemographic and psychiatric variables, as well as maternal history of drug abuse or disorder (Sonon, Richardson, Cornelius, Kim, & Day, 2015). The MHPCD study also revealed that school achievement at the age of 14 suffered in those with PCE (Goldschmidt, Richardson, Willford, Severtson, & Day, 2012). The Wechsler Individual Achievement Test Screener composite and reading scores were lower in adolescents with PCE (Table 17.4). These reduced scores were mediated by age performance at the age of 6, attention problems, depression symptoms at the age of 10, and early initiation of marijuana use (Goldschmidt et al., 2012). These results indicate that PCE is associated with adverse cognitive and behavioral outcomes that compromise offspring mental, emotional, and social development.

CONCLUSIONS

Cannabis is the most prevalent illicit drug used by pregnant women. Although most women abstain from use while pregnant, a surprisingly large proportion of women continue to use cannabis throughout pregnancy. These women tend to be younger, single, less educated, and have lower income. Definitive "diagnosis" of PCE by health-care professions relies primarily on patient self-report, and analytical detection methods, such as urine drug screening or hair analysis, which each have their own limitation, and are not always sufficiently sensitive or accurate for the time period of interest.

Long-term effects of PCE on offspring are controversial, but become more robust at higher levels of exposure, even after controlling for confounders. Although small, infrequent exposures seem to have little effect on offspring outcomes, heavier, more frequent PCE is clearly associated with negative effects. Where the line between "no effect" and "adverse effect" doses lies is unknown; therefore, pregnant women should be encouraged to quit their use of cannabis (Fig. 17.1).

MINI-DICTIONARY

Bayley Scales of Infant Development A norm-referenced assessment of early childhood development. The original version (1969) measured mental and motor development in infants aged

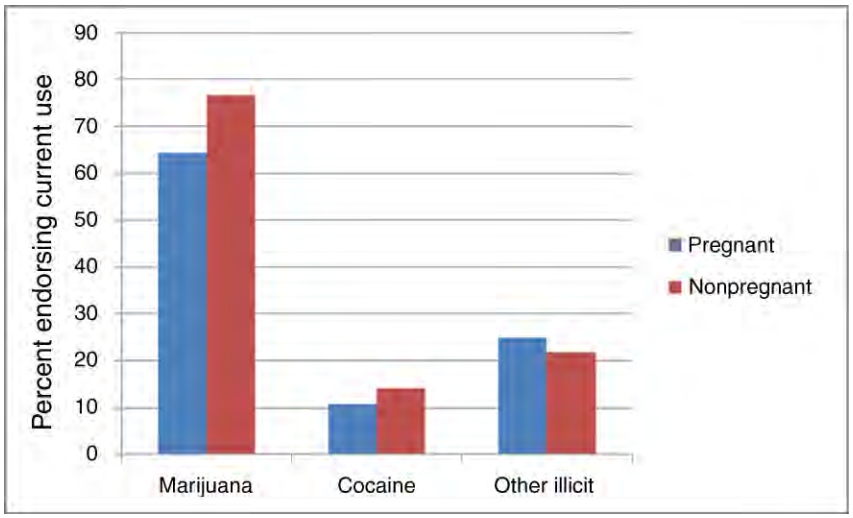


FIGURE 17.1 **Drugs used by pregnant and nonpregnant women who use any illicit drug.** This figure shows the choice of drug(s) used by women who use illicit drugs, indicating that cannabis use is highly prevalent in pregnant women in this population. *Source: (1996–1998) National Household Survey on Drug Abuse. Reproduced with permission from Ebrahim, S.H., and Gfroerer, J., 2003.*

3–28 months. The second edition (1993) extended the age range to 1–42 months, and added a behavioral rating scale. The third edition (2005) is composed of a core battery of five scales: child cognitive, motor and language interaction, and parental report of the child's social–emotional and adaptive behavior.

Cannabinoids A class of chemicals that bind to one or both known cannabinoid receptors: Cannabinoid 1 Receptor (CB1R) and the Cannabinoid 2 Receptor (CB2R). These chemicals may be isolated from the *Cannabis* plant (phytocannabinoids, eg, Δ^9-THC, the psychoactive component of cannabis), produced endogenously by a diverse array of Animalian species (endocannabinoids, eg, anandamide and 2-arachidonoylglycerol), or synthesized in laboratory conditions (synthetic cannabinoids, eg, JWH-018, WIN55, 212-2).

Cannabis A genus composed of three species of flowering plants (*C. sativa*, *C. indica* and *C. ruderalis*) that are best known for producing the psychoactive phytocannabinoid Δ^9-THC. Consumption by smoking, vaporizing or eating the Δ^9-THC-rich trichomes on the flowers of the plant results in alterations in mood, perception, and appetite. Resin extractions of the trichomes, such as, hashish and hash oil, contain high concentrations of Δ^9-THC, and are also consumed for their psychoactive effects. In addition to recreational purposes, *Cannabis* is also cultivated for industrial (eg, hemp made from the fibrous stalk of the plant), medicinal, and religious uses.

Hypertelorism A congenital facial malformation in which the eyes are abnormally spaced apart.

Meconium The earliest stools of the neonate that are composed solely of materials consumed in utero.

Pulsatility index The variability of flow velocity in a blood vessel measured by Doppler ultrasound, and calculated by subtracting the minimum diastolic velocity from the maximum systolic velocity, and dividing the difference by the mean flow velocity.

Resistance index A measure of flow in a blood vessel that is calculated by subtracting the end diastolic velocity from the peak systolic velocity, and dividing the difference by the peak systolic velocity. Abnormal umbilical or uterine artery resistance may indicate compromised fetal–placental circulation associated with adverse maternal–fetal conditions, such as, intrauterine growth restriction, or preeclampsia.

Severe epicanthus Overgrowth of the skin in the corners of the eyelids (ie, epicanthic folds).

Stanford-Binet Intelligence Scales An assessment that measures five domains of cognition in people aged 2 and older: fluid reasoning, knowledge, quantitative reasoning, visual-spatial processing, and working memory.

Wechsler Individual Achievement Test Screener An assessment of academic strengths and weaknesses in the areas of reading, math, written language, and oral language in people aged 4–85.

Wechsler Intelligence Scale for Children An assessment of cognitive abilities in children aged 6–16 that measures overall intellect ability, verbal comprehension, perceptual reasoning, processing speed, and working memory.

References

Barr, H. M., Streissguth, A. P., Martin, D. C., & Herman, C. S. (1984). Infant size at 8 months of age: relationship to maternal use of alcohol, nicotine, and caffeine during pregnancy. *Pediatrics*, *74*(3), 336–341.

Cascini, F., Aiello, C., & Di Tanna, G. (2012). Increasing delta-9-tetrahydrocannabinol (Delta-9-Thc) content in herbal cannabis over time: systematic review and meta-analysis. *Current Drug Abuse Reviews*, *5*(1), 32–40.

Conradt, E., Sheinkopf, S. J., Lester, B. M., Tronick, E., Lagasse, L. L., Shankaran, S., Bada, H., Bauer, C. R., Whitaker, T. M. Hammond, J. A., & Maternal Lifestyle Study. (2013). Prenatal substance exposure: neurobiologic organization at 1 month. *Journal of Pediatrics*, *163*(4), 989–94.e1.

Correia, M. A., Oliveria, A. P., Almeida, J. P., Sing, C. K., & Nobrega, J. D. (1995). Drug-addicted mothers. *Acta Medica Portuguesa*, *8*(1), 5–10.

Corse, S. J., & Smith, M. (1998). Reducing substance abuse during pregnancy. Discriminating among levels of response in a prenatal setting. *Journal of Substance Abuse Treatment*, *15*(5), 457–467.

Day, N. L., & Richardson, G. A. (1991). Prenatal marijuana use: epidemiology, methodologic issues, and infant outcome. *Clinics in Perinatology*, *18*(1), 77–91.

Day, N. L., Richardson, G. A., Goldschmidt, L., Robles, N., Taylor, P. M., Stoffer, D. S., Cornelius, M. D., & Geva, D. (1994). Effect of prenatal

marijuana exposure on the cognitive development of offspring at age three. *Neurotoxicology and Teratology*, *16*(2), 169–175.

Day, N. L., Richardson, G. A., Goldschmidt, L., & Cornelius, M. D. (2000). Effects of prenatal tobacco exposure on preschoolers' behavior. *Journal of Developmental and Behavioral Pediatrics*, *21*(3), 180–188.

Day, N. L., Leech, S. L., & Goldschmidt, L. (2011). The effects of prenatal marijuana exposure on delinquent behaviors are mediated by measures of neurocognitive functioning. *Neurotoxicology and Teratology*, *33*(1), 129–136.

Desilver, D. (2013). Nearly half of adults say they've tried marijuana, but not recently. Available from http://www.pewresearch.org/fact-tank/2013/06/03/more-than-a-third-of-adults-say-theyve-tried-pot-but-not-recently/

Ebrahim, S. H., & Gfroerer, J. (2003). Pregnancy-related substance use in the United States during 1996–1998. *Obstetrics and Gynecology*, *101*(2), 374–379.

El Marroun, H., Tiemeier, H., Jaddoe, V. W., Hofman, A., Mackenbach, J. P., Steegers, E. A., Verhulst, F. C., Van Den Brink, W., & Huizink, A. C. (2008). Demographic, emotional and social determinants of cannabis use in early pregnancy: the Generation R study. *Drug and Alcohol Dependence*, *98*(3), 218–226.

El Marroun, H., Tiemeier, H., Steegers, E. A., Jaddoe, V. W., Hofman, A., Verhulst, F. C., Van Den Brink, W., & Huizink, A. C. (2009). Intrauterine cannabis exposure affects fetal growth trajectories: the Generation R Study. *Journal of the American Academy of Child and Adolescent Psychiatry*, *48*(12), 1173–1181.

El Marroun, H., Tiemeier, H., Steegers, E. A., Roos-Hesselink, J. W., Jaddoe, V. W., Hofman, A., Verhulst, F. C., Van Den Brink, W., & Huizink, A. C. (2010). A prospective study on intrauterine cannabis exposure and fetal blood flow. *Early Human Development*, *86*(4), 231–236.

El Marroun, H., Hudziak, J. J., Tiemeier, H., Creemers, H., Steegers, E. A., Jaddoe, V. W., Hofman, A., Verhulst, F. C., Van Den Brink, W., & Huizink, A. C. (2011a). Intrauterine cannabis exposure leads to more aggressive behavior and attention problems in 18-month-old girls. *Drug and Alcohol Dependence*, *118*(2–3), 470–474.

El Marroun, H., Tiemeier, H., Jaddoe, V. W., Hofman, A., Verhulst, F. C., Van Den Brink, W., & Huizink, A. C. (2011b). Agreement between maternal cannabis use during pregnancy according to self-report and urinalysis in a population-based cohort: the Generation R Study. *European Addiction Research*, *17*(1), 37–43.

English, D. R., Hulse, G. K., Milne, E., Holman, C. D., & Bower, C. I. (1997). Maternal cannabis use and birth weight: a meta-analysis. *Addiction*, *92*(11), 1553–1560.

Falcon, M., Pichini, S., Joya, J., Pujadas, M., Sanchez, A., Vall, O., Garcia Algar, O., Luna, A., De La Torre, R., Rotolo, M. C., & Pellegrini, M. (2012). Maternal hair testing for the assessment of fetal exposure to drug of abuse during early pregnancy: Comparison with testing in placental and fetal remains. *Forensic Science International*, *218*(1–3), 92–96.

Fried, P. A. (1995). The Ottawa Prenatal Prospective Study (Opps): methodological issues and findings—it's easy to throw the baby out with the bath water. *Life Sciences*, *56*(23–24), 2159–2168.

Fried, P. A., & O'Connell, C. M. (1987). A comparison of the effects of prenatal exposure to tobacco, alcohol, cannabis and caffeine on birth size and subsequent growth. *Neurotoxicology and Teratology*, *9*(2), 79–85.

Fried, P. A., & Smith, A. M. (2001). A literature review of the consequences of prenatal marihuana exposure. An emerging theme of a deficiency in aspects of executive function. *Neurotoxicology and Teratology*, *23*(1), 1–11.

Fried, P. A., Watkinson, B., & Willan, A. (1984). Marijuana use during pregnancy and decreased length of gestation. *American Journal of Obstetrics and Gynecology*, *150*(1), 23–27.

Fried, P. A., Barnes, M. V., & Drake, E. R. (1985). Soft drug use after pregnancy compared to use before and during pregnancy. *American Journal of Obstetrics and Gynecology*, *151*(6), 787–792.

Fried, P. A., O'Connell, C. M., & Watkinson, B. (1992a). 60- and 72-month follow-up of children prenatally exposed to marijuana, cigarettes, and alcohol: cognitive and language assessment. *Journal of Developmental and Behavioral Pediatrics*, *13*(6), 383–391.

Fried, P. A., Watkinson, B., & Gray, R. (1992b). A follow-up study of attentional behavior in 6-year-old children exposed prenatally to marihuana, cigarettes, and alcohol. *Neurotoxicology and Teratology*, *14*(5), 299–311.

Fried, P. A., Watkinson, B., & Siegel, L. S. (1997). Reading and language in 9- to 12-year olds prenatally exposed to cigarettes and marijuana. *Neurotoxicology and Teratology*, *19*(3), 171–183.

Fried, P. A., Watkinson, B., & Gray, R. (1998). Differential effects on cognitive functioning in 9- to 12-year olds prenatally exposed to cigarettes and marihuana. *Neurotoxicology and Teratology*, *20*(3), 293–306.

Fried, P. A., Watkinson, B., & Gray, R. (1999). Growth from birth to early adolescence in offspring prenatally exposed to cigarettes and marijuana. *Neurotoxicology and Teratology*, *21*(5), 513–525.

Friguls, B., Joya, X., Garcia-Serra, J., Gomez-Culebras, M., Pichini, S., Martinez, S., Vall, O., & Garcia-Algar, O. (2012). Assessment of exposure to drugs of abuse during pregnancy by hair analysis in a Mediterranean island. *Addiction*, *107*(8), 1471–1479.

Goldschmidt, L., Day, N. L., & Richardson, G. A. (2000). Effects of prenatal marijuana exposure on child behavior problems at age 10. *Neurotoxicology and Teratology*, *22*(3), 325–336.

Goldschmidt, L., Richardson, G. A., Willford, J., & Day, N. L. (2008). Prenatal marijuana exposure and intelligence test performance at age 6. *Journal of the American Academy of Child and Adolescent Psychiatry*, *47*(3), 254–263.

Goldschmidt, L., Richardson, G. A., Willford, J. A., Severtson, S. G., & Day, N. L. (2012). School achievement in 14-year-old youths prenatally exposed to marijuana. *Neurotoxicology and Teratology*, *34*(1), 161–167.

Gray, T., & Huestis, M. (2007). Bioanalytical procedures for monitoring in utero drug exposure. *Analytical and Bioanalytical Chemistry*, *388*(7), 1455–1465.

Gray, K. A., Day, N. L., Leech, S., & Richardson, G. A. (2005). Prenatal marijuana exposure: effect on child depressive symptoms at ten years of age. *Neurotoxicology and Teratology*, *27*(3), 439–448.

Gray, T. R., Eiden, R. D., Leonard, K. E., Connors, G. J., Shisler, S., & Huestis, M. A. (2010). Identifying prenatal cannabis exposure and effects of concurrent tobacco exposure on neonatal growth. *Clinical Chemistry*, *56*(9), 1442–1450.

Griffith, D. R., Azuma, S. D., & Chasnoff, I. J. (1994). Three-year outcome of children exposed prenatally to drugs. *Journal of the American Academy of Child and Adolescent Psychiatry*, *33*(1), 20–27.

Hayatbakhsh, M. R., Najman, J. M., Bor, W., O'Callaghan, M. J., & Williams, G. M. (2009). Multiple risk factor model predicting cannabis use and use disorders: a longitudinal study. *The American Journal of Drug and Alcohol Abuse*, *35*(6), 399–407.

Hayatbakhsh, M. R., Kingsbury, A. M., Flenady, V., Gilshenan, K. S., Hutchinson, D. M., & Najman, J. M. (2011). Illicit drug use before and during pregnancy at a tertiary maternity hospital 2000–2006. *Drug and Alcohol Review*, *30*(2), 181–187.

Hayatbakhsh, M. R., Flenady, V. J., Gibbons, K. S., Kingsbury, A. M., Hurrion, E., Mamun, A. A., & Najman, J. M. (2012). Birth outcomes associated with cannabis use before and during pregnancy. *Pediatric Research*, *71*(2), 215–219.

Huizink, A. C. (2014). Prenatal cannabis exposure and infant outcomes: overview of studies. *Progress in Neuro-psychopharmacology & Biological Psychiatry*, *52*, 45–52.

Hurd, Y. L., Wang, X., Anderson, V., Beck, O., Minkoff, H., & Dow-Edwards, D. (2005). Marijuana impairs growth in mid-gestation fetuses. *Neurotoxicology and Teratology*, *27*(2), 221–229.

Jaddoe, V. W., Mackenbach, J. P., Moll, H. A., Steegers, E. A., Tiemeier, H., Verhulst, F. C., Witteman, J. C., & Hofman, A. (2006). The Generation R study: design and cohort profile. *European Journal of Epidemiology*, *21*(6), 475–484.

Johnson, G. (2014). U.S. drug policies fueling global push to legalize marijuana. *The Seattle Times*, February 16. Available from http://seattletimes.com/html/nationworld/2022928337_globalpotxml.html

Leech, S. L., Larkby, C. A., Day, R., & Day, N. L. (2006). Predictors and correlates of high levels of depression and anxiety symptoms among children at age 10. *Journal of the American Academy of Child and Adolescent Psychiatry*, *45*(2), 223–230.

Lozano, J., Garcia-Algar, O., Marchei, E., Vall, O., Monleon, T., Giovannandrea, R. D., & Pichini, S. (2007). Prevalence of gestational exposure to cannabis in a Mediterranean city by meconium analysis. *Acta Paediatrica*, *96*(12), 1734–1737.

Minnes, S., Robin, N. H., Alt, A. A., Kirchner, H. L., Satayathum, S., Salbert, B. A., Ellison, L., & Singer, L. T. (2006). Dysmorphic and anthropometric outcomes in 6-year-old prenatally cocaine-exposed children. *Neurotoxicology and Teratology*, *28*(1), 28–38.

Moore, D. G., Turner, J. D., Parrott, A. C., Goodwin, J. E., Fulton, S. E., Min, M. O., Fox, H. C., Braddick, F. M., Axelsson, E. L., Lynch, S., Ribeiro, H., Frostick, C. J., & Singer, L. T. (2010). During pregnancy, recreational drug-using women stop taking ecstasy (3,4-methylenedioxy-*N*-methylamphetamine) and reduce alcohol consumption, but continue to smoke tobacco and cannabis: initial findings from the Development and Infancy Study. *Journal of Psychopharmacology*, *24*(9), 1403–1410.

Ostrea, E. M., Jr, Ostrea, A. R., & Simpson, P. M. (1997). Mortality within the first 2 years in infants exposed to cocaine, opiate, or cannabinoid during gestation. *Pediatrics*, *100*(1), 79–83.

Porath, A. J., & Fried, P. A. (2005). Effects of prenatal cigarette and marijuana exposure on drug use among offspring. *Neurotoxicology and Teratology*, *27*(2), 267–277.

Richardson, G. A., Day, N. L., & Goldschmidt, L. (1995). Prenatal alcohol, marijuana, and tobacco use: infant mental and motor development. *Neurotoxicology and Teratology*, *17*(4), 479–487.

Saleh Gargari, S., Fallahian, M., Haghighi, L., Hosseinnezhad-Yazdi, M., Dashti, E., & Dolan, K. (2012). Maternal and neonatal complications of substance abuse in Iranian pregnant women. *Acta Medica Iranica*, *50*(6), 411–416.

SAMHSA. (2014). Results from the 2013 National Survey on Drug Use and Health: Summary of National Findings. NSDUH Series H-48, Hhs, Publication No.(SMA)14-4863. Rockville, Md: Substance Abuse and Mental Health Services Administration.

Scragg, R. K., Mitchell, E. A., Ford, R. P., Thompson, J. M., Taylor, B. J., & Stewart, A. W. (2001). Maternal cannabis use in the sudden death syndrome. *Acta Paediatrica*, *90*(1), 57–60.

Sherwood, R. A., Keating, J., Kavvadia, V., Greenough, A., & Peters, T. J. (1999). Substance misuse in early pregnancy and relationship to fetal outcome. *European Journal of Pediatrics*, *158*(6), 488–492.

Sidney, S. (2003). Comparing cannabis with tobacco—again. *British Medical Journal*, *327*(7416), 635–636.

Smith, A. M., Fried, P. A., Hogan, M. J., & Cameron, I. (2004). Effects of prenatal marijuana on response inhibition: an fMRI study of young adults. *Neurotoxicology and Teratology*, *26*(4), 533–542.

Smith, A. M., Fried, P. A., Hogan, M. J., & Cameron, I. (2006). Effects of prenatal marijuana on visuospatial working memory: an fMRI study in young adults. *Neurotoxicology and Teratology*, *28*(2), 286–295.

Sonon, K. E., Richardson, G. A., Cornelius, J. R., Kim, K. H., & Day, N. L. (2015). Prenatal marijuana exposure predicts marijuana use in young adulthood. *Neurotoxicology and Teratology*, *47*, 10.

Steinhausen, H. C., Blattmann, B., & Pfund, F. (2007). Developmental outcome in children with intrauterine exposure to substances. *European Addiction Research*, *13*(2), 94–100.

Varner, M. W., Silver, R. M., Rowland Hogue, C. J., Willinger, M., Parker, C. B., Thorsten, V. R., Goldenberg, R. L., Saade, G. R., Dudley, D. J., Coustan, D., Stoll, B., Bukowski, R., Koch, M. A., Conway, D., Pinar, H., & Reddy, U. M., Eunice Kennedy Shriver National Institute of Child Health and Human Development Stillbirth Collaborative Research Network. (2014). Association between stillbirth and illicit drug use and smoking during pregnancy. *Obstetrics and Gynecology*, *123*(1), 113–125.

Westfall, R. E., Janssen, P. A., Lucas, P., & Capler, R. (2006). Survey of medicinal cannabis use among childbearing women: patterns of its use in pregnancy and retroactive self-assessment of its efficacy against 'morning sickness'. *Complementary Therapies in Clinical Practice*, *12*(1), 27–33.

WHO. (2014). World Health Organization substance abuse facts—cannabis. Available from http://www.who.int/substance_abuse/facts/cannabis/en/

Williams, L. J., Correa, A., & Rasmussen, S. (2004). Maternal lifestyle factors and risk for ventricular septal defects. *Birth defects Research Part A*, *70*(2), 59–64.

Zuardi, A. W. (2006). History of cannabis as a medicine: a review. *Revista Brasileira de Psiquiatria*, *28*(2), 153–157.

CHAPTER

18

Cannabis and Clubbing: Relevance of Cannabis and Polydrug Use in the Clubbing Culture Today

D.A. Herzig, S. Bachmann

Clienia AG, Littenheid, Switzerland

SUMMARY POINTS

- Clubbing culture developed from raving culture in the late 1980s/early1990s.
- Drug use was and still is common during clubbing events.
- Cannabis use is elevated in users of clubbing drugs.
- Concomitant use of cannabis and clubbing drugs may have perceived short-term beneficial effects, but is associated with long-term problems.
- Since most studies to date do not address polydrug use, more research is needed on risk and resilience factors associated with concomitant cannabis and clubbing drug use.
- Certain individual (ie, variations in sensation seeking, impulsivity, and schizotypy), social (ie, variations in general communal attitude toward drug use), and biological factors (ie, variations in dopamine receptor availability) may explain the associations between cannabis and other drug use.
- More research is clearly warranted.

KEY FACTS OF CANNABIS AND CLUBBING DRUG USE

- Cannabis is often used concomitantly with clubbing drugs, especially ecstasy, amphetamines, and cocaine. For instance, in ecstasy users, rates of concomitant cannabis use range from 73% to 100%.
- Simultaneous use of clubbing drugs and cannabis may have acute perceived benefits, such as, masking or alleviating unwanted clubbing drug effects.
- Long-term problems may include higher risk for psychological, physical, and cognitive problems in polydrug users, compared to cannabis only and/or nonusers of illicit drug use.

LIST OF ABBREVIATIONS

GHB	Gamma-hydroxybutyrate
LSD	Lysergic acid diethylamide
MDMA	3,4-Methylenedioxy-*N*-methylamphetamine
NPS	New psychoactive substances

INTRODUCTION

The roots of clubbing culture go back to the first grassroot all-night dance events organized especially in the United Kingdom and United States, in the late 1980s/early 1990s, as a response of the generation X (born circa between 1960 and 1980) to economic alienation and conservatism common in mainstream culture (Anderson, 2009; Measham & Moore, 2009). As an opposition to the conservative mainstream culture and values of the time, drug use was common at rave parties, and early club studies identified higher levels of self-reported lifetime prevalence and current usage of illicit drugs among young attendees of electronic dance

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00020-X
Copyright © 2017 Elsevier Inc. All rights reserved.

events, than in individuals of similar age in the general population (Newcombe, 1992; Release, 1997).

These unlicensed dance events formed a threat to bureaucratic authority and conservative values of mainstream society, and political instruments (such as the Criminal Justice and Public Order Act 1994 in the United Kingdom, or the "War on Drugs" in the United States) were introduced to control noise, drug use, and handling of large amounts of people associated with these dance events revolving around electronic music (Hill, 2002; Measham, 2004). Consequently, raves were mostly transformed from unlicensed and outdoor dance events to licensed and indoor commercial venues (Measham & Moore, 2009). The definitions and terminologies to describe these events nowadays may differ (eg, electronic dance events, raves, night-time economy, clubbing, etc.), and the generalizability of drug use patterns amongst heterogeneous subcultures derived from the original rave culture has been questioned (Measham & Moore, 2009). Nevertheless, for ease of overview, we will refer to any all-night electronic dance event as "clubbing," and drugs used in the context of any of these events as "clubbing drugs" (Bowden-Jones, 2013).

Commonly described clubbing drugs include methamphetamine, cocaine, ketamine, ecstasy, GHB (gamma-hydroxybutyrate), and LSD (lysergic acid diethylamide) (Chinet, Stéphan, Zobel, & Halfon, 2007; Freese, Miotto, & Reback, 2002). Ecstasy/MDMA (3,4-methylenedioxy-*N*-methylamphetamine) is commonly used at dance clubs, techno parties, and all night raves (Parrott, 2006), and is therefore the most extensively studied drug in this context. Additionally, use of new psychoactive substances is common during clubbing events (Bowden-Jones, 2013). Cannabis is not typically listed as a clubbing drug (Moore & Miles, 2004). However, a high proportion of people using typical clubbing drugs is also likely to use cannabis, as will be outlined later. To note, cannabis may not be used during the clubbing event, but rather before or after (Moore & Miles, 2004). Some authors have also claimed that drug use during a clubbing event may be less harmful than consumption outside of these events (Forsyth, 1996), which includes problems associated with concomitant cannabis and clubbing drug use.

For these reasons, the epidemiology of, problems with, and reasons for concomitant cannabis and clubbing drug use will be elaborated in this chapter. Given the history and importance of clubbing culture, particularly in the United Kingdom, the literature cited in this chapter will be largely based on research conducted in the United Kingdom, and Europe more widely.

DRUG USE IN THE GENERAL POPULATION

Before elaborating on the rates of concomitant cannabis and other illicit drug use for comparative purposes it is useful to first establish the baseline rates (and trends, where available) for drug use in the general population.

Overall, European rates of cannabis use in the previous year seems to be relatively stable, over the past decade (Fig. 18.1), with 11.93% of young European adults (ages 15–34 years) having used cannabis in 2000, and 11.52% in 2011 (European Monitoring Centre for Drugs and Drug Addiction, 2013). In 2000 1.6% of young European adults have used ecstasy, compared to 1.68% in 2011 (Fig. 18.1). Amphetamines were used by 1.63% of young European adults in 2000, and 1.06% in 2011 (European Monitoring Centre for Drugs and Drug Addiction, 2013). Cocaine use has slightly increased in Europe (Fig. 18.1), with 1.4% of young European adults having used cocaine in 2000, and 2.4% in 2011 (European Monitoring Centre for Drugs and Drug Addiction, 2013). In 15–34 year olds, hallucinogen use in the previous year ranged between

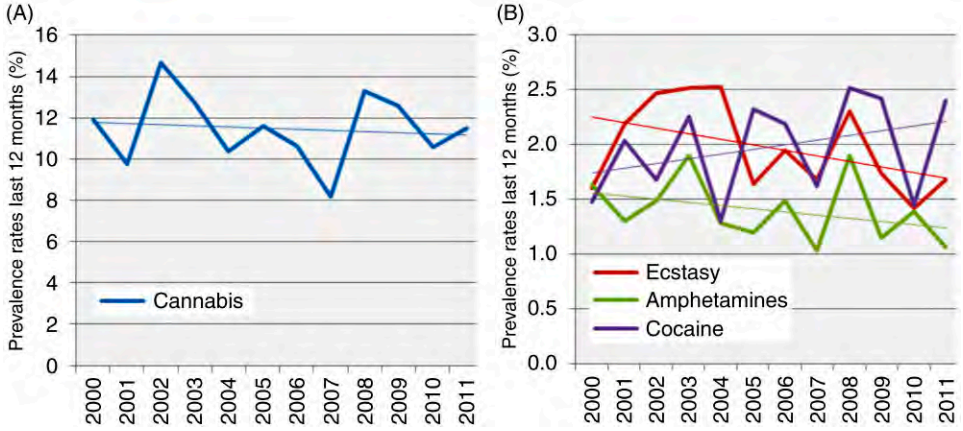


FIGURE 18.1 Prevalence rates of past 12-month use in European young adults and adolescents for (A) cannabis use, and (B) ecstasy, amphetamine, and cocaine use between 2000 and 2011. Only averages with three or more data points per year were included, based on survey data in a total of 24 European countries. The rates of cannabis and ecstasy use have remained relatively stable over the past decade, whereas amphetamine use has slightly increased, and cocaine use has increased. *Source: European Monitoring Centre for Drugs and Drug Addiction (2013).*

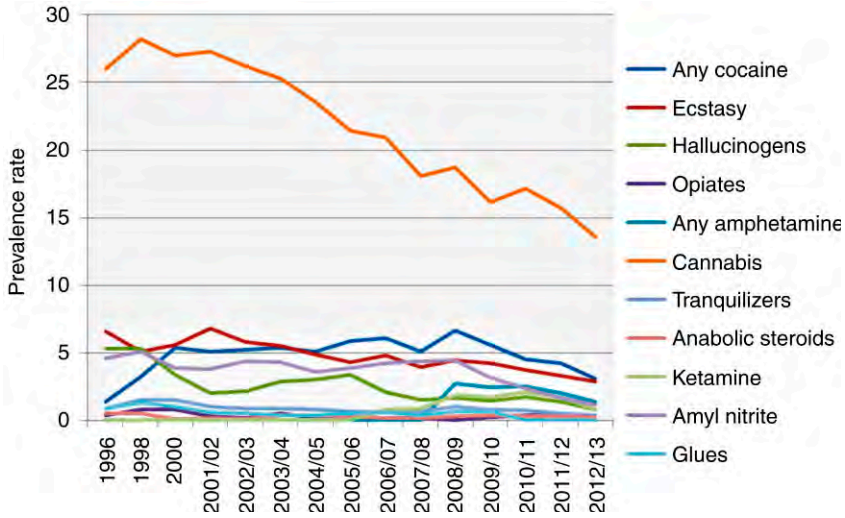


FIGURE 18.2 **Comparison to other drug trends in England and Wales in adolescents and young adults (ages 16–24).** In adolescents and young adults in England and Wales, cannabis, amphetamine, and ecstasy use have decreased between 1996 and 2013, whereas cocaine use has slightly increased. *Source: British Crime Survey (2012).*

0.2% and 5.9% in young European adults, whereas GHB use in the previous month was about 4.6% in specific surveys with preselected (and, therefore, likely more frequently using) samples (European Monitoring Centre for Drugs and Drug Addiction, 2011). The prevalence rate for opiate use (heroin) in the aforementioned sample ranged between 0.0% and 1.6% in 2011, depending on the country, with an average of 0.7% (UNODC, 2011). So far, data do not address the prevalence rates of ketamine use across young European adults yet.

In the United Kingdom, although cannabis use has steeply decreased from 26% in 1996 to 13.5% in 2012/2013 (Fig. 18.2), it is still the most commonly used illicit drug amongst young adults in the United Kingdom (British Crime Survey, 2012). Prevalence rates for amphetamine use in the previous year decreased from 2.7 in 2008/2009 to 1.4% in 2012/2013. Ecstasy was used in the previous year by 6.6% in 1996, and 2.9% in 2012/2013. Cocaine use increased from 1.4% in 1996 to 2.7% in 2012/2013. Opiate (heroin, methadone) use prevalence decreased from 0.4% in 1996 to 0.0% in 2012/2013 in young UK adults. Prevalence rates for ketamine use in the previous year in young UK adults remained stable (Fig. 18.2), at 1.8% in 2008 and 2012/2013 (British Crime Survey, 2012). No prevalence rates for GHB exist in the United Kingdom yet (British Crime Survey, 2012).

The number of identified new psychoactive substances (NPS) in the European Union rose from 14 in 2005 to 347 by the end of 2013 (UNODC, 2014). The amount of NPS has increased so much during this time that the amount of uncontrolled legal psychotropic substances (N = 347) now exceeds the amount of controlled substances (N = 234) for the first time (UNODC, 2014). On average, 5% of the European respondents admitted to having "ever" used an NPS but, in some countries, like the United Kingdom (8%), Latvia (9%), Poland (9%), and Ireland (16%), the prevalence of NPS use was considerably higher than in other countries, such as, Italy (1%), Finland (1%), Hungary (2%), Slovakia (3%), the Netherlands (3%), and the Czech Republic (4%) (van Amsterdam, Nutt, & van den Brink, 2013). The majority of currently identified NPS are synthetic cannabinoids (31%; see Table 18.1) (UNODC, 2014). Synthetic cannabinoids were used within the past 12 months by 6.5% of young

TABLE 18.1 Types and Proportion (in %) of Identified NPS Worldwide Until Dec. 2013[a]

Type	Proportion of identified NPS (%)
Synthetic cannabinoids	31
Phenethylamines	21
Synthetic cathinones	15
Others	11
Tryptamines	8
Plant-based substances	6
Piperazines	4
Ketamine and phencyclidine-type substances	3
Aminoindanes	1

NPS, new psychoactive substances.
[a]*The category with the highest rate of new psychoactive substances until Dec. 2013 was synthetic cannabinoids (ie, JWH-018 and JWH-073. JWH stands for John W. Huffman, one of the inventors of these cannabinoids), followed by phenethylamines and synthetic cathinones (ie, mephedrone, methylenedioxypyrovalerone, and methylone). Other substances included tryptamines, plant-based substances (kratom, khat, and salvia divinorum, and piperazines), piperazines [ie, m-chlorophenylpiperazine (mCPP), N-benzylpiperazine (BZP) and 1-(3-trifluoromethylphenyl) piperazine (TFMPP)], ketamine, and aminoindanes.*
UN Office on Drugs and Crime (UNODC, 2014).

adult respondents (age 19–28 years) to a worldwide online survey in 2011 (Winstock & Barratt, 2013).

While these statistics provide a baseline for drug use of a variety of clubbing drugs and cannabis, they do not indicate the rates of simultaneous or concomitant use.

RATES OF MULTIPLE SUBSTANCE USE, INCLUDING CANNABIS

Generally, this section elaborates on the rates of simultaneous or concomitant psychoactive substance use (Byqvist, 1999) in the general population, with a particular focus on cannabis and clubbing drug use (including new psychoactive substances) in young adults.

In 2009 the most frequently used additional illicit drug in the previous 12 months amongst 7178 European young adult cannabis users was cocaine, followed by ecstasy, and amphetamines (European Monitoring Centre for Drugs and Drug Addiction, 2009, Fig. 18.3).

In a similar survey of 1501 Spanish adolescents (12–17 years old) in 2007, 13.9% reported polydrug use. The majority of these polydrug users (9.4% of the total sample) indicated cannabis use together with tobacco and/or alcohol, in the past 6 months, and 0.5% used cannabis together with tobacco and/or alcohol, and cocaine, in the past 6 months (Font-Mayolas et al., 2013). The remaining adolescents either only used alcohol and tobacco (3.9% of the total sample), or consumed tobacco and cocaine, but not cannabis (0.1% of the total sample).

In 2000 a British survey of 8538 adults revealed that 32.92% (3.46% of the total sample; see Table 18.2) of those individuals using illicit drugs in the past year were polydrug users (Smith, Farrell, Bunting, Houston, & Shevlin, 2011). Interestingly, the majority of cannabis users reporting additional drug use were typically using clubbing drugs, such as, ecstasy, amphetamines and/or cocaine (Table 18.2).

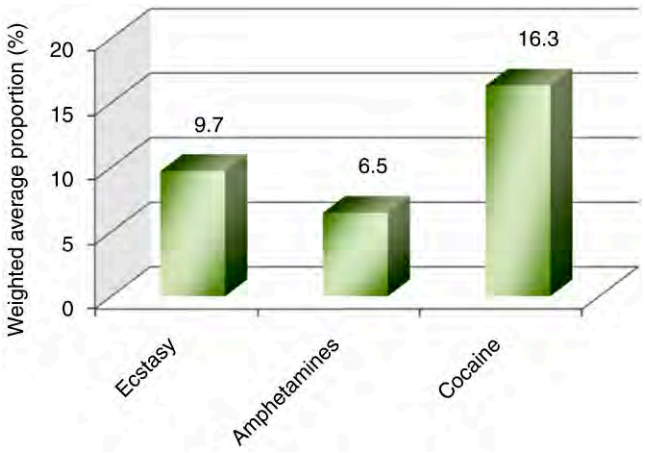


FIGURE 18.3 Average prevalence rate (based on weighted averages) of concomitant last year substance use in 7178 cannabis using European adolescents and young adults (ages 15–34) in 2009. The most popular drug used concomitantly with cannabis among European adolescents and young adults was cocaine, followed by ecstasy and then amphetamines. *Source: European Monitoring Centre for Drugs and Drug Addiction (2009).*

Rates of Cannabis Use in Clubbing Drug Users

Generally, the rates of cannabis use amongst MDMA users is vastly elevated, compared to the

TABLE 18.2 Patterns of Illicit Drug Use[a] in the Past Year[b]

Drug(s) used in the past year	#	% Total (*N* = 8538)	% CU (*N* = 683)
Ca[c]	536	6.28	78.5
Ca + E[d]	31	0.36	4.5
Ca + A[e]	29	0.34	4.25
Ca + Co[f]	29	0.34	4.25
Ca + Co + E	29	0.34	4.25
Ca + A + E	15	0.18	2.25
Ca + A + Co + E	14	0.16	2
A	23	0.27	N/A
T[g]	21	0.25	N/A

[a] *Based on weighted estimates.*
[b] *Most cannabis users only consumed cannabis in the past year. A minority used cannabis and ecstasy, followed by a combination of cannabis and amphetamines, cannabis and cocaine, or a combination of all three substances. Only very few people consumed all four substances, or only used amphetamines or tranquilizers.*
[c] *Cannabis.*
[d] *Ecstasy.*
[e] *Amphetamines.*
[f] *Cocaine.*
[g] *Tranquilizers.*
Smith et al. (2011).

TABLE 18.3 Drug Use Prevalence Rates (%) Among 980 Recent Users of Synthetic Cannabis[a]

Drug types	Last year (%)	Last month (%)
Alcohol	97.8	90.5
Cannabis (any)	95	88.4
Tobacco (any)	84.2	75.2
Energy drinks	76.6	61.4
Ecstasy (any)	50.7	26.2
Magic mushrooms	36.9	12.4
Cocaine	34	14.8
LSD	32.4	12.5
Benzodiazepines	31	18.5
Amphetamine (any)	28.2	14.8
Nitrous oxide	23.4	9.8
Ketamine	17.9	5.7
Caffeine tablets	16.5	8
Mephedrone	13.3	3.7
Heroin	4.8	2.7

LSD, lysergic acid diethylamide.
[a]*In recent users of synthetic cannabis, more than half used alcohol, plant-based cannabis, tobacco, energy drinks and/or ecstasy in the past year, as well. Approximately one-third had also used magic mushrooms, cocaine, LSD, benzodiazepines, and/or amphetamines. A minority had also consumed nitrous oxide, ketamine, caffeine tablets, mephedrone and/or heroin. Winstock and Barratt (2013).*

general population (see Butler & Montgomery, 2004; Sala & Braida, 2005 for overview), with concomitant cannabis and MDMA use ranging from 73% to 100%, depending on the recruited samples and referred time-frame of use (see Sala & Braida, 2005 for overview).

Additionally, another study in 76 GHB users revealed that all (100%) had a history of cannabis use, 78% used cannabis in the past 6 months, and 26% used cannabis in conjunction with GHB (Degenhardt, Darke, & Dillon, 2002), compared to 58% concomitantly using ecstasy, 37% alcohol, 36% amphetamines, and 21% ketamine. In another sample of 189 GHB users, the most frequently reported used substances were alcohol (96.8%) and cannabis (90.5%) (Sumnall, Woolfall, Edwards, Cole, & Beynon, 2008), with 68% combining GHB with other drugs simultaneously, including ecstasy (32.4%), alcohol (17.3%), cannabis (15.6%), and amphetamines (10.4%). Additionally, of 75 inpatients treated for GHB dependence, nearly half used cannabis (45%) in the past month (Brunt, Koeter, Hertoghs, van Noorden, & van den Brink, 2013), whereas the majority used alcohol (52%).

Rates of Cannabis Use in Users of New Psychoactive Substances

Regarding new psychoactive substances, only few studies address the issue of polydrug use. Only one group investigated concomitant drug use in synthetic cannabinoids consumers. As can be seen in Table 18.3, the majority of users of synthetic cannabinoids also used alcohol, tobacco, and ecstasy in the previous year.

Given the literature, cannabis and more typical club drugs should be investigated together, as they are likely to be used concomitantly (Moore & Miles, 2004).

PROBLEMS ASSOCIATED WITH POLYDRUG USE

Most research focus on the problems associated with use of one single drug. This section will, on the other hand, cover research addressing the influence of multiple substance use (including cannabis) on a variety of issues. Again, the combination of cannabis and ecstasy is the most extensively studied, and will be reviewed predominantly.

Cumulative multiple drug use, including concomitant cannabis and clubbing drug use, is associated with greater likelihood of addiction, poorer physical health, social and mental health problems (European Monitoring Centre for Drugs and Drug Addiction, 2011). For instance, cannabis has been shown to relate to an increased risk of mental health problems in regular ecstasy users, particularly depression and anxiety (Scott, Roxburgh, Bruno, Matthews, & Burns, 2012). Psychotic-like symptoms are also more severe in polydrug users, compared

to cannabis only users, and nonusers of illicit drugs (Van Dam, Earleywine, & DiGiacomo, 2008), and the risk of an earlier onset of psychosis (Stefanis et al., 2014) and schizophrenia (Power, Dragovic, Jablensky, & Stefanis, 2013) is higher in polydrug (including cannabis) than cannabis only users, and nonusers of illicit drugs. For instance, although cannabis may lead to earlier onset of psychosis, compared to nonusers, concomitantly using cannabis and amphetamines may lead to an even earlier age of onset of psychosis (Power et al., 2014). Moreover, early cannabis use is linked with the severity of cocaine-induced psychosis (Trape, Charles-Nicolas, Jehel, & Lacoste, 2014). Cannabis, in conjunction with MDMA, may also cause more severe cognitive problems than either substance alone (see Gouzoulis-Mayfrank & Daumann, 2006 for overview).

Cannabis can also potentiate the effects of MDMA on heart rate and temperature in humans (see Mohamed, Hamida, Cassel, de Vasconcelos, & Jones, 2011 for overview). In the majority of clubbers cousing stimulants, alcohol and cannabis lead to both heart rate and blood pressure increases to levels above those related to either cannabis or alcohol alone (see Mohamed et al., 2011 for overview). MDMA and cannabis couse can also lead to disadvantageous alterations in immunological functioning, compared to cannabis use only (Mohamed et al., 2011; Sala & Braida, 2005), and concomitant cannabis use can exacerbate MDMA-induced neuroendocrine abnormalities in recreational ecstasy users (see Mohamed et al., 2011 for overview).

Unsurprisingly, compared with non-MDMA using cannabis consumers, MDMA users were significantly more likely to have used other drugs, and to report more adverse health effects due to "polydrug use" (Shimane, Hidaka, Wada, & Funada, 2013).

THE REASONS FOR CANNABIS USE IN THE CLUBBING COMMUNITY

Short-Term Effects

The reasons for concomitant cannabis and other drug use, despite the problems listed previously, may consist in the acute psychological and physiological effects of drug interactions, of which the combination of cannabis and MDMA are the most extensively studied.

First, cannabis may mask or alleviate some of the adverse effects of clubbing drugs (see Mohamed et al., 2011 for overview). Cannabis, in combination with ecstasy, is commonly used during the "coming-down" phase (George, Kinner, Bruno, Degenhardt, & Dunn, 2010), to reverse stimulant-induced insomnia (Solowij, Hall, & Lee, 1992), as well as feelings of agitation and muscular tension which can persist after using stimulants, such as, MDMA (see Mohamed et al., 2011 for overview). Cannabis can also reduce the negative side effects of stimulating drugs, for example, it has been shown to alleviate sleep problems and pain (Walsh et al., 2013), as well as modulate stimulant-induced increases in body temperature, and oxidative stress (see Mohamed et al., 2011 for overview).

Psychological benefits of cannabis and stimulant couse may exist in the alleviation of stimulant-induced anxiety and depression (see Mohamed et al., 2011 for overview; Walsh et al., 2013), and moderate use of cannabis may also alleviate stimulant-induced aggression (see Mohamed et al., 2011 for overview).

Second, cannabis seems to enhance some desirable effects of stimulant drugs. For instance, 47% of ecstasy users reported concomitantly using cannabis, partially to prolong the feeling of "high" induced by the drug (Byqvist, 1999; Solowij et al., 1992). Additionally, 36% of cannabis users consume ecstasy simultaneously to improve its effects (Boys, Marsden, & Strang, 2001).

Gateway Theory

Regarding the reasons for concomitant cannabis and other drug use, it is possible that once an individual tries an illicit drug (eg, cannabis), he/she is more exposed. and hence more likely to use other illicit drugs (Kandel, Yamaguchi, & Klein, 2006), even when active drug-seeking behavior is controlled for (ie, Wilcox, Wagner, & Anthony, 2002). In this "gateway" theory, using one class of drug is a necessary, though not sufficient, facilitator of transition to use of the next class of drug, along the trajectory (Kandel, 2002). While cannabis is nowadays often referred to as a gateway drug, this role had originally been assigned to alcohol and tobacco (see Vanyukov et al., 2012 for overview). In other words, according to this theory, there is a natural progression from legal drugs, such as tobacco and alcohol, to illegal drugs including cannabis.

Few neurobiological studies support the role of cannabis as gateway drug by suggesting that cannabis acts on reward pathways in the brain, and changes the underlying neural connections in a way that predisposes to further drug use. However, evidence that cannabis induces changes predisposing to consumption of other illicit drugs is scarce, and not unanimously supported by animal models (see Rubino & Parolaro, 2008 for review). Some studies reported that, in rats, THC-pretreatment induced opioid receptor changes in reward-sensitive limbic structures, a fact that may explain the subsequently observed enhanced opioid use (see Rubino & Parolaro, 2008 for review). Additionally, activation of the cannabinoid CB1-receptor increased the reinforcing effects of heroin in rats (Solinas et al., 2005), suggesting that cannabis potentiates the hedonistic experience of opioids. On the other hand, amphetamine sensitivity in

THC-pretreated rats was not different to placebo-pretreated rats (see Rubino & Parolaro, 2008 for review). Solely based on neurobiological grounds, this might suggest that opiate use would likely follow cannabis use, if the gateway theory is supported, and prevalence rates for concomitant cannabis and opiate use should be relatively high, compared to other combinations of drugs of abuse. However, most studies suggest a relatively low rate (if any) of cannabis with concomitant opiate use, when compared to combinations of cannabis with stimulants and/or other drugs (see review previously). Furthermore, epidemiological studies in favor of the gateway theory support a trajectory from alcohol, cigarettes, cannabis, amphetamines, ecstasy, to opiates, such as, heroin (Pedersen & Skrondal, 1999).

Additionally, in some samples, illicit drug use is high in noncannabis users (see Degenhardt et al., 2010 for overview), and any relationship between cannabis and other illicit drug use seems to be short-termed, disappearing whilst cousers transition from adolescence to adulthood (Van Gundy & Rebellon, 2010). Therefore, it seems that cannabis does not unequivocally represent a gateway to other illicit drug use.

In recent years, it has also become apparent that even though different drugs use specific mechanisms to exert their effects, they all seem to affect similar reward-sensitive (ie, dopaminergic) neurotransmitter systems (see Degenhardt et al., 2010 for overview). This makes the identification of a specific gateway drug, unlikely which induces changes predisposing to further drug use. Unsurprisingly, epidemiological studies found that reduction of one potential gateway drug does not, per se, lead to a decreased consumption of any other illicit drugs (see Degenhardt et al., 2010 for overview). Furthermore, social environment and general communal attitude toward drug use may play a more important role than neurobiological factors to explain the association between cannabis and enhanced other drug use. For instance, in societies where cannabis use prevalence is relatively low, the association between cannabis use and transition to other illicit drugs is stronger than in societies where cannabis use is relatively frequent (see Degenhardt et al., 2010 for overview).

Common Liability to General Substance Use

Certain personality traits seem to be associated with both enhanced cannabis and polydrug use. For instance, a survey of 1076 northern Italian high school students revealed that cannabis use, illicit polydrug use, and alcohol abuse were each associated with high sensation seeking (Gerra et al., 2004). These findings concur with previous studies that showed a strong link between the trait of sensation seeking, and polydrug use including cannabis (Martins, Storr, Alexandre, & Chilcoat, 2008; Pedersen, Clausen, & Lavik, 1989). Novelty seeking, a concept similar to sensation seeking, has also been associated with drug use (see Bardo, Donohew, & Harrington, 1996 for review), and has been shown to relate to lower baseline availability of the neurotransmitter dopamine (see Bardo et al., 1996 for overview). Dopamine is involved in drug reinforcement and addiction, as well (see Volkow, Fowler, Wang, & Swanson, 2004 for review). Other personality traits include higher impulsivity for cannabis and polydrug users, as compared to nonusers (Martinotti et al., 2009), and schizotypy. Schizotypy, a personality trait representing schizophrenia symptoms in milder form in the general, healthy population (Mason, Claridge, & Jackson, 1995; Meehl, 1962), has been associated with increased vulnerability to psychosis and other mental health issues (van Os, Linscott, Myin-Germeys, Delespaul, & Krabbendam, 2009), as well as higher polydrug and cannabis use (Barkus & Murray, 2010; Herzig, Brooks, & Mohr, 2013).

Interestingly, novelty and sensation seeking, as well as impulsivity and schizotypy, have been linked with the dopamine D4 receptor gene (Grant, Gabriel, Kuepper, Wielpuetz, & Hennig, 2014; Munafò, Yalcin, Willis-Owen, & Flint, 2008 for metaanalysis), which is involved in the transmission of dopamine in the brain.

It is therefore possible that the higher rates of combined clubbing drug and cannabis use reflect a common liability to substance use (Morral, McCaffrey, & Paddock, 2002). However, one should note that, despite these assumed common liabilities, the majority of cannabis users do not consume other illicit drugs (see previous sections). Therefore, other factors are likely to influence the transition from cannabis to clubbing drug use. Moreover, polydrug use is diverse (Byqvist, 1999), and different clusters of polydrug use may differ in terms of outcome (Ives & Ghelani, 2006). Clearly, more research elucidating, for example, personal, social, and biological factors representing a risk of or resilience against illicit polydrug use and associated problems are needed in future.

MINI-DICTIONARY

Clubbing Clubbing typically refers to visiting venues (ie, dance clubs) hosting electronic dance music events.

Clubbing drugs These substances are typically consumed in the context of electronic dance music events.

Cocaine This psychoactive substance is extracted from the leaves of plants called *Erythroxylum coca*. Cocaine is a potent stimulant of the central nervous system, and can lead, for example, to reductions in appetite and sleep, and increases in euphoria, feeling energetic and mentally alert, restlessness, irritability, and anxiety. At higher and frequent doses, it can also cause paranoia and hallucinations, for example.

Depressants These substances decrease central nervous system functioning, resulting in reduction of physical, psychological, or cognitive function. They can also reduce anxiety and tension,

and have muscle relaxant properties. Examples are GHB, benzodiazepines, and opioids (eg, heroin).

Ecstasy 3,4-methylenedioxy-*N*-methylamphetamine (also known as MDMA) has stimulant and mild hallucinogenic properties, and induces feelings of well-being, empathy toward others, as well as decreased anxiety, and enhanced sensory perception, for example. Undesirable effects include anxiety, panic, or confusion.

GHB Gamma-hydroxybutyrate is a neuromodulator that was first synthesized in 1960, as an alternative anesthetic because of its ability to induce sleep and reversible coma. GHB has anxiolytic, sedative, and euphoric effects and, at higher doses, can induce, for example, seizures, confusion, respiratory problems, and memory lapses.

Hallucinogens These substances result in distortion of reality perception, and can induce feelings of depersonalization and derealization, as well as auditory and visual hallucinations. Examples are, LSD, magic mushrooms, and ketamine.

Ketamine This is an anesthetic drug used by veterinarians and medical professionals, but is also used in recreational settings as hallucinogen. It can induce, for instance, feelings of euphoria, relaxation, depersonalization and derealization, hallucinations, as well as disorganization, anxiety, panic, and paranoia.

Methamphetamine Methamphetamine is a potent stimulant of the central nervous system. It can reduce fatigue, appetite, and behavioral inhibition, for example, and increase arousal, euphoria, positive mood, cognitive performance, and anxiety, as well as psychotic episodes.

New psychoactive substances These substances are usually derived from known psychoactive compounds, and mimic their effects. However, they are often not controlled under any legislation. For this reason, many of these substances are legal to consume, despite their potential harmfulness.

Stimulants These substances stimulate the central nervous system, and can enhance physical, psychological, and cognitive function. Examples are, for example, (meth)amphetamines and cocaine.

References

Anderson, T. L. (2009). Understanding the alteration and decline of a music scene: observations from rave culture. *Sociological Forum*, *24*(2), 307–336.

Bardo, M. T., Donohew, R. L., & Harrington, N. G. (1996). Psychobiology of novelty seeking and drug seeking behavior. *Behavioural Brain Research*, *77*(1–2), 23–43.

Barkus, E., & Murray, R. M. (2010). Substance use in adolescence and psychosis: clarifying the relationship. *Annual Review of Clinical Psychology*, *6*(1), 365–389.

Bowden-Jones, O. (2013). 'Legal highs' and other 'club drugs': why the song and dance? *Psychiatrist*, *37*(6), 185–187.

Boys, A., Marsden, J., & Strang, J. (2001). Understanding reasons for drug use amongst young people: a functional perspective. *Health Education Research*, *16*(4), 457–469.

British Crime Survey. (2012). Drug misuse declared: findings from the 2012/13 Crime Survey for England and Wales. Available from https://www.gov.uk/government/publications/tables-for-drug-misuse-findings-from-the-2012-to-2013-csew

Brunt, T. M., Koeter, M. W., Hertoghs, N., van Noorden, M. S., & van den Brink, W. (2013). Sociodemographic and substance use characteristics of gamma hydroxybutyrate (GHB) dependent inpatients and associations with dependence severity. *Drug and Alcohol Dependence*, *131*(3), 316–319.

Butler, G. K. L., & Montgomery, A. M. J. (2004). Impulsivity, risk taking and recreational 'ecstasy' (MDMA) use. *Drug and Alcohol Dependence*, *76*(1), 55–62.

Byqvist, S. (1999). Polydrug misuse patterns in Sweden. Gender differences. *Substance Use & Misuse*, *34*(2), 195–216.

Chinet, L., Stéphan, P., Zobel, F., & Halfon, O. (2007). Party drug use in techno nights: a field survey among French-speaking Swiss attendees. *Pharmacology Biochemistry and Behavior*, *86*(2), 284–289.

Degenhardt, L., Dierker, L., Chiu, W. T., Medina-Mora, M. E., Neumark, Y., Sampson, N., et al. (2010). Evaluating the drug use "gateway" theory using cross-national data: consistency and associations of the order of initiation of drug use among participants in the WHO World Mental Health Surveys. *Drug and Alcohol Dependence*, *108*(1–2), 84–97.

Degenhardt, L., Darke, S., & Dillon, P. (2002). GHB use among Australians: characteristics, use patterns and associated harm. *Drug and Alcohol Dependence*, *67*(1), 89–94.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA, 2009). Polydrug use: patterns and responses. Retrieved on November 24th 2014 from http://www.emcdda.europa.eu/publications/selected-issues/polydrug-use/annex.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Annual Report 2011, The state of the drugs problem in Europe. Retrieved on November 24th 2014 from http://www.emcdda.europa.eu/publications/annual-report/2011

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Statistical Bulletin 2013. Retrieved on November 24th 2014 from http://www.emcdda.europa.eu/stats13#gps:displayCharts

Font-Mayolas, S., Gras, M. E., Cebrián, N., Salamó, A., Planes, M., & Sullman, M. J. M. (2013). Types of polydrug use among Spanish adolescents. *Addictive Behaviors*, *38*(3), 1605–1609.

Forsyth, A. J. M. (1996). Places and patterns of drug use in the Scottish dance scene. *Addiction*, *91*(4), 511–521.

Freese, T. E., Miotto, K., & Reback, C. J. (2002). The effects and consequences of selected club drugs. *Journal of Substance Abuse Treatment*, *23*(2), 151–156.

George, J., Kinner, S. A., Bruno, R., Degenhardt, L., & Dunn, M. (2010). Contextualising psychological distress among regular ecstasy users: the importance of sociodemographic factors and patterns of drug use. *Drug and Alcohol Review*, *29*(3), 243–249.

Gerra, G., Angioni, L., Zaimovic, A., Moi, G., Bussandri, M., Bertacca, S., et al. (2004). Substance use among high-school students: relationships with temperament, personality traits, and parental care perception. *Substance Use & Misuse*, *39*(2), 345–367.

Gouzoulis-Mayfrank, E., & Daumann, J. (2006). The confounding problem of polydrug use in recreational ecstasy/MDMA users: a brief overview. *Journal of Psychopharmacology*, *20*(2), 188–193.

Grant, P., Gabriel, F., Kuepper, Y., Wielpuetz, C., & Hennig, J. (2014). Psychosis-proneness correlates with expression levels of dopaminergic genes. *European Psychiatry*, *29*(5), 304–306.

Herzig, D. A., Brooks, R., & Mohr, C. (2013). Inferring about individual drug and schizotypy effects on cognitive functioning in polydrug using mephedrone users before and after clubbing. *Human Psychopharmacology*, *28*(2), 168–182.

Hill, A. (2002). Acid House and Thatcherism: noise, the mob, and the English countryside. *British Journal of Sociology*, *53*(1), 89–105.

Ives, R., & Ghelani, P. (2006). Polydrug use (the use of drugs in combination): a brief review. *Drugs*, *13*(3), 225–232.

Kandel, D. B. (2002). Examining the gateway hypothesis. Stages and pathways of drug involvement. In D. B. Kandel (Ed.), *Stages and pathways of drug involvement: Examining the gateway hypothesis* (pp. 3–15). New York, NY: Cambridge University Press.

Kandel, D. B., Yamaguchi, K., & Klein, L. C. (2006). Testing the Gateway Hypothesis. *Addiction*, *101*(4), 470–472.

Martinotti, G., Carli, V., Tedeschi, D., Di Giannantonio, M., Roy, A., Janiri, L., et al. (2009). Mono- and polysubstance dependent subjects differ on social factors, childhood trauma, personality, suicidal behaviour, and comorbid Axis I diagnoses. *Addictive Behaviors*, *34*(9), 790–793.

Martins, S. S., Storr, C. L., Alexandre, P. K., & Chilcoat, H. D. (2008). Adolescent ecstasy and other drug use in the National Survey of

Parents and Youth: the role of sensation-seeking, parental monitoring and peer's drug use. *Addictive Behaviors*, *33*(7), 919–933.
Mason, O., Claridge, G., & Jackson, M. (1995). New scales for the assessment of schizotypy. *Personality and Individual Differences*, *18*(1), 7–13.
Measham, F. (2004). Play space: historical and socio-cultural reflections on drugs, licensed leisure locations, commercialisation and control. *International Journal of Drug Policy*, *15*(5–6), 337–345.
Measham, F., & Moore, K. (2009). Repertoires of distinction: exploring patterns of weekend polydrug use within local leisure scenes across the English night time economy. *Criminology and Criminal Justice*, *9*(4), 437–464.
Meehl, P. E. (1962). Schizotaxia, schizotypy, schizophrenia. *American Psychologist*, *17*(12), 827–838.
Mohamed, W. M. Y., Hamida, S. B., Cassel, J. -C., de Vasconcelos, A. P., & Jones, B. C. (2011). MDMA: interactions with other psychoactive drugs. *Pharmacology Biochemistry and Behavior*, *99*(4), 759–774.
Moore, K., & Miles, S. (2004). Young people, dance and the sub-cultural consumption of drugs. *Addiction Research & Theory*, *12*(6), 507–523.
Morral, A. R., McCaffrey, D. F., & Paddock, S. M. (2002). Reassessing the marijuana gateway effect. *Addiction*, *97*(12), 1493–1504.
Munafò, M. R., Yalcin, B., Willis-Owen, S. A., & Flint, J. (2008). Association of the dopamine D4 receptor (DRD4) gene and approach-related personality traits: meta-analysis and new data. *Biological Psychiatry*, *63*(2), 197–206.
Newcombe, R. (1992). A researcher reports from the rave. In M. Ashton (Ed.), *The Ecstasy Papers: A collection of ISDD's publications on the dance drugs phenomenon* (pp. 22–28). London: ISDD.
Parrott, A. C. (2006). MDMA in humans: factors which affect the neuropsychobiological profiles of recreational ecstasy users, the integrative role of bioenergetic stress. *Journal of Psychopharmacology*, *20*(2), 147–163.
Pedersen, W., Clausen, S. E., & Lavik, N. J. (1989). Patterns of drug use and sensation-seeking among adolescents in Norway. *Acta Psychiatrica Scandinavica*, *79*(4), 386–390.
Pedersen, W., & Skrondal, A. (1999). Ecstasy and new patterns of drug use: a normal population study. *Addiction*, *94*(11), 1695–1706.
Power, B. D., Dragovic, M., Jablensky, A., & Stefanis, N. C. (2013). Does accumulating exposure to illicit drugs bring forward the age at onset in schizophrenia? *Australian and New Zealand Journal of Psychiatry*, *47*(1), 51–58.
Power, B. D., Stefanis, N. C., Dragovic, M., Jablensky, A., Castle, D., & Morgan, V. (2014). Age at initiation of amphetamine use and age at onset of psychosis: the Australian Survey of High Impact Psychosis. *Schizophrenia Research*, *152*(1), 300–302.
Release. (1997). *Release drugs and dance survey*. London, UK: Release.
Rubino, T., & Parolaro, D. (2008). Long lasting consequences of cannabis exposure in adolescence. *Molecular and Cellular Endocrinology*, *286*(1–2, Suppl. 1), S108–S113.
Sala, M., & Braida, D. (2005). Endocannabinoids and 3,4-methylenedioxymethamphetamine (MDMA) interaction. *Pharmacology Biochemistry and Behavior*, *81*(2), 407–416.
Scott, L. A., Roxburgh, A., Bruno, R., Matthews, A., & Burns, L. (2012). The impact of comorbid cannabis and methamphetamine use on mental health among regular ecstasy users. *Addictive Behaviors*, *37*(9), 1058–1062.
Shimane, T., Hidaka, Y., Wada, K., & Funada, M. (2013). Ecstasy (3, 4-methylenedioxymethamphetamine) use among Japanese rave population. *Psychiatry and Clinical Neurosciences*, *67*(1), 12–19.
Smith, G. W., Farrell, M., Bunting, B. P., Houston, J. E., & Shevlin, M. (2011). Patterns of polydrug use in Great Britain: findings from a national household population survey. *Drug and Alcohol Dependence*, *113*(2–3), 222–228.
Solinas, M., Panlilio, L. V., Tanda, G., Makriyannis, A., Matthews, S. A., & Goldberg, S. R. (2005). Cannabinoid Agonists but not inhibitors of endogenous cannabinoid transport or metabolism enhance the reinforcing efficacy of heroin in rats. *Neuropsychopharmacology*, *30*(11), 2046–2057.
Solowij, N., Hall, W., & Lee, N. (1992). Recreational MDMA use in Sydney: a profile of 'Ecstasy' users and their experiences with the drug. *British Journal of Addiction*, *87*(8), 1161–1172.
Stefanis, N. C., Dragovic, M., Power, B. D., Jablensky, A., Castle, D., & Morgan, V. A. (2014). The effect of drug use on the age at onset of psychotic disorders in an Australian cohort. *Schizophrenia Research*, *156*(2–3), 211–216.
Sumnall, H. R., Woolfall, K., Edwards, S., Cole, J. C., & Beynon, C. M. (2008). Use, function, and subjective experiences of gamma-hydroxybutyrate (GHB). *Drug and Alcohol Dependence*, *92*(1–3), 286–290.
Trape, S., Charles-Nicolas, A., Jehel, L., & Lacoste, J. (2014). Early cannabis use is associated with severity of cocaine-induced psychosis among cocaine smokers in Martinique, French West Indies. *Journal of Addiction Medicine*, *8*(1), 33–39.
UN Office on Drugs and Crime (UNODC), World Drug Report 2011, retrieved November 24th, 2014, from http://www.unodc.org/wdr/en/nps.html
UN Office on Drugs and Crime (UNODC), World Drug Report 2014. Retrieved November 24th, 2014, from http://www.unodc.org/wdr/en/nps.html
van Amsterdam, J., Nutt, D., & van den Brink, W. (2013). Generic legislation of new psychoactive drugs. *Journal of Psychopharmacology*, *27*(3), 317–324.
Van Dam, N. T., Earleywine, M., & DiGiacomo, G. (2008). Polydrug use, cannabis, and psychosis-like symptoms. *Human Psychopharmacology*, *23*(6), 475–485.
Van Gundy, K., & Rebellon, C. J. (2010). A Life-course perspective on the "Gateway Hypothesis". *Journal of Health and Social Behavior*, *51*(3), 244–259.
van Os, J., Linscott, R. J., Myin-Germeys, I., Delespaul, P., & Krabbendam, L. (2009). A systematic review and meta-analysis of the psychosis continuum: evidence for a psychosis proneness–persistence–impairment model of psychotic disorder. *Psychological Medicine*, *39*(02), 179–195.
Vanyukov, M. M., Tarter, R. E., Kirillova, G. P., Kirisci, L., Reynolds, M. D., Kreek, M. J., et al. (2012). Common liability to addiction and "gateway hypothesis": theoretical, empirical and evolutionary perspective. *Drug and Alcohol Dependence*, *123*(Suppl. 1(0)), S3–S17.
Volkow, N. D., Fowler, J. S., Wang, G. J., & Swanson, J. M. (2004). Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. *Molecular Psychiatry*, *9*(6), 557–569.
Walsh, Z., Callaway, R., Belle-Isle, L., Capler, R., Kay, R., Lucas, P., et al. (2013). Cannabis for therapeutic purposes: patient characteristics, access, and reasons for use. *International Journal of Drug Policy*, *24*(6), 511–516.
Wilcox, H. C., Wagner, F. A., & Anthony, J. C. (2002). Exposure opportunity as a mechanism linking youth marijuana use to hallucinogen use. *Drug and Alcohol Dependence*, *66*(2), 127–135.
Winstock, A. R., & Barratt, M. J. (2013). Synthetic cannabis: a comparison of patterns of use and effect profile with natural cannabis in a large global sample. *Drug and Alcohol Dependence*, *131*(1–2), 106–111.

CHAPTER

19

Cannabis and Sexual Behavior

G. Scimeca*, C. Chisari**, M.R.A. Muscatello*, C. Cedro*, G. Pandolfo*, R. Zoccali*, A. Bruno*

*Department of Biomedical, Dental Sciences and Morpho-functional Imaging, University of Messina, Messina, Italy
**University of York, York, United Kingdom

SUMMARY POINTS

- This chapter focuses on the effects of cannabis and other drugs on sexual behavior.
- Research has shown that cocaine at low doses have positive effects on sexual arousal. These effects include spontaneous erection and ejaculation, as well as multiple orgasms.
- On the other hand, chronic cocaine use is associated with erectile dysfunction and inhibited sexual desire.
- Additional research on opiates has shown that heroin has positive effects at low doses, whereas chronic use leads to reduced sexual desire, contributes to impotence, and interferes with ejaculation and orgasm.
- Methamphetamine users experience enhanced pleasure, and generally engage with many partners.
- However, long-term methamphetamine users experience sexual dysfunctions, such as, disorders of erection, ejaculation, and orgasm, as well as decreased sexual desire.
- Cannabis at low doses is associated with positive sexual effects, including improvement in the quality of the orgasm, and increased sexual pleasure and sexual satisfaction.
- Nevertheless, cannabis at high doses has been associated with erectile dysfunction, sterility, and reduction in testosterone levels.
- Additional research has also demonstrated that cannabis use was associated with slowing of temporal perception and, therefore, increased concentration on the sexual intercourse.
- Finally, it is also entirely plausible that the perceived positive effects of cannabis on sexual functioning are due to expectancy biases.

KEY FACTS OF COCAINE

- Cocaine usage increases dopamine levels, resulting in sexual excitement and pleasure, in animals and humans.
- Short-term cocaine use induces multiple orgasms, as well as spontaneous erection and ejaculation.
- Long-term cocaine use inhibits sexual arousal, as it leads to dopamine deficiency.
- Long-term cocaine use leads to delayed ejaculation, and difficulties in maintaining erection.
- Long-term cocaine use among women leads to difficulties in achieving orgasm, and diminished sexual desire.

KEY FACTS OF OPIATES

- Opiates redirect blood away from the genitals, thus contributing to sexual dysfunction.
- Heroin at low doses triggers sexual arousal, and enhances sexual performance.
- Heroine at high doses reduces sexual desire, contributes to impotence, and interferes with ejaculation and orgasm.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00021-1
Copyright © 2017 Elsevier Inc. All rights reserved.

- Heroin suppresses testosterone production, thus inhibiting sexual desire.

KEY FACTS OF PSYCHOLOGICAL CONSEQUENCES OF DRUG ABUSE

- Drug abusers have difficulties in establishing committed and intimate relationships with their partners.
- Drug abusers are mainly characterized by instrumental sexual behavior, as they show the tendency to view the partner as a sexual object.
- Drug addicts are more prone to seek sex only for pure physical pleasure, and to appreciate masturbation and pornography rather than sexual intercourses with a partner.
- Drug addicts often experience depression, anxiety, and anger.
- Men who abuse drugs are at increased risk of inflicting sexual violence on women.

KEY FACTS OF CANNABIS

- Chronic cannabis smokers have significantly reduced plasma testosterone levels, and exhibit sterility.
- Cannabis users are more likely to get sexually transmitted diseases, such as, gonorrhea and chlamydia.
- At low doses cannabis has disinhibiting effects, and enhances sexual arousal.
- Chronic cannabis use has inhibitory effects on sexual behavior, and leads to erectile dysfunction.
- Cannabis use has anxiolytic effects, resulting in more relaxation during the sexual encounter.
- Cannabis consumption slows temporal perception.

LIST OF ABBREVIATIONS

MA Metamphetamine
THC Phytocannabinoid Δ^9-tetrahydrocannabinol

INTRODUCTION

Among a range of side effects of drug abuse, sexual behavior has been an unexplored area in the clinical care of drug addicts until recently, although highly important. On gross inspection, heavy drug use leads to impotence, painful sex, inability to ejaculate, reduced sexual interest and satisfaction, or premature ejaculation (Johnson, Phelps, & Cottler, 2004). In addition to these sexual disorders, researchers are interested in studying the effects of specific psychoactive drugs on human libido. In particular, an extensively studied drug in relation to sexual behavior that will be the main focus of this chapter is marijuana. Previously thought to be an aphrodisiac, marijuana is now recognized to be a drug that enhances sexual motivation in low doses, but that can lead to reduced libido and difficulties in reaching orgasm, in high doses (Johnson et al., 2004). Moreover, many studies have shown that marijuana consumption is also associated with health-related risky sexual behavior (Li et al., 2011).

Research on drugs, such as, stimulants suggests that these tend to increase libido and sexual satisfaction (Semple, Zians, Strathdee, & Patterson, 2009). Among them, cocaine has been fairly studied, showing mixed findings, as it can lead to both increased and inhibited sexual desire (Warner, 1993). Finally, amphetamine and methamphetamine (MA) tend to increase libido and pleasure by lowering inhibitions in both low and acute doses (Feucht, 1993). However, high and chronic doses reduce sexual motivation and satisfaction (Smith, Wesson, & Apter-Marsh, 1984).

This chapter will explore the various effects of marijuana on sexual behavior. It will describe its effects in high and low doses, and will also look at gender differences. Finally, this chapter will illustrate the effects of other drugs (cocaine, heroin, MA) on sexual behavior, in order to have a more comprehensive understanding of this area.

DRUGS AND SEXUAL BEHAVIOR

Studies looking at the relationships between specific drugs and sexual behavior have shown that cocaine, a stimulant with anesthetic features (Buffum, Moser, & Smith, 1988), increases levels of dopamine, norepinephrine, serotonin, and is often linked to sexual desire (Rawson, Washton, Domier, & Reiber, 2002) (Table 19.1). Increased dopamine levels have been found to enhance sexual excitement in animals, and are associated with feelings of pleasure (Leavitt, 1995). Research shows that short-term cocaine use causes spontaneous erection and ejaculation, as well as multiple orgasms (Buffum, 1982). However, despite the positive sexual effects associated with short-term cocaine use, many studies claim that long-term cocaine consumption results in several sexual dysfunctions. In fact, long-term cocaine use causes dopamine deficiency, resulting in inhibited sexual arousal (Cocores, Miller, Pottash, & Gold, 1988). Additionally, problems like delayed ejaculation and difficulties in maintaining an erection are common among chronic cocaine addicts. Gold (1997) argues that impotency and other sexual difficulties may continue after cocaine consumption has stopped. Additional studies have also shown that, among women, chronic cocaine use leads to difficulties in achieving orgasm and diminished sexual desire (Henderson, Boyd, & Whitmarsh, 1995).

TABLE 19.1 Type of Drug and Sexual Disorders

Type of drug	Sexual effects
Short-term cocaine use	Spontaneous erection, spontaneous ejaculation, multiple orgasms
Long-term cocaine use	Inhibited sexual arousal, delayed ejaculation, difficulties in maintaining erection, impotency, difficulties in achieving orgasm, diminished sexual desire
Heroin at low doses	Enhanced sexual arousal, enhanced sexual performance
Heroin at high doses and chronic use	Diminished sexual desire, impotence, difficulties in achieving orgasm
Amphetamine and methamphetamine at low and acute doses	Increased libido and pleasure, unprotected sex, delayed orgasm
Amphetamine and methamphetamine at high and chronic doses	Reduced sexual motivation and satisfaction, problems with erection, ejaculation, diminished sexual desire

There are different types of drugs, and these have different effects on the sexual disorders.

Studies on opiates (eg, heroin, morphine, methadone) have demonstrated that their effects on sexual function results from a complex mix of psychological, pharmacological, and sociocultural factors. Opiates are central nervous system depressants that relieve pain, acting on various neurotransmitters in the brain (Pinger, Payne, Hahn, & Hahn, 1998). Opiates also redirect blood away from the genitals, a factor that contributes to sexual dysfunction (Gay & Sheppard, 1973). Among opiates, heroin is a drug whose effect on sexual behavior has been fairly researched. Researchers suggest that small amounts of heroin enhance sexual arousal and sexual performance (Jaffe, Knapp, & Ciraulo, 1997). However, high doses and chronic use diminish sexual desire, interfere with ejaculation, and lead to impotence in men. Further, heroin also suppresses testosterone production (Buffum et al., 1988), thus inhibiting sexual desire.

Users of MA, a stimulant associated with sexual activity, reported to have numerous several sexual partners, and to engage in unprotected sex more often than other drug users (Molitor et al., 1999). Buffum (1982) also claims that MA reportedly increases sexual desire, and delays orgasm. Despite these positive effects, long-term MA users experience problems with erection, ejaculation, orgasms, as well as a decrease in sexual excitement (Buffum et al., 1988).

In addition to the physical consequences of heavy drug use, drug addicts also tend to have problems with their partners, which, in turns, affects their sexual lives (Reiss & Leik, 1989). This topic has been studied by Bruno et al. (2012), who evaluated sexual attitudes in a sample of polydrug addicted subjects (opiates, cocaine, amphetamine, inhalants, marijuana/sedatives, or hallucinogens abusers) compared with 90 nonsubstance abusing subjects. Their results showed that drug users were particularly inclined to search for sexual intercourse, and more open to different kinds of sexual experiences. However, they showed difficulties in forming emotionally close relationships with their partners, and tended to consider them as sexual objects, giving a fundamental importance to their sexual expertise. This study also showed that drug addicts did not feel the need to give love, and sought sex only for pure physical pleasure. In fact, they preferred a solitary sexual pleasure like pornography, rather than having sex with a partner (Bruno et al., 2012) (Table 19.2). Carroll, McGinley, and Mack (2001), who also studied the emotional difficulties experienced by drug users, found that maladaptive sexual behavior (eg, feeling guilty or overthinking about sex) was common in most of the examined subjects.

Additional research also reports that drug addicts often experience depression, anxiety, and anger (Liang, Chikritzhs, & Lenton, 2011), factors that dramatically affect their sexual life (Muscatello et al., 2010). Addiction has also been shown to affect self-concept (Carroll, 1982), and the ability to form intimate relationships with other people (Amaro & Hardy-Fanta, 1995).

Another documented consequence of substance abuse is also intimate partner violence (Table 19.3). Several studies have found that men who abuse drugs are at increased risk of inflicting sexual violence on women (Abbot, Johnson, Koziol-McLain, & Lowenstein, 1995). Drug abuse in females has also been associated with events of either physical or sexual violence, especially when the partners are also drug addicts (El-Bassel, Gilbert, & Rajah, 2000).

CANNABIS AND SEXUAL BEHAVIOR

Throughout history, cannabis consumption has been associated with positive effects on sexual behavior, a belief which is still present today (Abel, 1981). This relationship has been extensively studied in the last years, showing that phytocannabinoid Δ^9-tetrahydrocannabinol (THC) is the psychoactive component responsible for changes of sexual functioning in drug addicts.

The first suggestion that cannabinoids might affect sexual functioning was reported in a study on rats exposed to smoke containing radiolabeled THC

TABLE 19.2 Effect of Drugs on Sexual Behavior

Sexual behavior	Sexual effects
Sexual excitement	Enhanced easiness and degree of arousal in response to sexual stimuli
Sexual satisfaction	Reduced general satisfaction that a person derives from his/her sex life
Sexual shyness	Discomfort felt in the presence of the opposite sex
Impersonal sex	Tendency to consider the partner as a sexual object, neglecting his/her personality and the importance of establishing an emotional close relationship with him/her
Sexual nervousness	Inability to achieve sufficient sexual gratification due to disturbing thoughts, emotions, and actions coming from sex life
Pornography	Appreciation of explicit erotic images, both of verbal and graphic nature
Sexual permissiveness	A very tolerant attitude toward sexuality and its rules
Sexual disgust	Disgust of some sexual practices, even when loving the usual sexual partner
Physical sex	Giving fundamental importance to the partner's sexual expertise, ignoring other personality traits
Aggressive sex	Hostility during the sexual act

Drugs have different effects on various aspects of sexual behavior and attitudes.

TABLE 19.3 Psychological and Relational Problems Experienced by Substance Users

Psychological effects present in drug addicts	
Difficulties in establishing committed and deep relationships with their partners	Depression, anxiety, anger
Intimate partner violence	Tendency to feel guilty, worried and to overthink about sex
Self-concept instability	Tendency to neglect the importance of giving love to the partner
Tendency to neglect the partner's feelings and personality	

Drug addicts tend to have different kinds of problems with their partners that, in turn, affects their sexual lives.

(Ho et al., 1970). Tissue analysis showed a very high concentration of radiolabeled THC in the testicles, suggesting that cannabinoids play a role in testicular processes. Following this evidence, a series of intense investigations took place in the early 1970s. For instance, Kolodny, Masters, Kolodner, and Toro (1974) demonstrated that plasma testosterone levels were significantly reduced in chronic cannabis addicts (Table 19.4). Furthermore, this reduction was dose-dependent. In fact, when cannabis users were subdivided by the intensity of their usage, heavy users (more than 10 cannabis joints per week) showed a greater reduction in testosterone levels than moderate users (5–9 joints per week). In the same study, further examinations also evidenced that 35% of the sample exhibited sterility, as their sperm counts were less than 30 million per ml. However, this reduction was only present in heavy users.

Additional studies conducted among convicted adolescents (Kingree, Braithwaite, & Woodring, 2000) and in samples of homosexual men (Clutterbuck, Gorman, McMillan, Lewis, & Macintyre, 2001) showed that cannabis use was also associated with less condom use (Table 19.5). However, among young heterosexual adults (Brodbeck, Matter, & Moggi, 2006), and adults in a substance use jail diversion program (Leigh, 2002), cannabis use was not associated with condom use. Beyond condom use, a significant amount of research has been conducted to assess whether cannabis users are more likely to get sexually transmitted diseases. Evidence has shown that cannabis smokers were more likely to get sexually transmitted diseases, and adolescents who regularly used cannabis were also more likely to test positive for gonorrhea and chlamydia (Liau, Diclemente, & Wingood, 2002). Finally, it is important to stress that sexual risk behavior among cannabis users may be due to more stable personality traits that stimulate both drug use and sexual risk (Brodbeck et al., 2006).

Despite the negative effects of cannabis on sexual functioning, additional studies showed that low-dose cannabis has enhancing effects on sexual arousal. For instance, a study by Sumnall, Beynon, Conchie, Riley, and Cole (2007) revealed that over 30% of a sample of 270 adults in the United Kingdom reported using cannabis in order to improve their sexual performance.

Self-report surveys on male samples also support the notion that cannabis has a positive effect on sexual experience. Kolodny et al. (1979) interviewed 800 male subjects aged between 18 and 30; in their sample, 83%

TABLE 19.4 Main Studies Addressing Cannabis and Sexual Behavior

Authors	Year of publication	Study and result	Participants
Kolodny et al.	1974	Reduced levels of testosterone in chronic cannabis smokers	20 males
Koff	1974	In females, self-reported increase of sexual desire after smoking one marijuana joint (71%), or after smoking two or more joints (50%)	128 females and 123 males
Halikas et al.	1982	Self-reported increased duration of sexual intercourse (39%), orgasm improvement (68%), increased sexual pleasure and satisfaction (75%)	61 males and 39 females
Liau et al.	2002	Higher probability of sexually transmitted diseases (gonorrhoea, Chlamydia) in cannabis users	522 adolescent males and females
Smith et al.	2010	Recurrent cannabis use is associated with an increased number of sexual partners for both men and women, and inability to orgasm as desired	8650 people

There have been very few studies that have investigated effects of cannabis on sexual behavior.

TABLE 19.5 Positive and Negative Effects of Cannabis on Sexual Behavior

Positive effects (low doses)	Negative effects (high doses)
Enhanced sexual desire	Reduction in testosterone and sperm
Enhanced sexual pleasure and satisfaction	Sterility
Enhanced erection maintenance and ejaculatory control	Less condom use, getting sexually transmitted diseases
	Diminished sexual desire, impotence, difficulties in achieving orgasms
	Erectile dysfunction

Cannabis is associated with both positive and negative effects on sexual behavior depending on dose (low vs. high).

reported that cannabis enhanced sexual pleasure. However, it seemed that this effect was not due to changes in erection maintenance or ejaculatory control; rather, cannabis was found helpful in easing a general relaxation that allowed more attention and focus on sexual pleasure, thus making sex more enjoyable. Another study on male cannabis addicted (Halikas, Weiler, & Morse, 1982) showed that 39% of the sample reported that cannabis use prolonged the duration of sexual intercourse, 68% reported that it improved the quality of their orgasm, and 75% reported that it increased sexual pleasure and sexual satisfaction. Finally, considering the effects of drug dose on sexual behavior, Koff (1974) found that 71% of female cannabis users reported an increase in sexual desire after smoking no more than one marijuana joint. However, this number decreased to 50% for females who smoked two or more joints. As a result, Koff concluded that as the marijuana dose goes beyond one marijuana joint, the positive effects on sex decreased. In the same study, a similar pattern was also found for sexual pleasure. In fact, women reported increased sexual pleasure after smoking one marijuana joint, but decreased sexual desire with exceeding doses. Overall, these results suggest that in low-dose, cannabis enhances sexual pleasure, whereas higher doses cause a reduction in sexual pleasure. Chopra and Jandu (1976) conducted a similar study, and reported comparable results in a large Indian sample of chronic male users.

Additional research has also shown that chronic marijuana consumption leads to erectile dysfunction. In fact, evidence of erectile dysfunction caused by chronic and acute cannabis use have been reported in North African, North American, and Indian samples (Scher, 1970). Further, many have argued that cannabis facilitates sexual arousal by enhancing touch awareness, in lower doses (Halikas et al., 1982). However, this effect seems questionable because in nonsexual contexts marijuana has either no effect, or actually lowers touch sensitivity and tactile perception (Reese, 1977).

Studies exclusively conducted on women failed to show negative effect of cannabis on sexual behavior. For instance, a study by Smith et al. (2010) examined the presence of sexual difficulties in a sample of 4350 women cannabis smokers. The sexual difficulties were considered only if they lasted for more than 1 month, and included low sexual desire, orgasmic dysfunction, lack of vaginal lubrication, sexual pain, and anxiety. The results of this study did not indicate a correlation between sexual difficulties and frequency of cannabis use.

TABLE 19.6 Proposed Explanations for Positive Effects of Cannabis at Low Doses

Explanations	Sexual experience
Altered sensation and/or perception	Increased tactile sensitivity
Anxiolytic effects	Enhanced relaxation during sexual acts
Slowing of temporal perception	Sexual activities are perceived to last longer than they do
Increased awareness	Mindfully focusing on the body and its sensations during sexual intercourse
Disinhibited behavior	Lack of restrictions exhibited before and during sexual act
Placebo effects	Belief that cannabis is an aphrodisiac substance
Correlation with other psychoactive substances	Effects of other drugs taken in conjunction with cannabis during sexual intercourse

There are various kinds of explanations for the effects of cannabis on sexual behavior at low doses, ranging from psychological, to chemical, or neurological mechanisms.

Several researches have tried to assess why low-dose cannabis has positive effects on sexual behavior (Table 19.6). It has been suggested that cannabis may alter perception in a way that improves sexual experiences (Abel, 1981; Adams & Martin 1996). Halikas et al. (1982) proposed that cannabis positively impacts sexual functioning by enhancing tactile sensitivity. In support of this, Dawley, Winstead, Baxter, and Gay (1979) studied this aspect in a sample of experienced marijuana addicts. Their results show that 48% of the sample reported that marijuana had a beneficial effect on sensations during sex. Conversely, a study by Reese (1977) found that marijuana resulted in either negative or no effects on tactile sensitivity, in nonsexual situations. This leads to doubts as to whether marijuana use increases tactile sensitivity in sexual contexts.

Alternatively, sexual function may be indirectly enhanced through the effects of cannabinoids on anxiety. Given the well-known anxiolytic effects of cannabinoids (Adams & Martin, 1996), when people smoke cannabis they are more able to relax and, therefore, to enjoy the sexual intercourse more. This hypothesis has been supported by Kolodny, Masters, and Johnson (1979), who found that while 81% of their sample of 500 subjects reported that cannabis use resulted in enhanced sexual pleasure, this pleasure was attributed to the effects of increased relaxation, and not to any direct effects of cannabis on sexual desire.

Alternatively, it seems plausible that cannabis smokers perceive an improvement of their sexual activity due to a slowing of temporal perception while "high," therefore sexual activities seem to last longer than they actually do (Jarvik & Brecher 1977; Melges, Tinklenberg, Hollister, & Gillespie, 1971). Melges et al. (1971) showed that slowing of temporal perception resulted in increased concentration on the actual moment.

Another possibility is that cannabis use results in disinhibition that, in turn, leads to more relaxation and sensory focus (Dawley et al., 1979; Kolodny et al., 1979; McKay, 2005). Additionally, it is plausible that given cannabis' reputation as an aphrodisiac, its positive effects on sex are due to expectancy effects (ie, placebo effect). Several studies support the idea that the sexual experiences of cannabis addicts are influenced by their sexual expectations from the substance (Crenshaw & Goldberg, 1996; Rosen, 1991). Therefore, the perceived relationship between cannabis and sexual behavior might be purely correlational, with cannabis having neither direct nor indirect effects on sexual functioning.

Finally, it has been argued that the correlation between cannabis and sexual function may be the result of the fact that alcohol and other psychoactive substances are often taken in conjunction with cannabis. These other substances may affect sexual function, but their effects may be wrongly attributed to cannabis (Gorzalka & Hill, 2006).

MINI-DICTIONARY

Cannabinoids Family of compounds (phytocannabinoids, derivatives of *Cannabis sativa*, endocannabinoids, and synthetic cannabinoids) whose action in the CNS is mediated through specific interactions with G-protein-coupled receptors, named CB1 and CB2. CB1 receptor is predominantly located in the brain, and its activation inhibits neurotransmitter release, and modulates both excitatory and inhibitory neuronal activity in the CNS. CB2 is mainly expressed in immune tissue, and it is probably involved in cannabinoid-mediated immune responses.

Cannabis A psychoactive drug whose main constituent is Δ^9-tetrahydrocannabinol (THC). It also contains other cannabinoids, including cannabidiol (CBD), cannabigerol (CBG), Δ^9-tetrahydrocannabivarin (9-THCV), and cannabidivarin (CBDV).

Cocaine Cocaine is a central nervous system stimulant with anesthetic properties that alters the levels of dopamine, serotonin, and noradrenaline.

Dopamine A catecholamine neurotransmitter that plays a number of important roles in the human and animal nervous system. These include learning and reward evaluation, as well as sexual behavior.

Heroin An extremely addictive narcotic drug derived from morphine. Heroin is classified as an opiate.
Methamphetamine A stimulant drug included in the amphetamine family.
Neurotransmitters Chemical substances released at synapses that mediate signaling between neurons. These include dopamine, norepinephrine, etc.
Opiates Central nervous system depressants that cause sedation and relieve pain. These act on several neurotransmitters in the brain, including endorphins. Opiates include heroin, morphine, methadone, codeine, etc.
Testosterone A sex hormone and anabolic steroid found in humans and many animals. In humans, testosterone is secreted by the testicles in males, and by ovaries in females.
THC The phytocannabinoid Δ^9-tetrahydrocannabinol (THC) is the psychoactive component responsible for changes of sexual functioning in drug addicts

References

Abbot, J., Johnson, R., Koziol-McLain, J., & Lowenstein, S. R. (1995). Domestic violence against women. Incidence and prevalence in an emergency department population. *JAMA*, *273*, 1763–1767.

Abel, E. (1981). Marihuana and sex: a critical survey. *Drug and Alcohol Dependence*, *8*, 1–22.

Adams, I. B., & Martin, B. R. (1996). Cannabis: pharmacology and toxicology in animals and humans. *Addiction*, *91*, 1585–1614.

Amaro, H., & Hardy-Fanta, C. (1995). Gender relations in addiction and recovery. *Journal of Psychoactive Drugs*, *27*, 325–337.

Brodbeck, J., Matter, M., & Moggi, F. (2006). Association between cannabis use and sexual risk behavior among young heterosexual adults. *AIDS and Behaviour*, *10*, 5.

Bruno, A., Scimeca, G., Marino, A. G., Mento, C., Micò, U., Romeo, V. M., Pandolfo, G., Zoccali, R., & Muscatello, M. R. A. (2012). Drugs and sexual behavior. *Journal of Psychoactive Drugs*, *44*, 359–364.

Buffum, J., Moser, C., & Smith, D. (1988). Street drugs and sexual function. In J. M. A. Sitsen, J. Money, & H. Musaph (Eds.), *Handbook of sexology* (pp. 462–477). Amsterdam, Netherlands: Elsevier Science Publishers.

Buffum, J. J. (1982). Pharmacosexology: the effects of drugs on sexual function a review. *Psychoactive Drugs*, *14*, 5–44.

Carroll, J. F., McGinley, J. J., & Mack, S. E. (2001). Exploring the self-reported sexual problems and concerns of drug-dependent males and females in modified, therapeutic community treatment. *Journal of Substance Abuse Treatment*, *20*, 245–250.

Carroll, J. F. X. (1982). Personality and psychopathology: a comparison of alcohol and drug-dependent persons. In J. Solomon, & K. A. Keeley (Eds.), *Perspectives in alcohol and drug abuse: similarities and differences* (pp. 59–88). Boston, MA: John Wright PSG Inc.

Chopra, G. S., & Jandu, B. S. (1976). Psychoclinical effects of long-term marijuana use in 275 Indian chronic users. A comparative assessment of effects in Indian and USA users. *Annals of the New York Academy of Sciences*, *282*, 95–108.

Clutterbuck, D. J., Gorman, D., McMillan, A., Lewis, R., & Macintyre, C. C. A. (2001). Substance use and unsafe sex amongst homosexual men in Edinburgh. *AIDS Care*, *13*, 527–535.

Cocores, J. A., Miller, N. S., Pottash, A. C., & Gold, M. S. (1988). Sexual dysfunction in abusers of cocaine and alcohol. *American Journal of Drug Abuse*, *14*, 169–173.

Crenshaw, T. L., & Goldberg, J. P. (1996). *Sexual pharmacology: drugs that affect sexual functioning*. New York, NY: W.W. Norton & Company.

Dawley, H. H., Jr., Winstead, D. K., Baxter, A. S., & Gay, J. R. (1979). An attitude survey of the effects of marijuana on sexual enjoyment. *Journal of Clinical Psychology*, *35*, 212–217.

El-Bassel, N., Gilbert, L., & Rajah, V. (2000). Fear and violence: raising the HIV stakes. *AIDS Education and Prevention*, *12*, 154–170.

Feucht, T. E. (1993). Prostitutes on crack cocaine: addiction, utility and marketplace economics. *Deviant Behavior*, *14*, 91–108.

Gay, G. R., & Sheppard, C. W. (1973). Sex-crazed dope fiends-myths or realities? *Drug Forum*, *2*, 125–140.

Gold, M. S. (1997). Cocaine (and crack): clinical aspects. In J. H. Lowinson, P. Ruiz, R. B. Millman, & J. G. Langrod (Eds.), *Substance abuse: A comprehensive textbook* (3rd ed., pp. 181–199). Baltimore, MD: Williams & Wilkins.

Gorzalka, B. B., & Hill, M. N. (2006). Cannabinoids, reproduction, and sexual behavior. *Annual Review of Sex Research*, *17*, 132–161.

Halikas, J., Weiler, R., & Morse, C. (1982). Effects of regular marijuana use on sexual performance. *Journal of Psychoactive Drugs*, *14*, 59–70.

Henderson, D. J., Boyd, C. J., & Whitmarsh, J. (1995). Women and illicit drugs: sexuality and crack cocaine. *Health Care for Women International*, *16*, 113–124.

Ho, B. T., Fritchie, G. E., Kralik, P. M., Englert, L. F., Mclsaac, W. M., & Idanpaan-Heikkila, J. (1970). Distribution of tritiated l-delta-9-tetrahydrocannabinol in rat tissues after inhalation. *Journal of Pharmacy and Pharmacology*, *22*, 538–539.

Jaffe, J. H., Knapp, C. M., & Ciraulo, D. A. (1997). Opiates: clinical aspects. In J. H. Lowinson, P. Ruiz, R. B. Millman, & J. G. Langrod (Eds.), *Substance abuse: A comprehensive textbook* (3rd ed., pp. 158–166). Baltimore, MD: Williams & Wilkins.

Jarvik, M., & Brecher, E. (1977). Drugs and sex: inhibition and enhancement. In J. Money, & H. Musaph (Eds.), *Handbook of sexology* (pp. 1095–1106). Amsterdam, Netherlands: Elsevier/North Holland Biomedical Press.

Johnson, S. D., Phelps, D. L., & Cottler, L. B. (2004). The association of sexual dysfunction and substance use among a community epidemiological sample. *Archives of Sexual Behavior*, *33*, 55–63.

Kingree, J. B., Braithwaite, R., & Woodring, T. (2000). Unprotected sex as a function of alcohol and marijuana use among adolescent detainees. *Journal of Adolescent Health*, *27*, 179–185.

Koff, W. (1974). Marijuana and sexual activity. *Journal of Sex Research*, *10*, 194–204.

Kolodny, R. C., Masters, W. H., & Johnson, V. (1979). *Textbook of sexual medicine*. Boston, MA: Little, Brown & Co.

Kolodny, R. C., Masters, W. H., Kolodner, R. M., & Toro, G. (1974). Depression of plasmatestosterone levels after chronic intensive marihuana use. *New England Journal of Medicine*, *290*, 872–874.

Leavitt, F. (1995). *Drugs & behavior* (3rd ed.). Thousand Oaks, CA: SAGE Publications, Inc.

Leigh, B. C. (2002). Alcohol and condom use: a meta-analysis of event-level studies. *Sexually Transmitted Diseases*, *29*, 476–482.

Li, J., Liu, H., Li, J., Luo, J., Koram, N., & Detels, R. (2011). Sexual transmissibility of HIV among opiates users with concurrent sexual partnerships: an egocentric network study in Yunnan, China. *Addiction*, *106*, 1780–1787.

Liang, W., Chikritzhs, T., & Lenton, S. (2011). Affective disorders and anxiety disorders predict the risk of drug harmful use and dependence. *Addiction*, *106*, 1126–1134.

Liau, A., Diclemente, R. J., & Wingood, G. M. (2002). Associations between biologically confirmed marijuana use and laboratory-confirmed sexually transmitted diseases among African American adolescent females. *Sexually Transmitted Diseases*, *29*, 387–390.

McKay, A. (2005). Sexuality and substance use: the impact of tobacco, alcohol, and selected recreational drugs on sexual function. *Canadian Journal of Human Sexuality*, *14*, 41–56.

Melges, F. T., Tinklenberg, J. R., Hollister, L. E., & Gillespie, H. K. (1971). Marihuana and the temporal span of awareness. *Archives of General Psychiatry*, *24*, 564–567.

Molitor, F., Ruiz, J. D., Flynn, N., Mikanda, J. N., Sun, R. K., & Anderson, R. (1999). Methamphetamine use and sexual and injection risk behaviors among out-of-treatment injection drug users. *American Journal of Drug and Alcohol Abuse*, *25*, 475–493.

Muscatello, M. R., Bruno, A., Scimeca, G., Pandolfo, G., Paduano, R., Mico', U., Bellinghieri, P. M., Di Nardo, F., Iannuzzo, G., Cofini, V.,

Di Orio, F., & Zoccali, R. (2010). The relationship between anger and heterosexual behavior. An investigation in a nonclinical sample of urban Italian undergraduates. *Journal of Sexual Medicine*, *7*, 3899–3908.

Pinger, R. R., Payne, W. A., Hahn, D. B., & Hahn, E. J. (1998). *Drugs: issues for today* (3rd ed.). Boston, MA: McGraw-Hill.

Rawson, R. A., Washton, A., Domier, C. P., & Reiber, C. (2002). Drugs and sexual effects: role of drug type and gender. *Journal of Substance Abuse and Treatment*, *22*, 103–108.

Reese, C. R. (1977). Neurophysiological studies of cannabis in human subjects. *Journal of Substance Use and Abuse*, *4*, 118–127.

Reiss, I. L., & Leik, R. K. (1989). Evaluating strategies to avoid AIDS: number of partners vs. use of condoms. *Journal of Sex Research*, *26*, 411–433.

Rosen, R. C. (1991). Alcohol and drug effects on sexual response: human experimental and clinical studies. *Annual Review of Sex Research*, *2*, 119–179.

Scher, J. (1970). The marijuana habit. *Journal of the American Medical Association*, *214*, 1120.

Semple, S. J., Zians, J., Strathdee, S. A., & Patterson, T. L. (2009). Sexual marathons and methamphetamine use among HIV-positive men who have sex with men. *Archives of Sexual Behavior*, *38*, 583–590.

Smith, A. M. A., Ferris, J. A., Simpson, J. M., Shelley, J., Pitts, M. K., & Richters, J. (2010). Cannabis use and sexual health. *Journal of Sexual Medicine*, *7*, 787–793.

Smith, D. E., Wesson, D. R., & Apter-Marsh, M. (1984). Cocaine-and alcohol-induced sexual dysfunction in patients with addictive disease. *Journal of Psychoactive Drugs*, *16*, 359–361.

Sumnall, H. R., Beynon, C. M., Conchie, S. M., Riley, S. C. E., & Cole, J. C. (2007). An investigation of the subjective experiences of sex after alcohol or drug intoxication. *Journal of Psychopharmacology*, *21*, 525–537.

Warner, E. A. (1993). Cocaine abuse. *Annals of Internal Medicine*, *119*, 226–235.

CHAPTER

20

Friendships and Cannabis Use

*J.H. Boman IV**, *C. Heck***

***Department of Criminal Justice, University of Wyoming, Laramie, WY, United States**
****City and County of Denver, Crime Prevention and Control Commission, Denver, CO, United States**

SUMMARY POINTS

- This chapter focuses on the impact that friends have on marijuana use.
- Friends, and particularly the marijuana use patterns of friends, have a strong impact on a person's choices to begin and continue using marijuana.
- Friends are most influential during the developmental stages of late childhood, early, middle, and late adolescence, and emerging adulthood. As a person enters their late 20s and beyond, it is likely that their marijuana use is not strongly influenced by the use patterns of friends.
- Marijuana is frequently used in small, intimate friendship groups during adolescence and emerging adulthood.
- American Courts have responded to shared usage by implementing nonassociation orders, or the situation when a court orders a person to not spend time with others who are known to use marijuana.
- With changing marijuana legislation in the United States and the world, the extent to which friends influence marijuana could potentially change going into the future.

KEY FACTS OF FRIENDS' INFLUENCE ON MARIJUANA USE DURING LATE CHILDHOOD AND ADOLESCENCE

- Late childhood is the first developmental period where there is a notable increase in the number of people initiating marijuana use.
- Most people who will use marijuana will initiate during adolescence or emerging adulthood, with the most common age being 17 years old.
- Users frequently use with their friends in small, intimate peer groups, during late childhood, adolescence, and emerging adulthood.
- Friends, and especially the marijuana use patterns of friends, are one of (if not the most) important causes of marijuana use during the late childhood, and into emerging adulthood. The impact that friends exert on marijuana use begins to fade in the early 20s.

KEY FACTS OF FRIENDS' INFLUENCE ON MARIJUANA USE DURING MIDDLE ADULTHOOD AND BEYOND

- Marijuana use becomes much less common toward the end of emerging adulthood, and is increasingly uncommon as a person ages into middle adulthood and beyond.
- Those who continue to use marijuana during the adult portions of their lives make conscious decisions to use, and are not influenced extensively by the marijuana use patterns of their friends.
- Some research suggests that there may be a resurgence of peer influence for alcohol among retired persons. With increasingly lenient marijuana use patterns in the United States and the world, a resurgence of the peer effect could potentially occur with respect to marijuana use.

KEY FACTS OF AMERICAN COURT RESPONSES TO SHARED MARIJUANA USE BETWEEN FRIENDS

- It is well understood that persons, and particularly young persons, tend to use marijuana in intimate peer groups.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00022-3
Copyright © 2017 Elsevier Inc. All rights reserved.

- American courts, and particularly specialized drug courts, have implemented "nonassociation orders" in response to the fact that many people use with others.
- Nonassociation orders are court orders prohibiting a user from spending time with other marijuana users.
- With relaxing marijuana legislation that has recently begun in the United States, American courts are increasingly shying away from hearing marijuana cases. This could affect how nonassociation orders are applied in the future in marijuana use cases.

LIST OF ABBREVIATIONS

DEA	Drug Enforcement Administration
NADCP	National Association of Drug Court Professionals
ONDCP	Office of National Drug Control Policy

INTRODUCTION

It has long been known that the influence of friends is one of the most robust correlates of a person's own marijuana (cannabis) use. In US culture, the importance of peer influence for marijuana use was realized many decades ago, when a marijuana culture which had originally grown out of the jazz music scene in the early 1900s expanded to a much wider audience, in the 1920s and 1930s. To provide just one example, an adolescent was driven to marijuana use—and ultimately homicide—because of his choice to associate with friends who also used marijuana, in the propaganda film *Reefer Madness* (1936). Research also recognized the importance of peers on marijuana use, especially after the social movements of the 1960s in the United States (Kandel, 1978). Currently, there is no serious debate about whether peers influence marijuana use. Instead, this is accepted as a fact (Akers & Cochran, 1985; Maxwell, 2002).

Perhaps because of the extent to which personal use is influenced by friends' use, marijuana is commonly seen as a social drug. On one level, this is largely correct since marijuana is commonly used with friends and/or because of friends. One potential conclusion from this fact is that peers—and particularly the marijuana use patterns of peers—are the leading cause of marijuana use. Though this is commonly thought to be the case, we caution against such a conclusion, and instead argue that peers exert a strong impact on marijuana use only at certain ages.

In this chapter, our general focus is on the extent to which friends impact marijuana use. We begin by offering some generalizations about peer influence and discuss the importance of the construct to the scientific discipline of criminology, as well as the practice of marijuana use. Following, we emphasize that the peer effect on marijuana use is not the same at all points of a person's life-course, and keep a keen eye on how peers impact marijuana use in different ways, at different ages. Next, we discuss how the American Criminal Justice System has implemented judicial responses to shared marijuana usage among friends. Finally, we discuss some future directions, where more friend-based marijuana research is necessary.

As a point of clarification, there is a difference between "peers" and "friends" (Kreager, 2004). "Peers" describe those with whom a person is similarly situated, while "friends" describe those with whom the person shares close and affectionate ties. While we do understand that there is a difference between these two groups of individuals, this chapter is about the influence of friends on marijuana use. With this explicitly noted, we will use the terms "peers" and "friends" interchangeably at times, simply to avoid monotony.

THE NATURE OF PEER INFLUENCE

The opinions, characteristics, and behavior of friends are widely recognized as one of the leading correlates and causes of personal behavior, in a wide variety of social science disciplines, including (but not limited to) fields concerned with psychological, sociological, developmental, and criminological matters. In the criminological context, the first scholar to place a central focus on the importance of friends was Edwin Sutherland, in the 1930s. Sutherland, who has been credited with founding criminology as a distinct discipline (Cullen, 2011), was a student of the Chicago School of sociological thought (Shaw, Zorbaugh, McKay, & Cottrell, 1929). Scholars of the Chicago School were primarily focused on investigating the apparent link between criminal behavior and detrimental structural conditions, such as, community-wide socioeconomic disadvantage, a lack of legitimate opportunities for employment, and social disorganization (Shaw & Becker, 1930; Shaw & McKay, 1942).

At the time when Sutherland began writing his most influential peer-based work (the 1930s–40s), Chicago School criminologists had already acknowledged that friends may be important for behavior. However, they did not believe that friends were a central cause of crime or substance use, a point to which Sutherland strongly disagreed. Instead of continuing research within the structural-level context of the Chicago School, Sutherland developed "differential association theory" (Sutherland, 1939, 1942, 1947), a theory of individual behavior which assumed that friends could be a leading correlate and cause of deviant and conforming behavior. Though Sutherland left many of the concepts within differential association theory broad and open to

interpretation, the central hypothesis of the theory was that the more a person is exposed to peers who engage in deviant behavior, the more likely he/she is to engage in deviance him/herself. Overall, this hypothesis has received overwhelming support in research, as hundreds of studies have found that those who have deviant friends are highly likely to commit deviant behavior themselves (Pratt et al., 2010).

Perhaps due to the overwhelming empirical support of differential association theory, the notion that peers affect behavior has become engrained as a central concept to criminologists. This has been aided by scholars who have expanded upon Sutherland's theory, particularly the extension of differential association offered by Akers's "social learning theory" (Akers, 2009).

Regardless of the theoretical framework through which it is applied, the behavior of peers consistently shows a strong, positive, and significant relationship with the behavior of individuals (Pratt et al., 2010; Warr, 2002). Despite the coefficient of peer behavior nearly always being significantly related to the behavior of an individual, the magnitude by which the coefficients vary depends on the type of behavior that is under consideration. Though still significant, the weakest coefficients are typically found in the associations between peer shoplifting and peer violence, and an individual's shoplifting and violent behaviors, respectively (Warr, 2002). On the other hand, the strongest coefficients are typically found in the associations between peer substance use and vandalism, and the individual's substance use and vandalism patterns, respectively (Warr, 2002). The difference in the coefficient sizes of these relationships is likely at least partially attributable to cooffending, or the situation where the actor and his/her peers all engage in a behavior together. Stated differently, people are more likely to vandalize property and use substances with friends, than they are to steal from stores and be violent with friends.

In short, evidence strongly supports the notion that the substance use patterns of friends are overwhelmingly important for one's personal use (Warr, 2002). As such, it has been claimed that the substance use of friends is the primary correlate and/or cause of one's own substance use (Agnew, 1991).

With the importance of friends emphasized, the question then becomes "which substances are most influenced by peer use?" The short answer to this question is social drugs, which generally encompass alcohol and marijuana, but can also include other "club drugs," such as ecstasy, cocaine, and methamphetamines (DEA, 2001). But such a simple answer may be somewhat misleading, due to strong evidence that suggests that there are different causes of substance use at different stages of one's life-course. We now turn our focus to such developmental matters, with a keen eye specifically on the impact that friends have on marijuana use.

HOW DO FRIENDS INFLUENCE MARIJUANA USE?

Generally, research has demonstrated that a person's behavior is influenced by different types of people at different ages (Warr, 1993). Due to the need for care, infants and young children share close relationships with their caregivers (usually parents), and are strongly influenced by them (Hartup, 1996). However, the influence parents have on behavior wanes, as children enter late childhood and early adolescence (Hartup, 1996). By the time an actor reaches late childhood, peers replace parents as the main agent of behavioral influence (Hartup, 1996). Peers remain the primary mechanism of behavioral influence until people reach their mid-to-late 20s, at which point many people are settling down into committed relationships (Warr, 1998).

The period during which friends have the most influence on marijuana use is the period of late childhood, adolescence, and emerging adulthood (Warr, 2002). Conveniently, these are also the developmental periods when marijuana use increases rapidly (late childhood and adolescence), peaks (adolescence to emerging adulthood), and declines rapidly (emerging adulthood) in the general population (Moffitt, 1993; Nagin & Tremblay, 2005). Thus, the developmental stages during which marijuana use is the highest in the overall population are the same developmental stages when friends influence use the most.

Although there are exceptions to this general trend, most people who choose to use marijuana use at the highest rate of their lives during adolescence and emerging adulthood (Warr, 2002; Moffitt, 1993). Accordingly, the developmental time period of adolescence is extremely important for the onset and continuance of marijuana use. Additionally, the influence that peers exert on marijuana use in adolescence shifts considerably during this tumultuous time in life. As such, we review the impact that friends have on marijuana use during several distinct developmental periods—late childhood, early adolescence, middle adolescence, late adolescence, emerging adulthood, and middle adulthood and beyond.

Late Childhood and Early Adolescence

Late childhood and early adolescence are generally thought of as the time when a person is between 9 and 12 years of age (Kastenbaum, 1993). In addition to the number of physical and hormonal changes that happen during this period of life, late childhood is also marked by a very important social shift. By the time most children enter late childhood—and certainly by the time a child turns 11 or 12—the most important influence for behavior no longer comes from parents and/or caregivers, but instead comes from friends (Hartup, 1996).

This shift in social influence from caregivers to friends carries important consequences for marijuana use. Late childhood is the beginning of a period of several years when numerous people will choose to use marijuana for the first time. This "initiation" or "experimentation" with the substance almost never occurs in a manner where a child acquires marijuana and uses it on his/her own. Instead, the onset of use during this age almost always happens because of some form of peer pressure or peer influence. One common scenario is a child's friend acquires marijuana and chooses to share it with the individuals within his/her friendship group (Anderson, 1999). Another common scenario is the child, or the child's friend(s), is acquainted with a slightly older individual (including older siblings) through whom he/she is able to acquire marijuana for use immediately or later (Ellickson, Tucker, Klein, & Saner, 2004).

Regardless of the means through which marijuana is first ingested, peers play a central role in instigating the onset of use during late childhood and early adolescence. To a great extent, this observation is also true during the developmental stage of middle adolescence.

Middle Adolescence

Middle adolescence is defined as the time when a person is between 13 and 15 years old (Kastenbaum, 1993). When individuals transition from the late childhood/early adolescence time period to middle adolescence, most people who will eventually use marijuana have not yet begun using (ONDCP, 2011). However, many future users will initiate use during middle adolescence (ONDCP, 2011).

Like during the period of late childhood and early adolescence, evidence suggests that the marijuana use patterns of friends are the most important correlate of the onset of experimentation with marijuana during the ages of 13–15 (Kandel, 1978). However, marijuana use trends frequently change with the onset of the early teenage years. In particular, a common pattern of use that arises during middle adolescence is the beginning of a transition from experimentation with marijuana to steady, regular patterns of marijuana use that will persist through middle and late adolescence, and into emerging adulthood. Depending on the user, regular, steady use, which can be referred to as "use maintenance" (Ellickson et al., 2004; Gilchrist, Gillmore, & Lohr, 1990), is something that can last from a very short period of time (eg, several months) to many years (eg, a lifetime; Windle & Wiesner, 2004).

Those who enter the maintenance stage of use during middle adolescence are very likely to have friends who also regularly use marijuana (Warr, 1993), although not necessarily in similar quantities (Kandel & Davies, 1991). Most peer groups (Fig. 20.1 for an example of a social network) during this age period have individuals embedded with them who use substances frequently, those who use occasionally, and those who do not use at all (Haynie, 2002). Stated differently, peer networks in middle adolescence generally contain people who engage in a wide range of different behaviors (Haynie, 2002). Regardless, evidence routinely suggests that the use of friends is extremely highly correlated with marijuana use maintenance during middle adolescence (Kandel & Davies, 1991).

Several authors have investigated the relationship between marijuana use and/or general drug use and friendship quality, during middle adolescence. Using emerging networks of recreational drug users (including marijuana users), Kandel and Davies (1991) explored the relationship between substance use and friends' social bonds, concluding that users were more closely tied to one another than nondrug-users. Using data on adolescents who were at high risk for criminal behavior and drug use, Krohn and Thornberry (1993) reached a similar conclusion. Additionally, the authors concluded that drug users were willing to alter their friendship networks so that they were more closely tied with peers who also used drugs (also see Giordano, Lonardo, Manning, & Longmore, 2010; Piehler & Dishion, 2007). Using the Add Health Data, this latter finding was replicated by McGloin (2009), who concluded that deviant individuals will drop friends, and select into friendships with those who behave more similarly to themselves.

Despite some significant results, other studies fail to demonstrate a relationship between marijuana use and/or general drug use and friendship quality (Giordano, Cernkovich, & Pugh, 1986). Perhaps part of the reason for this is that some studies have concluded that drug users who are in middle adolescence experience more conflict with one another than those who do not use drugs (Kandel, 1978; Krohn & Thornberry, 1993). Thus, it certainly appears that there are mixed results regarding how drug use impacts friendship quality during middle adolescence. More studies focusing specifically on marijuana, use as opposed to "general" drug use, seem necessary.

Overall, the period of middle adolescence is a time when the use of friends exerts a heavy influence on the onset and maintenance of marijuana use. This is in spite of the fact that there is substantial friendship turnover during this time (McGloin, 2009; Urberg, 1999), and mixed evidence on how friendship quality is impacted by marijuana use, and vice versa (Giordano et al., 1986; Krohn & Thornberry, 1993).

Late Adolescence

Persons are considered to be in late adolescence during the ages of 16–18 years of age (Kastenbaum, 1993). During this period of time, the peak of marijuana use

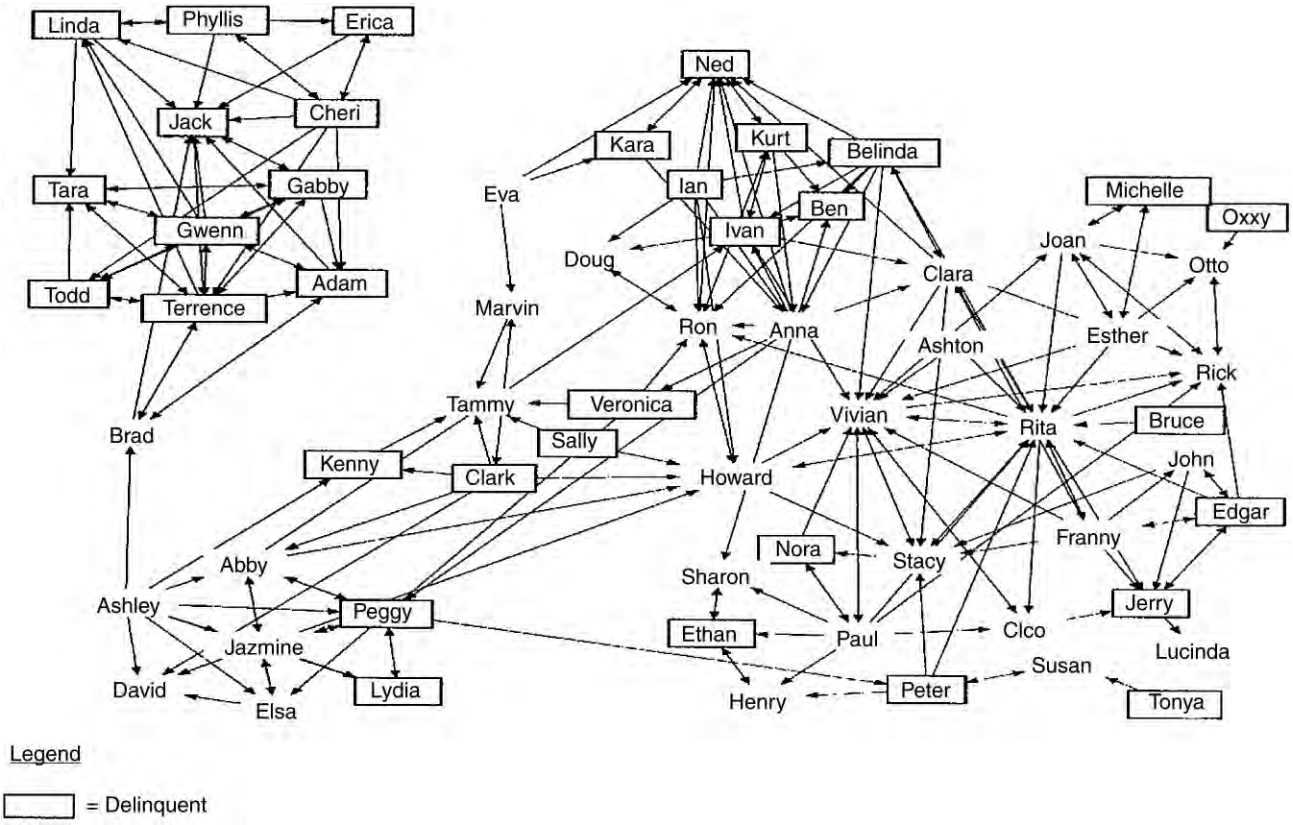


FIGURE 20.1 **An example of a social network during adolescence.** Outgoing arrows signify one nominated another as a friend. Incoming arrows mean the person was nominated as a friend by another. Double-headed arrows mean both members of the friendship nominated each other (reciprocal friendship). *Source: Taken from Haynie (2002), p. 115.*

in the overall population begins to occur, and will continue for several years into emerging adulthood (Moffitt, 1993; ONDCP, 2011; Warr, 2002).[a] Part of the reason for this is that late adolescence (17.5 years of age, in particular) is the most common time in the overall US population for people to begin experimenting with marijuana (ONDCP, 2011). Like it was in late childhood and middle adolescence, the use of marijuana is strongly influenced by the marijuana use patterns of one's friends at this time (Akers & Cochran, 1985).

Despite the marijuana use patterns of persons during late adolescence being heavily influenced by peers, the extent to which friends influence marijuana use is not getting any stronger during this time (Warr, 1998, 2002). For many people, late adolescence is the last developmental stage where peer influence is the primary social factor that relates to deviance and marijuana use (Warr, 1998).

There is mixed evidence on how friendship quality and marijuana use relate to one another in late adolescence (Giordano et al., 1986, 2010; Kandel & Davies, 1991; Piehler & Dishion, 2007). However, marijuana users in late adolescence do appear to change friendships more frequently than nonusers do (McGloin, 2009). There is ample evidence to suggest that this so called "friend shopping" is partially caused by users wanting to associate with peers who also use (McGloin, 2009). Despite this, friendship networks are still quite diverse during this time period, as the majority of them are comprised of those who do and do not use marijuana (Haynie, 2002).

Emerging Adulthood

Despite a person being considered an adult legally in the United States, and many other countries, at the time they turn 18 years old, few people at this age carry full responsibilities as an adult (eg, financial independence, full-time job, etc.). As such, developmental scholars have stressed that those who are in their late teens and early twenties cannot be fully considered adults (Arnett, 2000). Accordingly, the developmental period of emerging adulthood has been emphasized in research, and is defined as the time when a person is between 18 and 25 years of age (Arnett, 2000).

[a]Heading into the future, this age range could potentially change with the legalization movement of marijuana in the United States and other countries. We comment on this in the concluding remarks of this chapter.

In consideration of how friends affect marijuana use, emerging adulthood is the beginning of a period of great change. The reason for this is because the influence that friends exert on marijuana use dramatically decreases during this time, particularly toward a person's mid-20s (Warr, 1998). This is despite the fact that people tend to use marijuana in quite different patterns, or trajectories, during this age (Windle & Wiesner, 2004). While many persons are using marijuana steadily, others use only sporadically. Additionally, some will use solely in poly-drug contexts, some will experiment with marijuana only, and many will refrain from use entirely.

A consequence, or perhaps cause, of the decrease in peer influence during emerging adulthood is a shift in how people use marijuana that increases especially quickly as people reach their mid-20s. During the previous developmental stages of middle and late adolescence, an extremely common way for marijuana to be used was in a peer group where two or more people jointly use marijuana (Kandel, 1978; Kandel & Davies, 1991). During emerging adulthood, and particularly its latter years, there is a sharp decrease in co-use of marijuana with friends (Kandel & Davies, 1991; Warr, 1998).

Despite a drop in peer group-based usage during emerging adulthood, there is some evidence that suggests that friendship quality may be aided by marijuana use during this time. Using dyadic friendship data, Boman, Stogner, and Miller (2013) found that individuals who are in friendships in which both persons use marijuana have very high quality friendships. However, despite having higher overall quality friendships than nonusers, these individuals also experienced more conflict with one another. The latter finding is similar to other studies using general measures of substance use in adolescent samples, perhaps indicating some extent of cross generalizability across substances, and different developmental periods (Hawkins & Fraser, 1985; Kandel & Davies, 1991; Krohn & Thornberry, 1993; also refer to Haynie, 2001, 2002; McGloin, 2009 for some generalizations about deviance, peer networks, and network balance).

In sum, the amount to which marijuana use is affected by the use patterns of friends is rapidly decreasing during emerging adulthood. Users instead are becoming more independent, and use frequently occurs without some form of peer influence. However, persons who continue to use with friends tend to experience very high quality friendships, although the occasional conflictual encounter may occur.

Early Adulthood, Middle Adulthood, and Beyond

As people transition to the mid-to-late 20s, 30s, and into their 40s, the same trend that was observed during emerging adulthood is continuing, but to a greater extent. During these developmental stages, research suggests that persons who use marijuana do so for reasons that are not peer related (Warr, 1998; Nagin & Tremblay, 2005).

That is, the use of peers does not seem to contribute substantially to a person's decisions to use marijuana during their late 20s, 30s, 40s, or 50s. Despite this, individuals do still tend to use marijuana with some frequency (Fig. 20.2).

The reasons for the lack of a significant peer effect during adulthood are numerous. A major reason is that most peoples' focus shifts to nonpeer-based activities, such as, employment, finances, child rearing, and family (Warr, 1998). Additionally, adults have less free time than

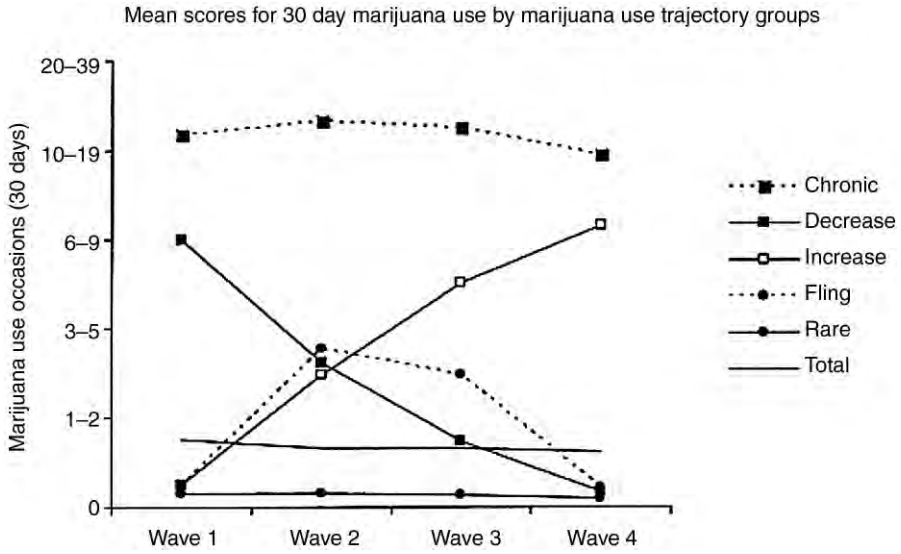


FIGURE 20.2 **Trajectories of marijuana use for those in early adulthood (ages 18–24).** *Source: Taken from Schulenberg et al. (2005), p. 263.*

Individual characteristics	Intervention	Short-term outcomes	Outcomes
Risk and need factors	Treatment, judicial oversight, intensive supervision, drug testing	Program completion, clean drug tests, employment and educational attainment	Sobriety, reduced criminality, improved social function

FIGURE 20.3 **The American drug court model which was originally developed in Dade County, Florida, United States.**

those in adolescence and emerging adulthood, meaning that finding time to gather groups of friends together for marijuana use is considerably more difficult than it was during earlier developmental stages.

Despite the peer effect dissolving during adulthood, there is some research that suggests that a resurgence in the importance of peer behavior may occur. Studying senior citizens, Akers, La Greca, Cochran, and Sellers (1989) found that the alcohol consumption patterns of same-age friends reemerge as strong and significant correlates of one's own alcohol use. Unlike those who are raising children, retirees experience a strong drop in responsibilities, and have much more discretion in how they spend their abundance of free time. Although alcohol is a much different intoxicant than marijuana, its perception as a "social drug" may suggest that this finding could cross-generalize in future research. This may be especially true, considering that many regions of the world are relaxing legislation regarding the use and sales of marijuana.

AMERICAN COURT RESPONSES TO SHARED USE

As we have discussed, marijuana is a social drug that most frequently is initiated and continued by the marijuana use patterns of one's friends. One important attempt to legally address the issue of marijuana and other drug use among friends began with some overworked professionals in Dade County, Florida, United States. These professionals came together from applied legal, criminal justice, and substance abuse treatment backgrounds, with the goal of solving the difficult issue of repeat offenders with substance abuse problems clogging the criminal justice system. The group determined that a possible solution to these problems would be a complex model linking the authority of the court, and the supervision and drug testing services provided by probation, with substance abuse treatment. This program has since become known as the "drug court model" (Fig. 20.3).

As part of the drug court program (Fig. 20.4), one of the most important elements of the drug court model relates to probation conditions that are enforced through frequent judicial contact. A commonly used condition is called the "nonassociation order." This order is frequently given to substance users who are caught by the police, and have been known to associate with other users. In essence, the nonassociation order is a court-mandated condition of probation that states that a marijuana (or other drug) user may have no contact with his/her friends who are known to also use illicit substances.

While all the courts and service agencies had worked together prior to the creation of the drug court model, its implementation called for a higher and more formalized level of cooperation. The model worked, and has continued to work, and more than 2400 programs across

Referral process	Program admission	Program activities	Graduation requirements
County attorney, judges, defense bar, law enforcement sources	Legal criteria	Intensive supervision	Completion of probation requirements
Referred for review	Substance abuse criteria/assessment	Random drug testing	Clean and sober living
Team decision to begin admission process	Community safety criteria	Intensive substance abuse treatment	Employment or educational goals
–	Participant agreement	Weekly court appearances supporting behavioral model	Stable living arrangement

FIGURE 20.4 **The program agenda for the drug court model.**

the United States and globally have been started using this model, in a way in which its core has been changed very little (Huddleston, Marlowe, & Casebolt, 2008). The drug court model has revolutionized the system of justice for many substance abusing and addicted offenders.

The intuitive appeal of the drug court model is obvious to those who work in the American Criminal Justice System. It makes sense that the authority of the court should be brought to bear on the daily activities of substance abusing offenders. The drug court model has brought effectiveness to substance abuse treatment, filling in where other historical programs—which have very low levels of retention for users—have failed (Stark, 1992).

To provide just one example of a failing treatment program, the extent to which probation services and incarceration are ineffective at changing substance abusing behaviors is well documented. The United States has been on a prison building binge that has one in every 134 residents of the United States incarcerated at midyear 2009 (West, 2009), despite reduced levels of offending in nearly every category of crime. Of these prisoners, over half report substance abuse or dependence, at the time of incarceration (Mumola & Karberg, 2004). The drug court model was designed with the goals of keeping offenders in community-based treatment, thus reducing the need for incarceration. And, after 25+ years of operation, the empirical evidence supporting the drug court model goes well beyond the initial intuitive appeal. There is a strong body of scientific research that supports the use of drug courts, as opposed to traditional criminal justice mechanisms with substance abusing offenders (Huddleston et al., 2008).

Though drug courts are tasked with controlling substance abuse generally, they have placed a considerable amount of effort into combating marijuana use, specifically. The United States' National Association of Drug Court Professionals has taken a hard line stance against marijuana use, claiming that marijuana has a negative impact on the brain, and addictive properties (NADCP Board of Directors, 2012). And, drug courts have proven to reduce marijuana use among participants, as well as those who graduate from the program (Bureau of Justice Assistance, 2010). While the intensity of the treatment model is too much for some, drug court professionals believe the drug court model has positively impacted the lives of many marijuana users, by reducing or eliminating their use of marijuana (Huddleston & Marlowe, 2011).

FUTURE DIRECTIONS OF RESEARCH AND CONCLUSIONS

Friends, and especially the marijuana use patterns of friends, exert a substantial impact on an individual's marijuana use during late childhood, adolescence, and emerging adulthood. Around the time a person enters their mid to late 20s, friend influence wanes, and those who continue to use marijuana do so for reasons other than having friends who use.

With the implementation of the drug court model and nonassociation orders, American courts have acknowledged that peers do matter for marijuana use. However, considering the increasingly dubious legal status of marijuana in the United States, the US court system finds itself at somewhat of an impasse. Several states in the United States have legalized, or have referendums that propose to legalize, marijuana. This means that marijuana can be purchased legally, and taxed, in these jurisdictions.

Given the recent push for legalization in the United States, the current state of knowledge on how friends influence marijuana could certainly change, and for a number of reasons. To provide one possibility, marijuana legalization could lead to a wider audience using the substance, since it is now legal and extremely easy to obtain (see the study by Lynne-Landsman, Livingston, & Wagenaar, 2007 on how medical marijuana legislation in the United States has changed adolescent marijuana use patterns). This may change the contexts and patterns of how people use marijuana, thus potentially altering what we know about how friends influence marijuana use. There are a number of possibilities as to what could happen. One potential implication could be that shared use becomes more common for adults, and especially for those who have some level of expendable income, and free time. If this were the case, group-based usage of marijuana could become as common among adults as it currently is for adolescents. This could mean an emergence of a significant friend effect for those who would have otherwise "aged out" of the developmental periods traditionally associated with friend influence. This could potentially also occur in a cohort effect, where adolescents who grew up regularly using marijuana continue to use in a manner where peers still influence marijuana use patterns at adult ages. Other possibilities exist too, though it is difficult to speculate on what they may be, given the absence of empirical data at the time this chapter was written.

This discussion points to perhaps the main future direction of necessary research on marijuana and friends. As marijuana becomes increasingly legally available across the United States, and other worldwide jurisdictions, what will happen to the marijuana use patterns of people of different ages? We already know that people tend to follow different trajectories of marijuana use (Windle & Wiesner, 2004), but will these trajectories—and the reasons that people follow them—change? To further complicate matters, any change in how friends impact marijuana use could depend on the actions of worldwide governments in the upcoming years.

One way or the other, research needs to focus specifically on peer relationships and marijuana use during this transition. Further, we stress the necessity for research to focus solely on marijuana. Much of the literature we have reviewed is on "drug use" or "offending" generally, and not specific to cannabis. As marijuana legislation changes, researchers must focus specifically on how marijuana affects friendships, and how these friendships in turn impact marijuana use.

Regardless of what is to come with the state of marijuana legislation in the United States, another area of research that is needed is the exploration of how friends affect marijuana use (or if they do) among elderly populations. The fact that peer influence may reemerge as a significant correlate of alcohol use among the retired (Akers et al., 1989) begs the question if this is also the case with marijuana.

Further, it would be useful to explore how friendship quality affects, and/or is affected by, marijuana use generally, but especially during the adult years. With younger populations, the two most common findings are (1) marijuana use does not affect friendship quality, or (2) improves it, but leads to conflict. The equivocal findings may in part be due to the lack of stability that friendships have in adolescence, compared to adulthood (McGloin, 2009).

In conclusion, the future of our understanding on how friends influence marijuana use could change substantially, heading into the near future. Regardless, there is enough research (Pratt et al., 2010) to suggest that the peer effect that exists during late childhood, adolescence, and emerging adulthood will not dissipate, despite what happens with legislation. Succinctly, friends are too important in causing experimentation with, and continuation of, marijuana use at a variety of ages for the effect to completely dissolve just because of legislative reasons.

MINI-DICTIONARY

Differential association An extensively supported theory of crime developed by Edwin Sutherland in the 1930s and 1940s. It generally hypothesizes that a person can be pulled in and out of crime and substance use by the definitions they develop from the extent to which their peers are involved in crime and substance use.

Drug court An American-based model that was designed to remove drug offenders from the criminal justice system, and place them in a court which only deals with drug offenses.

Friend A person with whom a target individual shares close, interpersonal ties. Peers can be friends, but not all peers are friends. Friends are perceptual; that is, if the target individual perceives they are friends with another, that other person is a friend in the mind of the target individual. Thus, the other person does not have to reciprocate the friendship label.

Friend influence Generally, also known as peer influence. The extent to which friends exert a behavioral, social, or emotional impact on a target individual.

Nonassociation order An American court order that states a target individual may not spend time with another person; the other person is generally a known substance user.

Peer A person who is similarly situated to a target individual in terms of age and social status. Not necessarily a friend, although we use the terms friend and peer interchangeably to avoid monotony.

Peer group Also known as a friendship group, or friend group. A subset of a person's social network consisting of individuals with whom he/she perceives affectionate ties, and considers a friend. Peer groups are generally smaller than social networks, though they can still be quite sizable.

Shared marijuana use Also known as co-use. The situation when a target individual engages in marijuana use in the presence of at least one other person who is also using marijuana.

Social network A person's entire group of peers with whom he/she spends time. Persons in the peer network do not need to be identified as friends. Generally consists of many individuals, especially during the time when adolescents are enrolled in school.

References

Agnew, R. (1991). The interactive effects of peer variables on delinquency. *Criminology*, *29*, 47–72.

Akers, R. L. (2009). *Social learning and social structure: A general theory of crime and deviance*. Brunswick, NJ: Transaction.

Akers, R. L., & Cochran, J. (1985). Adolescent marijuana use: a test of three theories of deviant behavior. *Deviant Behavior*, *6*, 323–346.

Akers, R. L., La Greca, A. J., Cochran, J., & Sellers, C. S. (1989). Social learning theory and alcohol behavior among the elderly. *Sociological Quarterly*, *30*, 635–638.

Anderson, E. (1999). *Code of the street: Decency, violence, and the moral life of the inner city*. New York, NY: W. W. Norton and Co.

Arnett, J. J. (2000). Emerging adulthood: a theory of development from the late teens through the twenties. *American Psychologist*, *55*, 469–480.

Boman, J. H., IV, Stogner, J., & Miller, B. L. (2013). Binge drinking, marijuana use, and friendships: the relationship between similar and dissimilar usage and friendship quality. *Journal of Psychoactive Drugs*, *45*, 218–226.

Bureau of Justice Assistance. (2010). What have we learned from evaluations of drug courts? Available from https://www.bja.gov/evaluation/program-substance-abuse/drug2.htm

Cullen, F. T. (2011). Beyond adolescence-limited criminology: choosing our future—the American Society of Criminology 2010 Sutherland Address. *Criminology*, *49*, 287–330.

DEA. (2001). *Drug intelligence brief: Club drugs: An overview*. Drug Enforcement Agency Report No. 01026. Available from http://www.usdoj.gov/dea/pubs/intel/01026/index.htm

Ellickson, P. L., Tucker, J. S., Klein, D. J., & Saner, H. (2004). Antecedents and outcomes of marijuana use initiation during adolescence. *Preventive Medicine*, *39*, 976–984.

Gilchrist, L. D., Gillmore, M. R., & Lohr, M. J. (1990). Drug use among pregnant adolescents. *Journal of Consulting and Clinical Psychology*, *58*, 402–407.

Giordano, P. C., Cernkovich, S. A., & Pugh, M. D. (1986). Friendships and delinquency. *American Journal of Sociology*, *91*, 1170–1202.

Giordano, P. C., Lonardo, R. A., Manning, W. D., & Longmore, M. A. (2010). Adolescent romance and delinquency: a further exploration of Hirschi's "cold and brittle" relationships hypothesis. *Criminology*, *48*, 919–946.

Hartup, W. W. (1996). The company they keep: friendships and their developmental significance. *Child Development*, *67*, 1–13.

Hawkins, J. D., & Fraser, M. W. (1985). Social networks of street drug users: a comparison of two theories. *Social Work Research and Abstracts*, *21*, 3–12.

Haynie, D. L. (2001). Delinquent peers revisited: does network structure matter? *American Journal of Sociology*, *106*, 1013–1057.

Haynie, D. L. (2002). Friendship networks and delinquency: the relative nature of peer delinquency. *Journal of Quantitative Criminology*, *18*, 99–134.

Huddleston, C. W., III, & Marlowe, D. B. (2011). *Painting the current picture: A national report on drug courts and other problem-solving court programs in the United States*. Washington, DC: National Drug Court Institute.

Huddleston, C. W., III, Marlowe, D. C., & Casebolt, R. (2008). *Painting the current picture: A national report card on drug courts and other problem-solving court programs in the United States*. Washington, DC: National Drug Court Institute.

Kandel, D. B. (1978). Similarity in real-life adolescent friendship pairs. *Journal of Personality and Social Psychology*, *36*, 306–312.

Kandel, D. B., & Davies, M. (1991). Friendship networks, intimacy, and illicit drug use in young adulthood: a comparison of two competing theories. *Criminology*, *29*, 441–469.

Kastenbaum, R. (1993). *Encyclopedia of adult development*. Phoenix, AZ: Oryx Press.

Kreager, D. A. (2004). Strangers in the halls: isolation and delinquency in school networks. *Social Forces*, *83*, 351–390.

Krohn, M. D., & Thornberry, T. P. (1993). Network theory: a model for understanding drug abuse for African-American and Hispanic youth. In M. R. De La Rosa, & J. L. R. Adrados (Eds.), *Drug abuse among minority youth: Methodological issues and recent research advances*. Rockville, MD: National Institute on Drug Abuse.

Lynne-Landsman, S. D., Livingston, M. D., & Wagenaar, A. C. (2007). Effects of state medical marijuana laws on adolescent marijuana use. *American Journal of Public Health*, *103*, 1500–1506.

Maxwell, K. A. (2002). Friends: the role of peer influence across adolescent risk behaviors. *Journal of Youth and Adolescence*, *31*, 267–277.

McGloin, J. M. (2009). Delinquency balance: revisiting peer influence. *Criminology*, *47*, 439–477.

Moffitt, T. E. (1993). Adolescence-limited and life course persistent antisocial behavior: developmental taxonomy. *Psychological Review*, *100*, 674–701.

Mumola, C. J., & Karberg, J. C. (2004). *Drug use and dependence, state and federal prisoners, 2004*. Washington, DC: Bureau of Justice Assistance.

NADCP Board of Directors. (2012). Position Statement on Marijuana. National Association of Drug Court Professionals. Available from http://www.nadcp.org/sites/default/files/nadcp/NADCP%20Board%20Position%20Statement%20-%20Marijuana.pdf

Nagin, D. S., & Tremblay, R. E. (2005). Developmental trajectory groups: fact or a useful statistical fiction? *Criminology*, *43*, 873–904.

ONDCP. (2011). Answers to frequently asked questions about marijuana. Office of National Drug Control Policy. Available from http://www.whitehouse.gov/ondcp/frequently-asked-questions-and-facts-about-marijuana#initiation

Piehler, T. F., & Dishion, T. J. (2007). Interpersonal dynamics within adolescent friendship: dyadic mutuality, deviant talk, and patterns of antisocial behavior. *Child Development*, *78*, 214–224.

Pratt, T. C., Cullen, F. T., Sellers, C. S., Winfree, L. T., Jr, Madensen, T. D., Daigle, L. E., Fearn, N. E., & Gau, J. M. (2010). The empirical status of social learning theory: a meta-analysis. *Justice Quarterly*, *27*, 765–802.

Reefer Madness. (1936). Film. Motion Picture Ventures.

Schulenberg, J. E., Merline, A. C., Johnston, L. D., O'Malley, P. M., Bachman, J. G., & Laetz, V. B. (2005). Trajectories of marijuana use during the transition to adulthood: the big picture based on national panel data. *Journal of Drug Issues*, *35*, 255–279.

Shaw, C. R., & Becker, H. S. (1930). *The Jack-Roller: a delinquent boy's own story*. Chicago, IL: University of Chicago Press.

Shaw, C. R., & McKay, H. D. (1942). *Juvenile delinquency in urban areas*. Chicago, IL: University of Chicago Press.

Shaw, C. R., Zorbaugh, H., McKay, H. D., & Cottrell, L. S. (1929). *Delinquency areas*. Chicago, IL: University of Chicago Press.

Stark, M. J. (1992). Dropping out of substance abuse treatment: a clinically oriented review. *Clinical Psychology Review*, *12*, 93–116.

Sutherland, E. H. (1939). *Principles of criminology* (3rd ed.). Philadelphia, PA: Lippincott.

Sutherland, E. H. (1942). The development of the concept of differential association. *Ohio Valley Sociologist*, *XV*, 3–4.

Sutherland, E. H. (1947). *Principles of criminology* (4th ed.). Philadelphia, PA: Lippincott.

Urberg, K. A. (1999). Introduction: some thoughts about studying the influence of peers on children and adolescents. *Merrill-Palmer Quarterly*, *45*, 1–12.

Warr, M. (1993). Age, peers, and delinquency. *Criminology*, *36*, 17–40.

Warr, M. (1998). Life-course transitions and desistance from crime. *Criminology*, *36*, 183–216.

Warr, M. (2002). *Companions in crime: the social aspects of criminal conduct*. New York, NY: Cambridge University Press.

West, H. (2009). *Prison inmates at midyear 2009*. Washington, DC: Bureau of Justice Statistics.

Windle, M., & Wiesner, M. (2004). Trajectories of marijuana use from adolescence to young adulthood: predictors and outcomes. *Development and Psychopathology*, *16*, 1007–1027.

CHAPTER

21

Students' Knowledge of Cannabis

M. Drozd, J. Sobczyński

Department of Pharmaceutics, Medical University of Lublin, Lublin, Poland

SUMMARY POINTS

- The chapter focuses on cannabis-related knowledge among students (adolescents and young adults), as well as the role of knowledge in recreational and medical cannabis use.
- Students with broader and more nuanced knowledge on cannabis are less likely to use it, which is a basis for including cannabis-related knowledge in school prevention programs and university courses.
- Successful school-based drug prevention comprises of repeated courses that aim to present the key facts on cannabis health effects, address the issue of peer pressure, and develop the refusal skills.
- Internet, media, and friends are the most popular sources of information that students approach when seeking information on illicit drugs.
- University students know of positive, medically useful cannabis effect on human health, but don't know of specific cannabis uses in medicine.
- Evaluation of cannabis-related knowledge among nonhealth professionals may help to develop better patient education programs in order to address misconceptions on medical cannabis and, consequently, improve medication adherence.

KEY FACTS ON CANNABIS USE AMONG ADOLESCENTS AND YOUNG ADULTS

- It was estimated that 14.6 million (11.2 % of European young adults aged 15–34) used cannabis in 2013.
- Regular cannabis use in adolescence adversely affects mental health in young adults, increasing risk of psychotic disorders and symptoms.
- Adolescent regular cannabis users are more likely to use other illicit substances as compared to nonusers, and they may also experience decreased cognitive functions.

KEY FACTS ON MEDICAL CANNABIS AND CANNABINOIDS

- Cannabis has been used to treat diseases and alleviate their symptoms since antiquity, and is now approved for medical use in some American states.
- Sativex, Cemet and Marinol are cannabinoid-based medication registered for use by patients suffering from certain chronic diseases and cancer, in Europe and the United States.

KEY FACTS ON MEDICATION COMPLIANCE

- While around 20–30% of patients don't adhere to medication regimens that aim to cure diseases or relieve symptoms, 30–40% fail to follow regimens designed to prevent health problems, and 50% do not comply with long-term medication regimens.
- In 2001, it was estimated that poor adherence generates annual approximate direct and indirect health-care costs of $177 billion, in the United States.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00023-5
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ADR	Adverse drug reaction
ESPAD	European School Survey Project on Alcohol and Other Drugs
MS	Multiple sclerosis
NIDA	National Institute of Drug Abuse
THC	Tetrahydrocannabinol

INTRODUCTION

Cannabis has been widely used for recreational purposes, especially in the last 50 years. Cannabis use among adolescents and young adults gained particular scientific interest since, together with alcohol, it is the most popular psychoactive agents among youth (see the Key facts on cannabis use among adolescents and young adults; Hall & Degenhardt, 2009). Previous reports showed that cannabis use among students is associated with lower educational attainment, low religiosity, adverse life events, and increased reported use of illicit drugs other than cannabis (Engs & Mullen, 1999; Pacula et al., 2000). Moreover, low socioeconomic status in childhood, as well as various intrapersonal variables, were associated with higher cannabis use prevalence (von Sydow, Lieb, Pfister, Höfler, & Wittchen, 2002). The research identified a group of adolescents that are more likely to use cannabis in the future (vulnerable nonusers; Crano, Siegel, Alvaro, Lac, & Hemovich, 2008) that should primarily be addressed by drug prevention programs. The reader is referred to a comprehensive review, where the influence of a number of various factors on cannabis use is described (Macleod et al., 2004). However, the level of cannabis-related knowledge among students, and its impact on cannabis use prevalence and patterns of use, has rarely been studied. Geramian, Akhavan, Gharaat, Tehrani, and Farajzadegan, 2012 analyzed knowledge of cannabis and other psychoactive agents among 6998 Iranian students (aged 14–18), from both urban and rural areas (Geramian et al., 2012). They found that female students had higher knowledge on health consequences of drug use. Heckman, Dykstra, and Collins, 2011 evaluated cannabis-related knowledge as a means to assess the efficacy of a "drugs and behavior" university course (Heckman et al., 2011). Newton, Teesson, Vogl, and Andrews (2010) measured cannabis-related knowledge using a 16-questions quiz in order to determine the level of knowledge before and after students' participation in a cannabis and alcohol school prevention program (Newton et al., 2010). However, there does not seem to be one universal scale, test, or quiz to properly evaluate the level of knowledge, so each study yields results that cannot be compared.

Apart of their psychoactive properties, the active ingredients of cannabis are reported to possess a distinct pharmacological effect on the human body (see the Key facts on medical cannabis and cannabinoids; Leung, 2011; Borgelt, Franson, Nussbaum, & Wang, 2013). Medical cannabis use is approved in certain American states as a treatment modality for patients suffering from, for example, cancer, glaucoma, multiple sclerosis (MS), chronic pain, Crohn disease, Alzheimer disease, Parkinson disease (Marcoux, Larrat, & Vogenberg, 2013). As the cannabinoid administration in form of smoked herb may have negative impact on the respiratory tract, several pharmaceutical cannabinoid preparations have been developed (Machado Rocha, Stefano, De Cassia Haiek, Rosa Oliveira, & Da Silveira, 2008). While nabilone (Cesamet) is utilized in the United States to control chemotherapy-induced vomiting and nausea, in patients suffering from cancer, dronabinol (Marinol) is used for the same indications by Acquired Immunodeficiency Syndrome (AIDS) patients. Nabiximols (distributed in several European countries, Canada, and New Zealand under its brand name Sativex) is employed to relieve the symptoms of MS, for example, neuropathic pain, spasticity, and overactive bladder (Sativex, 2014). However, the use of cannabinoids is still not widely recognized in medicine, and does not correspond to their therapeutic potential. Previous studies aimed to assess cannabis-related knowledge and attitudes among health-care professionals, as it influences prescribing registered cannabis products, admission into medical cannabis programs, and affects patient care in hospice settings (Charuvastra, Friedmann, & Stein, 2005; Kondrad & Reid, 2013; Uritsky, McPherson, & Pradel, 2011). However, knowledge, attitude, and perception of medical cannabis has not been thoroughly evaluated among the general public. The only study exploring this topic is the work of Taylor (1972), and our recent paper (Sobczyński et al., 2013). Taylor (1972) examined the risk perception and attitude toward cannabis' legal status among 372 Canadian freshmen students. It was found that while the views on legal status were not affected by the responder's gender, women were more likely to recognize the risk associated with cannabis use.

CANNABIS-RELATED KNOWLEDGE, ATTITUDE, AND RISK PERCEPTION IN THE CONTEXT OF RECREATIONAL USE

It has been found that adolescents who reported higher levels of parental monitoring and control were less likely to be past or current cannabis users (Ramirez et al., 2004). However, as the influence of parental control and supervision is expected to dwindle with the student's age, high-school students are more likely to be

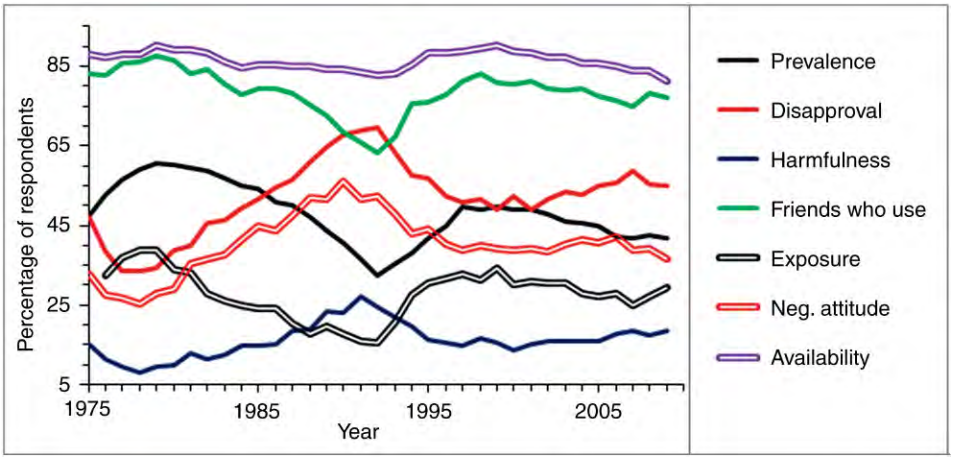


FIGURE 21.1 **Cannabis use and views.** Trends among American students in the period 1975–2009. Self-reported cannabis use, attitude, perception of risk, and exposure among American 12th graders (aged 17–18). *Availability*, percentage of respondents who considered cannabis "very easy" or "fairly easy" to get if desired; *Disapproval*, percentage of respondents who answered positively when asked: "Do you disapprove of people (≥ 18 years old) trying marijuana once or twice?;" *Exposure*, percentage of respondents who have often been around people taking cannabis during last 12 months; *Friends who use*, percentage given by respondents, when asked: "How many of your friends smoke marijuana?;" *Harmfulness*, percentage of respondents who considered that use of marijuana (once or twice) is associated with great risk; *Negative attitude*, percentage of respondents who answered positively when asked: "Do you think that people (≥ 18 years old) should be prohibited by law from smoking marijuana in private?;" *Prevalence*, self-reported lifetime use of cannabis. *Source: Based on the report of Johnston et al., 2010.*

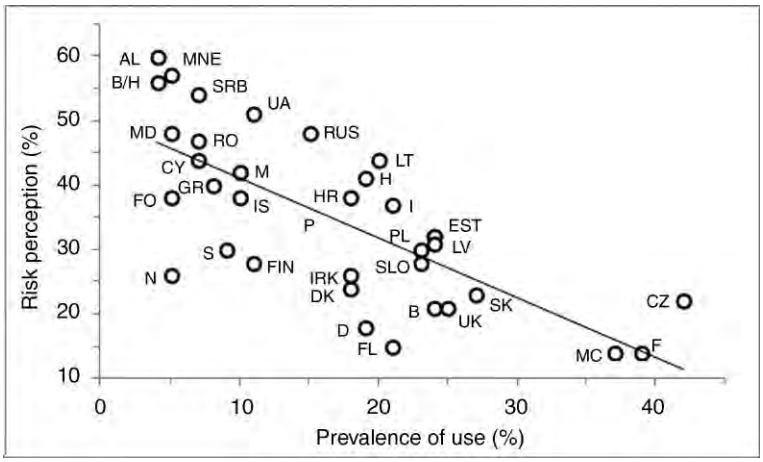


FIGURE 21.2 **The relationship between self-reported cannabis use prevalence and risk perception among European students.** Correlation between self-reported lifetime use of cannabis and the percentage of students (aged 15–16, n = 100,000) answering that use of cannabis (once or twice) is associated with great risk. A trend line was implemented using Microsoft Office Excel 2007 (correlation coefficient = 0.73). *AL*, Albania; *B*, Belgium; *B/H*, Bosnia and Herzegovina; *BG*, Bulgaria; *C*, Croatia; *CY*, Cyprus; *CZ*, Czech Republic; *D*, Germany; *DK*, Denmark; *EST*, Estonia; *F*, France; *FIN*, Finland; *FL*, Liechtenstein; *FO*, Faroe Islands; *GR*, Greece; *H*, Hungary; *IS*, Iceland; *IRL*, Ireland; *I*, Italy; *LV*, Latvia; *LT*, Lithuania; *M*, Malta; *MD*, Moldova; *MC*, Monaco; *MNE*, Montenegro; *N*, Norway; *PL*, Poland; *P*, Portugal; *RO*, Romania; *RUS*, Russian Federation (Moscow); *SRB*, Serbia; *SK*, Slovak Republic; *SLO*, Slovenia; *S*, Sweden; *UA*, Ukraine; *UK*, United Kingdom. *Source: Based on the report of Hibell et al., 2012.*

influenced by their closest friends and classmates, as previously reported (Iannotti & Bush, 1992; Lewis & Clemens, 2008). The National Institute of Drug Abuse (NIDA) study envisaged the impact of social norms among young adults on self-reported cannabis use (Johnston, O'Malley, Bachman, & Schulenberg, 2010). Cannabis use among students was elevated when the perception of exposure and cannabis consumption by friends was high (Fig. 21.1). A similar trend was observed in Europe, in a survey performed under the auspices of the European School Survey Project on Alcohol and Other Drugs (ESPAD; Hibell et al., 2012), showing a clear negative correlation between perception of risk associated with cannabis use, and self-reported use prevalence was reported (Fig. 21.2). The Eurobarometer telephone-based survey showed that the health risk associated with cannabis use was lower among young people who have previously used cannabis (one or two times; 52 % listed medium or high risk), as compared to those that never used it (94% listed medium or high risk), as similar to NIDA

and ESPAD studies (Eurobarometer, 2008). While 91% of respondents recognized the health risk associated with regular cannabis use, only 30% of study participants claimed that incidental use of cannabis posed a medium or high risk. As similar to the ESPAD report, the Eurobarometer study showed significant differences in risk perception among young people from different EU member states, arising from differences in social norms in countries more favorable to cannabis use (eg, Denmark, Netherlands, or Czech Republic), as compared to others, where even incidental cannabis use is considered harmful (eg, Cyprus and Romania). In contrary to the NIDA study, the ESPAD and Eurobarometer reports showed a positive correlation between perceived low availability and perceived high risk of cannabis use.

Ramirez et al. (2004) interviewed a group of 1094 American fourth-through 12th-grade students (Anglo Americans and Hispanic Americans), and distinguished a group of adolescents who were less likely to use cannabis. They exhibited stronger bonds with parents, and had more profound cannabis-related knowledge (presumably gained from relatives). Seeking cannabis-related knowledge from friends, especially the ones being regular cannabis users, may encourage cannabis consumption (Figs. 21.1, 21.2), as the risk perception among users is generally lower than among nonusers (Danseco, Kingery, & Coggeshall, 1999). Moreover, more frequent users considered frequent use (ie, ≥2–3 times per week) to be less harmful than nonfrequent users (O'Callaghan, Reid, & Copeland, 2006). It has also been found that even these users that are aware of cannabis risk were more likely to recognize psychological problems (30.3%; concerning, for example, concentration, memory, motivation), followed by physical and general health issues (21.5%; eg, respiratory problems, cancer, impotency), rather than legal and life issues. However, the serious social personal consequences (eg, impaired performance at university or work, and problems with relationships) were listed only when interviewees were prompted, and some issues like addiction were given more emphasis (O'Callaghan et al., 2006).

Taking into consideration the conflicting influence of parents, friends, teachers, etc. on cannabis use among students, it is important to determine the possible sources of knowledge that young people consult when seeking information on cannabis. Eurobarometer responders listed the same potential sources of information on illicit drugs, in various countries (the three most popular answers were identical), and obtained information from similar sources in the period of the previous year (Fig. 21.3). As the results showed that internet is an important source of knowledge for students (and it is becoming even more significant, as compared to previous Eurobarometer results from 2008), internet-based prevention measures may be useful in educating young people on cannabis. While adolescents were more likely to be informed on illicit drugs by school prevention programs (63%), young adults (aged 22–24) obtained information from media campaigns (54%). Whereas school campaigns showed to be an important measure in forging the students' perception of drugs, many students, especially those that tried cannabis, turned to friends.

CANNABIS-RELATED KNOWLEDGE IN THE CONTEXT OF SUBSTANCE USE PREVENTION PROGRAMS

As mentioned in the previous section, media campaigns and school-based drug prevention programs are important sources of cannabis-related knowledge for students. Different approaches have been utilized in order to address the risk factors that lead to cannabis use, on the individual adolescent's level (summarized in Fig. 21.4). Skill-based programs aim to develop resistance skills and assertiveness, among sensation-seekers, addressing, for example, stress management, communication, decision-making, and goal-setting abilities. The knowledge-centered programs will provide students with information in order to make better choices. Programs offer information on consequences of cannabis use, effects of cannabis products on the individual's health and behavior, especially in the context of polydrug use. The goal of giving a thorough description of psychological drug action is to build a negative attitude toward drugs and, consequently, decrease their use. The discussion of drugs' addictive potential and outcomes of developing an addiction may be used to dissuade students from using cannabis. Knowledge-based programs also affect students' perception of cannabis use, as the difference between perceived and real prevalence of drug use, in the context of peer pressure, is explained. It has been shown that perception of cannabis use prevalence among peers is overestimated (McCabe, 2008; Franca, Dautzenberg, Falissard, & Reynaud, 2010; Wolfson, 2000). As students that have a false impression that cannabis is very popular among the youth may be more likely to try it (see the previous section), presenting the statistics of drug use may help to clear up this misconception. Providing information on different cannabis products (eg, their potency, short- and long-term health risks), as well as the legal and financial consequences of drug use, may also discourage drug use among adolescents and young adults. Finally, the positive attitude to nonuse is enhanced by presenting alternatives to drug use in regard to situations when adolescents would typically use cannabis (Fig. 21.4). The latter approach is mostly addressed to young people taking drugs as a way to deal with bad mood, or personal problems.

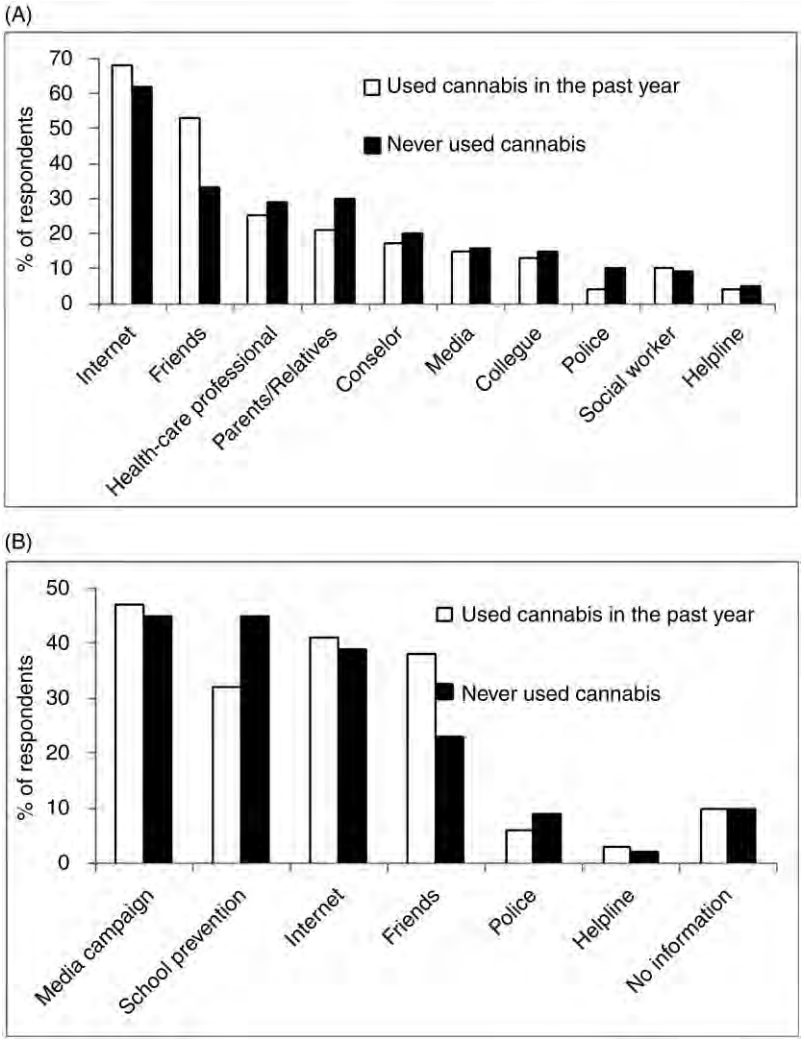


FIGURE 21.3 Sources of information on illicit drugs, reported by European young people. Potential sources of information about illicit drugs and drug use, reported by students (aged 15–24, $n = 12{,}315$) who never used cannabis and students who used cannabis at least once in the last year. A and B present answers for following questions: *A*, "If you wanted to have information about illicit drugs and drug use in general, who would you turn to?;" *B*, "Through which channels, have you been informed over the past year about the effects and risks of the use of illicit drugs?." *Source: Based on data from Eurobarometer (2011).*

It has been found that skill-base programs are somehow more efficient in discouraging drug use (Faggiano et al., 2005). However, the increased knowledge can help some students to understand the link between drug action and its harmful effect. As many factors presented in Fig. 21.4 are intertwined, the most efficient programs will integrate knowledge elements, and develop useful interpersonal skills (Coggans, 2006). There is an ongoing discussion regarding the content, as well as delivery measures, of the ideal substance use prevention program. The reader is referred to a review of school prevention programs and guidelines by Sloboda (2008). Evaluation of students' cannabis-related knowledge may be useful in the development of knowledge-based prevention programs and university courses tailor-made for a group of students, considering their socio-demographic characteristics. It may show, for example, where the gaps in education are, envisage students' understanding of the delivered content, or evaluate how much basic knowledge courses should include. Some elements that were found to play crucial role in respect to the efficacy of drug prevention programs are summarized in Fig. 21.5. An example of a drug prevention course can be found in paper by Newton et al. (2010). As similar to the development of school-oriented programs, reports concerning students' knowledge may serve as a basis for establishing and improving education tools for specialized drug counselors and health professionals, as well as internet-based drug prevention programs and media campaigns (Derzon & Lipsey, 2001; Tossman & Leuschner, 2009).

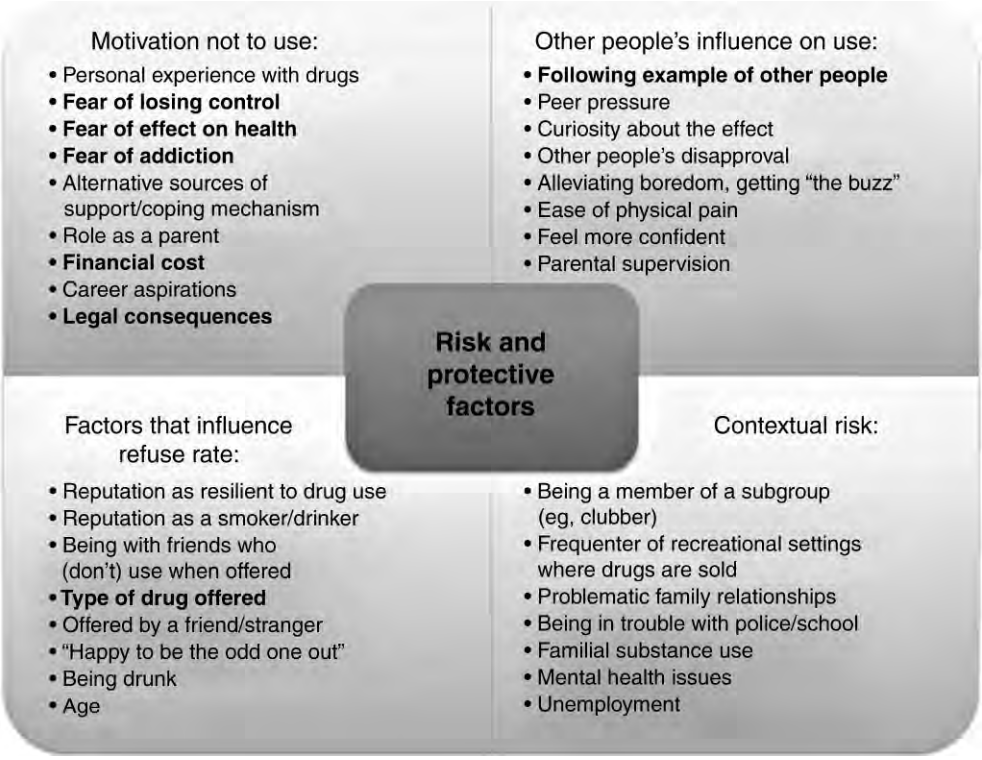


FIGURE 21.4 **Risk and protection factors influencing substance use.** Diagram summarizing various risk and protection factors. Factors that can be influenced by knowledge-based interventions are highlighted (bold). *Source: Based on report of UK Home Office (2007).*

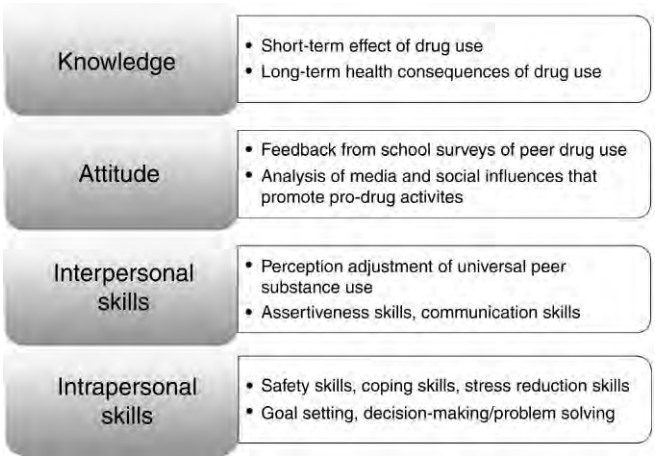


FIGURE 21.5 **Content of successful drug prevention program at school.** Content to include in school-based drug prevention programs. *Source: Based on review of Tobler, Lessard, Marshall, Ochshorn, and Roona (1999).*

CANNABIS-RELATED KNOWLEDGE AND ATTITUDES IN THE CONTEXT OF MEDICAL USE OF CANNABIS

Since more cannabinoid-based pharmaceutical products are expected to be developed, it's interesting to look at one of the issues that has significant impact on the drugs' clinical treatment outcomes (ie, treatment efficacy and safety), namely the medication compliance (see the Key facts on medication compliance; PGEU, 2008). Medication compliance is defined as the extent to which a patient correctly follows medical advice (eg, treatment regimen, lifestyle tips, advice concerning disease management). Causes of low medication compliance include: socioeconomic factors (eg, age, economic status, treatment cost), patient-related factors (eg, forgetfulness, misunderstood instructions, treatment anxiety, fear of developing dependence on medication), drug-related factors (eg, length and complexity of treatment, adverse reactions (ADRs—see the Mini-dictionary), as well as specific, condition-related factors (Vermeire, Hearnshaw, Van Royen, & Denekens, 2001). Some problems hindering the therapeutic use of cannabis and cannabinoids (eg, patient substituting pharmaceutical cannabinoid dosage forms with a cannabis product) were mentioned by Grinspoon (2001). Moreover, patients with a history of substance use may try to induce a euphoric effect by exceeding the therapeutic dose, or polydrug use (Grant, Atkinson, Gouaux, & Wilsey, 2012). Finally, patients may undergo drug tests at their workplace, which aim to identify persons involved in illicit substance use. However, tests would not discriminate between recreational and medical cannabis use (Neavyn, Blohm, Babu, & Bird, 2014).

In order to initiate the treatment, patients must believe that (1) they suffer from the disease, (2) the disease will most likely negatively impact their wellbeing, and (3)

the severity or threat of the disease will be diminished or abolished, considering they follow health recommendations. There are many concerns and misconceptions arising from patients' inadequate knowledge on cannabis that are expected to affect medication compliance negatively. Firstly, patients may be concerned about becoming dependent on their medication, in the long run, due to the addictive potential of tetrahydrocannabinol (THC). This has previously been observed for methylphenidate, a remedy for attention-deficit hyperactivity disorder (Berger, Dor, Nevo, & Goldzweig, 2008). Secondly, patients may be worried about other ADRs associated with cannabinoids, especially when the treatment of an off-label indication is considered (Wang, Collet, Shapiro, & Ware, 2008). THC-induced intoxication and euphoria, as well as other nervous system disorders, may be particularly annoying in elderly patients (Grant et al., 2012). The most frequently reported ADRs of Sativex, that is, drowsiness, dizziness, and fatigue, have already been identified as a reason for low medication compliance in the treatment of muscle spasms with baclofen (Halpern, Gillard, Graham, Varon, & Zorowitz, 2013; Sativex, 2014). Ware, Doyle, Woods, Lynch, and Clark (2003) surveyed almost 3000 patients using medical cannabis. Out of 403 patients that discontinued the treatment, 60 reported euphoria or other ADRs as a reason for discontinuing medicinal cannabis. Moreover, cannabinoids may interact with other drugs taken by patients (eg, antidepressants, neuroleptic and analgesic agents), leading to ADR potentiation (Seamon, Fass, Maniscalco-Feichtl, & Abu-Shraie, 2007).

When faced with a necessity of adhering to a treatment regimen, patients confront the health-care professional's advice with their own set of beliefs (Vermeire et al., 2001). The proposed therapeutic schedule is confronted with perceived group and social norms, as well as the perceived social consequences regarding the behavior and its acceptability (van Dulmen et al., 2007). The opinion of patients' family and friends will influence the medication compliance in the form of negative or positive social influence. Social support to medication compliance includes increased motivation, confidence, and internalization of social norms that promote healthy choices. A nonsupportive social influence can interfere with beneficial health behavior, and discourage following the medication regimen (DiMatteo, 2004). We hypothesize that if the patient has low cannabis-related knowledge, perceives cannabis as a dangerous substance with detrimental effect on human health, and associates it primarily with drug abuse, he or she will be reluctant to initiate and follow treatment with cannabinoids. Considering the perceived risk of using given medication is high, patients would try to minimize the possible damage. Patients may postpone the treatment initiation, cease to follow the medication regimen (eg, reduce the dose or frequency), or stop using a drug. Chong et al. (2006) have previously surveyed 254 MS patients using medical cannabis (Chong et al., 2006). One-quarter of respondents expressed concerns on possible social stigma (25%), a percentage being comparable with patients who were worried by becoming dependent (31%), or suffering from drug-drug interactions (27%).

The issue of nonadherence should be addressed early on (by, for example, patient education), when introducing treatment with medical cannabis or synthetic cannabinoids, as pointed out by Grant et al. (2012) . Education programs aim to increase patient compliance, promote healthy choices, change disease and treatment perception, and improve disease management (van Dulmen et al., 2007). Evaluation of students' cannabis-related knowledge and attitude may be used to analyze the expected knowledge of a nonmedical professional, in order to build up pharmaceutical care education and counseling. It will also serve as a background for studies concerning cannabinoid medication compliance, and predicting the compliance pattern in a given country. In this regard, several issues can be addressed when developing a survey. Firstly, the level of both basic knowledge on cannabis and knowledge on specific cannabis uses, as well as sources of knowledge on cannabis, should be studied. Moreover, the knowledge on side effects and risk perception, as well as attitude toward medical cannabis, should be evaluated. Currently, there is a limited number of such reports.

Our web-based study attempted to assess the level of university students' knowledge concerning positive and negative effects of cannabis on human health, as well as sources of knowledge on cannabis (Sobczyński et al., 2013). Although respondents knew about the possible medical use of cannabis, and recognized the potentially useful impact of cannabis on human health, they were more likely to associate cannabis with its psychoactive rather than therapeutic use (Fig. 21.6). While around 40% of respondents knew that cannabis could be used by patients suffering from cancer or MS, students didn't know of registered pharmaceutical cannabinoids, probably due to lack of registered cannabis products in Polish pharmacies in 2011. There was a distinct difference in knowledge on cannabis and cannabis perception, depending on the respondents' gender (Fig. 21.6). Women were more likely to recognize the health risk associated with cannabis use, as similar to previous reports (Taylor, 1972). Men listed more different sources of cannabis-related knowledge, and were more likely to list textbooks and personal experience with cannabis. More men than women recognized the medical use of cannabis, and knew the term "medical marijuana." In a follow-up study, performed in 2014, we surveyed another sample of university students

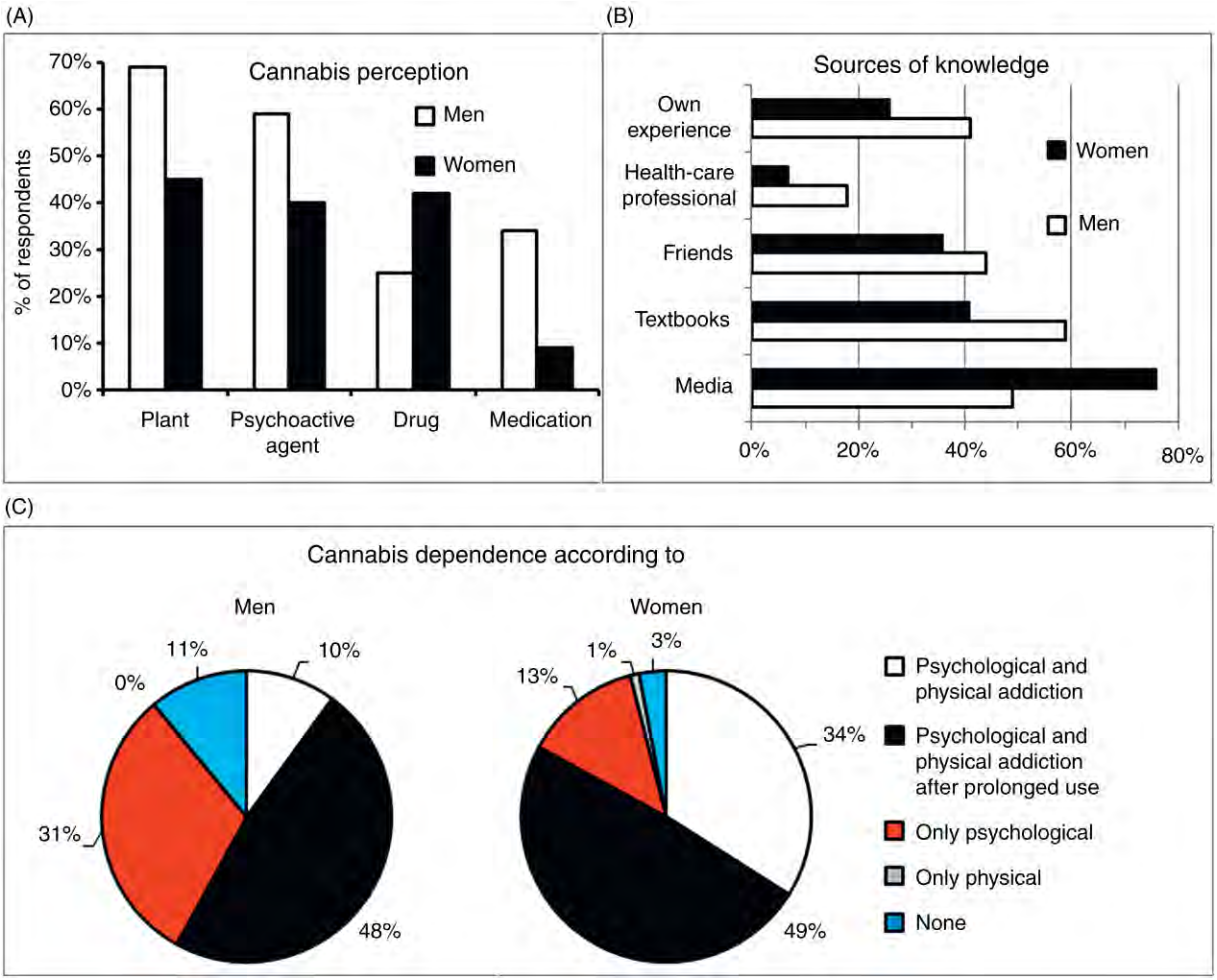


FIGURE 21.6 **Gender differences in cannabis-related knowledge and risk perception among Polish students.** Cannabis perception (A), sources of cannabis-related knowledge (B), and perception of cannabis addictive potential (C), according to male and female university students (aged 18–23, *n* =176). *Source: Based on Ostapkiewicz (2012), and reproduced from Sobczyński et al. (2013), with permission of the copyright owner.*

(Sobczyński, Drozd, manuscript in preparation). In the period of 2011–2014, Polish media addressed the topic of legalization of cannabis use in some American states, and the first cannabinoid pharmaceutical preparation, Sativex, obtained market authorization in Poland (Sativex, 2014). This was presumably the reason for an increase in self-reported positive cannabis effects on human health, and in a higher recognition rate of medical cannabis (Fig. 21.7).

MINI-DICTIONARY

Adverse drug reaction Undesired, harmful reaction, following taking medication in the prescribed dose that may be predictable, dependent (majority of cases) or independent of dose.
Cannabis product Cannabis products (eg, cannabis herbal preparation or resin) are derived from the cannabis plant; cannabis products are characterized by different composition, THC content, and subsequently different potency due to variety in plant genetics, mode of cultivation, and processing.
Cannabis-related knowledge An individual's knowledge of short- and long-term effects of cannabis on health and behavior, as well as the addictive potential of cannabis products.
Health-care professional Person who, by education, training, certification, or licensure is qualified to, and engaged in, providing health-care (eg, doctor, nurse, pharmacist).
Medical cannabis This term denotes the use of cannabis or its active ingredients—cannabinoids such as THC and cannabidiol (CBD)—in the treatment of diseases, or for alleviating symptoms.
Medication compliance The patient's conscious decision to undertake treatment medication as prescribed.
Pharmaceutical cannabinoid products Pharmaceutical product in the form in which it is intended for use (eg, a tablet, spray, capsule), with an active component—cannabinoid drug(s)—and inactive components, facilitating formulation.
Polydrug use Refers to concomitant or consecutive use of two psychoactive agents in order to potentiate their action, or lessen side effects.
School-based drug prevention program These programs may include school-day or afterschool classes delivered by school teachers or specialized personnel, and may be mandatory or voluntary. Their main goal is to deter or to delay the onset of drug use by providing students with the skills and knowledge to address the problem.

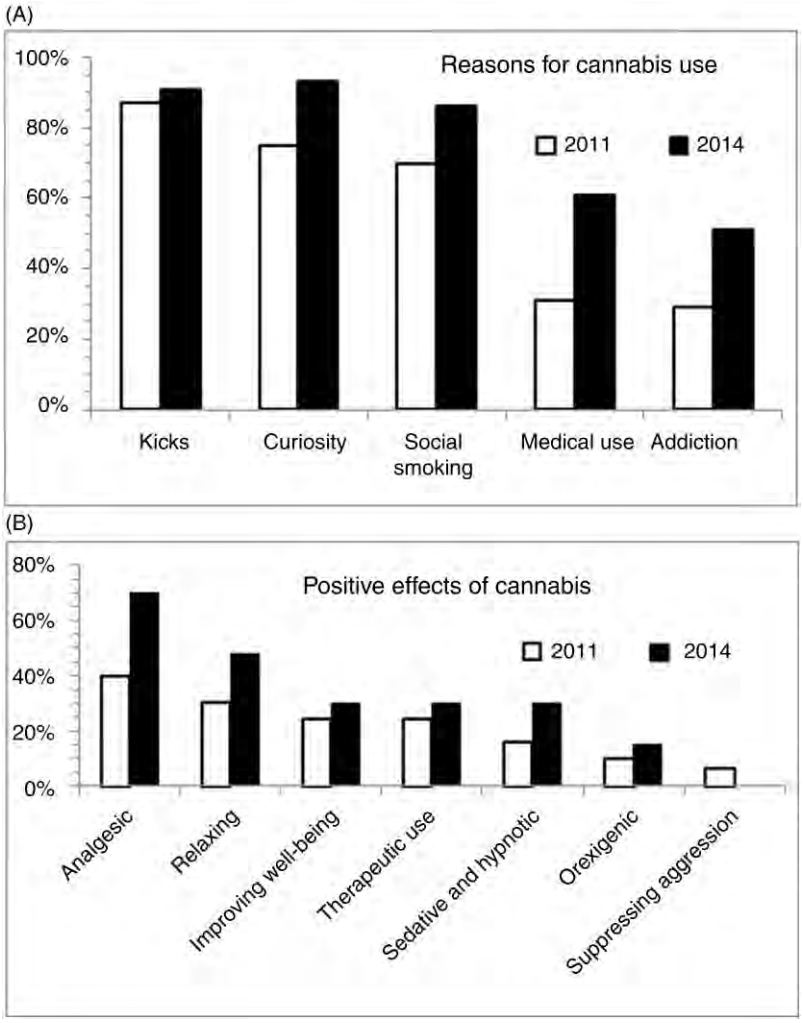


FIGURE 21.7 Difference in students' perception of cannabis in relation to medical use, in 2011 and 2014. Reasons for cannabis use (A), and self-reported positive effects of cannabis on human health (B). *Source: Based on our previous work (Sobczyński et al., 2013; n = 176) and a survey, performed in 2014 (unpublished data; n = 114). Adapted and reproduced with permission of the copyright owner.*

References

Berger, I., Dor, T., Nevo, Y., & Goldzweig, G. (2008). Attitudes toward attention-deficit hyperactivity disorder (ADHD) treatment: parents' and children's perspectives. *Journal of Child Neurology*, *23*(9), 1036–1042.

Borgelt, L. M., Franson, K. L., Nussbaum, A. M., & Wang, G. S. (2013). The pharmacologic and clinical effects of medical cannabis. *Pharmacotherapy*, *33*(2), 195–209.

Charuvastra, A., Friedmann, P. D., & Stein, M. D. (2005). Physician attitudes regarding the prescription of medical marijuana. *Journal of Addictive Diseases*, *24*(3), 87–93.

Chong, M. S., Wolff, K., Wise, K., Tanton, C., Winstock, A., & Silber, E. (2006). Cannabis use in patients with multiple sclerosis. *Multiple Sclerosis*, *12*(5), 646–651.

Coggans, N. (2006). Drug education and prevention: has progress been made? *Drugs: Education, Prevention, and Policy*, *13*(5), 417–422.

Crano, W. D., Siegel, J. T., Alvaro, E. M., Lac, A., & Hemovich, V. (2008). The at-risk adolescent marijuana nonuser: expanding the standard distinction. *Prevention Science*, *9*(2), 129–137.

Danseco, E. R., Kingery, P. M., & Coggeshall, M. B. (1999). Perceived risk of harm from marijuana use among youth in the USA. *School Psychology International*, *20*(1), 39–56.

Derzon, J. H., & Lipsey, M. W. (2001). A meta-analysis of the effectiveness of mass-communication for changing substance-use knowledge, attitudes, and behavior. In W. D. Crano, M. Burgoon, & S. Oskamp (Eds.), *Mass media and drug prevention: Classic and contemporary theories and research* (pp. 231–256). Mahwah, NJ: Psychology Press.

DiMatteo, M. R. (2004). Social support and patient adherence to medical treatment: a meta-analysis. *Health Psychology*, *23*(2), 207–218.

Engs, R. C., & Mullen, K. (1999). The effect of religion and religiosity on drug use among a selected sample of post secondary students in Scotland. *Addiction Research and Theory*, *7*(2), 149–170.

Faggiano, F., Vigna-Taglianti, F. D., Versino, E., Zambon, A., Borraccino, A., & Lemma, P. (2005). School-based prevention for illicit drugs' use. *Cochrane Database Systematic Reviews*, *2*, 1–98.

Franca, L. R., Dautzenberg, B., Falissard, B., & Reynaud, M. (2010). Peer substance use overestimation among French university students: a cross-sectional survey. *BMC Public Health*, *10*(1), 169–179.

Geramian, N., Akhavan, S., Gharaat, L., Tehrani, A. M., & Farajzadegan, Z. (2012). Determinants of drug abuse in high school students and their related knowledge and attitude. *Journal of the Pakistan Medical Association*, *62*(Suppl. 2), 62–66.

Grant, I., Atkinson, J. H., Gouaux, B., & Wilsey, B. (2012). Medical marijuana: clearing away the smoke. *Open Neurology Journal*, *6*, 18–25.

Grinspoon, L. (2001). On the pharmaceuticalization of marijuana. *International Journal of Drug Policy*, *12*(5), 377–383.
Hall, W., & Degenhardt, L. (2009). Adverse health effects of non-medical cannabis use. *Lancet*, *374*(9698), 1383–1391.
Halpern, R., Gillard, P., Graham, G. D., Varon, S. F., & Zorowitz, R. D. (2013). Adherence associated with oral medications in the treatment of spasticity. *Physical Medicine and Rehabilitation*, *5*(9), 747–756.
Heckman, C. J., Dykstra, J. L., & Collins, B. N. (2011). Substance-related knowledge, attitude, and behaviour among college students. Opportunities for health education. *Health Education Journal*, *70*(4), 383–399.
Hibell, B., Guttormsson, U., Ahlström, S., Balakireva, O., Bjarnason, T., Kokkevi, A., & Kraus, L. (2012). *The 2011 ESPAD report. Substance use among students in 36 European countries*. Stockholm, Finland Sweden: The Swedish Council for Information on Alcohol and other Drugs.
Iannotti, R. J., & Bush, P. J. (1992). Perceived vs. actual friends' use of alcohol, cigarettes, marijuana, and cocaine: which has the most influence? *Journal of Youth and Adolescence*, *21*(3), 375–389.
Johnston, L. D., O'Malley, P. M., Bachman, J. G., & Schulenberg, J. E. (2010). *Monitoring the future: national survey results on drug use, 1975-2009. Volume I: Secondary school students*. Rockville, MD: National Institute on Drug Abuse (NIDA), NIH Publication No. 10-7584.
Kondrad, E., & Reid, A. (2013). Colorado family physicians' attitudes toward medical marijuana. *Journal of the American Board of Family Medicine*, *26*(1), 52–60.
Leung, L. (2011). Cannabis and its derivatives: review of medical use. *Journal of the American Board of Family Medicine*, *24*(4), 452–462.
Lewis, T. F., & Clemens, E. (2008). The influence of social norms on college student alcohol and marijuana use. *Journal of College Counseling*, *11*(1), 19–31.
Machado Rocha, F. C., Stefano, S. C., De Cassia Haiek, R., Rosa Oliveira, L. M. Q., & Da Silveira, D. X. (2008). Therapeutic use of *Cannabis sativa* on chemotherapy-induced nausea and vomiting among cancer patients: systematic review and meta-analysis. *European Journal of Cancer Care*, *17*(5), 431–443.
Macleod, J., Oakes, R., Copello, A., Crome, I., Egger, M., Hickman, M., Oppenkowski, T., Stokes-Lampard, H., & Smith, G. D. (2004). Psychological and social sequelae of cannabis and other illicit drug use by young people: a systematic review of longitudinal, general population studies. *Lancet*, *363*(9421), 1579–1588.
Marcoux, R. M., Larrat, E. P., & Vogenberg, F. R. (2013). Medical marijuana and related legal aspects. *Pharmacy and Therapeutics*, *38*(10), 612.
McCabe, S. E. (2008). Misperceptions of non-medical prescription drug use: a web survey of college students. *Addictive Behaviors*, *33*(5), 713–724.
Neavyn, M. J., Blohm, E., Babu, K. M., & Bird, S. B. (2014). Medical marijuana and driving: a review. *Journal of Medical Toxicology*, 1–11.
Newton, N. C., Andrews, G., Teesson, M., & Vogl, L. E. (2009). Delivering prevention for alcohol and cannabis using the internet: a cluster randomized controlled trial. *Preventive Medicine*, *48*(6), 579–584.
Newton, N. C., Teesson, M., Vogl, L. E., & Andrews, G. (2010). Internet-based prevention for alcohol and cannabis use: final results of the Climate Schools course. *Addiction*, *105*(4), 749–759.
O'Callaghan, F., Reid, A., & Copeland, J. (2006). Risk perception and cannabis use in a sample of young adults. *Journal of Substance Use*, *11*(2), 129–136.
Ostapkiewicz, A. (2012). *Evaluation of students' knowledge of cannabis influence on human health*. Lublin, Poland: Medical University of Lublin, Master thesis.
Pacula, R. L., Grossman, M., Chaloupka, F. J., O'Malley, P. M., Johnston, L., & Farrelly, M. C. (2000). *Marijuana and youth*. Cambridge, MA: National Bureau of Economic Research, No. w7703.
PGEU. (2008). *Targeting Adherence. Improving Patient Outcomes in Europe through Community Pharmacists' Intervention*. Brussels, Belgium: Pharmaceutical Group of the European Union (PGEU).
Ramirez, J. R., Crano, W. D., Quist, R., Burgoon, M., Alvaro, E. M., & Grandpre, J. (2004). Acculturation, familism, parental monitoring, and knowledge as predictors of marijuana and inhalant use in adolescents. *Psychology of Addictive Behaviors*, *18*(1), 3.
Sloboda, Z. (2008). Moving towards evidence-based practice: school-based prevention of substance use in the USA. In S. Rödner Sznitman, B. Olsson, & R. Room (Eds.), *A cannabis reader: global issues and local experiences. Monograph series 8 (Vol. 1)*. Lisbon, Portugal: EMCDDA.
Sativex. (2014). *Public Assessment Report. Decentralised Procedure. Sativex Oromucosal Spray*. London, UK: GW Pharma Limited.
Seamon, M. J., Fass, J. A., Maniscalco-Feichtl, M., & Abu-Shraie, N. A. (2007). Medical marijuana and the developing role of the pharmacist. *American Journal of Health-System Pharmacy*, *64*(10), 1037–1044.
Sobczyński, J., Drozd, M., Wośko, S., Wielgus, S., Ostapkiewicz, A., Kochaniec, M., & Szymańska, J. (2013). Evaluation of students' knowledge of cannabis influence on human health. *Current Issues in Pharmacy and Medical Sciences*, *26*(2), 235–239.
Sobczyński, J., Drozd, M. (2016). Knowledge and perception regarding cannabis among Polish students. Unpublished manuscript.
Taylor, H. (1972). Opinions on marijuana: sex differences at a Western Canadian university. *Canadian Journal of Counselling and Psychotherapy*, *6*(2), 116–119.
The European Commission: Eurobarometer. (2008). Young people and drugs among 15-24 year-olds. Analytical report. Brussels, Belgium: Directorate of General Communication.
The European Commission: Eurobarometer. (2011). *Youth attitudes on drugs. Analytical report*. Brussels, Belgium: Directorate of General Communication.
Tobler, N. S., Lessard, T., Marshall, D., Ochshorn, P., & Roona, M. (1999). Effectiveness of school-based drug prevention programs for marijuana use. *School Psychology International*, *20*(1), 105–137.
Tossman, H. -P., & Leuschner, F. (2009). *Internet-based drug treatment interventions. Best practice and applications in EU Member States*. Lisbon, Portugal: EMCDDA, EMCDDA Insights No 10.
UK Home Office (2007), Identifying and exploring young people's experiences of risk, protective factors and resilience to drug use, London: Home Office Development and Practice Report, Stationery Office.
Uritsky, T. J., McPherson, M. L., & Pradel, F. (2011). Assessment of hospice health professionals' knowledge, views, and experience with medical marijuana. *Journal of Palliative Medicine*, *14*(12), 1291–1295.
Wang, T., Collet, J. P., Shapiro, S., & Ware, M. A. (2008). Adverse effects of medical cannabinoids: a systematic review. *Canadian Medical Association Journal*, *178*(13), 1669–1678.
Ware, M. A., Doyle, C. R., Woods, R., Lynch, M. E., & Clark, A. J. (2003). Cannabis use for chronic non-cancer pain: results of a prospective survey. *Pain*, *102*(1), 211–216.
Wolfson, S. (2000). Students' estimates of the prevalence of drug use: evidence for a false consensus effect. *Psychology of Addictive Behaviors*, *14*(3), 295.
van Dulmen, S., Sluijs, E., van Dijk, L., de Ridder, D., Heerdink, R., & Bensing, J. (2007). Patient adherence to medical treatment: a review of reviews. *BMC Health Services Research*, *7*(1), 55–68.
Vermeire, E., Hearnshaw, H., Van Royen, P., & Denekens, J. (2001). Patient adherence to treatment: three decades of research. A comprehensive review. *Journal of Clinical Pharmacy and Therapeutics*, *26*(5), 331–342.
von Sydow, K., Lieb, R., Pfister, H., Höfler, M., & Wittchen, H. U. (2002). What predicts incident use of cannabis and progression to abuse and dependence? A 4-year prospective examination of risk factors in a community sample of adolescents and young adults. *Drug and Alcohol Dependence*, *68*(1), 49–64.

CHAPTER

22

Childhood Trauma and Cannabis: Risk Factors in Severe Mental Disorders?

M. Aas, I. Melle

NORMENT, KG Jebsen Centre for Psychosis Research, Division of Mental Health and Addiction, Oslo University Hospital & Institute of Clinical Medicine, University of Oslo, Oslo, Norway

SUMMARY POINTS

- This chapter focuses on the role of childhood trauma and cannabis as potential risk factors in severe mental disorders, focusing on schizophrenia spectrum, or bipolar illness.
- A history of childhood trauma is associated with use of cannabis in adolescence and early adulthood.
- The literature supports the additive effects of having both cannabis abuse and childhood trauma, and more severe clinical features of schizophrenia or bipolar disorders.
- This chapter has also discussed the putative idea behind the cooccurrence of cannabis use in individuals with high levels of childhood trauma, focusing on reported opposite effects on the body's stress system, the HPA axis.
- This chapter focuses on the importance of gathering information on early adverse events, as well as cannabis use, when investigating mechanisms behind severe mental illness.

KEY FACTS OF HYPOTHALAMIC PITUITARY-ADRENAL (HPA) AXIS

- The HPA axis is the main endocrine system involved in the stress response.
- The main stress hormone, cortisol, is affected by a variety of physiological and psychological stressors.
- Acute stressful experiences mobilize adaptive changes in the bodily processes and performance that help to meet the strain of environmental challenges, and protect against treats to the internal homeostasis (Sapolsky, 2000; Selye, 1975).
- While long-term stress can lead to maladaptive changes in bodily processes and performance, they also have a negative effect on health, cognition and mood (McEwen, 1998).
- Patients with severe mental illness, such as psychosis have an abnormal HPA axis. Both increased cortisol levels during the day, as well as a blunted awakening response is described in the literature (Mondelli, 2014).

LIST OF ABBREVIATIONS

CBD	Cannabidiol
CI	Confidence interval
CTQ	Childhood Trauma Questionnaire
HPA axis	Hypothalamic-pituitary-adrenal (HPA) axis
OR	Odds ratio
SCID	Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (DSM)
SD	Standard deviation
THC	Tetrahydrocannabinol

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00024-7
Copyright © 2017 Elsevier Inc. All rights reserved.

INTRODUCTION

Several studies demonstrate that childhood trauma and cannabis use are associated with increased risk for schizophrenia and bipolar disorders, as well as increased symptom severity. Could it be that a history of childhood trauma increases the risk of using cannabis, which is then linked to psychopathology in vulnerable individuals? This chapter will cover the literature on childhood trauma and psychopathology, and cannabis use and psychopathology, and will aim to answer the question aforementioned. This chapter will also investigate the relationship between a history of childhood trauma and cannabis use, with the development of mental disorders, focusing on schizophrenia spectrum, and bipolar disorders.

CHILDHOOD TRAUMA AND MENTAL DISORDERS

The literature demonstrates associations between a history of childhood trauma and mental disorders in adulthood, both by increasing the risk for developing a mental disorder, and by increasing illness severity. For example, childhood trauma is well documented in the literature as a potential risk factor for developing psychosis. A recent large scale metaanalysis concluded that childhood trauma is a strong environmental risk factor for developing psychosis, with an overall effect of OR = 2.78 (95% CI = 2.34–3.31) (Varese et al., 2012). Subjects (45,441) were included in the metaanalysis gathered from case control studies, prospective studies, and cross-sectional studies. Across the different studies, it was concluded that a history of childhood trauma was more frequently reported in high-risk individuals, compared to healthy controls, and also associated with the development of psychopathology severity (Sahin et al., 2013; Thompson et al., 2010). In our own work, we have previously shown that childhood trauma is associated with reduced cognitive function, in two independent psychosis studies (Aas et al., 2011a, 2012c). We have also demonstrated a relationship between a history of childhood trauma and brain volume reduction of limbic areas of the amygdala and hippocampus (Aas et al., 2012b), as well as an interaction with genetic risk variants in psychosis (Aas et al., 2012a, 2013b).

Another recent metaanalysis also confirmed that childhood trauma is associated with bipolar disorders, both by increased prevalence, and more severe clinical characteristics (Daruy-Filho, Brietzke, Lafer, & Grassi-Oliveira, 2011). These findings are supported by two recent large studies (Aas et al., 2013a; Etain et al., 2013). The study by Etain et al. (2013; $n = 587$), demonstrated that a history of childhood trauma was associated with more than 5 years earlier age at onset, in the patients reporting three types of childhood trauma (emotional, sexual, and physical abuse), compared to those reporting no childhood abuse, in a dose-dependent manner (Fig. 22.1). Moreover, a history of childhood trauma has also been linked to a rapid cycling course (Garno, Goldberg, Ramirez, & Ritzler, 2005; Etain et al., 2013), affective lability (Aas et al., 2014; Fig. 22.2), psychotic features (Bebbington et al., 2004; Janssen et al., 2004; Shevlin, Dorahy, & Adamson, 2007), number of lifetime mood episodes (Brown, McBride, Bauer, & Williford, 2005; Nemeroff, 2004; Weber et al., 2008; Etain et al., 2013), and suicide ideation and attempts (Alvarez et al., 2011; Etain et al., 2013).

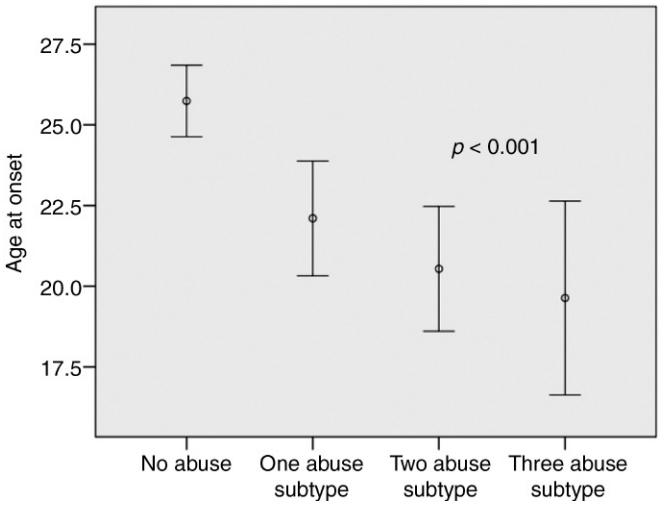


FIGURE 22.1 Age of onset in years (mean, and standard error) and childhood abuse (Etain et al., 2013). Fig. 22.1 illustrates a dose relationship between childhood abuse and earlier age at onset. The more abuse subtypes the patient report, the lower the age at onset. Kruskal–Wallis Test; $p < 0.001$; no abuse: $n = 361$, mean ± SD = 25.76 ± 10.54; one abuse subtype: $n = 114$, mean ± SD = 22.11 ± 9.49; two abuse subtypes: $n = 61$, mean ± SD = 20.54 ± 7.55; three abuse subtypes: $n = 22$, mean ± SD = 19.64 ± 7.05. *Source: Figure published in Etain, B., Aas, M., Andreassen, O. A., Lorentzen, S., Dieset, I., Gard, S., et al. (2013). Childhood trauma is associated with severe clinical characteristics of bipolar disorders.* Journal of Clinical Psychiatry, 74, *991–998.*

A history of childhood trauma has also been associated with unipolar depression (Heim, Newport, Mletzko, Miller, & Nemeroff, 2008b), including long-term changes in the hypothalamic-pituitary-adrenal (HPA) axis, as well as long-term epigenetic changes (Heim, Mletzko, Purselle, Musselman, & Nemeroff, 2008a; Heim et al., 2008b; Nemeroff & Binder, 2014). Long-term changes in the HPA axis are also found in patients with psychosis reporting high levels of childhood trauma (Mondelli et al., 2010). Therefore, there is reasonably consistent evidence that childhood trauma is associated with an increased risk for development of severe mental disorders in adulthood, by interacting with specific genes, as well as having a long-term influence on specific biomarkers, such as the HPA axis.

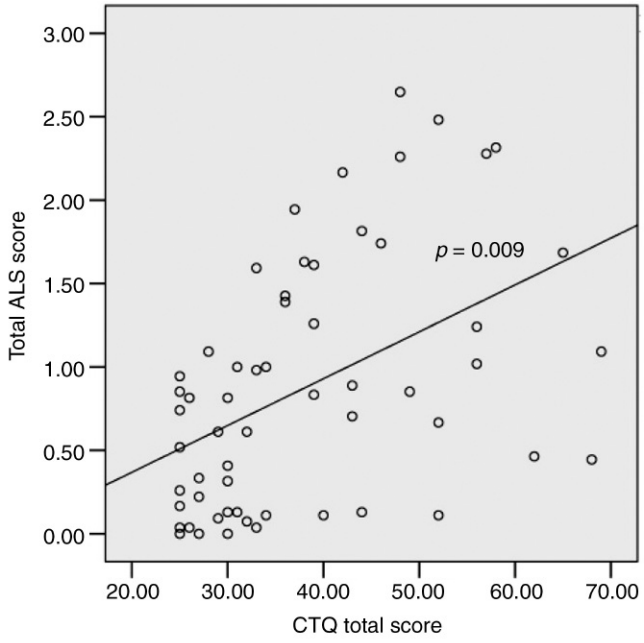


FIGURE 22.2 Affective lability score (ALS) and CTQ total score (Aas et al., 2014). Fig. 22.2 illustrates that childhood trauma is associated with higher score on the affective liability scale. Spearman's correlation: $r = 0.40$, $p = 0.009$, CTQ = Childhood Trauma Questionnaire. *Source: Figure published in Aas, M., Aminoff, S. R., Vik, L. T., Etain, B., Agartz, I., Andreassen, O. A., et al. (2014). Affective lability in patients with bipolar disorders is associated with high levels of childhood trauma. Psychiatry Research, 218* (1–2), 252–255.

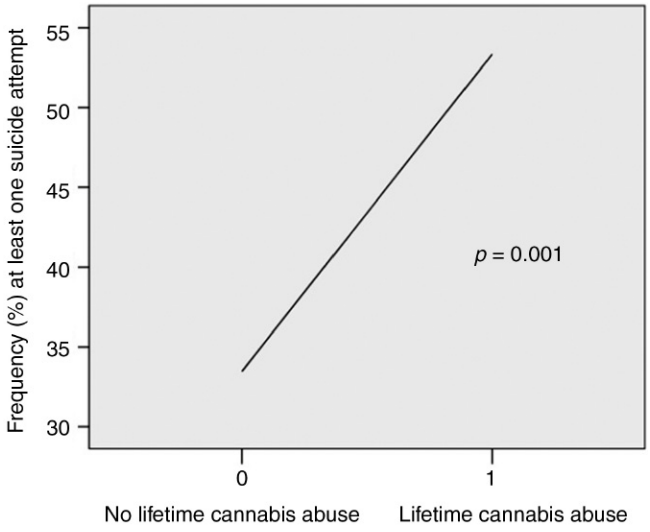


FIGURE 22.3 Cannabis abuse and suicide attempt (wish to die) (Aas et al., 2013a). Fig. 22.3 illustrates that cannabis abuse is associated with higher prevalence of having at least one suicide attempt (wish to die) in bipolar patients, compared to cases without cannabis abuse. Chi-square, $p = 0.001$ "no lifetime cannabis abuse," $n = 368$; "lifetime cannabis abuse," $n = 211$. *Source: Figure published in Aas, M., Etain, B., Bellivier, F., Henry, C., Lagerberg, T., Ringen, A., et al. (2013a). Additive effects of childhood abuse and cannabis abuse on clinical expressions of bipolar disorders. Psychological Medicine, 1–10.*

CANNABIS AND MENTAL DISORDERS

In addition to childhood trauma, one of the most consistently reported environmental risk factors for psychotic disorders is cannabis use. Several studies demonstrate that the use of cannabis increases the risk of schizophrenia (Andreasson, Allebeck, Engstrom, & Rydberg, 1987; van Os et al., 2002), with further reports of similar findings for bipolar disorders (Lev-Ran, Le, McKenzie, George, & Rehm, 2013). Moreover, cannabis has been shown to correlate with severity of illness in psychotic disorders. In addition, among both patients with schizophrenia and in bipolar disorders, those who report cannabis use have an earlier age at onset (Di Forti et al., 2014; Lagerberg et al., 2011, 2014). Moreover, in a large study of bipolar disorders (n = 471) and healthy controls (n = 1761), bipolar patients were 6.8 times more likely to report a lifetime history of cannabis abuse, than healthy controls (Agrawal, Nurnberger, & Lynskey, 2011). Agrawal et al. (2011) also reported associations between cannabis abuse and increased suicide attempts, mixed episodes, and greater disability, with similar findings found in the study by Aas et al. (2013a); see Fig. 22.3. Post (2010) supports these findings by linking drug abuse to increased sensitization, and vulnerability to recurrent episodes, thus driving illness progression in bipolar disorders. However, research on this field is sparse, and more studies are needed.

ARE PEOPLE WHO EXPERIENCE CHILDHOOD TRAUMA MORE PRONE TO ILLICIT DRUG USE, INCLUDING CANNABIS, LATER IN LIFE?

Individuals reporting a history of childhood trauma are more prone to substance abuse in adulthood (Aas et al., 2013a; Etain et al., 2013; Fergusson, Boden, & Horwood, 2008; Fig. 22.4). A 25 year-long prospective study examining the development of illicit drug use, from a birth cohort of 1265 New Zealand children, found that childhood sexual and physical abuse are the main predictors for illicit drug use at ages 16–25 (Fergusson et al., 2008). A national longitudinal study of adolescent Heath (AddHealth) of 12,748 adolescents found that maltreatment in childhood is significantly associated with increased binge drinking in adolescence, with 12.4% prevalence of binge drinking, compared to 9.9% among those reporting no maltreatment (Shin, Edwards, & Heeren, 2009).

Both illicit drug use, including cannabis use, and a history of childhood trauma are associated with increased risk of psychosis, and both should be taken into account when investigating risk factors for psychosis (Houston, Murphy, Shevlin, & Adamson, 2011). As described in the paper by Aas et al. (2013a), the possible

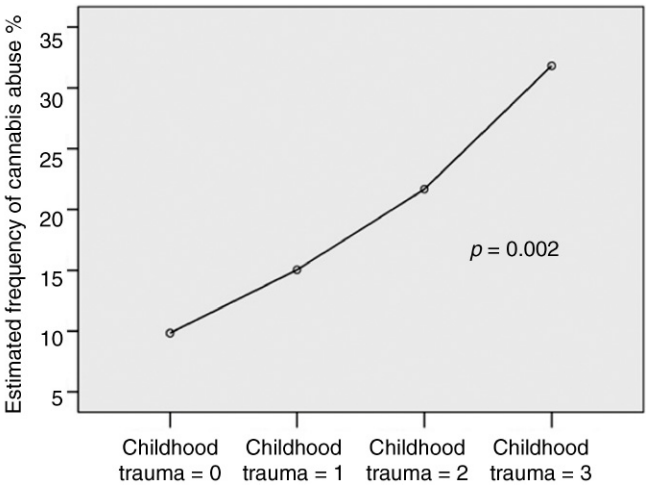


FIGURE 22.4 Substance abuse and childhood trauma abuse dose effect (Aas et al., 2013a,b). Fig. 22.4 illustrates a dose effect of number of childhood trauma experiences and use of cannabis: $X^2 = 14.52$, df = 3, $p = 0.002$. Based on Bernstein's cutoff scores for moderate to severe abuse; no abuse, $n = 366$; one abuse subtype, $n = 113$; two abuse subtypes, $n = 60$; three abuse subtypes, $n = 22$. *Source: Figure published in Etain, B., Aas, M., Andreassen, O. A., Lorentzen, S., Dieset, I., Gard, S., et al. (2013). Childhood trauma is associated with severe clinical characteristics of bipolar disorders.* Journal of Clinical Psychiatry, 74, *991–998.*

mechanism behind the cooccurrence of childhood abuse and cannabis abuse could be related to their having opposite effects on the HPA axis (Heim et al., 2008a; van Leeuwen et al., 2011). Cannabis and substance use have been linked to a reduction in HPA activity (van Leeuwen et al., 2011), whilst a history childhood trauma has been linked to increased HPA axis, specifically in depression (Heim et al., 2008a,b). Thus, it could be hypothesized that cannabis or substance use in individuals with a history of childhood trauma may be viewed as a way of "regulating" the HPA axis. Further studies here are needed.

WHAT IS THE LINK BETWEEN A HISTORY OF CHILDHOOD TRAUMA, CANNABIS USE, AND SEVERE MENTAL DISORDERS?

Both childhood trauma and cannabis use are linked to bipolar disorders, both by increased frequency, and severity of clinical characteristics. The question is whether they influence the clinical picture separately or in tandem. We have shown previously that individuals reporting childhood trauma tend to show increased risk of substance abuse in adulthood, compared to individuals without childhood trauma, thus indicating possible interactions or additive effects. It may also be that relationships to clinical expressions are driven, or mediated by, either cannabis abuse or childhood trauma. A recent paper by Morgan et al. (2014) aimed to answer this question from a dataset consisting of 1680 individuals, as part of the South East London Community Heath Study (SELCoh). The authors found a strong association between childhood abuse and other environmental risk factors for psychosis, including cannabis use, and between later life events and an increased odds ratio of psychotic experiences. Moreover, the additive effects of different risk factors were larger than when investigating them separately. The large study ($n = 7403$) from the Adult Psychiatric Morbidity Survey 1997, from the United Kingdom, supports the findings previously; the participants reporting both nonconsensual sexual experiences in childhood, and cannabis consumption, had more than a 7 times (OR 7.84) greater likelihood to be diagnosed with psychosis, compared to participants without these experiences. The OR sank to 6.10, when investigating only nonconsensual sexual experiences in childhood, without taking cannabis use into consideration (Houston et al., 2011). Another study of adolescents in an urban environment from Ireland ($n = 211$) found that combined exposure to nonconsensual sexual experiences in childhood, and cannabis use, increased the likelihood of having a diagnosis of psychosis by almost 12 times (Harley et al., 2010); however, the sample size was small, with only $n = 5$ scoring positive for psychosis and childhood trauma, and $n = 4$ scoring positive for cannabis and psychosis.

In bipolar disorders, additive effects of childhood trauma and cannabis use (here a SCID verified diagnosis of cannabis abuse) have been associated with clinical features of the disorder (Aas et al., 2013a). In a sample of 587 participants with a bipolar disorder, from France and Norway, cannabis abuse was significantly associated with childhood abuse, specifically emotional and sexual abuse. Cannabis abuse moderated the prevalence of having at least one suicide attempt in the patients with childhood sexual abuse (Aas et al., 2013a); see Fig. 22.5. Additive effects were also observed between cannabis abuse and childhood trauma on earlier age at onset, and increased prevalence of rapid cycling (Aas et al., 2013a), see Fig. 22.6.

Although a relationship is observed between increased risk of substance abuse in individuals experiencing childhood trauma, the literature does not support clear mediation effects between childhood trauma, cannabis abuse, and the development of psychopathology. The literature supports additive effects and moderation effects in the direction of having both increases in the risk of developing a mental disorder, as well as increasing severity of clinical features. More studies are required to investigate this further.

Limitations of this type of research are many: first, childhood trauma is often measured retrospectively, for example, using the Childhood Trauma Questionnaire (CTQ), with the inherent weaknesses of retrospective interviews. Ideally, when investigating childhood trauma and risk of schizophrenia or bipolar disorder,

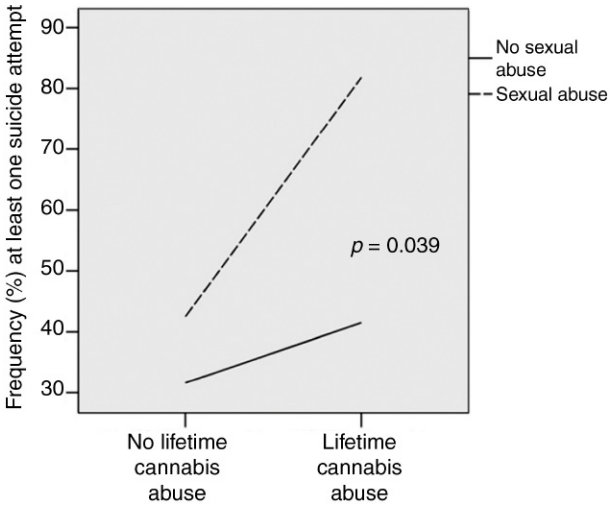


FIGURE 22.5 Interaction between childhood sexual abuse, cannabis abuse, and a history of at least one suicide attempt (Aas et al., 2013a,b). Fig. 22.5 illustrates interaction (p = 0.039) between sexual abuse, cannabis abuse, and a history of lifetime suicide attempt. Binary logistic regression. "No lifetime cannabis abuse," n = 368; "lifetime cannabis abuse," n = 211; sexual abuse dichotomized into trauma versus no trauma, based on moderate to severe cutoff scores by Bernstein et al. (2003), "no sexual abuse," n = 475; "sexual abuse," n = 104. *Source: Figure published in Aas, M., Etain, B., Bellivier, F., Henry, C., Lagerberg, T., Ringen, A., et al. (2013). Additive effects of childhood abuse and cannabis abuse on clinical expressions of bipolar disorders.* Psychological Medicine, *1–10.*

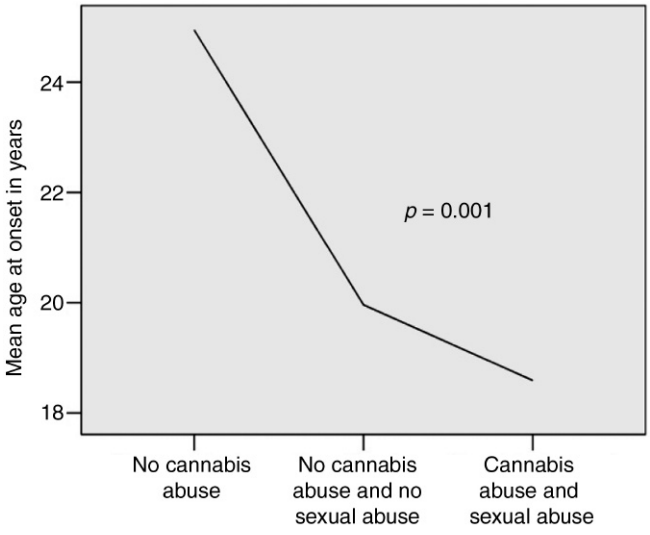


FIGURE 22.6 Age at onset, substance abuse, and childhood trauma abuse dose effect (Aas et al., 2013a). Fig. 22.6 illustrates significant additive effect for sexual abuse and cannabis abuse on earlier age at onset (p < 0.001), with the earliest age at onset in the group reporting both cannabis abuse and childhood trauma (mean ± SD of 18.6 ± 7.3 years; compared to no cannabis and no childhood abuse: mean ± SD of 25.0 ± 10.5 years). Kruskal-Wallis Test, p = 0.001, "no lifetime cannabis abuse," n = 499; "lifetime cannabis abuse and no sexual abuse," n = 53,·"lifetime cannabis abuse and sexual abuse," n = 22. *Source: Figure published in Aas, M., Etain, B., Bellivier, F., Henry, C., Lagerberg, T., Ringen, A., et al. (2013a). Additive effects of childhood abuse and cannabis abuse on clinical expressions of bipolar disorders.* Psychological Medicine, *1–10.*

prospective studies are conducted following a large cohort, over many years. The challenge here is the N required, as these illnesses are quite rare (1–3% of the population), as well as the time required, and resources in general. Second, several factors should be taken into account when investigating cannabis use, specifically the potency, frequency, and duration. The potency of cannabis is defined as the concentration of tetrahydrocannabinol (THC), the major active principle of the cannabis plant. Apart from THC, cannabis and cannabis resin contain many other cannabinoids. One of these (cannabidiol, CBD) has attracted recent interest because it is believed to show antipsychotic properties (Home Office Cannabis Potency Study, 2008). THC/CBD levels vary in the different types of cannabis, with synthetic skunk showing the highest THC levels, compared to the herbal version. The component of THC varies in the different drugs used (synthetic vs herbal), which may challenge comparisons between studies. Frequency of use is also important to take into account; is cannabis only tried a few times, or used regularly? A recent study investigated daily use, age first started (after or before the age of 15), as well as THC levels, in order to capture a broader, and more complete picture of how cannabis may increase risk of psychosis (Di Forti et al., 2014); however, most of the studies in the literature do not include such detailed information on cannabis use.

CONCLUSIONS

Childhood trauma and cannabis use are reported as risk factors in severe mental disorders, such as schizophrenia and bipolar disorder, both by increasing frequency, and by association with more severe clinical features of the disorder. Furthermore, several studies show that a history of childhood trauma is associated with cannabis use. More studies are needed to explore the association between childhood trauma and cannabis use in severe mental illness. So far, the literature supports additive affects, as well as increased odds ratio, when including both risk factors together in multivariate models, compared to measuring the risk factors independently. This chapter has also discussed the putative idea behind the mechanism of the cooccurrence of cannabis use in individuals with high levels of childhood trauma as a possible consequence of having opposite effects on the HPA axis, thus potentially related to some sort of "self-medication" aiming to normalize the HPA axis. To return to the question in the introduction, namely whether a history of childhood trauma increases the risk of using cannabis, the literature review covered in this chapter supports this link. Furthermore, this chapter supports a link between childhood trauma and cannabis use, and more severe clinical psychopathology in vulnerable individuals.

MINI-DICTIONARY

Additive effect A variable that has an additive effect can be added to other terms in a model to determine its effect on the independent variable.
Bipolar disorder Bipolar disorder is a mental illness characterized by periods of elevated mood (mania or hypomania), often followed by periods of depression.
Childhood trauma A history of childhood trauma covers different types of adverse experiences in childhood, such as: emotional abuse, physical abuse, sexual abuse, physical neglect, and/or emotional neglect. A frequently used questionnaire to capture information on childhood trauma in the literature is the Childhood Trauma Questionnaire (CTQ), a self-report retrospective questionnaire of 28 items (Bernstein et al., 2003). Another frequently used self-report measure of childhood trauma is, for example, the Childhood Experience of Care and Abuse Questionnaire (CECA.Q) (Bifulco, Bernazzani, Moran, & Jacobs, 2005). The CECA.Q includes physical abuse by the main mother and father figures (usually, but not necessarily, the biological parents), sexual abuse by any adult or an individual at least 5 years older than the recipient, parental antipathy (hostility, rejection, or coldness), and emotional or physical neglect. Both the physical and sexual abuse sections of the CECA.Q begin with screening questions, and then positive responses are followed up with more detailed questions to elicit concrete examples. Childhood trauma may also cover loss or separation of a parent.
Hypothalamic-pituitary-adrenal (HPA) axis The HPA axis is the main endocrine system involved in the stress response. The main stress hormone, cortisol, is affected by a variety of psychological stressors, including: trauma, fever, anxiety, exercise, and exposure to new situations (Pariante & Miller, 2001).
Mediation statistical analysis In statistics, a mediation model is an analysis that seeks to identify the mechanism that underlies an observed relationship between an independent variable and a dependent variable, via the inclusion of a third explanatory variable, known as a mediator variable.
Odds ratio (OR) An OR is a measure of association between an exposure and an outcome. The OR represents the odds that an outcome will occur, given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure.
Schizophrenia disorder Schizophrenia is a mental disorder often characterized by abnormal behavior, and failure to recognize what is real. Common symptoms include delusions, hallucinations, confused or unclear thinking, inactivity, and reduced social engagement and emotional expression.
Standard deviation Standard deviation measures the amount of variation from the average. A low standard deviation indicates that the data points tend to be very close to the mean; a high standard deviation indicates that the data points are spread out.
Tetrahydrocannabinol (THC) THC is the major psychoactive ingredient in cannabis, and the component of cannabis associated with psychotic illness (Di Forti et al., 2014).

Acknowledgments

Figures included in this chapter: we thank the respective journals for their cooperation and willingness to share their figures for illustration purposes in this chapter. The names of the respective journals are included beside each figure.

References

Aas, M., Aminoff, S. R., Vik, L. T., Etain, B., Agartz, I., Andreassen, O. A., et al. (2014). Affective lability in patients with bipolar disorders is associated with high levels of childhood trauma. *Psychiatry Research*, *218*, 252–255.

Aas, M., Dazzan, P., Fisher, H. L., Morgan, C., Morgan, K., Reichenberg, A., et al. (2011a). Childhood trauma and cognitive function in first-episode affective and non-affective psychosis. *Schizophrenia Research*, *129*, 12–19.

Aas, M., Djurovic, S., Athanasiu, L., Steen, N. E., Agartz, I., Lorentzen, S., et al. (2012a). Serotonin transporter gene polymorphism, childhood trauma, and cognition in patients with psychotic disorders. *Schizophrenia Bulletin*, *38*, 15–22.

Aas, M., Etain, B., Bellivier, F., Henry, C., Lagerberg, T., Ringen, A., et al. (2013a). Additive effects of childhood abuse and cannabis abuse on clinical expressions of bipolar disorders. *Psychological Medicine*, *44*, 1653–1662.

Aas, M., Haukvik, U. K., Djurovic, S., Bergmann, O., Athanasiu, L., Tesli, M. S., et al. (2013b). BDNF val66met modulates the association between childhood trauma, cognitive and brain abnormalities in psychoses. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *46C*, 181–188.

Aas, M., Navari, S., Gibbs, A., Mondelli, V., Fisher, H. L., Morgan, C., et al. (2012b). Is there a link between childhood trauma, cognition, and amygdala and hippocampus volume in first-episode psychosis? *Schizophrenia Research*, *137*, 73–79.

Aas, M., Steen, N. E., Agartz, I., Aminoff, S. R., Lorentzen, S., Sundet, K., et al. (2012c). Is cognitive impairment following early life stress in severe mental disorders based on specific or general cognitive functioning? *Psychiatry Research*, *198*, 495–500.

Agrawal, A., Nurnberger, J. I., Jr, & Lynskey, M. T. (2011). Cannabis involvement in individuals with bipolar disorder. *Psychiatry Research*, *185*, 459–461.

Alvarez, M. J., Roura, P., Oses, A., Foguet, Q., Sola, J., & Arrufat, F. X. (2011). Prevalence and clinical impact of childhood trauma in patients with severe mental disorders. *Journal of Nervous and Mental Disease*, *199*, 156–161.

Andreasson, S., Allebeck, P., Engstrom, A., & Rydberg, U. (1987). Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. *Lancet*, *2*, 1483–1486.

Bebbington, P. E., Bhugra, D., Brugha, T., Singleton, N., Farrell, M., Jenkins, R., et al. (2004). Psychosis, victimisation and childhood disadvantage: evidence from the second British National Survey of Psychiatric Morbidity. *British Journal of Psychiatry*, *185*, 220–226.

Bernstein, D. P., Stein, J. A., Newcomb, M. D., Walker, E., Pogge, D., Ahluvalia, T., et al. (2003). Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse and Neglect*, *27*, 169–190.

Bifulco, A., Bernazzani, O., Moran, P. M., & Jacobs, C. (2005). The childhood experience of care and abuse questionnaire (CECA.Q): validation in a community series. *British Journal of Clinical Psychology*, *44*, 563–581.

Brown, G. R., McBride, L., Bauer, M. S., & Williford, W. O. (2005). Impact of childhood abuse on the course of bipolar disorder: a replication study in U.S. veterans. *Journal of Affective Disorders*, *89*, 57–67.

Daruy-Filho, L., Brietzke, E., Lafer, B., & Grassi-Oliveira, R. (2011). Childhood maltreatment and clinical outcomes of bipolar disorder. *Acta Psychiatrica Scandinavica*, *124*, 427–434.

Di Forti, M., Sallis, H., Allegri, F., Trotta, A., Ferraro, L., Stilo, S. A., et al. (2014). Daily use, especially of high-potency cannabis, drives the earlier onset of psychosis in cannabis users. *Schizophrenia Bulletin*, *40*, 1509–1517.

Etain, B., Aas, M., Andreassen, O. A., Lorentzen, S., Dieset, I., Gard, S., et al. (2013). Childhood trauma is associated with severe clinical characteristics of bipolar disorders. *Journal of Clinical Psychiatry*, *74*, 991–998.

Fergusson, D. M., Boden, J. M., & Horwood, L. J. (2008). The developmental antecedents of illicit drug use: evidence from a 25-year longitudinal study. *Drug and Alcohol Dependence*, *96*, 165–177.

Garno, J. L., Goldberg, J. F., Ramirez, P. M., & Ritzler, B. A. (2005). Impact of childhood abuse on the clinical course of bipolar disorder. *British Journal of Psychiatry*, *186*, 121–125.

Harley, M., Kelleher, I., Clarke, M., Lynch, F., Arseneault, L., Connor, D., et al. (2010). Cannabis use and childhood trauma interact additively to increase the risk of psychotic symptoms in adolescence. *Psychological Medicine*, *40*, 1627–1634.

Heim, C., Mletzko, T., Purselle, D., Musselman, D. L., & Nemeroff, C. B. (2008a). The dexamethasone/corticotropin-releasing factor test in men with major depression: role of childhood trauma. *Biological Psychiatry*, *63*, 398–405.

Heim, C., Newport, D. J., Mletzko, T., Miller, A. H., & Nemeroff, C. B. (2008b). The link between childhood trauma and depression: insights from HPA axis studies in humans. *Psychoneuroendocrinology*, *33*, 693–710.

Houston, J. E., Murphy, J., Shevlin, M., & Adamson, G. (2011). Cannabis use and psychosis: re-visiting the role of childhood trauma. *Psychological Medicine*, *41*, 2339–2348.

Janssen, I., Krabbendam, L., Bak, M., Hanssen, M., Vollebergh, W., de, G. R., et al. (2004). Childhood abuse as a risk factor for psychotic experiences. *Acta Psychiatrica Scandinavica*, *109*, 38–45.

Lagerberg, T. V., Kvitland, L. R., Aminoff, S. R., Aas, M., Ringen, P. A., Andreassen, O. A., et al. (2014). Indications of a dose-response relationship between cannabis use and age at onset in bipolar disorder. *Psychiatry Research*, *215*, 101–104.

Lagerberg, T. V., Sundet, K., Aminoff, S. R., Berg, A. O., Ringen, P. A., Andreassen, O. A., et al. (2011). Excessive cannabis use is associated with earlier age at onset in bipolar disorder. *European Archives of Psychiatry and Clinical Neurosciences*, *261*, 397–405.

Lev-Ran, S., Le, F. B., McKenzie, K., George, T. P., & Rehm, J. (2013). Bipolar disorder and co-occurring cannabis use disorders: characteristics, co-morbidities and clinical correlates. *Psychiatry Research*, *209*, 459–465.

McEwen, B. S. (1998). Protective and damaging effects of stress mediators. *The New England Journal of Medicine*, *338*, 171–179.

Mondelli, V. (2014). From stress to psychosis: whom, how, when and why? *Epidemiology and Psychiatric Sciences*, *23*, 215–218.

Mondelli, V., Dazzan, P., Hepgul, N., Di, F. M., Aas, M., D'Albenzio, A., et al. (2010). Abnormal cortisol levels during the day and cortisol awakening response in first-episode psychosis: the role of stress and of antipsychotic treatment. *Schizophrenia Research*, *116*, 234–242.

Morgan, C., Reininghaus, U., Reichenberg, A., Frissa, S., Hotopf, M., & Hatch, S. L. (2014). Adversity, cannabis use and psychotic experiences: evidence of cumulative and synergistic effects. *British Journal of Psychiatry*, *204*, 346–353.

Nemeroff, C. B. (2004). Neurobiological consequences of childhood trauma. *Journal of Clinical Psychiatry*, *65*(Suppl. 1), 18–28.

Nemeroff, C. B., & Binder, E. (2014). The preeminent role of childhood abuse and neglect in vulnerability to major psychiatric disorders: toward elucidating the underlying neurobiological mechanisms. *Journal of the American Academy of Child and Adolescent Psychiatry*, *53*, 395–397.

Pariante, C. M., & Miller, A. H. (2001). Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. *Biological Psychiatry*, *49*, 391–404.

Post, R. M. (2010). Mechanisms of illness progression in the recurrent affective disorders. *Neurotoxicity Research*, *18*, 256–271.

Sahin, S., Yuksel, C., Guler, J., Karadayi, G., Akturan, E., Gode, E., et al. (2013). The history of childhood trauma among individuals with ultra high risk for psychosis is as common as among patients with first-episode schizophrenia. *Early Intervention in Psychiatry*, *7*, 414–420.

Sapolsky, R. M. (2000). The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death. *Biological Psychiatry*, *48*, 755–765.

Selye, H. (1975). Stress and distress. *Comprehensive therapy*, *1*(8), 9–13.

Shevlin, M., Dorahy, M. J., & Adamson, G. (2007). Trauma and psychosis: an analysis of the National Comorbidity Survey. *American Journal of Psychiatry*, *164*, 166–169.

Shin, S. H., Edwards, E. M., & Heeren, T. (2009). Child abuse and neglect: relations to adolescent binge drinking in the national longitudinal study of Adolescent Health (AddHealth) Study. *Addictive Behaviors*, *34*, 277–280.

Thompson, A., Nelson, B., McNab, C., Simmons, M., Leicester, S., McGorry, P. D., et al. (2010). Psychotic symptoms with sexual content in the "ultra high risk" for psychosis population: frequency and association with sexual trauma. *Psychiatry Research*, *177*, 84–91.

van Leeuwen, A. P., Creemers, H. E., Greaves-Lord, K., Verhulst, F. C., Ormel, J., & Huizink, A. C. (2011). Hypothalamic-pituitary-adrenal axis reactivity to social stress and adolescent cannabis use: the TRAILS study. *Addiction*, *106*, 1484–1492.

van Os, J., Bak, M., Hanssen, M., Bijl, R. V., de Graaf, R., & Verdoux, H. (2002). Cannabis use and psychosis: a longitudinal population-based study. *American Journal of Epidemiology*, *156*, 319–327.

Varese, F., Smeets, F., Drukker, M., Lieverse, R., Lataster, T., Viechtbauer, W., et al. (2012). Childhood adversities increase the risk of psychosis: a meta-analysis of patient-control, prospective- and cross-sectional cohort studies. *Schizophrenia Bulletin*, *38*, 661–671.

Weber, K., Rockstroh, B., Borgelt, J., Awiszus, B., Popov, T., Hoffmann, K., et al. (2008). Stress load during childhood affects psychopathology in psychiatric patients. *BMC Psychiatry*, *8*, 63.

CHAPTER

23

Parent's Influence on Children's Cannabis Use

S. Miller, J.T. Siegel, W.D. Crano

Division of Behavioral and Organizational Sciences, School of Social Science, Policy and Evaluation, Claremont Graduate University, Claremont, CA, United States

SUMMARY POINTS

- This chapter focuses on how parental behavior can influence children's use of cannabis.
- Recent changes in public sentiment regarding the acceptability of cannabis have coincided with an increase in the level of adolescent cannabis use.
- Cannabis use in adolescence has been linked to significant cognitive developmental delay in young adults, which is all the more reason to emphasize how parents can prevent adolescent cannabis use.
- Parental use has been shown to change the expectancies children perceive regarding their own cannabis use, in such a way that they will be more likely to experiment with cannabis if their own parents are cannabis users.
- Authoritative parenting, characterized by setting high expectations and showing affection, has been shown to be the most effective parenting style for preventing adolescent cannabis use.
- Effective communication between parents and their children is based on feelings of trust and acceptance which facilitate the willful disclosure of information from the child to the parent.
- Parental monitoring is one of the most effective ways that parents can prevent adolescent cannabis use, by virtue of having knowledge about their child's activities, whereabouts, and peer groups.
- Increasing parent's feelings of competence to talk to their children about cannabis use is an area where policy makers could have an impact by designing more targeted media campaigns.

KEY FACTS OF OUTCOME EXPECTANCIES

- Outcome expectancies are the beliefs an individual has concerning what they will experience if they engage in a particular behavior.
- Expectancies are formed in a number of ways, either from previous experience, second-hand accounts, media portrayals, educational mediums, and other informational sources.
- The expectancies an individual has regarding a specific behavior can be positive, negative, neutral and, in many cases, a mix of all three.
- The more favorably a person perceives the outcomes of a behavior to be, the more likely they will perform that behavior, if given the opportunity.
- Outcome expectances are based on *perceptions* of reality and, therefore, do not have to conform to what is most likely to happen (if the behavior is performed) to be influential.

The aforementioned points outline the core facets of outcome expectancies, what they are, how they're formed, and how they function to guide behavior.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00025-9
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

NSPY National Survey of Parents and Youth

INTRODUCTION

In the 1980s, there was a now (in)famous commercial which was part of an antidrug media campaign targeting adolescent cannabis use. A hostile parent confronts his son with a stash of cannabis he found in the child's room, and starts peppering the child with questions, trying to learn out how this could come to pass. Finally the parent demands to know, "Who taught you how to do this stuff?" The visibly distraught child shoots back with, "You, alright! I learned it by watching you!" A solemn voiceover intones, "Parents who use drugs have children who use drugs." Clearly, the commercial was trying to persuade parents not to use drugs, if for nothing else than the sake of their children. While it is true that parents who are cannabis users are more likely to have children who use cannabis (Miller, Siegel, Hohman, & Crano, 2013), what is often overlooked is why adolescent cannabis use is a bad thing. Parents should be concerned with their child's cannabis use for two main reasons. First, children who use drugs are, by that very behavior, at greater risk for delinquency, and entering into the criminal justice system, as most countries enforce a prohibition against cannabis use by minors. Second, there is mounting evidence (Dougherty et al., 2013; Gruber, Sagar, Dahlgren, Racine, & Lukas, 2012; Lisdahl & Price, 2012) indicating that children who use cannabis are at greater risk for developmental delay. Meier et al. (2012), for example, showed up to an eight points deficit in IQ scores among young adults who were regular cannabis users as children. These findings are made all the more relevant, given recent trends indicating an increase in adolescent cannabis use (SAMHSA, 2010; Fig. 23.1).

PARENTAL INFLUENCE ON ADOLESCENT CANNABIS USE

Researchers, prevention specialists, and policy makers have been concerned, for many years, with the processes by which parents influence children's illicit drug use, as exemplified by the commercial described at the start of this chapter. This concern suggests that children may infer the boundaries of substance usage from their parents' drug use behaviors. The evidence showing that parents who use cannabis are more likely to have children who themselves become cannabis users supports this possibility (Duncan, Duncan, Hops, & Stoolmiller, 1995; McDermott, 1984). This is a troubling finding. It implies that children are destined to make the same mistakes as their parents—and, further, that there is nothing the parent can do about it. However, the explanation is not so simple. Many parental factors have been shown to affect the likelihood of their children's initiating substance misuse, and these factors appear to hold, independent of parents' prior usage. Of course, current, ongoing parental drug use is a major risk factor for children (Miller et al., 2013), and may well trump the ameliorative factors that will be discussed.

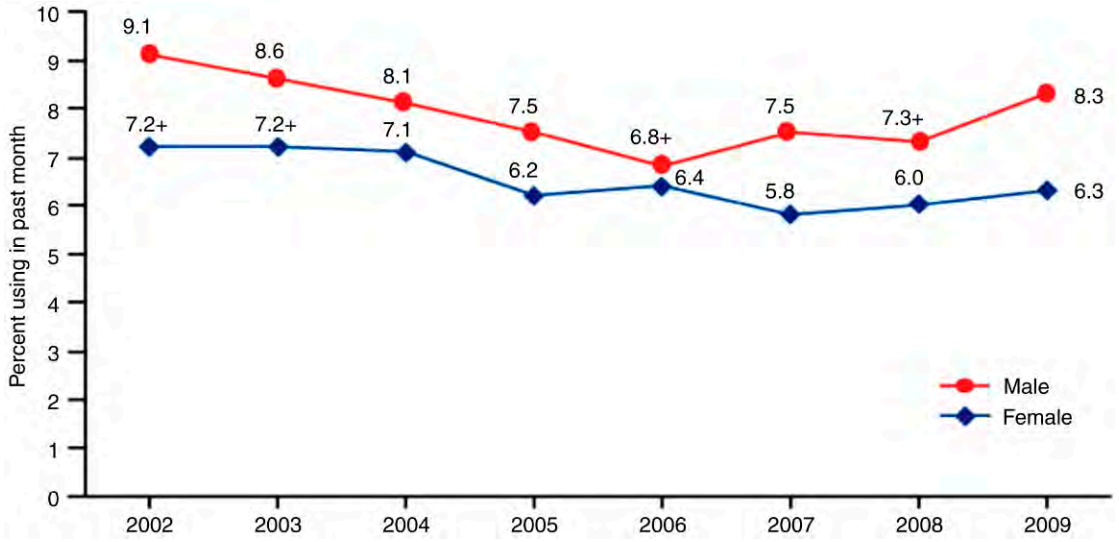


FIGURE 23.1 **Past-month marijuana use among youth, age 12–17, by gender: 2002–09.** Among youth age 12–17 in 2009, males had a higher rate than females of current marijuana use (8.3% for males vs 6.3% for females). +, Difference between this estimate and the 2009 estimate is statistically significant at the 0.05 level. *Source: From SAMHSA (2010; Fig. 2.9).* Results from the 2009 National Survey on Drug Use and Health: Vol. I. Summary of National Findings. *Rockville, MD. Reprinted with permission.*

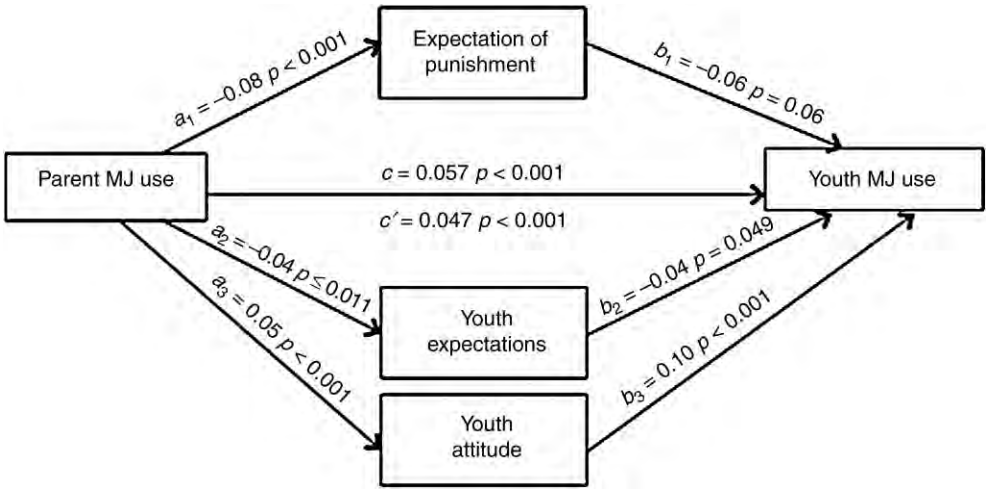


FIGURE 23.2 Multiple mediation model of parent cannabis use influencing child cannabis use. This figure shows how expectations of punishment, outcome expectations of cannabis use, and attitudes toward cannabis are influenced by the recency of parent cannabis use. *Note*. All variables were measured at R1, except youth marijuana use, which was measured at R2, and the effects were controlled for age measured at R1. *Source: From Miller et al. (2013, Fig. 1, p. 851). Copyright 2013 by the American Psychological Association. Reprinted with permission.*

Until recently, there was a dearth of good data available to examine the psychosocial processes behind this relationship. Either there were data about parental drug use that did not address the drug use habits of the child or, more likely, there were data on the child but not the parent. Even when data regarding the drug use habits of both parent and child were available, it was likely to be cross-sectional, making it impossible to test hypotheses regarding the development of the process of influence over time. This changed with the National Survey of Parents and Youth (NSPY), which included information from parents and children on their own drug use behavior (David, Hornik, & Maklan, 2010). In a recent analysis of the NSPY dataset, Miller et al. (2013) identified three key factors that mediated the relation between parental cannabis use and their adolescent children's use (Fig. 23.2).

RECENCY OF PARENTS' CANNABIS USE AND CHILDREN'S INITIATION

The research of Miller et al. (2013) was concerned with parental factors that might influence how adolescent nonusers of cannabis came to be users. It showed that the parents' substance use behaviors were associated with their children's initiation of use. Even more predictive, regarding drug use, were the child's *perceptions* of what their parents knew or did. Analysis indicated that children's attitudes toward cannabis were significantly related to parental usage. If a parent had ever used cannabis, the child was more likely to do so. Further, and more importantly, the recency of parental usage also was significantly related to children's behaviors: the more recent the parental use, the more positive were children's attitudes toward cannabis. Two other factors mediated the relation between parental usage and children's initiation. One of these had to do with the child's expectation of punishment for cannabis use. The more recent the parent's usage, the lower was the child's expectations of punishment for use. The third mediating factor between recency of parental use and children's initiation was the child's expectations regarding the effects of cannabis. Table 23.1 shows the correlations between parental use, child use, and the mediating factors associated with both. The more recent the parent's usage, the more positive were the child's expectations regarding the effects of cannabis. For more on how outcome expectancies influence behavior see Section "Key Facts of Outcome Expectancies.".

TABLE 23.1 Correlations Between Child Marijuana Use, Parent Marijuana Use, Expectation of Punishment, Attitude, and Expectations

Item	V1	V2	V3	V4
V1. Child marijuana use	—			
V2. Parent marijuana use	0.13***	—		
V3. Punish	–0.14***	–0.13***	—	
V4. Attitude	0.18***	0.08**	–0.19***	—
V5. Expectations	–0.13***	–0.07*	0.24***	0.34***

This table illustrates how each of the youth variables concerning attitudes and expectations of use (ie, V3, V4, and V5) are related to parent cannabis use at Time 1, and child cannabis use at Time 2. In essence, the variables that predicted child cannabis use at Time 2 were predicted by parent cannabis use at Time 1. All variables were measured at R1, except child marijuana use, which was measured at R2. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
From Miller et al. (2013, Table 1, p. 850). Copyright 2013 by the American Psychological Association. Reprinted with permission.

Clearly, the child's impressions have implications for their own usage. Research has established that adolescents with more positive attitudes toward cannabis are more likely to try the drug (Hohman, Crano, Siegel, & Alvaro, 2014). On the other hand, expectations of punishment can be a strong deterrent to use. The analysis indicated that such expectations were attenuated as a function of recency of parental use. Expectations regarding the effects of cannabis also were shown to affect children's usage intentions. These expectations can range from the physiological (eg, feelings of euphoria, brain damage), to the social (eg, increased popularity, stoner label), and they, too, mediated the association between parental use and children's initiation.

Obviously, these results suggest that parents should never have initiated cannabis use. For many parents, of course, this admonition comes too late. However, children's *perceptions* of their parents' past usage, attitudes toward cannabis, the punishment in store for usage, and the gains and losses of cannabis usage can be affected by present (vs past, perhaps distant past) parental behaviors, and these behaviors can be learned or modified by appropriate training. How this can be done is indicated in a number of recent studies.

PARENTAL WARMTH AND MONITORING (KNOWLEDGE OF CHILD'S ACTIONS AND WHEREABOUTS)

Two parental variables that have received considerable research attention are parental monitoring, and parental warmth. Parental monitoring can mean many things, but generally is subdivided into two categories: surveillance and knowledge gained from the child's self-disclosure of actions to parents. Both appear to have protective effects on children's substance misuse, but knowledge is clearly the preferred modality, as it suggests a warm and accepting family context in which the child feels free to disclose issues to parents, whose advice is actively sought, rather than imposed. Early survey research by Ramirez et al. (2004) showed that youth (fourth through 12 graders) who perceived higher degrees of parental surveillance were less likely to use illicit substances (cannabis and inhalants). The same result was found as a function of perceived family warmth: the greater warmth perceived by the child, the less likely was he or she to use either substance. Both of these results were qualified by an interaction with knowledge; that is, effects of surveillance and family warmth were enhanced as a function of the child's knowledge of the consequences of drug use.

These results were reinforced by a meta-analysis concerned with the link between parental monitoring and cannabis use in youth. Lac and Crano (2009) found a statistically significant inverse relation between parental monitoring and cannabis use, across 17 studies with more than 35,000 independent observations. The results were strongest when monitoring was defined in terms of parental knowledge (presumably the result of children's self-disclosure). The data were unequivocal. A file drawer analysis indicated that more than 7000 studies of nil effect would be needed to render the results nonsignificant.

A formal model of the way in which parental knowledge and warmth might affect adolescents' use of cannabis, framed within the theory of planned behavior (Ajzen, 1991), was proposed by Lac, Alvaro, Crano, and Siegel (2009). This research made use of data from the restricted version of the National Survey of Parents and Youth, which was conducted to evaluate the National Youth Antidrug Media Campaign (National Institute on Drug Abuse, 2006). The sample consisted of 2141 adolescents (12–18 year olds) and their parents. The data from this archive were entered into a structural equation model. Parental warmth and parental knowledge were hypothesized to affect attitudes toward cannabis, subjective norms regarding cannabis use, and perceived behavioral control. Results showed that parental knowledge affected all three of these endogenous variables; warmth affected the first two, but not perceived behavior control. Attitudes, subjective norms, and perceived behavioral control, in turn, were significantly associated with adolescents' intentions to use cannabis, which significantly predicted actual cannabis use 1 year later. This study revealed a strong and statistically significant linkage from parental monitoring and warmth to adolescent beliefs, and consequent behaviors. It indicates clearly the importance of parents in moderating use. The research supported the majority of hypotheses. Parental knowledge predicted more negative attitudes toward cannabis, stronger subjective norms against usage, and higher levels of perceived behavioral control in avoiding cannabis. These factors, in turn, attenuated usage intentions and actual use.

A conceptual replication and extension of the research of Lac et al. (2009) was advanced by Hemovich, Lac, and Crano (2011), who were interested in the ways that family structure (single or dual parents) were associated with adolescents' attitudes and friendship patterns, and the consequent effects of these predictors on illicit substance use (alcohol, tobacco, and cannabis). The study involved 4173 child–parent pairs (adolescents ranged from 12 to 18 years of age), who were assessed over 2 years. The data were used in a path analytic model in which family structure and family income affected parental monitoring behavior, but not family warmth. Lower family income was observed in single-parent families, as were lower levels of monitoring. The authors speculated that single parents' need to be engaged in the labor force, of necessity, detracted from the time available to monitor children, but would not necessarily impinge on

the warmth they extended to their children. Monitoring and warmth, in turn, affected two critical variables: children's attitudes toward illicit substance use, and the attitudes and the choice of the friends with whom they associated. The link between attenuated parental monitoring and children's substance use was hypothesized to operate through the adolescents' social ties and attitudes toward illicit substances. This hypothesis was supported. The analysis of the path model revealed that lower monitoring and warmth predicted more drug-friendly attitudes on the part of the adolescents, and their choice of friends—who were more likely to be involved in delinquent behaviors, including substance misuse. These two factors, in turn, were significantly related to the adolescent's own use of illicit substances, 1 year later. It is important to note that only substance-free adolescents were used in the first year of the study.

Further studies on parental monitoring designed to explain *why* monitoring matters have revealed that parental monitoring affects adolescents' perceived norms regarding the acceptability of using cannabis, their attitudes toward cannabis, and their intentions to use cannabis (Yabiku et al., 2010). Further, evidence suggests that strong parental monitoring may mitigate negative peer influence (Tragesser, Beauvais, Swaim, Edwards, & Oetting, 2007), and an evaluation of a parental monitoring intervention found that parents' knowledge of adolescent activities was more accurate when exposed to the monitoring intervention (Li et al., 2002).

It stands to reason that parents who are more knowledgeable about their children and their activities will be better equipped to recognize and address potential risk factors associated with cannabis or other substance misuse. The research cited here and elsewhere supports this conclusion. However, it is important to note that the effects of parental monitoring, much like communication, do not exist in a vacuum. Monitoring is most effective when parental warmth and communication are present, as well (Fletcher, Steinberg, & Williams-Wheeler, 2004; Patrick, Snyder, Schrepferman, & Snyder, 2005).

PARENTAL WARMTH AND PARENTING STYLE: SOME CONCEPTUAL OVERLAP

Parental warmth is conceptualized in the literature as the love and affection shown to children throughout their developmental years. Warmth more broadly has to do with children's *perceptions* of parental acceptance or rejection (Khaleque, 2013). As discussed, research on the relationship between parental warmth and adolescent cannabis use has shown a negative association (Hemovich et al., 2011; Lac et al., 2009). This finding suggests that mutual trust between parent and child has a strong protective effect from substance involvement, and has received empirical support in the literature concerning child delinquency (Kerr, Stattin, & Trost, 1999). It is likely that trust, rooted in perceptions of love and acceptance, facilitates the willful disclosure of information from the child to the parent (leading to parental knowledge). With this trust also comes a greater willingness to accept the attitudes and norms of the parent, regarding cannabis use (Lac et al., 2009).

Parenting style is similar to parental warmth, but it incorporates warmth into a broader framework of parenting. In her seminal work, Baumrind (1967) identified four key elements of parenting that have to do with how controlling, demanding, communicative, and nurturing parents were with their children. This research led to the theoretical identification of three distinct parenting styles: authoritarian, authoritative, and permissive (Baumrind, 1991), and later a fourth style known as "uninvolved" (Maccoby & Martin, 1983), which primarily has to do with neglectful or absent parents. In a review of the literature on parenting styles and drug use, Becona et al. (2012) found that the authoritative style was the style most consistently associated with lower rates of adolescent drug use (including cannabis use), while the uninvolved style was linked to increased likelihood of adolescent drug use. The authoritative style is characterized by a "firm but fair" approach to parenting, in which parents have very high expectations of their children, but also show warmth, and recognize their child's need for independence.

PARENTAL COMMUNICATION AND CHILDREN'S SUBSTANCE USE

While showing love and acceptance helps to build a solid relationship between parents and their children, communicating the dangers of cannabis use before children have an opportunity to experiment on their own also has positive preventive implications. Although parents have a demonstrably important role to play in the prevention of their children's substance misuse, surprisingly little research has been devoted to enhancing parental preventive communication. The focus on parental-child substance prevention communication may provide important insights into prevention, and so it is reasonable to hope that more research on this topic will be forthcoming. A recent study that merits close consideration was performed by Huansuriya, Siegel, and Crano (2014). In this 4-year panel study, parents' exposure to an ongoing antidrug media campaign was assessed. Higher levels of ad exposure were associated with parents' attitudes toward communicating with their children about drugs, and higher levels of perceived social approval from significant others for doing so. These variables, along with perceived behavioral control, anticipated parents' intentions to discuss drug avoidance with their children.

These intentions were associated with children's reports of drug communication with parents, on a measure taken 1 year later. The frequency of communication with parents was associated with children's attenuated intentions to use drugs. This study was replicated in an analysis of the third and fourth year of the panel study, with identical results.

This study strongly suggests that adolescents view parents as a credible source on drugs. The results of Huansuriya et al. (2014), however, indicated that many parents did not know how to communicate about drugs with their children. Their perceived behavioral control to discuss drugs with their children was not affected by the ad campaign. This may be due to the fact that the great majority of the ads used in the campaign were directed toward youth, and provided little information that parents could use in gaining knowledge and confidence about drug-relevant communication with their children. The children's perceived behavioral control also was not a strong component in their substance avoidance. Both of these findings suggest the importance of providing more usable facts to parents in their conversations with children about the dangers of drug misuse. Further support for this contention was supplied by Lamb and Crano (2014), who showed that although parents were generally aware of their children's usage behavior, most children, even those engaged in marijuana use, assumed their parents would not know if they were using drugs.

The implications to be drawn from both Lamb and Crano (2014) and Huansuriya et al. (2014) are that parents are willing actors in facilitating drug prevention in their children, but may not know the most effective ways of doing so and, as such, may avoid speaking about drug prevention, even if they know or suspect their children are at risk. We know parents can be an effective source of antidrug communication for adolescents (Kafka & London, 1991; Tobler & Komro, 2010), but it is not enough to tell parents to tell their children to avoid drugs. They must be informed about the proper ways of communicating antidrug messages in ways that are informed and persuasive. Assuming that parents already know what to say is not supported by the relevant literature. Teaching parents how to communicate about the complex and ever-changing landscape of available substances may be challenging, and the job is complicated by the fact that a one-size-fits-all strategy may not be optimal. After learning the relevant facts, parents will need to modify their communication strategies, depending on the nature of the child, and their relationship with him or her.

To summarize, we have addressed four key parental behaviors shown to be related to adolescent cannabis use. They are: parental use of cannabis, parental warmth and style, parental communication, and, finally, parental monitoring. These behaviors have been shown to influence adolescent norms, attitudes, and intentions regarding cannabis use, and have further been found to yield influence synergistically, with one behavior often facilitating the efficacy of another. So, for parents who are concerned about their children experimenting with drugs, Table 23.2 summarizes "best practices" for how to raise children who remain abstinent from using cannabis.

TABLE 23.2 Best Practices for Raising Drug-Free Children

1. Refrain from using cannabis, or quit if you already do.
2. Adopt an authoritative parenting style.
3. Communicate with your child about the dangers of cannabis.
4. Monitor your children's activities and peer groups, and be aware of potential risk factors associated with cannabis use.

This table lists four of the most established research findings linking parental behavior to adolescent cannabis use. Research suggests that parents who follow these practices in their own behavior and in their interactions with their children will be less likely to have children who use cannabis.

While there is no magic bullet for preventing adolescent cannabis use, these parenting practices collectively represent the clearest evidence we have on how to mitigate the risks that lead to cannabis use in adolescents.

CONCLUSIONS

Parents can play a key role in lowering the risk of their children's illicit substance use. In fulfilling this role, parents need to understand (1) how their behavior affects their children's cannabis use, and (2) how best to communicate with their children about the potential dangers of cannabis. Research examining effective ways to teach parents how and what to communicate to their children about cannabis use may prove important in preventing adolescent substance misuse. It is important to learn which parental variables should be targeted. In addition, there are many more avenues to explore in the field of parental influence than are covered here (suggestions for "Future Directions" are outlined in Table 23.3). Many of

TABLE 23.3 Future Directions in the Field of Parental Influence

- Researchers, in conjunction with policy advisors and governmental agencies, should begin to study the impact of decriminalization and legalization of cannabis laws on adolescents.
- More neurocognitive evidence on the developmental effects of cannabis use during adolescence is needed to better inform parents and children about potential long-term, negative consequences.
- Studies on how to increase parents' perceived behavioral control to talk to their children about cannabis use are needed to better inform antidrug media campaigns, and other interventions focused on the parent.
- Continuing to adapt representative and longitudinal surveys (eg, NSPY) will be necessary to reflect the changing landscape of adolescent drug use.

This table outlines brief suggestions for expanding the knowledge base in the field of child cannabis use. The four points are based on the literature to date, and are responsive to the changing societal norms concerning the legal status of cannabis.

the constructs reviewed here (eg, parental warmth, parental style, family structure, etc.) are of theoretical interest, to be sure, but, more importantly, provide important insights into informational and attitudinal interventions that may lead to positive outcomes.

MINI-DICTIONARY

Attitude The positive, negative, or neutral evaluation of an object, where an "object" can be a person, a thing, or a behavior. Attitudes have the power to influence behavior.

Delinquency Often used in reference to adolescents, delinquency is characterized by antisocial behavior, such as, drug use, school absence (or school dropout), and other illegal or dangerous acts.

Expectancies of use The perceived beliefs surrounding the use of a particular substance, in this case, cannabis. Expectancies can be positive or negative, and will influence the likelihood a person will experiment with cannabis.

Parental monitoring The degree to which a parent has knowledge of their child's activities, whereabouts, and friend groups.

Parental preventative communication The specific conversations that parents have with their children about engaging in risky and/or illegal activities, specifically drug use.

Parental warmth The affection and acceptance shown to a child by the parent. Warmth facilitates trust within the context of the parent–child relationship.

Parenting style The manner in which parents relate to their children with regard to expectations of behavior, communication, and punishment. Parents typically adopt one of four broad categories of parenting style: authoritarian, authoritative, permissive, and uninvolved, with authoritative being the most protective against child substance abuse.

Perceived behavioral control Also known as "self-efficacy," the degree to which people feel they are able to complete a specific task.

Subjective norms Also known as "injunctive norms," subjective norms are the perceived expectations people close to us have for our behavior. For example, if a person's entire family loathes smoking, there is a strong subjective norm for that person to not smoke.

Theory of planned behavior A theory proposed by Icek Ajzen that describes how attitudes, subjective norms, and perceived behavioral control are factors that influence a person's intention to engage in a specific behavior (Ajzen, 1991).

References

Ajzen, I. (1991). The theory of planned behavior. *Organizational Behavior and Human Decision Processes*, *50*(2), 179–211.

Baumrind, D. (1967). Child care practices anteceding three patterns of preschool behavior. *Genetic Psychology Monographs*, *75*, 43–88.

Baumrind, D. (1991). The influence of parenting style on adolescent competence and substance use. *The Journal of Early Adolescence*, *11*, 56–95.

Becona, E., Martinez, U., Calafat, A., Juan, M., Fernandez-Hermida, J. R., & Secades-Villa, R. (2012). Parental styles and drug use: a review. *Drugs: Education, Prevention & Policy*, *19*, 1–10.

David, S., Hornik, R., & Maklan, D. (2010). *National Survey of Parents and Youth (NSPY), 1998–2004—Restricted Use Files [Computer file]. ICPSR 27868-v1*. Ann Arbor, MI: Inter-university Consortium for Political and Social Research.

Dougherty, D. M., Mathias, C. W., Dawes, M. A., Furr, R., Charles, N. E., Liguori, A., & … Acheson, A. (2013). Impulsivity, attention, memory, and decision-making among adolescent marijuana users. *Psychopharmacology*, *226*, 307–319.

Duncan, T. E., Duncan, S. C., Hops, H., & Stoolmiller, M. (1995). An analysis of the relationship between parent and adolescent marijuana use via generalized estimating equation methodology. *Multivariate Behavioral Research*, *30*, 317–339.

Fletcher, A. C., Steinberg, L., & Williams-Wheeler, M. (2004). Parental influences on adolescent problem behavior: revisiting Stattin and Kerr. *Child Development*, *75*, 781–796.

Gruber, S. A., Sagar, K. A., Dahlgren, M., Racine, M., & Lukas, S. E. (2012). Age of onset of marijuana use and executive function. *Psychology of Addictive Behaviors*, *26*(3), 496–506.

Hemovich, V., Lac, A., & Crano, W. D. (2011). Understanding early-onset drug and alcohol outcomes among youth: the role of family structure, social factors, and interpersonal perceptions of use. *Psychology, Health & Medicine*, *16*, 249–267.

Hohman, Z. P., Crano, W. D., Siegel, J. T., & Alvaro, E. M. (2014). Attitude ambivalence, friend norms, and adolescent drug use. *Prevention Science*, *15*(1), 65–74.

Huansuriya, T., Siegel, J. T., & Crano, W. D. (2014). Parent–child drug communication: pathway from parents' ad exposure to youth's marijuana use intention. *Journal of Health Communication*, *19*(2), 244–259.

Kafka, R. R., & London, P. (1991). Communication in relationships and adolescent substance use: the influence of parents and friends. *Adolescence*, *26*(103), 587–598.

Kerr, M., Stattin, H., & Trost, K. (1999). To know you is to trust you: parents' trust is rooted in child disclosure of information. *Journal of Adolescence*, *22*(6), 737–752.

Khaleque, A. (2013). Perceived parental warmth, and children's psychological adjustment, and personality dispositions: a meta-analysis. *Journal of Child and Family Studies*, *22*(2), 297–306.

Lac, A., Alvaro, E. M., Crano, W. D., & Siegel, J. T. (2009). Pathways from parental knowledge and warmth to adolescent marijuana use: an extension to the theory of planned behavior. *Prevention Science*, *10*, 22–32.

Lac, A., & Crano, W. D. (2009). Monitoring matters: meta-analytic review reveals the reliable linkage of parental monitoring with adolescent marijuana use. *Perspectives on Psychological Science*, *4*, 578–586.

Lamb, C. S., & Crano, W. D. (2014). Parents' beliefs and children's marijuana use: evidence for a self-fulfilling prophecy effect. *Addictive Behaviors*, *39*, 127–132.

Li, X., Stanton, B., Galbraith, J., Burns, J., Cottrell, L., & Pack, R. (2002). Parental monitoring intervention: practice makes perfect. *Journal of the National Medical Association*, *94*(5), 364–370.

Lisdahl, K. M., & Price, J. S. (2012). Increased marijuana use and gender predict poorer cognitive functioning in adolescents and emerging adults. *Journal of the International Neuropsychological Society*, *18*(4), 678–688.

Maccoby, E. E., & Martin, J. A. (1983). Socialization in the context of the family: parent–child interaction. In P. H. Mussen, & E. M. Hetherington (Eds.), *Handbook of child psychology: Vol. 4. socialization, personality, and social development* (4th ed.). New York, NY: Wiley.

McDermott, D. (1984). The relationship of parental drug use and parents' attitude concerning adolescent drug use to adolescent drug use. *Adolescence*, *XIX*(73), 89–97.

Meier, M. H., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R. E., & …, Moffitt, T. E. (2012). Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proceedings of the National Academy of Sciences of the United States Of America*, *109*(40), E2657–E2664.

Miller, S. M., Siegel, J. T., Hohman, Z., & Crano, W. D. (2013). Factors mediating the association of the recency of parent's marijuana use and their adolescent children's subsequent initiation. *Psychology of Addictive Behaviors*, *27*(3), 848–853.

National Institute of Drug Abuse [NIDA]. (2006). *User's guide for the evaluation of the national youth anti-drug media campaign*. Rockville, MD: Westat.

Patrick, M. R., Snyder, J., Schrepferman, L. M., & Snyder, J. (2005). The joint contribution of early parental warmth, communication and tracking, and early child conduct problems on monitoring in late childhood. *Child Development*, *76*(5), 999–1014.

Ramirez, J. R., Crano, W. D., Quist, R., Burgoon, M., Alvaro, E. M., & Grandpre, J. (2004). Acculturation, familism, parental monitoring, and knowledge as predictors of marijuana and inhalant use in adolescents. *Psychology of Addictive Behaviors*, *18*, 3–11.

SAMHSA. (2010). *Results from the 2009 National Survey on Drug Use and Health: Vol. I. Summary of National Findings*. Rockville, MD: Center for Behavioral Health Statistics and Quality.

Tobler, A. L., & Komro, K. A. (2010). Trajectories or parental monitoring and communication and effects on drug use among urban young adolescents. *Journal of Adolescent Health*, *46*(6), 560–568.

Tragesser, S. L., Beauvais, F., Swaim, R. C., Edwards, R. W., & Oetting, E. R. (2007). Parental monitoring, peer drug involvement, and marijuana use across three ethnicities. *Journal of Cross Cultural Psychology*, *38*(6), 670–694.

Yabiku, S. T., Marsiglia, F. F., Kulis, S., Parsai, M. B., Becerra, D., & Del-Colle, M. (2010). Parental monitoring and changes in substance use among Latino/a and non-Latino/a preadolescents in the southwest. *Substance Use & Misuse*, *45*(14), 2524–2550.

CHAPTER

24

Cannabis Users and Premorbid Intellectual Quotient

L. Ferraro, L. Sideli**, D. La Barbera***

***Biomedical Department of Internal and Specialist Medicine; Department of Experimental Biomedicine and Clinical Neuroscience, School of Medicine, University of Palermo, Palermo, Italy**
****Department of Experimental Biomedicine and Clinical Neuroscience, School of Medicine, University of Palermo, Palermo, Italy**

SUMMARY POINTS

- The chapter focuses on premorbid Intellectual Quotient (IQ) in cannabis users, one of the most controversial topics in studies on the harmful effects of cannabis use on cognition.
- Several studies have ascertained acute and residual effects of cannabis use in the memory domain, but only a minimal general cognitive effect after a long-term period of abstinence.
- A number of longitudinal studies were able to obtain IQ measures before and after cannabis consumption, but they yielded discordant findings.
- Authors, however, observed a relationship between higher premorbid IQ and recreational or discontinued use while a lower premorbid IQ resulted as a predictor for regular or heavy cannabis use.
- Studies on cannabis use and cognitive impairment associated with schizophrenia paradoxically found better IQ among patients with cannabis use lifetime.
- At the end of the chapter, authors proposed a conceptual table to summarize the complex relationship between premorbid IQ and cannabis use in determining different outcomes, by sustaining the hypothesis of a shared genetic-early environment vulnerability to psychosis and cannabis use, which is reflected on premorbid IQ and able to influence the contact with the substance.
- In turn, cannabis may be a trigger for psychosis towards this continuum of neurodevelopmental vulnerability.

KEY FACTS OF IQ

- The Intellectual Quotient (IQ) is a standardized measure, able to estimate the intelligence of an individual starting from the assessment of verbal and not-verbal skills.
- Each person's IQ is compared to a norming sample composed of other people from the same population. The medium IQ is conventionally defined as a score of 100 and the majority of the population belong to an IQ range between 70 and 130.
- IQ scores variance is due to the interaction between genes and environmental factors.

KEY FACTS OF PREMORBID IQ

- Dementia is a well-known cause of the decline from premorbid IQ and other cognitive domains. However, also other factors, such as severe mental illnesses, traumatic brain injuries, brain cancer, stroke and hypoxia may determinate a detrimental effect on premorbid IQ.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00026-0
Copyright © 2017 Elsevier Inc. All rights reserved.

- Two principal ways in which a decline from premorbid IQ could occur were suggested: an alteration in the development of the brain linked to a genetic vulnerability that frequently appears in childhood or in adolescence and an age-related structural and functional degeneration of the brain that may be physiological or pathological.
- Premorbid IQ has been suggested as a good predictor for individuals at risk of developing psychosis and as an important prognostic factor to be taken into account.

LIST OF ABBREVIATIONS

CBD Cannabidiol
CNS Central nervous system
FEP First episode psychosis
IQ Intellectual quotient
THC Tetrahydrocannabinol

INTRODUCTION

The harmful effects of cannabis use are a contentious debate topic in research and public health, especially in the context of its legalization and medical use. One of the most widely-studied and critical points of this issue concerns neuropsychological-specific and global impairment, that is, the impact of cannabis use on individual psychological functions (memory, attention, executive functions), and its effects on the general intellectual quotient (IQ). High efficiency, speed, and multitasking capacities are in great demand in today's world, and detrimental effects on brain functions have been identified as risk factors for different psychiatric (Bora, Yücel, & Pantelis, 2010; Hatch et al., 2007) and medical illnesses (Calvin et al., 2011). These considerations augment the significance and urgency surrounding the study of the problem, since cannabis is one of the commonly-used illegal drugs, and the rates have increased in the last few years (SAMHSA, 2013). The National Survey on Drug Use and Health has identified cannabis as the leading drug associated with the initiation of illecit-drug use (SAMHSA, 2013). It also estimates that about 2.4 million people aged 12 or older had started using cannabis by 2012, and 5.4 million use marijuana on a daily basis (SAMHSA, 2013). All this data suggests that there is a significant proportion of very young people at risk, during this most critical period of brain maturation. While several studies report an acute adverse effect of cannabis on cognition, with obvious consequences on personal and public health (Asbridge, Hayden, & Cartwright, 2012; Kalant, 2004), the long-term effects of cannabis are still unclear (Grant et al., 2003; Schreiner & Dunn, 2012). This state of uncertainty is mostly attributed to the difficulty in controlling for relevant variables—first of all, premorbid cognition—in naturalistic designs, thus preventing the drawing of firm conclusions about the casual direction of positive results. To further complicate the matter, studies on cannabis use and cognitive impairment associated with schizophrenia have yielded controversial results (Yücel et al., 2012; Potvin, Joyal, Pelletier & Stip, 2008; Rabin, Zakzanis, Daskalakis, & George, 2011). These considerations lead to the conclusion that premorbid conditions are the most appropriate indicator in determining the observed effects of cannabis on cognition among clinical and nonclinical samples.

CANNABIS AND COGNITION: APPROACHING THE "PRE" PROBLEM

The association between cannabis use and cognitive performance has been fully-studied. Table 24.1 shows a nonexhaustive summary of the principal reviews on this subject, with the authors' conclusions. It started from 1995, when Pope, Gruber, and Yurgelun-Todd (1995) first tried to distinguish between the acute and residual effects of cannabis in individuals without any psychiatric disorders.

Even differences between heavy users and controls had very modest results, or were absent in this study (Pope et al., 1995), so the authors argued that it would be very important to try to exclude the virtual continuous effect of acute intoxication, and the confounding effect of withdrawal syndrome. With this consideration in mind, they later tried to define the neurotoxicity of cannabis as its long-term residual effect lasting after 28 days of abstinence and tested people who had used cannabis on an almost daily basis (Pope, Gruber, & Yurgelun-Todd, 2001a; Pope et al., 2001b; Pope, Gruber, Hudson, Huestis, & Yurgelun-Todd, 2002). Their results suggested deficits in memory and attention present for days after discontinuing use, but mainly reversible after 4–5 weeks (Pope et al., 2001a), regardless of the cumulative amount of lifetime cannabis use (Pope et al., 2002). Gonzalez et al. (2002) examined the methodology adopted in the previous literature on the basis of the correspondence to a priori criteria, and their results were consistent with those from Pope and coworkers. Despite methodological rigor, very few studies among those considered assessed premorbid neuropsychological abilities, and they, usually, looked at vocabulary or verbal fluency tests (Table 24.2). The same group performed a metaanalysis in 2003 (Grant et al., 2003) by including 1014 subjects, and the average global neurocognitive effect was small, and was expressly observed in memory and forgetting/retrieval tests.

TABLE 24.1 Reviews and Metaanalysis on Cannabis and Cognition

Authors	Study type	Number of studies included (years of publication)	Cannabis effect targeted	Impaired domains
Pope, Gruber, and Yurgelun-Todd (1995)	Review	42 (1972–93)	RE = 12–24 HA LTE > 24 HA	RE: attention, psychomotor tasks, short-term memory LTE: minimal
Pope, Gruber, Hudson, Huestis, and Yurgelun-Todd (2001b)	Review	8 (1996–2001) RE 7 (1989–2000) LTE	RE < 28 DA LTE > 28 DA	RE: memory and selective attention LTE: minimal
Gonzalez, Carey, and Grant (2002)	Review	40 (1973–2002)	RE > 24 HA	RE: attention and working memory
Grant, Gonzalez, Carey, Natarajan, and Wolfson (2003)	Metaanalysis	11 (1977–2002)	RE > 24 HA	RE: global performance, learning and forgetting
Gonzalez (2007)	Review	20 (1980–2007) AE 19 (1999–2006) RE	AE ≤ 12 HA RE > 24 HA	AE: memory retrieval RE: memory
Jacobus, Bava, Cohen-Zion, Mahmood, and Tapert (2009)	Review	16 (1989–2008)	RE < 28 DA LTE > 90 DA	RE: attention, processing speed, verbal learning and memory LTE: none
Solowij and Pesa (2010)	Review	116 (2000–2010)	AE ≤ 24 HA RE = 24 HA-28 DA	AE and RE: attention, inhibition, verbal memory and other memory processes
Crean, Crane, and Mason (2011)	Review	12 (1970–2009) AE; 14 (1995–2010) RE; 11 (1995–2006) LTE	AE ≤ 6 HA RE = 7–20 DA LTE > 21 DA	AE: working memory, attention and inhibition RE: decision making and risk taking LTE: none
Schreiner and Dunn (2012)	Metaanalysis	33 (2000–2011) RE; 13 of them on LTE	RE > 4 HA LTE > 25 DA	RE: global performance, most cognitive domains LTE: none
Crane, Schuster, Fusar-Poli, and Gonzalez (2013)	Review	34 (2007–2012) AE; 61 (2007–2012) RE	AE < 8 HA RE > 8 HA	AE: learning and memory, attention, concentration and working memory RE: episodic memory, attention, concentration, risk taking and decision making

The table presents a summary of the principal reviews about cannabis use, and acute, residual, and long-term effects on cognition with authors' findings.
Abbreviations: AE, acute effect; RE, residual effect; LTE, long-term effect; HA, hours of abstinence; DA, days of abstinence.

A small effect has also been observed by Gonzalez (2007) in frequent and heavy cannabis users, and this was not necessarily matched with changes in brain functioning. However, results from Gonzalez and Grant's group appear not to be able to overcome the question about long-term neurotoxicity, given that most of the cannabis users included in their studies had been abstinent for a maximum of 2 weeks. In fact, in a recent update of Grant's work, published by Schreiner and Dunn (2012), most of the cognitive domains assessed, as well as the overall performance, resulted significantly impaired when subjects were tested within the first 25 days of abstinence, but not after. This study indicated a small negative residual effect, and no lasting effects on neuropsychological performance, regardless of the duration of use.

Besides the amount of cannabis used and abstinence length, another crucial point, when considering the harmful effects of cannabis on cognition, is the age of first cannabis use. Jacobus et al. (2009) addressed this point in a review, and concluded that adolescents who use marijuana heavily tend to show disadvantages in attention, processing speed, verbal learning, and memory that persist beyond 1 month of abstinence, but largely remit after 3 months of sustained abstinence. Once again, without any measure of premorbid IQ, it is difficult to establish whether a causal direction exists between cannabis use and cognition.

Di Forti, Morrison, Butt, and Murray (2007) addressed "the chicken or the egg" question by claiming the role of an earlier age of onset in using cannabis as a determinant in the detrimental effect of the substance on cognition and hypothesizing a gene–environment pattern of common vulnerability to cannabis effect and psychosis, where neuropsychological deficits underlay a profile of people at-risk to develop psychosis without the need for the additional risk factor of cannabis use. Other reviews have ascertained impairment in memory during the acute phase (Ranganathan & D'Souza, 2006), and in the long-term (Solowij & Battisti, 2008), together with attention and inhibition, depending on duration, frequency, dose, and onset of cannabis use in adolescence (Solowij & Pesa, 2010). Schoeler and Bhattacharyya (2013) have recently highlighted the role of the mix of different cannabinoids present in the cannabis used on cognition. Other executive functions have received more attention in recent years (Crane et al., 2013; Crean et al., 2011) as is shown in Fig. 24.1.

Most of the reviews concentrate on residual and long-term effects. Residual effects are the most-represented

TABLE 24.2 Instruments for Assessing Premorbid IQ

Type of test	Examples	Task	Strengths and limitations
Verbal subtests of the Wechsler Adult Intelligence Scale (WAIS)[a]	• Vocabulary	To provide the definition of a list of increasingly difficult words	They are considered less sensitive to the impact of age and various forms of brain damage. However, they are strongly affected by the integrity of long-term memory, and might be poorly reliable in individuals with impaired memory
	• Information	To answer a list of questions regarding general culture (ie, in what continent is Argentina?)	
Verbal fluency	• Controlled Oral Word Association Test (COWAT) from the Halstead-Reitan neuropsychological test battery[b]	To generate as many words as he/she can, beginning with a particular letter (ie, F, A, and S) or belonging to a particular semantic category (ie, animals) within a limited time	They depend on the ability to retrieve and organize information of the semantic memory, considered the most resistant memory register. However, reduced speed and a deficit in verbal memory could compromise the test
Reading test	• Wechsler Test for Adult Reading, WTAR[c] • National Adult Reading Test, NART[d]	To read aloud a list of irregularly spelled words (whose transcriptions do not correspond to their pronunciation)	Since general spelling rules cannot be applied to irregular words, the correct pronunciation mainly relies on remote simple learning of vocabulary. However, they are not available for nonirregular languages

The table provides a critical summary of the principal instruments used to assess premorbid IQ in cannabis studies. Abbreviation: IQ: intellectual quotient.
[a] *Wechsler, D. (Ed.) (1997).* WAIS-III Administration and Scoring Manual. *San Antonio, TX: The Psychological Corporation.*
[b] *Reitan, R. M., & Wolfson, D. (1993).* The Halstead-Reitan neuropsychological test battery: Theory and clinical interpretation. *Tucson, AZ: Neuropsychology Press.*
[c] *Holdnack, H. A. (2001).* Wechsler Test of Adult Reading. *San Antonio, TX: The Psychological Corporation.*
[d] *Nelson, H. E., & Willison, J. (Eds.), (1991).* National Adult Reading Test (NART), Test Manual *(2nd ed.). Windsor: NFER Nelson.*

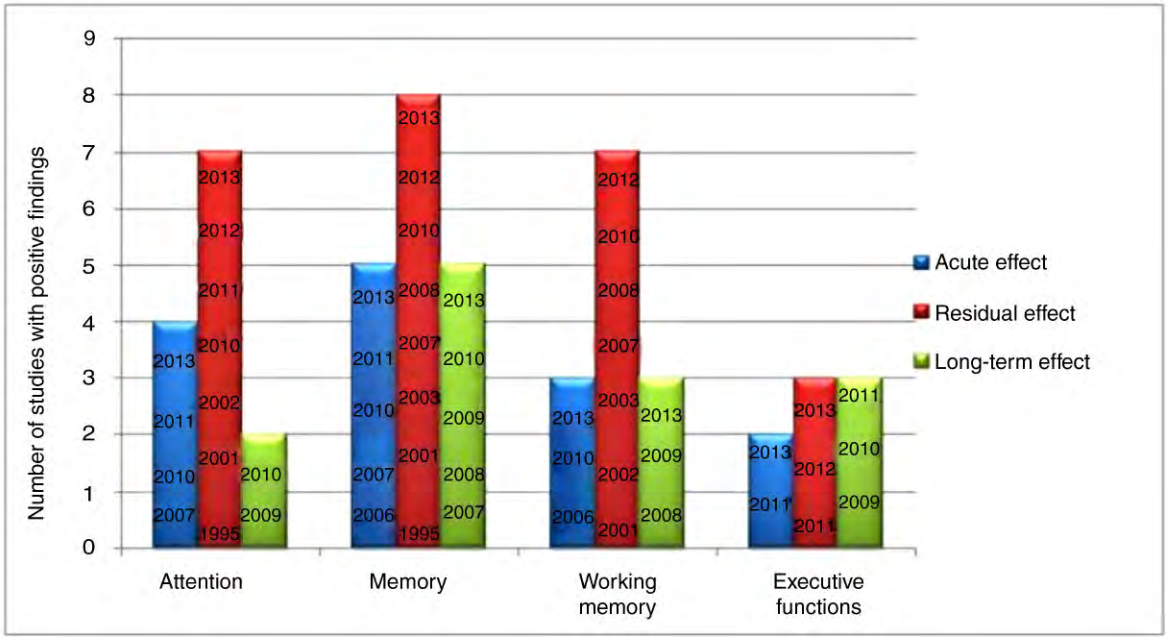


FIGURE 24.1 **Cannabis use and cognitive functioning.** This histogram provides an overview of the main findings on cannabis use and cognition mentioned in this chapter. Cognitive functions are on the *x*-axis, and the number of studies with positive findings on deterioration for each domain, divided according to acute effect, residual effect, and long-term effect, are counted on the *y*-axis. Each bar cumulates studies on the field indicated, by referring to their year of publication—from the oldest to the newest. Original material.

findings over the years, especially in the memory domain, and positive results on long-term effects of cannabis probably depend on ameliorated research designs. Very few papers from those mentioned previously considered general IQ as a measure of interest, and generally found a small residual effect on overall impairment, but not after a long-term period of abstinence (Grant et al., 2003; Schreiner & Dunn, 2012).

LONGITUDINAL STUDIES ON PREMORBID IQ: WHICH "PRE" PREDICTS WHAT "POST"

Table 24.2 provides a critical summary of the principal instruments used to assess premorbid IQ in cannabis studies.

Solowij and Battisti (2008) reported three studies showing lower vocabulary scores in cannabis users, compared to controls. Pope et al. (2001b) estimated premorbid IQ by using a vocabulary test which resulted in lower scores among heavy users than controls, additionally attenuating differences in cognitive performance between the two groups, when entered as covariates (Pope et al., 2001b).

Schreiner and Dunn (2012) indicated a similar impairment on overall performance in the verbal/language domain—assessed by vocabulary, verbal fluency, and naming tests—within the first 25 days of abstinence, but not after this period of time. These results suggest that the vocabulary and verbal fluency tests behave more as measures of actual capacities, than as an estimate of premorbid IQ. Longitudinal studies are probably the best way to address this issue, by measuring cognitive performance before and after a period of life in which cannabis is consumed. Otherwise, in the absence of these possibilities, reading tests have been suggested as a better method because they are able to estimate the general ability before eventual impairment, as opposed to other tests (Griffin, Rivera-Mindt, Rankin, Ritchie, & Scott, 2002). Table 24.3 simplifies methods and conclusions of the principal longitudinal studies on premorbid IQ and cannabis use.

In the early 1980s, Kellam, Ensminger, and Simon (1980) tested a large cohort of African-American adolescents from Woodlawn, an urban, poor community on the South Side of Chicago, at their first degree school, and retested them 10 years later, by revealing that people with higher IQ, social adaptive capacities, and readiness-for-school scores were more likely to use cannabis in their teenage years. Fleming et al. (1982) later revealed on the same population that boys with higher IQ and readiness-for-school tended to initiate substance use at an earlier age, and girls who were rated as shy or having learning problems tended to initiate use at a later age. Ensminger, Juon, and Fothergill (2002) reinterviewed 952 subjects from this original cohort at age 32, and showed that males having superior IQ scores in childhood were more likely to initiate cannabis use in adolescence, but were also more likely to discontinue this behavior as young adults, while to be shy and aggressive was a risk factor for becoming a persistent cannabis user as an adult.

Fried and coworkers (Fried, Watkinson, & Gray, 2005; Fried, Watkinson, James, & Gray, 2002) had the opportunity to follow-up on a group of young subjects, as part of the Ottawa Prenatal Prospective Study, and assessed their IQ before, during, and after cessation of regular marijuana use. Young adults with heavy actual or former use started with a lower IQ, compared to nonusers and light users, but only actual heavy use was related to a lower IQ, when compared to the other groups. However, similar deficits were no longer apparent 3 months after cessation of regular use. Opposite findings were derived from two different British cohort studies by White and coworkers (White, Gale, & Batty, 2012a). They found that a higher childhood IQ is related to an increased risk of illegal drug use in adolescence and adulthood, even when correcting for material disadvantage, antisocial behavior, social distress, and anxiety. However, the same group (White, Mortensen, & Batty, 2012b) found out the opposite result on a group of males US Vietnam veterans with IQ assessed at the time of the military service enrollment. That is, subjects with higher IQ scores had a lower probability to be habitual users of cannabis, during active service and in civilian life, with a moderating effect of socioeconomic status in adulthood.

Meier et al. (2012) had recently published a paper where people from the Dunedin Cohort Study were followed-up from childhood to the age of 38. Subjects with more persistent cannabis dependence (that had started in adolescence) presented greater IQ decline, not specific to any domains, when compared to study members who never used cannabis. This latter group experienced only a slight increase in IQ. Results stayed significant after ruling out possible confounders, such as, education, diagnosis of schizophrenia, other drug dependence, and past-week cannabis consumption. Interestingly, people who reported a lifetime recreational use of cannabis, but not dependence, started with a higher IQ than people that never used cannabis. These two groups had similar IQ levels at the age of 38. However, the authors did not test the study subjects after a period of abstinence of at least 4 weeks. Therefore, their findings are likely to be more reliable for the residual effect of cannabis, than the long-term effect. This study has raised a debate surrounding other potential confounders of the results. For example, Rogeberg (2013a) argues that socioeconomic status alone can potentially explain the IQ decline observed by Meier et al. (2012), and recalls the Flynn–Dickens model of IQ (Dickens & Flynn, 2001) that emphasizes a two-way causality between IQ and environment, with both a direct influence on IQ from the genotype, and the opposite. That is because people with

TABLE 24.3 Longitudinal Studies on Premorbid IQ and Cannabis Use

Authors	Study	Number of subjects	Pattern of cannabis use as described by authors	Measures (age in years in which measures are taken)	Conclusions
a) Kellam et al. (1980)	1966–1967 first graders Woodlawn, African Americans—United States a),b),c)	705 a),b)	Lifetime a),b)	IQ (6; 16) a),b)	Higher IQ, social adaptive capacities and readiness-for-school scores in childhood predict cannabis use in teenage years
b) Fleming, Kellam, and Brown (1982)					Subjects with higher IQ and readiness-for-school in childhood tend to initiate cannabis use at an earlier age. Shy girls or girls with learning problems tend to start use later
c) Ensminger et al. (2002)		952	Discontinue; Persistent	IQ (6; 32)	Males having higher IQ in childhood are more likely to initiate in adolescence, but also to discontinue use as young adults. Low-scoring females are less predisposed to persistent use
d) Fried et al. (2002)	Ottawa Prenatal Prospective Study (OPPS)—Canada d),e)	70	Light current regular; heavy current regular; former regular; none d),e)	IQ (12; 17–20)	Overall IQ, and other domains, impaired in heavy current users, but for no longer than 3 months after cessation of regular use d),e)
e) Fried et al. (2005)		113		IQ, processing speed, memory, vocabulary, attention, abstracting abilities (12; 17–20)	
f) White and Batty (2011)	1970 British Cohort Study—UK	7,946	Occasional	IQ (5; 10)	High childhood IQ increases the risk of use in adolescence and adulthood, especially in women
g) White et al. (2012a)	1958 National Child Development Survey—UK	6,713	Recreational	IQ (11; 42)	Use in middle age is more probable in children with a greater IQ, especially women
h) White et al. (2012b)	Vietnam Experience Study—USA	14,362	Habitual	IQ (22; 40)	Subjects with higher IQ at 22 are less likely to be habitual users, during active service and in civilian life. This association is attenuated by confounding variables
i) Meier et al. (2012)	Dunedin Cohort Study—New Zealand	1,037	Persistent	IQ (7–13; 38)	A drop from childhood to adult average full-scale IQ especially in adolescent-onset users

In this table is offered an overview of longitudinal studies that expressly assess IQ before and after cannabis use. Abbreviation: IQ, intellectual quotient.

a higher IQ tend to be involved into more cognitively challenging environments that further increase IQ. Daly (2013) intervenes in the debate saying that the personality trait of "conscientiousness" may be protective for the same people. The cut and thrust of this discussion is in two successive letters in the same volume of the Proceedings of the National Academy of Science (Moffit, Meier, Caspi, & Poulton, 2013; Rogeberg, 2013b). In conclusion, all these discordant findings show that IQ is not only a complex proxy for cognitive functions, but also one for social adaptation, environmental factors, and genes. It is far from exhaustive of, or independent from, these other measures, and is determined by a consensus on conventional and standardized elements. Interestingly, all these studies present a point of consistency, as indicated in Fig. 24.2. That is, the relationship between higher premorbid IQ and recreational or discontinued use, while a lower premorbid IQ results as a predictor for regular or heavy cannabis use.

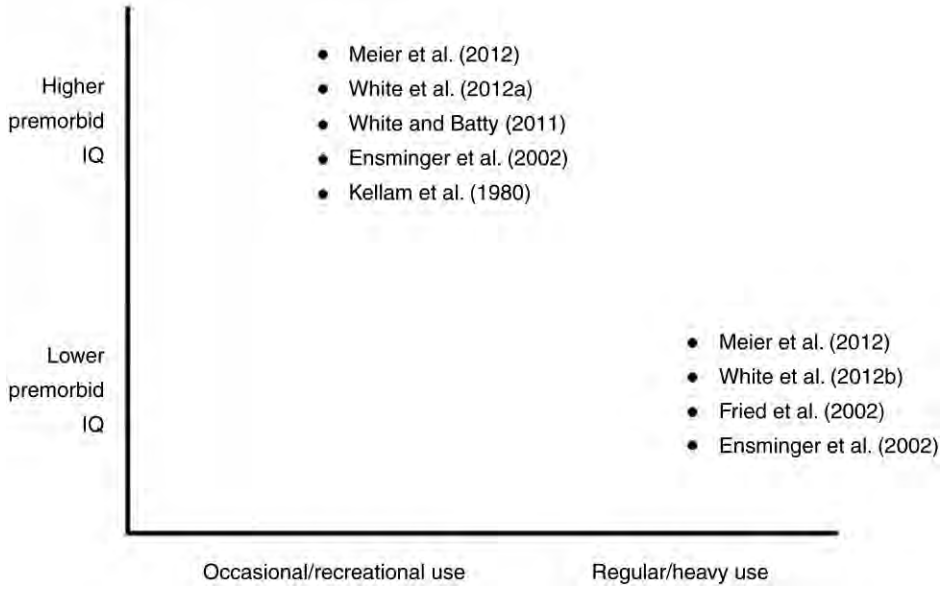


FIGURE 24.2 **Premorbid IQ and cannabis: main findings from longitudinal studies.** This graph provides a simplification of the results of longitudinal studies on premorbid IQ, categorized into "higher" and "lower" on the *y*-axis, and differentiated by patterns of cannabis use, recreational versus regular use, in the *x*-axis.

CANNABIS AND PREMORBID IQ: WHAT'S THE REAL RISK?

When looking at the previously mentioned studies, it seems less likely that cannabis use influences IQ, and more likely that premorbid IQ is linked to cannabis use, toward complex mechanisms. Nevertheless, it is very difficult to believe that a psychotropic substance acting on important neurotransmitters such as acetylcholine, dopamine, GABA, glutamate, serotonin (Grotenhermen, 2004), does not have neuropsychological effects at all, in the long run. It was suggested that its effects are so subtle that they are difficult to detect (Gonzalez, 2007), otherwise cannabis may be relatively innocuous for most people, but quite toxic for the occasional individual (Pope et al., 2001b), depending on dose, type of cannabis used, age of onset (Solowij & Pesa, 2010), and gender (Crane et al., 2013). Cannabis use has been ascertained as a risk factor for the development of psychosis (Moore et al., 2007; Potvin & Amar, 2008), and may lead to an earlier age of onset of the illness, especially if cannabis use starts in early adolescence, with a pattern of daily use of high potency cannabis (Di Forti et al., 2014). Surprisingly, three different metaanalyses on neurocognition and cannabis among schizophrenic patients found better cognitive performance among patients with a lifetime use of cannabis (Potvin, Joyal, Pelletier, & Stip, 2008; Rabin et al., 2011; Yücel et al., 2012). This counterintuitive finding, coupled with the fact that most psychotic patients suffer from cognitive impairment (Reichenberg et al., 2009) make it more difficult to understand the mismatch between this two risk factors.

In a recent study, our group (Ferraro et al., 2013) found strong evidence for a better premorbid IQ, and a minor deterioration (difference between IQ and premorbid IQ) in FEP patients who referred to have used cannabis in their lifetime. Such differences were not witnessed among controls, with a significant interaction between group and cannabis use. Cases of our group were more likely to have preferred skunk, and to have smoked on a daily basis, than controls so we suggested that the better cognitive performance of FEP patients who had used cannabis, compared with those who had not, was due to the better premorbid IQ of the former. Moreover, psychotic cannabis users reported a social use of cannabis in similar proportions to those in controls (69%). Thus, these findings are compatible with the view that, among psychotic patients, those who used cannabis were less neurodevelopmentally impaired than those who did not, and their better premorbid cognition was likely to have facilitated their use of the drug in a typical recreational way, sharing it with their friends (Ferraro et al., 2013).

Data observation suggests that cannabis is a dangerous substance because, while studies on animal models could precisely measure the proportion between tetrahydrocannabinol (THC) and cannabidiol (CBD), the types of cannabis that are encountered by real-world users have a wide range of THC:CBD ratios (see also Niesink & van Laar, 2013). Also, healthy people who smoke higher THC concentration cannabis are more likely to develop psychosis-like symptoms (such as paranoia) (Morgan & Curran, 2008), even with a recreational pattern of use (Morgan et al., 2012). Among the tiny percentage of smokers

who develop psychosis, the preferred variety used is skunk (Di Forti et al., 2009), which has a relatively high concentration of THC. Therefore, there is not enough evidence to sustain the possibility that paradoxical effects of recreational cannabis use frequently observed in naturalistic studies with psychotic patients are derived from a neuroprotective action of CBD on the brain.

So, what about premorbid IQ and cannabis use? What is the real risk that people who smoke cannabis are facing? In Fig. 24.3, the authors speculated about a possible

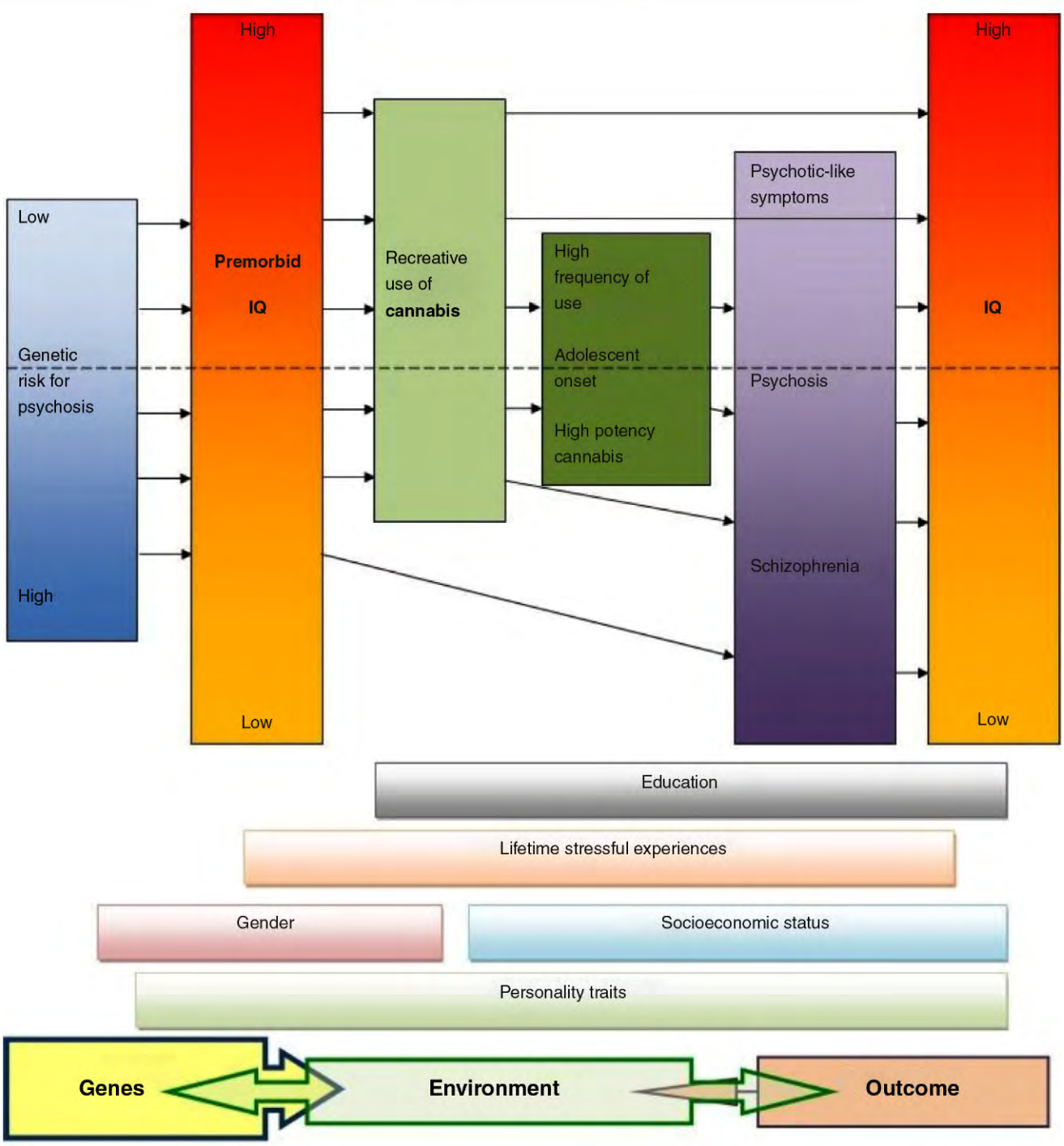


FIGURE 24.3 A possible explanation of the link between premorbid IQ, cannabis use, and psychosis. This conceptual table proposes a shared genetic vulnerability to psychosis and cannabis use that may cause psychotic-like symptoms in healthy people, or may be a trigger for psychosis in people predisposed—toward different pattern of cannabis use—on a continuum of vulnerability that involves premorbid IQ, and is reflected in actual IQ. Education, socioeconomic status, gender, and personality traits are shown as examples of moderator factors. The lower part of the figure indicates the main fields where each factor belongs.

explanation of the link between premorbid IQ, cannabis use, and the risk of psychosis.

A pattern of genetic-early environment vulnerability to psychosis could predispose subjects to cannabis use, as was suggested in a very recent study on 2082 healthy individuals, conducted by Power et al. (2014). THC may, in turn, cause psychotic-like symptoms in healthy people with some predisposition to psychosis, even if consumed in a recreational way (Morgan et al., 2012; Sideli et al., 2015). This pattern of common vulnerability could explain why cannabis users are impaired in many of the same type of cognitive tests in which people with schizophrenia are impaired (Solowij & Michie, 2007).

Going down toward the continuum, the greater the predisposition to psychosis, the stronger is the association with lower premorbid IQ. This relationship operates across the entire range of intellectual abilities, in a progression of neurodevelopmental risk (Khandaker, Barnett, White, & Jones, 2011). However, only people near to a normal premorbid IQ range (ie, lower limit of normality, 90–100) have the opportunity to stay in contact with the illegal substance, sharing it with friends (Ferraro et al., 2013) and *this* constitutes an additional risk factor for cannabis use, among patients and healthy people. *This*, coupled with a pattern that usually presents a high risk (ie, frequent use, onset of use in adolescence, preference for cannabis with high TCH concentration), but that could be harmful even in a recreational way, falling down the continuum of the neurodevelopmental impairment, perhaps because it encounters a major sensitiveness to the THC effect (Verdejo-Garcia et al., 2013). Even more neurodevelopmentally compromised individuals could develop psychosis, without the need for the additional risk factor of cannabis use (Di Forti et al., 2007), and they could also have major cognitive deterioration at their first episode because they have less cognitive reserve (Leeson, Harrison, Ron, Barnes, & Joyce, 2012). As already mentioned, other factors can moderate (and can be influenced by) the contact with an illegal substance and the outcome, in different complex ways. For example, gender, personality traits, education, and lifetime stressful experiences, including childhood trauma (Sideli et al., 2015) could play a role. However, the authors only have at their disposal the previous literature, and a point of view from which to generate this streamlined model, and they are aware that further investigation is needed in order to understand this complex mechanism.

CONCLUSIONS

Cognitive abilities of subjects who smoked cannabis are reliable only if assessed after a prolonged time of abstinence. Eventual long-term impairment observed after this period could reflect the consequence of a complex premorbid gene—early environmental predisposition. This predisposition can influence the contact with the substance, and a particular pattern of cannabis use, moderated by other environmental factors. This could be true even for clinical samples since, according to the neurodevelopmental theory of schizophrenia (Murray & Lewis, 1987), the neurocognitive impairment in psychosis remains stable after the onset of the illness (Bora & Murray, 2014). Moreover, cannabis use could be a trigger for a neurodevelopmental predisposition.

So, is cannabis safe? The answer is no. Since we do not know which special premorbid cocktail was prepared for us, cannabis could act like a "Russian roulette" placed against the brain: the more you play, the higher is the risk you are taking but, sometimes, a single game could be enough.

MINI-DICTIONARY

Acute cannabis effects Effects measured within 24 h from cannabis administration.
First episode of psychosis (FEP) An individual affected by a psychotic disorder (schizophrenia, major depression or bipolar I disorder with psychotic features) presenting for the first time to mental health services to receive medical treatment.
Intellectual quotient (IQ) An estimate of general intellectual functioning of an individual, including both verbal and practical skills. It is, usually, assessed using multidimensional tests and by comparing the individual's performance with those of the people of similar age and gender in a determinate population.
Literature review and meta-analysis A critical summary of the literature that discusses a particular research question, which may include a quantitative estimation of the evidence of interest (metaanalysis).
Long-term cannabis effect Effects lasting after at least 28 days of cannabis abstinence.
Longitudinal/prospective study A study designed to compare repeated observations of the same individuals collected at different time-points, that is before and after the exposure to a risk factor or the administration of a treatment.
Neurodevelopment impairment A pathological alteration of the primary brain function (attention, memory, learning, language, and emotion) that arises in childhood as consequence of genetic and environmental exposures.
Premorbid IQ An estimate of general intellectual functioning of an individual before the occurrence of any event potentially detrimental for his mental health, such as the onset of a mental disorder or the use of any psychoactive substance.
Residual cannabis effect Effects lasting beyond the first 24 and up to 28 days after the last cannabis administration.
Schizophrenia A severe mental disorder characterized by delusions, hallucinations, and disorganized language and behavior.

References

Asbridge, M., Hayden, J. A., & Cartwright, J. L. (2012). Acute cannabis consumption and motor vehicle collision risk: systematic review of observational studies and meta-analysis. *BMJ*, *9*, 344–536.
Bora, E., & Murray, R. M. (2014). Meta-analysis of cognitive deficits in ultra-high risk to psychosis and First-Episode Psychosis: do the cognitive deficits progress over, or after, the onset of psychosis? *Schizophrenia Bulletin*, *40*(4), 744–755.

Bora, E., Yücel, M., & Pantelis, C. (2010). Cognitive impairment in schizophrenia and affective psychoses: implications for DSM-V criteria and beyond. *Schizophrenia Bulletin*, *36*, 36–42.

Calvin, C. M., Deary, I. J., Fenton, C., Roberts, B. A., Der, G., Leckenby, N., & Batty, G. D. (2011). Intelligence in youth and all-cause-mortality: systematic review with meta-analysis. *International Journal of Epidemiology*, *40*(3), 626–644.

Crane, N. A., Schuster, R. M., Fusar-Poli, P., & Gonzalez, R. (2013). Effects on cannabis on neurocognitive functioning: recent advances, neurodevelopmental influences, and sex differences. *Neuropsychology Review*, *23*(2), 117–137.

Crean, R. D., Crane, N. A., & Mason, B. (2011). An evidence-based review of acute and long-term effects of cannabis use on executive cognitive functions. *Journal of Addiction Medicine*, *5*(1), 1–8.

Daly, M. (2013). Personality may explain the association between cannabis use and neuropsychological impairment. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(11), E979.

Di Forti, M., Morrison, P. D., Butt, A., & Murray, R. M. (2007). Cannabis use and psychiatric and cognitive disorders: the chicken or the egg? *Current Opinion in Psychiatry*, *20*, 228–234.

Di Forti, M., Morgan, C., Dazzan, P., Pariante, C., Mondelli, V., Marques, T. R., Handley, R., Luzi, S., Russo, M., Paparelli, A., Butt, A., Stilo, S., Wiffen, B., Powell, J., & Murray, R. M. (2009). High-potency cannabis and the risk of psychosis. *British Journal of Psychiatry*, *195*, 488–491.

Di Forti, M., Sallis, H., Allegri, F., Trotta, A., Ferraro, L., Stilo, S., Marconi, A., La Cascia, C., Reis Marques, T., Pariante, C., Dazzan, P., Mondelli, V., Paparelli, A., Kolliakou, A., Prata, D., Gaughran, F., David, A. S., Morgan, C., Stahl, D., Khondoker, M., MacCabe, J. H., & Murray, R. M. (2014). Daily use, especially of high-potency cannabis, drives the earlier onset of psychosis in cannabis users. *Schizophrenia Bulletin 40*(6), 1509–1517.

Dickens, W. T., & Flynn, J. R. (2001). Heritability estimates versus large environmental effects: the IQ paradox resolved. *Psychological Review*, *108*(2), 346–369.

Ensminger, M. E., Juon, H. S., & Fothergill, K. E. (2002). Childhood and adolescent antecedents of substance use in adulthood. *Addiction*, *97*, 833–844.

Ferraro, L., Russo, M., O'Connor, J., Wiffen, B., Falcone, M. A., Sideli, L., Gardner-Sood, P., Stilo, S., Trotta, A., Dazzan, P., Mondelli, V., Taylor, H., Friedman, B., Sallis, H., La Cascia, C., La Barbera, D., David, A. S., Reichenberg, A., Murray, R. M., & Di Forti, M. (2013). Cannabis users have higher premorbid IQ than other patients with first onset psychosis. *Schizophrenia Research*, *150*, 129–135.

Fleming, J. P., Kellam, S. G., & Brown, C. H. (1982). Early predictors of age at first use of alcohol, marijuana, and cigarettes. *Drug and Alcohol Dependence*, *9*, 285–303.

Fried, P. A., Watkinson, B., James, D., & Gray, R. (2002). Current and former marijuana use: preliminary findings of a longitudinal study of effects on IQ in young adults. *Canadian Medical Association Journal*, *166*(7), 887–891.

Fried, P. A., Watkinson, B., & Gray, R. (2005). Neurocognitive consequences of marijuana – a comparison. *Neurotoxicology Teratololgy*, *27*, 231–239.

Gonzalez, R. (2007). Acute and non-acute effects of cannabis on brain functioning and neuropsychological performance. *Neuropsychology Review*, *17*, 347–361.

Gonzalez, R., Carey, C., & Grant, I. (2002). Nonacute (residual) neuropsychological effects of cannabis use: a qualitative analysis and systematic review. *Journal of Clinical Pharmacology*, *42*, 48S–57S.

Grant, I., Gonzalez, R., Carey, C., Natarajan, L., & Wolfson, T. (2003). Non-acute (residual) neurocognitive effects of cannabis use: a meta-analytic study. *Journal of the International Neuropsychological Society*, *9*, 679–689.

Griffin, S. L., Rivera-Mindt, M., Rankin, E. J., Ritchie, A. J., & Scott, J. G. (2002). Estimating premorbid intelligence. Comparison of traditional and contemporary methods across the intelligence continuum. *Archives of Clinical Neuropsychology*, *17*, 497–507.

Grotenhermen, F. (2004). Pharmacology of cannabinoids. *Neuroendocrinology Letters*, *25*(1–2), 14–23.

Hatch, S. L., Jones, P. B., Kuh, D., Hardy, R., Wadsworth, M. E., & Richards, M. (2007). Childhood cognitive ability and adult mental health in the British 1946 birth cohort. *Social Science and Medicine*, *64*(11), 2285–2296.

Jacobus, J., Bava, S., Cohen-Zion, M., Mahmood, O., & Tapert, S. F. (2009). Functional consequences of marijuana use in adolescents. *Pharmacology Biochemistry and Behavior*, *92*(4), 559–565.

Kalant, H. (2004). Adverse effects of cannabis on health: an update of the literature since 1996. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *28*(5), 849–863.

Kellam, S. G., Ensminger, M. E., & Simon, M. B. (1980). Mental health in first grade and teenage drug, alcohol, and cigarette use. *Drug and Alcohol Dependence*, *5*, 273–304.

Khandaker, G. M., Barnett, J. H., White, I. R., & Jones, P. B. (2011). A quantitative meta-analysis of population-based studies of premorbid intelligence and schizophrenia. *Schizophrenia Research*, *132*(2–3), 220–227.

Leeson, V. C., Harrison, I., Ron, M. A., Barnes, T. R., & Joyce, E. M. (2012). The effect of cannabis use and cognitive reserve on age at onset and psychosis outcomes in first-episode schizophrenia. *Schizophrenia Bulletin*, *38*(4), 873–880.

Meier, M., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R. S., McDonald, K., Ward, A., Poulton, R., & Moffitt, T. E. (2012). Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(40), E 2657–E 2664.

Moffitt, T. E., Meier, M. H., Caspi, A., & Poulton, R. (2013). Reply to Rogeberg and Daly: no evidence that socioeconomic status or personality differences confound the association between cannabis use and IQ decline. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(11), E980–E982.

Moore, T. H., Zammit, S., Lingford-Hughes, A., Barnes, T. R. E., Jones, P. B., Burke, M., & Lewis, G. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet*, *370*, 319–328.

Morgan, C. J., & Curran, H. V. (2008). Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. *British Journal of Psychiatry*, *192*, 306–307.

Morgan, C. J., Gardener, C., Schafer, G., Swan, S., Demarchi, C., Freeman, T. P., Warrington, P., Rupasinghe, I., Ramoutar, A., Tan, N., Wingham, G., Lewis, S., & Curran, H. V. (2012). Sub-chronic impact of cannabinoids in street cannabis on cognition, psychotic-like symptoms and psychological well-being. *Psychological Medicine*, *42*(2), 391–400.

Murray, R. M., & Lewis, S. W. (1987). Is schizophrenia a neurodevelopmental disorder? *British Medical Journal (Clinical Research Edition)*, *295*, 681–682.

Niesink, R. J. M., & van Laar, M. W. (2013). Does cannabidiol protect against adverse psychological effects of THC? *Frontiers in Psychiatry*, *4*, 1–8.

Pope, H. G., Jr., Gruber, A. J., & Yurgelun-Todd, D. (1995). The residual neuropsychological effects of cannabis: the current status of research. *Drug and Alcohol Dependence*, *30*, 25–34.

Pope, H. G., Jr., Gruber, A. J., & Yurgelun-Todd, D. (2001a). Residual neuropsychological effects of cannabis. *Current Psychiatry Reports*, *3*, 507–512.

Pope, H. G., Jr., Gruber, A. J., Hudson, J. I., Huestis, M. A., & Yurgelun-Todd, D. (2001b). Neuropsychological performance in long-term cannabis users. *Archives of General Psychiatry*, *58*, 909–915.

Pope, H. G., Jr., Gruber, A. J., Hudson, J. I., Huestis, M. A., & Yurgelun-Todd, D. (2002). Cognitive measures in long-term cannabis users. *Journal of Clinical Pharmacology*, *42*, 41S–47S.

Potvin, S., & Amar, M. B. (2008). Review: cannabis use increases the risk of psychotic outcomes. *Evidence Based Mental Health*, *11*, 28.

Potvin, S., Joyal, C. C., Pelletier, J., & Stip, E. (2008). Contradictory cognitive capacities among substance-abusing patients with schizophrenia: a meta-analysis. *Schizophrenia Research*, *100*, 242–251.

Power, R. A., Verweij, K. J., Zuhair, M., Montgomery, G. W., Henders, A. K., Heath, A. C., Madden, P. A., Medland, S. E., Wray, N. R., & Martin, N. G. (2014). Genetic predisposition to schizophrenia associated with increased use of cannabis. *Molecular Psychiatry*, *11*, 1201–1204.

Rabin, R. A., Zakzanis, K. K., Daskalakis, J. Z., & George, T. P. (2011). The effects of cannabis use on neurocognition in schizophrenia: a meta-analysis. *Schizophrenia Research*, *128*, 111–116.

Ranganathan, M., & D'Souza, D. C. (2006). The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology*, *188*, 425–444.

Reichenberg, A., Harvey, P. D., Bowie, C. R., Mojtabai, R., Rabinowitz, J., Heaton, R. K., & Bromet, E. (2009). Neuropsychological function and dysfunction in schizophrenia and psychotic affective disorders. *Schizophrenia Bulletin*, *35*, 1022–1029.

Rogeberg, O. (2013a). Correlations between cannabis use and IQ change in the Dunedin cohort are consistent with confounding from socioeconomic status. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(11), 4251–4254.

Rogeberg, O. (2013b). Reply to Moffitt et al. Causal inference from observational data remains difficult. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(11), E983.

SAMHSA (Substance Abuse and Mental Health Services Administration). (2013). *Results from the 2012 National Survey on Drug Use and Health: Summary of National Findings*. Rockville, MD: SAMHSA, NSDUH Series H-46, HHS Publication No. (SMA) 13-4795.

Schoeler, T., & Bhattacharyya, S. (2013). The effect of cannabis use on memory function: an update. *Substance Abuse and Rehabilitation*, *4*, 11–27.

Schreiner, A. M., & Dunn, M. E. (2012). Residual effects of cannabis use on neurocognitive performance after prolonged abstinence: a meta-analysis. *Experimental and Clinical Psychopharmacology*, *20*, 420–429.

Sideli, L., Fisher, H. L., Murray, R. M., Sallis, H., Russo, M., Stilo, S., Paparelli, A., Wiffen, B., O'Connor, J. A., Pintore, S., Ferraro, L., La Cascia, C., La Barbera, D., Morgan, C., & Di Forti, M. (2015). Interaction between cannabis consumption and childhood abuse in psychotic disorders: the role of different patterns of cannabis use. *Early Intervention in Psychiatry*. (Epub ahead of print).

Solowij, N., & Battisti, R. (2008). The chronic effects of cannabis on memory in humans: a review. *Current Drug Abuse Reviews*, *1*, 81–98.

Solowij, N., & Michie, P. (2007). Cannabis and cognitive dysfunction: parallels with endophenotypes of schizophrenia? *Journal of Psychiatry and Neuroscience*, *32*(1), 30–52.

Solowij, N., & Pesa, N. (2010). Cognitive abnormalities and cannabis use. *Revista Brasileira de Psiquiatria*, *32*(Suppl. I), S31–S40.

Verdejo-Garcia, A., Fagundo, A. B., Cuenca, A., Rodriguez, J., Cuyas, E., Langohr, K., de Sola Llopis, S., Civit, E., Farré, M., Peña-Casanova, J., & de la Torre, R. (2013). COMT val158met and 5-HTTLPR genetic polymorphisms moderate executive control in cannabis users. *Neuropsychopharmacology*, *38*, 1598–1606.

White, J. W., & Batty, G. D. (2011). Intelligence across childhood in relation to illegal drug use in adulthood: 1970 British Cohort Study. *Journal of Epidemiology and Community Health*, *66*(9), 767–774.

White, J. W., Gale, C. R., & Batty, G. D. (2012a). Intelligence quotient in childhood and the risk of illegal drug use in middle-age: the 1958 National Child Development Survey. *Annals of Epidemiology*, *22*, 654–657.

White, J. W., Mortensen, L. H., & Batty, G. D. (2012b). Cognitive ability in early adulthood as a predictor of habitual drug use during later military service and civilian life: the Vietnam Experience Study. *Drug and Alcohol Dependence*, *125*, 164–168.

Yücel, M., Bora, E., Lubman, D. I., Solowij, N., Brewer, W. J., Cotton, S. M., Conus, P., Takagi, M. J., Fornito, A., Wood, S. J., McGorry, P. D., & Pantelis, C. (2012). The impact of cannabis use on cognitive functioning in patients with schizophrenia: a meta-analysis of existing findings and new data in a first-episode sample. *Schizophrenia Bulletin*, *38*, 316–330.

CHAPTER

25

Cannabis and Traffic Accidents

*R.B. De Boni**, *R.P. Limberger***, *T.R.V. Sousa*†

**INI Evandro Chagas, FIOCRUZ, Rio de Janeiro, Rio de Janeiro, Brazil
**LABTOXICO: Laboratory of Toxicology, Faculty of Pharmacy, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil
†Center for Drug and Alcohol Research, Hospital de Clinicas de Porto Alegre, Federal University of Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

SUMMARY POINTS

- This chapter presents insight on epidemiological studies, techniques, and rationale for laboratory testing, and the legislation regarding driving under the influence of cannabis.
- Cannabis was the most common illicit drug found on the three largest roadside surveys that objectively evaluated the presence of cannabis, and used probability samples.
- The prevalence of cannabis among injured drivers varies widely, based on geographic region and epidemiological methods, but most studies have found that cannabis use is higher among men and young adults.
- A metaanalysis estimated that driving under the influence of cannabis increased the risk of fatal crashes.
- Different toxicological methods used to detect cannabis, and the interpretations of their results, are a challenge on the comparison of epidemiological findings.
- Whole blood sampling is still the most effective way to confirm the presence of tetrahydrocannabinol (THC) in the body, although the most accessible matrix for the roadside detection is oral fluid.
- The combination of immunological methods with GC/MS, LC-MS/MS, or both, seems to be the best approach for toxicological analysis.
- The laws that regulate driving under the influence of illegal drugs also regulate driving under the influence of cannabis.
- Most countries that have drug driving legislation apply zero-tolerance laws, with regard to illegal substances.

KEY FACTS

- Cannabis is the most frequent illicit drug detected among drivers.
- The prevalence of driving under the influence of cannabis ranged from 0.6% to 6.5%, but data are not entirely comparable across studies, given the methodological differences among them.
- Driving under the influence of cannabis increased the risk of fatal crashes (OR = 2.10; 95% CIs = 1.31–3.36) (Asbridge et al., 2012).
- Punishments vary, given the country in European Union, but may include suspended licenses, fines, and prison sentences (EMCDDA, 2014).

LIST OF ABBREVIATIONS

BAC	Blood alcohol concentration
DRUID	Driving under the influence of drugs, alcohol and medicines
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00027-2
Copyright © 2017 Elsevier Inc. All rights reserved.

TAs Traffic accidents
THC Δ^9-Tetrahydrocannabinol
WHO World Health Organization

INTRODUCTION

Traffic accidents (TAs) are a public health problem, and the World Health Organization (WHO) estimates that they are the cause of 1.24 million fatalities worldwide each year (WHO, 2013). Certain risk factors have been well established for TAs, and these factors can be categorized as environmental, vehicular, or individual. For example, environmental risk factors include city infrastructures, climate conditions, and legislation. Vehicular risk factors include vehicular conditions, and the presence of safety equipment, such as seat belts, and air bags. Individual risk factors include driver sex and age, as well as driving experience, driving behavior, and the use of alcohol, drugs, or both. Approximately 90% of all TAs are related to individual risk factors. The use of alcohol has been associated with an increased likelihood of crashes since the 1960s (Borkenstein, Crowther, Shumate, Ziel, & Zylman, 1974; Compton et al., 2002), and, as scientific evidence has increased, the laws that restrict the use of alcohol prior to driving have also increased. According to the Global Status Report on Road Safety, 89% of the world's countries (comprising 66% of the world's population) have laws that restrict drinking and driving (WHO, 2013). This legislation and its enforcement have contributed to the decrease in the number of TAs within many high-income countries, over recent decades (Fell & Voas, 2006).

Evidence regarding how drugs other than alcohol affect driving and TAs has accrued more recently. In 2004, a comprehensive review of the scientific literature showed that few data were being obtained with regard to the prevalence of drug-influenced driving among the general population because most studies have addressed injured or deceased drivers (Kelly, Darke, & Ross, 2004). These authors also highlighted that the distribution of drugs found among injured drivers typically follows the distribution found in the general population; thus, cannabis is one of the most frequent illicit drugs detected among drivers because it is the most frequently consumed illicit substance in many countries (UNODC, 2013). More recently, Hartman and Huestis (2013) reviewed epidemiological, culpability, and experimental studies over the last two decades, and noted that most studies found a positive association between cannabis use and TAs. The authors highlighted the importance of toxicological techniques, since most studies that did not found association have used self-reports or urine samples to detect drug use.

Furthermore, experimental studies have demonstrated that cannabis use affects many driving skills, including reaction time, divided attention, and lane position. Different designs can be used to assess the effects of cannabis on driving, and its consequences for TAs. In 2008, a panel of experts proposed a set of guidelines for research on driving under the influence of drugs (Walsh, Verstraete, Huestis, & Mørland, 2008). These guidelines provide recommendations in three major areas of knowledge: behavioral, epidemiological, and toxicological, and were a landmark for research on driving under the influence of drugs. Given that the present book has a specific chapter about the effects of cannabis on behavior and the ability to drive, addressed via experimental studies, this chapter focuses on the epidemiological studies that were conducted in order to evaluate the association between cannabis use and TAs, the techniques and rationale for laboratory testing, and the legislation regarding driving under the influence of cannabis.

EPIDEMIOLOGY OF DRIVING UNDER THE INFLUENCE OF CANNABIS

This section discusses the three major types of observational studies that have been conducted to evaluate the association between cannabis and TAs: roadside surveys, hospital studies, and fatal crash studies (Walsh et al., 2008). These designs answer different research questions, and are important for exploring associations and their magnitudes. In most cases, however, they cannot determine causality due to their cross-sectional and observational natures. The prevalence of cannabis use is typically lower among the general population of drivers (according to roadsides surveys) than among injured or fatally injured drivers (according to hospital and fatal crash studies); furthermore, most case-control studies have found an increased chance of TAs among individuals who drive under the influence of cannabis. Tables 25.1–25.3 provide some examples of studies conducted over the last 10 years. These examples include studies that were published in peer-reviewed journals, employed objective measurements of cannabis use, and provided results for cannabis use alone (ie, rather than cannabis with alcohol, or other drug combinations). In addition, only one study was included per country (generally the most recent), and we searched for at least one study from low- and middle-income countries.

Roadside Surveys

Roadside surveys are an important tool for establishing the prevalence of driving under the influence of drugs within the general population of drivers. This methodology surpasses classic general population surveys because it presents the possibility of an objective

TABLE 25.1 Prevalence of Cannabis and Methods on Selected Roadside Surveys

References	Country(ies)	Year	Sample	Method for detection[a]	Prevalence of cannabis (%)
Houwing et al. (2011)	Belgium, Czech Republic, Denmark, Finland, Hungary, Italy, Lithuania, Netherlands, Norway, Poland, Portugal, Spain, Sweden	2008	Probability sample *N* = 50,000	Oral fluid and/or blood	1.38
Lacey et al. (2009)	United States	2007	Stratified three-stage random sample of weekend nighttime drivers + Friday daytime *N* = 7,719	Oral fluid and/or blood	Daytime = 3.9 Nighttime = 6.5
Gjerde et al. (2008)	Norway	2005–06	Stratified two stage cluster sample *N* = 10,835	Oral fluid	0.6
Ingsathit et al. (2009)	Thailand	2005–06	Nonprobabilistic *N* = 1,635	Urine	1.1

[a] *Toxicological analysis may be performed using different techniques and cutoffs, irrespectively of the biological matrix.*

onsite measure (as opposed to self-reports), thereby reducing social desirability and recall bias. In addition, roadside surveys restrict their study populations to drivers who are actually on the road, because many people with driver's licenses do not drive, and individuals might drive without a valid driver's license, in countries with lax enforcement. However, roadside surveys are expensive and challenging to execute because they generally require the presence of police officers, and must extensively address ethical issues in order to protect participant privacy. As with any mobile population (Sudman, Sirken, & Cowan, 1988), designing representative probability samples of drivers is not an easy task, but this is a crucial step in study planning, if we are to provide valid estimates for the survey population.

The three largest roadside surveys that objectively evaluated the presence of cannabis among drivers, and that benefited from probabilistic samples, were the DRUID project (http://www.druid-project.eu/Druid/EN/Home/home_node.html), which included more than 50,000 drivers from 13 European countries; the Norwegian roadside survey, which included 10,000 drivers; and the US 2007 National Roadside Survey of Alcohol and Drug Use by Drivers (http://www.nhtsa.gov/Driving+Safety/Research+&+Evaluation/2007+National+Roadside+Survey+of+Alcohol+and+Drug+Use+by+

TABLE 25.2 Selected Hospital Studies Conducted to Evaluate Cannabis Among Traffic Crashes Victims

Reference	Country(ies)	Design	Sample	Prevalence of cannabis
Asbridge et al. (2014)	Canada	Case-crossover	*N* = 860	11% increased odds of injury: OR 4.1 (1.98–8.52)
Legrand, Silverans, de Paepe, Buylaert, and Verstraete (2013)	Belgium	Cross-sectional	*N* = 1078	5.3%
Wiese Simonsen et al. (2013)	Denmark	Cross-sectional	*N* = 840	3.7%
Drummer et al. (2012)	Australia	Cross-sectional	*N* = 1714	9.8%
De Boni et al. (2011)	Brazil	Cross-sectional	*N* = 640	13.3%
Bogstrand et al. (2011)	Norway	Cross-sectional	*N* = 1272	6.2%
Santamariña-Rubio et al. (2009)	Spain	Cross-sectional	*N* = 387	17.0% (men) and 3.8% (women)
Movig et al. (2004)	Netherlands	Case-control	Cases: *N* = 110 Controls: *N* = 816	12% (cases) 6% (controls) Adjusted OR 1.22 (0.55–2.73)
Mura et al. (2003)	France	Case-control	Cases: *N* = 900 Controls: *N* = 900	10% (cases) 5% (controls)

TABLE 25.3 Selected Studies on Fatal Crashes Conducted to Evaluate Cannabis Among Traffic Crashes Victims

References	Country(ies)	Year	Design	Sample	Prevalence of cannabis
Li, Brady, and Chen (2013)	United States	2007	Population based case-control	Cases: *N* = 737 Controls: *N* = 7,719	9.8% (cases) 5.6% (controls) OR 1.83 (1.39–2.39)
Gjerde, Normann, Christophersen, Samuelsen, and Mørland (2011)	Norway	2003–08	Case-control	Cases: *N* = 204 Controls: *N* = 10,540	No significant association between cannabis and fatal road accidents
Mravcík, Vorel, and Zábranský (2007)	Czech Republic	2003–05	Cross-sectional	*N* = 1,213	2.2%
Laumon, Gadegbeku, Martin, and Biecheler (2005)	France	2001–03	Case-control	Cases: *N* = 6,776 Controls: *N* = 3,006	7.0% cannabis associated with responsibility OR 3.32 (2.63–4.18)
Drummer et al. (2004)	Australia	2004	Cross-sectional	*N* = 3,398	13.5% cannabis associated with being culpable OR 2.7 (1.02–7.0)

Drivers), which included approximately 8500 drivers. All of these studies included objective screening of multiple drugs. As expected, the drug use findings followed the patterns of consumption found among the general population. Specifically, cannabis was the most common nonprescribed drug found, and the drivers who tested positive were mostly young men. Table 25.1 provides an example of the methods and results from selected roadside surveys conducted around the world. The prevalence of driving under the influence of cannabis ranged from 0.6% to 6.5%, but these data are not entirely comparable across nations, given the methodological differences among the studies. The most important differences were the days and hours of interviews, as well as the toxicological methods used to establish cannabis use. In fact, although challenges and controversies exist, the detection of cannabis via roadside surveys is one of the greatest advances in the field, which is described in the section "Cannabis Detection and Traffic."

Importantly, some countries (eg, Australia) already test for the presence of drugs in the saliva of drivers, during routine random tests conducted by police. The results of these tests are not comparable with those from roadside surveys, because driver selection is not probabilistic, but they are important as surveillance on the trends of cannabis use among drivers. Davey, Armstrong, and Martin (2014) analyzed a dataset from the Queensland Roadside Drug-testing Program, since its implementation (from 2007 to 2012; more than 80,000 samples). They found that cannabis, after methamphetamine, was the most common illicit drug found among drivers. Because THC is the most commonly consumed illicit drug in Australia, these authors argued that their findings might have been related to differences in the detection windows for cannabis and methamphetamine.

Hospital Studies

The literature investigating the effects of substance use among individuals in emergency settings is older than roadside surveys, but challenges remain with regard to ethical issues (see Goldim, Fernandes, & Pechansky, 2011 for a review). Hospital studies are important to determine the prevalence of cannabis use among injured drivers. These studies might also be designed as case-control studies and, therefore, estimate the likelihood of TAs among drivers under the influence of cannabis, compared with drug-free drivers. In addition, they can also explore the associations between cannabis use and injury severity (from progression to death), and its economic costs.

The prevalence of cannabis use among injured drivers varies widely, based on geographic region and epidemiological methods, but most results indicate that cannabis use is higher among men and young adults (compared with women and older drivers, respectively). Most case-control designs have shown that the likelihood of TAs is higher for drivers who used cannabis, compared with nonusers (Table 25.2).

Fatal Crash Studies

Finally, the last type of observational study to be discussed includes the studies that evaluate fatally injured drivers. The prevalence of cannabis among fatally injured drivers is similar to that among those nonfatally injured. The metaanalysis conducted by Asbridge,

Hayden, and Cartwright (2012) revealed that driving under the influence of cannabis increased the risk of fatal crashes (OR = 2.10; 95% CIs = 1.31–3.36), and this effect size was higher than that found with regard to culpability in nonfatal crash studies. Table 25.3 shows some of the results from fatal crash studies. As with roadside surveys and hospital studies, however, these results are not entirely comparable, due to methodological differences.

An important piece of information that can be obtained from this design relates to trends over time, and their associations with availability and legal aspects. For example, Salomonsen-Sautel, Min, Sakai, Thurstone, and Hopfer (2014) performed a time-series analysis comparing the pre- and postcommercialization of medical marijuana in Colorado (United States), between 1994 and 2011. The authors found a significant increase in the prevalence of cannabis among fatally injured drivers (from 4.5% in 1994 to 10% in 2009). Given the possibility of evaluating such outcomes, these types of studies expand beyond drug-influenced driving, and are fundamental to monitor the social harms related to cannabis availability and its legalization.

CANNABIS DETECTION AND TRAFFIC

Epidemiological findings are difficult to compare for many reasons; some of the most important are the different toxicological methods used to detect cannabis, and the interpretation of their results. Delta-9-tetrahydrocannabinol (Δ^9-tetrahydrocannabinol or THC) is the major psychoactive constituent of cannabis, formed from multiple THC acids that are present in *Cannabis sativa* L. in vivo. THC is metabolized via hydroxylation to an active metabolite, 11-hydroxy-THC (11-OH-THC) which, in turn, is oxidized to an inactive 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) (Cone & Huestis, 2007).

One of the key issues for toxicologists is to establish the association of cannabis use and the impairment on driving skills. The blood-concentration-time profile of THC shows a dose effect on driving performance; however, it is difficult to establish a relationship between THC blood concentration and impairment, once the cannabinoids possess unconventional pharmacokinetics (Armentano, 2013). Unlike other psychoactive drugs, the peak THC blood levels do not typically correspond with the participants' maximum levels of behavioral impairment. THC shows a counter-clockwise hysteresis; in other words, the effects of the psychoactive substance lag behind those observed at maximal drug concentrations (Gotenhermen, 2003; Hartman & Huestis, 2013).

In general, plasma elimination half-life (t½) is used to estimate how long it takes a drug to leave the body, but the half-life of THC differs for regular versus infrequent users. Thus, the presence of THC, its metabolites, or both, in blood, particularly at low levels, is an inappropriate indicator of impairment. Although high blood concentrations of THC (greater than 5 ng/mL) are indicative of recent use, low concentrations (below 2 ng/mL) do not necessarily suggest that cannabis use has not been recent. Naive or infrequent users of cannabis can be more severely affected at low THC blood concentrations than regular users of the drug (Poulsen, Moar, & Pirie, 2014). In addition, THC is a lipophilic compound that accumulates rapidly in body fat, where it is stored and slowly redistributed throughout the blood. Although occasional consumers of cannabis will likely test negative for the presence of THC in blood, within 12 h following inhalation, THC's lipid solubility can lead to positive test results for residual concentrations of THC, even after several days of abstinence, among certain chronic users. The potential presence of residual, low levels of THC in the blood, combined with the possibility of periodic increases in THC blood levels, absent concomitant use, might confound the ability of toxicologists to interpret whether the presence of THC in one blood sample is evidence of recent cannabis consumption by an occasional consumer, or indicative of past consumption by a more frequent cannabis user (Toennes, Ramaekers, Theunissen, Moeller, & Kauert, 2008).

Another challenge on the driving field is with regard to the choice of biological matrices. Whole blood is the gold standard for threshold setting because it relates best to the scientific evidence on driving impairment. However, THC has a rapid metabolism and, if the time between the traffic stop/accident and blood sampling is delayed, the blood concentration might decrease markedly (based on a THC t½ of 1.5 h). Small amounts of THC, as well as its metabolites, THC-COOH and 11-OH-THC, are typically found in the blood of users, and the detection of both is used to demonstrate cannabis consumption. The detection window for THC is narrow and, as it was mentioned previously, it depends on dosage, and chronic versus occasional use. The 11-OH-THC metabolite is present in the blood at low levels, almost immediately after cannabis inhalation, and possesses a relatively short detection period in blood. Thus, the presence of 11-OH-THC, particularly in substantial quantities, might indicate recent cannabis ingestion, and possible behavioral impairments (Armentano, 2013).

Most studies on the association of cannabis use and TAs that have found no association have used urine as the biological matrix to detect cannabis (Hartma & Huestis, 2013). Drug concentrations in urine are typically not relevant with regard to their effects on driving impairment, and urinary drug concentrations are not generally accepted as indicators of the effects on immediate driving safety. Although urinalyses are advantageous—because they are fairly easy to collect in large volumes, the technology for drug screening is well established,

and numerous studies have described how to interpret these drug findings (Gotenhermen, 2003)—the interpretation of urinalyses on the road safety area is often complex, and show great variability.

The use of oral fluid (of which, saliva is a key constituent) as a matrix for cannabis testing has gained increasing interest because it is easy to obtain, and the advances in technology have enabled the collection of small amounts of oral fluid using commercial devices. In comparison to urine, oral fluid has stronger correlations with blood measures and impairment (Toennes et al., 2008). However, contamination of the buccal cavity is an issue for detecting cannabis use, given the usual administration route of the drug. Following the recent use of cannabis, "shallow deposits" can accumulate in the buccal cavity, producing elevated concentrations in oral fluid, for several hours after ingestion. Unfortunately, cannabinoids do not pass readily from the blood into saliva, and the detection of THC in oral fluid may be attributed to the contamination of the oral cavity following smoking. THC and small amounts of THC-COOH may be found in oral fluid, following the consumption of smoked or orally ingested cannabis. The confirmed presence of both THC and 11-OH-THC in oral fluid is useful to substantiate use (Cone & Huestis, 2007).

In most instances, when one is looking for cannabis in biological specimens, screening tests by immunoassay are employed, or maybe a broad-based screen, such as gas chromatography mass spectrometry (GC/MS). Screening tests must be appropriate, and validated for the type of biological specimens being analyzed. For example, immunoassays used on whole blood must be appropriately validated for that purpose. If the results of preliminary, unconfirmed screening tests are included on the final report, the report must clearly state that the results are unconfirmed. As a general matter of scientific and forensic principle, the detection or initial identification should be confirmed by a second technique, based on a different chemical principle. Where possible, the use of mass spectrometry is recommended as the confirmatory technique, where possible and practical (SOFT/AAFS Forensic Laboratory Guidelines, 2006). A minimum of 5% of negative screening tests should be analyzed by mass spectrometry to evaluate false negative results (Walsh et al., 2008). Gas chromatography coupled to mass spectrometry (GC/MS) is the method of choice to assess cannabinoids. The high performance liquid chromatography coupled to mass detector (LC-MS or LC-MS/MS) makes possible the analysis of acidic and neutral cannabinoids, without requiring any derivatization (protective reactions of polar functional groups) or previous decarboxylation, and allowed the multidrug methods.

The prevalence of cannabis use reported in the literature relies on which cannabis components were analyzed, and whether the results originated from the analysis of blood, oral fluid, or urine via different techniques. Therefore, some variation in the results of different studies is to be expected. In summary, whole blood sampling is the most effective way to confirm the presence of THC in the body because it is most related to the scientific evidence regarding driving performance, given that it is more related to recent use. The most accessible matrix for the roadside detection of drugs is oral fluid because it is easy to obtain. The debate continues with regard to whether oral fluid THC concentrations are correlated with blood THC concentrations and, therefore, an indirect indicator of driving impairment (Wolff & Johnston, 2014). Hence, the choice of fluid for roadside screening is under discussion, and numerous confounds have emerged; more studies are needed to clarify toxicological aspects. The combination of immunological methods with GC/MS, LC-MS/MS, or both, seems to be the best approach for toxicological analysis. The cutoffs applied for each matrix should be carefully evaluated, based on international guidelines.

LEGISLATION

Given the difficulties in establishing impairment on driving skills based on toxicological tests, countries have struggled to enact legislation that regulates driving under the influence of cannabis. Overall, the laws that regulate driving under the influence of illegal drugs also regulate driving under the influence of cannabis. Three approaches to legislation exist, regarding drug-influenced driving: establishing the legally allowed limit, zero-tolerance laws, and impairment laws (EMCDDA, 2012, 2014; Verstraete et al., 2011).

- Establishing a maximum fixed limit for a substance. Most legislation regarding driving under the influence of alcohol establishes the allowable blood alcohol concentration (BAC) under which it is legal to drive. If a driver's BAC is above the legal limit, then he or she is considered as an offender, regardless of whether he or she shows signs of impairment.
- Zero-tolerance laws. These laws are a subgroup of legal limit laws that do not allow any substance concentrations in the blood. That is, any detectable amount of psychoactive substances in the driver's body is a violation of the law.
- Impairment legislation. In these cases, it is necessary to evaluate each case to determine whether an individual's ability to drive is compromised.

Most countries that already have drug-consumption and driving legislation apply zero-tolerance laws with

regard to illegal substances, but the toxicological approach for determining recent use is not always clearly regulated. In the next paragraphs, some examples of countries that have established drug-related driving laws are provided.

Australia

Australia was the first country to routinely assess the use of drugs among drivers. Testing began in Victoria (2003) with the amendment of the Road Safety Act (1986), which implemented random drug screenings, and created new offences for failing or refusing a drug test (Australian Capital Territory, nd; Dixon, 2007; Government of Victoria, 2014). Currently, all states and territories in Australia can legally conduct roadside drug testing. In the Northern Territory, the saliva test can be conducted when a driver is involved in a TA, driving a large vehicle, suspected to be under the influence of alcohol, drugs, or both, or is stopped for a random breath test. All other states and territories can conduct routine drug testing using oral fluid for illegal drugs (Rowden, Mazurski, Withaneachi, & Stevens, 2011; Northern Territory, 2014). Differences exist with regard to the penalties among the states, but they can include disqualification from driving, heavy fines, imprisonment, or some combination therein. Prison sentences can vary from 9 months to 2 years for driving under the influence, depending on whether it is a first, second, or additional offense. Refusing to take the test can also result in any of these penalties (Western Australia, 2014).

Canada

Impaired driving (due to alcohol or other drugs) is an offense under the Criminal Code of Canada, which added more sanctions and mandatory programs to the various highway traffic acts, in most of its provinces and territories (Canadian Center on Substance Abuse, 2013). According to Beirness and Beasley (2010), the Criminal Code of Canada was amended in July 2008 to provide police with the authority to demand that drivers submit to drug impairment testing. It is also an offense to fail or refuse a test of physical coordination (ie, standard field sobriety tests), receive an evaluation that determines a drug influence by a certified drug recognition expert, or refuse to provide samples of breath, blood, urine, or oral fluid for analysis of alcohol and drug content (Minister of Justice of Canada, 2014). The penalties can range from 10 years in prison to lifetime imprisonment, if the impaired driving results in injury or deaths, respectively. The provincial and territorial laws impose immediate short-term license suspensions, and additional sanctions can include license reinstatement fees, mandatory participation in driver rehabilitation programs, and vehicle impoundment (Ministry of Justice of British Columbia, 2014).

European Union

According to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA, 2012), as well as Verstraete et al. (2011), a variety of legal mechanisms related to drug control and road traffic control laws, drug tolerance levels, and enforcement are used to sanction drug-influenced driving in European countries. Zero-tolerance laws for illicit drugs, such as cannabis were introduced in Belgium, Portugal, and Sweden, in 1999, and in France and Finland in 2003 (EMCDDA, 2007), but many differences exist with regard to how countries manage their legislation and enforcement. In some countries (eg, Finland), drivers with traces of illicit drugs in their systems during sobriety testing can be prosecuted for illicit drug consumption; in other countries (eg, Belgium), specific clauses exist in the road traffic laws that prohibit the results of the tests from being used in other criminal investigations. To detect drug driving, some countries (eg, Belgium, Spain, Estonia, and Denmark) randomly stop drivers to test them, whereas other countries (eg, Germany, Greece, France, and Italy) require that police officers first suspect that the driver is impaired. Punishments vary, given the country, but may include suspended licenses, fines, and prison sentences (EMCDDA, 2014).

United States

In the United States, legislation on driving under the influence of drugs varies by state, but not all states present laws addressing it. On those that present, legislation is mostly zero-tolerance for illicit substances. In California, New York, and Hawaii the law separate the driving under the influence of alcohol, under the influence of a drug, and under the combined influence of alcohol and a drug—as part of their impaired driving stature; other States have training for law enforcement programs that certifies drug recognition experts. According to the Governors Highway Safety Association (2014), the state of Washington has a specific determination on cannabis use: following the state's legalization of marijuana for recreational use, in November 2012, people with a THC blood content of 5 ng/mL or higher cannot drive (Kay & Logan, 2011; Washington State Legislature, 2014). Ohio (Ohio State, 2014) and Nevada defined 2 ng THC/mL as sufficient for impairment, whereas Pennsylvania's health department has a guideline of 5 ng that can be used in driving violation cases. Blood testing by medical professionals is used to identify THC

in Washington drivers, after the police suspect impaired driving. The penalties for drug-influenced driving in the United States differ by state, but can include imprisonment, fines, license suspension, or revocation for up to 3 years.

CONCLUSIONS

This chapter reviewed epidemiological, toxicological, and legal aspects related to the use of cannabis and driving. Most epidemiological studies have found a positive association between cannabis use and TAs. The biological matrices most appropriate for detecting cannabis use, in the context of TAs, are blood (the gold standard) and oral fluid (especially in screenings during roadside surveys). Legislation regarding driving under the influence of drugs has advanced over the last decade. Some important challenges remain in all areas. Finally, we argue that using comparable methodologies is one of the most fundamental issues for continuing with the research agenda in this field.

MINI-DICTIONARY

Biological matrices Fluids or body parts used in laboratory tests to detect diseases or substances.
Delta-9-tetrahydrocannabinol (Δ^9-tetrahydrocannabinol or THC) Principal psychoactive constituent (or cannabinoid) of cannabis.
Gas chromatography mass spectrometry (GC/MS) Analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample.
Hydroxylation Addition or replacement of hydroxyl in a molecule.
11-hydroxy-THC (11-OH-THC) Main active metabolite of THC formed in the body after cannabis consumption.
Immunological methods Biochemical test that measures the presence or concentration of a macromolecule in a solution through the use of an antibody or immunoglobulin.
Liquid chromatography coupled to mass detector (LC-MS or LC-MS/MS) Analytical chemistry technique that combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry.
11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) Metabolite formed in the body by oxidation of the 11-hydroxy-THC.
Screening tests by immunoassay Diagnostic kits aimed the early detection of substances by antibodies.
THC metabolites THC metabolites are the intermediates and products of metabolism of THC.

References

Armentano, P. (2013). Should per se limits be imposed for cannabis? Equating cannabinoid blood concentrations with actual driver impairment: practical limitations and concerns. *Humboldt Journal of Social Relations*, *35*, 45–55, http://norml.org/pdf_files/per_se_limits_for_cannabis.pdf.

Asbridge, M., Hayden, J. A., & Cartwright, J. L. (2012). Acute cannabis consumption and motor vehicle collision risk: systematic review of observational studies and meta-analysis. *British Medical Journal*, *344*, e536.

Asbridge, M., Mann, R., Cusimano, M. D., Trayling, C., Roerecke, M., Tallon, J. M., Rehm, J., et al. (2014). Cannabis and traffic collision risk: findings from a case-crossover study of injured drivers presenting to emergency departments. *International Journal of Public Health*, *59*(2), 395–404.

Australian Capital Territory. (n.d.). Road Transport (Alcohol and Drugs) Act 1977 amended on May 2014. http://www.legislation.act.gov.au/a/1977-17/20140523-57853/pdf/1977-17.pdf

Beirness, D., & Beasley, E. (2010). A roadside survey of alcohol and drug use among drivers in British Columbia. *Traffic Injury Prevention*, *11*(3), 215–221.

Bogstrand, S. T., Normann, P. T., Rossow, I., Larsen, M., Mørland, J., & Ekeberg, Ø. (2011). Prevalence of alcohol and other substances of abuse among injured patients in a Norwegian emergency department. *Drug and Alcohol Dependence*, *117*(2–3), 132–138.

Borkenstein, R. F., Crowther, R. F., Shumate, R. P., Ziel, W. B., & Zylman, R. (1974). The role of the drinking driver in traffic accidents: the Grand Rapids Study. *Blutalkohol*, *11*(Suppl. 1), 1–132.

Canadian Center on Substance Abuse. (2013). *Impaired driving in Canada. Summary*. Ottawa, ON: CCSA, http://ccsa.ca/Resource Library/CCSA-Impaired-Driving-in-Canada-2013-en.pdf

Compton, R., Blomberg, R., Moskowitz, H., Burns, M., Peck, R., & Fiorentino, D. (2002). Crash risk of alcohol-impaired driving. In D. R. Mayhew, & C. Dussault (Eds.), *Drugs and Traffic Safety-T 2002: 16th International Conference on Alcohol, Drugs and Traffic Safety* (pp. 38–44). Monreal, QC: ICADTS, http://www.saaq.gouv.qc.ca/t2002/actes/pdf/(06a).pdf

Cone, E. J., & Huestis, M. A. (2007). Interpretation of oral fluid tests for drugs of abuse. *Annals of the New York Academy of Sciences*, *1098*, 51–103.

Davey, J., Armstrong, K., & Martin, P. (2014). Results of the Queensland 2007–2012 roadside drug testing program: the prevalence of three illicit drugs. *Accident, Analysis and Prevention*, *65*, 11–17.

De Boni, R., Bozzetti, M. C., Hilgert, J., Sousa, T., Von Diemen, L., Benzano, D., Pechansky, F., et al. (2011). Factors associated with alcohol and drug use among traffic crash victims in southern Brazil. *Accident, Analysis and Prevention*, *43*(4), 1408–1413.

Dixon, N. (2007). *Random drug driving tests under the Transport Legislation and Another Act Amendment Bill 2006*. Brisbane, QLD: Queensland Parliament, http://www.parliament.qld.gov.au/documents/explore/ResearchPublications/ResearchBriefs/2007/RBR200704.pdf

Drummer, O. H., Gerostamoulos, J., Batziris, H., Chu, M., Caplehorn, J., Robertson, M. D., & Swann, P. (2004). The involvement of drugs in drivers of motor vehicles killed in Australian road traffic crashes. *Accident, Analysis and Prevention*, *36*(2), 239–248.

Drummer, O. H., Kourtis, I., Beyer, J., Tayler, P., Boorman, M., & Gerostamoulos, D. (2012). The prevalence of drugs in injured drivers. *Forensic Science International*, *215*(1–3), 14–17.

European Monitoring Centre for Drugs and Drug Addiction. (2007). *Drugs and driving: Selected issues*. Lisbon, Portugal: EMCDDA, http://www.drugs.ie/resourcesfiles/ResearchDocs/Europe/Research/2007/TDSI07003ENC_WEB.pdf

European Monitoring Centre for Drugs and Drug Addiction. (2012). *Driving under the influence of drugs, alcohol and medicines in Europe—Findings from the DRUID Project*. Luxembourg: Publications Office of the European Union.

European Monitoring Centre for Drugs and Drug Addiction. (2014). *Legal approaches to drugs and driving*. Lisbon, Portugal: EMCDDA, http://www.emcdda.europa.eu/html.cfm/index19034EN.html

Fell, J., & Voas, R. (2006). The effectiveness of reducing illegal blood alcohol concentration (BAC) limits for driving: evidence for lowering the limit to .05 BAC. *Journal of Safety Research*, *37*(3), 233–243.

Gjerde, H., Normann, P. T., Pettersen, B. S., Assum, T., Aldrin, M., Johansen, U., Øiestad, E. L., Christophersen, A. S., & Mørland, J. (2008). Prevalence of alcohol and drugs among Norwegian motor vehicle drivers: a roadside survey. *Accident, Analysis and Prevention*, *40*(5), 1765–1772.

Gjerde, H., Normann, P. T., Christophersen, A. S., Samuelsen, S. O., & Mørland, J. (2011). Alcohol, psychoactive drugs and fatal road traffic accidents in Norway: a case-control study. *Accident, Analysis and Prevention*, *43*(3), 1197–1203.

Goldim, J. R., Fernandes, M. S., & Pechansky, F. (2011). Ethical, legal and social issues related to alcohol and drug research. *Current Opinion in Psychiatry*, *24*, 181–185.

Gotenhermen, F. (2003). Pharmacokinetics and pharmacodynamics of cannabinoids. *Clinical Pharmacokinetics*, *42*, 327–360.

Government of Victoria. (2014). *Road Safety Act 1986 amended on March 2013*. Melbourne, VIC: Government of Victoria, Australia, http://www.legislation.vic.gov.au/domino/Web_Notes/LDMS/LTObject_Store/LTObjSt7.nsf/DDE300B846EED9C7CA257616000A3571/9F75ACDE5475A0BBCA257B3B00760AF5/$FILE/86-127a151bookmarked.pdf

Governors Highway Safety Association. (2014). *Drug impaired driving laws*. Washington, DC: GHSA, http://www.ghsa.org/html/stateinfo/laws/dre_perse_laws.html

Hartman, R. L., & Huestis, M. (2013). Cannabis effects on driving skills. *Clinical Chemistry*, *59*(3), 1–25.

Houwing, S., Hagenzieker, M., Mathijssen, R., Bernhoft, I. M., Hels, T., Janstrup, K., … Verstraete, A. G. (2011). Annex 4. Deliverable 2.2.3 Prevalence of alcohol and other psychoactive substances in general traffic. In: I. M., Bernhoft (Ed.), Deliverable D2.4.1 Results from epidemiological research—prevalence, risk and characteristics of impaired drivers (pp. 48–53). DRUID-Driving under the influence of drugs, alcohol and medicines. http://www.druid-project.eu/Druid/EN/deliverales-list/deliverables-list-node.html;jsessionid4D041765EE5A22ABF6D7B8F4A07BB778.live2052

Ingsathit, A., Woratanarat, P., Anukarahanonta, T., Rattanasiri, S., Chatchaipun, P., Wattayakorn, K., Suriyawongpaisal, P., et al. (2009). Prevalence of psychoactive drug use among drivers in Thailand: a roadside survey. *Accident, Analysis and Prevention*, *41*(3), 474–478.

Kay, G. G., & Logan, B. K. (2011). *Drugged driving expert panel report: A consensus protocol for assessing the potential of drugs to impair driving*. Washington, DC: National Highway Traffic Safety Administration.

Kelly, E., Darke, S., & Ross, J. (2004). A review of drug use and driving: epidemiology, impairment, risk factors and risk perceptions. *Drug and Alcohol Review*, *23*(3), 319–344.

Lacey, J. H., Keley-Baker, T., Furr-Holden, D., Voas, R., Romano, E., Ramirez, A., … Berning, A. (2009). 2007 National roadside survey of alcohol and drug use by drivers—drug results. Washington, DC: National Highway Traffic Safety Administration.

Laumon, B., Gadegbeku, B., Martin, J.-L., & Biecheler, M.-B. (2005). Cannabis intoxication and fatal road crashes in France: population based case-control study. *British Medical Journal (Clinical Research Edition)*, *331*(7529), 1371.

Legrand, S.-A., Silverans, P., de Paepe, P., Buylaert, W., & Verstraete, A. G. (2013). Presence of psychoactive substances in injured Belgian drivers. *Traffic Injury Prevention*, *14*(5), 461–468.

Li, G., Brady, J. E., & Chen, Q. (2013). Drug use and fatal motor vehicle crashes: a case-control study. *Accident, Analysis and Prevention*, *60*, 205–210.

Minister of Justice of Canada. (2014). *Evaluation of impaired operation (drugs and alcohol) regulations*. Ottawa, ON: Ministry of Justice, http://laws-lois.justice.gc.ca/eng/regulations/SOR-2008-196/page-1.html

Ministry of Justice of British Columbia. (2014). *The various alcohol & drug related prohibitions and suspensions*. Victoria, BC: Ministry of Justice of British Columbia, http://www2.gov.bc.ca/gov/content/transportation/driving-and-cycling/driving-prohibitions-suspensions/prohibitions-and-suspensions/alcohol-and-drug-related-suspensions

Movig, K. L. L., Mathijssen, M. P. M., Nagel, P. H. A., van Egmond, T., de Gier, J. J., Leufkens, H. G. M., & Egberts, A. C. G. (2004). Psychoactive substance use and the risk of motor vehicle accidents. *Accident, Analysis and Prevention*, *36*(4), 631–636.

Mravcík, V., Vorel, F., & Zábranský, T. (2007). Drugs and fatal traffic accidents in the Czech Republic. *Central European Journal of Public Health*, *15*(4), 158–162.

Mura, P., Kintz, P., Ludes, B., Gaulier, J. M., Marquet, P., Martin-Dupont, S., Pourrat, O., et al. (2003). Comparison of the prevalence of alcohol, cannabis and other drugs between 900 injured drivers and 900 control subjects: results of a French collaborative study. *Forensic Science International*, *133*(1–2), 79–85.

Northern Territory of Australia. (2014). *Traffic Act 2012*. Darwin, NT: Northern Territory Government, http://notes.nt.gov.au/dcm/legislat/legislat.nsf/d989974724db65b1482561cf0017cbd2/653d4667b9c70efa69257f4b007c2110/$FILE/ATTF6ER8.pdf/Rept009.pdf

Ohio State. (2014). *Traffic Law 4511.19—Operating vehicle under the influence of alcohol or drugs—OVI*. Columbus, OH: The State of Ohio, http://codes.ohio.gov/orc/4511.19.

Poulsen, H., Moar, R., & Pirie, R. (2014). The culpability of drivers killed in New Zealand road crashes and their use of alcohol and other drugs. *Accident, Analysis and Prevention*, *67*, 119–128.

Rowden, P., Mazurski, E., Withaneachi, D., & Stevens, M. (2011). Roadside Drug Testing in New South Wales. In *Australasian Road Safety Research, Policing, and Education Conference, Perth*. Canberra, ACT: ACRS, http://acrs.org.au/files/arsrpe/Roadside%20Drug%20Testing%20in%20New%20South%20Wales.pdf

Salomonsen-Sautel, S., Min, S. -J., Sakai, J. T., Thurstone, C., & Hopfer, C. (2014). Trends in fatal motor vehicle crashes before and after marijuana commercialization in Colorado. *Drug and Alcohol Dependence*, *140*, 137–144.

Santamariña-Rubio, E., Pérez, K., Ricart, I., Rodríguez-Sanz, M., Rodríguez-Martos, A., Brugal, M. T., & Suelves, J. M. (2009). Substance use among road traffic casualties admitted to emergency departments. *Injury Prevention*, *15*(2), 87–94.

SOFT/AAFS. (2006). *Forensic Toxicology Laboratory Guidelines, 2006 Version*. Mesa, AZ: Society of Forensic Toxicology, http://www.soft-tox.org/files/Guidelines_2006_Final.pdf

Sudman, S., Sirken, M. G., & Cowan, C. D. (1988). Sampling rare and elusive populations. *Science*, *240*, 991–996.

Toennes, S. W., Ramaekers, J. G., Theunissen, E. L., Moeller, M. R., & Kauert, G. F. (2008). Comparison of cannabinoid pharmacokinetic properties in occasional and heavy users smoking a marijuana or placebo joint. *Journal of Analytical Toxicology*, *32*, 470–477.

UNODC. (2013). *World Drug Report*. Vienna: United Nations.

Verstraete, A., Knoche, A., Jantos, R., Skopp, G., Gjerde, H., Mørland, J., et al. (2011). *Per se limits: Methods of defining cut-off values for zero tolerance*. Bergisch Gladbach, Germany: DRUID, http://hdl.handle.net/1854/LU-1988464

Walsh, J. M., Verstraete, A. G., Huestis, M. A., & Mørland, J. (2008). Guidelines for research on drugged driving. *Addiction (Abingdon, England)*, *103*(8), 1258–1268.

Washington State Legislature. (2014). *RCW 46.61.502—Driving under the influence*. Olympia, WA: Washington State Legislature, http://apps.leg.wa.gov/rcw/default.aspx?cite=46.61.502

Western Australia. (2014). *Road Traffic Act 1974 amended on 2007*. Perth, WA: Government of Western Australia, http://www.slp.wa.gov.au/pco/prod/FileStore.nsf/Documents/MRDocument:5095W/$FILE/RoadTrfcAmDrugsAct2007_00-00-02.doc?OpenElement

WHO. (2013). *Global status report on road safety 2013 supporting a decade of action*. Geneva, Switzerland: WHO, www.who.int

Wiese Simonsen, K., Steentoft, A., Bernhoft, I. M., Hels, T., Rasmussen, B. S., & Linnet, K. (2013). Psychoactive substances in seriously injured drivers in Denmark. *Forensic Science International*, *224*(1–3), 44–50.

Wolff, K., & Johnston, A. (2014). Cannabis use: a perspective in relation to the proposed UK drug-driving legislation. *Drug Testing Analysis*, *6*, 143–154.

Page left intentionally blank

CHAPTER

e2

Cannabis Use in Youth Subcultures

*M. Pawson**, *B.C. Kelly***

***Department of Sociology, The Graduate Center, City University of New York, New York, NY, United States**

****Department of Sociology, Purdue University, West Lafayette, IN, United States**

SUMMARY POINTS

- Cannabis is used widely across many youth cultures.
- Cannabis use is a common and normative behavior within subcultural scenes.
- Subcultural systems influence and inform cannabis consumption practices, and the meanings associated with them.
- The use of blunts has come to be a defining feature of marijuana smoking within the hip-hop subculture, and is laden with specific symbolic meaning.
- Informed by the subculture's ethos of generosity, marijuana sharing practices play an integral role in forming and sustaining a sense of community within the jam band scene.
- The use of marijuana to modulate the effects of club drugs, within the EDM scene, highlights how specific subcultural practices like drug sequencing shape marijuana consumption.

KEY FACTS OF HIP-HOP CULTURE

- Hip-hop emerged in America among Black youth during the 1970s, and flourished during the 1980s.
- The genre is typically performed by MCs rapping over beats-driven music.
- Hip-hop culture grew to encompass other elements of Black American youth culture, including fashion, art, and dance.
- Blunts are the means by which marijuana is commonly smoked within hip-hop subcultures.

KEY FACTS OF JAM BAND CULTURE

- The jam band culture emerged from summer festivals of rock bands, such as, the Grateful Dead.
- The genre currently relies upon live performances involving extended musical improvisations.
- Subcultural participants often traveled to festivals and lived in makeshift communities.
- The jam band subculture is communally oriented.
- The sharing of cannabis is an important means of networking and enhancing solidarity within the jam band scene.

KEY FACTS OF ELECTRONIC DANCE MUSIC CULTURE

- Electronic dance music (EDM) emerged early on in the form of "house" music, and was more widely popularized following the birth of the rave scene in the United Kingdom during the late 1980s.
- The musical genre has spawned many subgenres of EDM, including techno, drum-n-bass, and dubstep.
- Dancing under the influence of drugs became a common, though not universal, routine for rave participants and nightclub attendees.
- Cannabis is often used by members of the EDM scene to influence the effects of other drugs.

LIST OF ABBREVIATIONS

EDM	Electronic dance music
MDMA	The drug methylenedioxymethamphetamine, commonly known as ecstasy

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00019-3
Copyright © 2017 Elsevier Inc. All rights reserved.

INTRODUCTION

As the most commonly used illicit drug in many Western nations, marijuana has been incorporated into the lifestyles of a wide range of individuals (Hathaway, 1997). Adolescents and young adults are among the largest group of consumers of cannabis; this remains a persistent trend across many countries. The normalization of its use is especially visible within youth subcultures (Parker, Aldridge, & Measham, 1999). This is apparent in the significant cultural accommodations toward the use of marijuana commonly found within particular subcultures, as well as the drug's pervasive availability and relatively high rates of regular recreational use.

Youth cultures are important guiding forces in the lives of many adolescents and young adults. A youth culture is essentially a cluster of young people who share activities, mindsets, tastes, and styles on the basis of their subcultural affiliation. While styles are often the most recognizable facets of these subcultures, they are merely outward representations of the symbolic system of meanings that is inherent within each subculture. In this respect, a youth culture provides a way of thinking about the world for the young people who participate in it. Yet, it also extends beyond mere psychology to the ways in which young people act and interact. The broader cultural framework of a youth culture shapes the ways in which young people who participate in them behave. Importantly, youth cultures function differently and, therefore, establish different patterns of drug use across them, both with respect to the substances used, and the meanings underlying their use (Kelly et al., 2015). Indeed, studies have shown that musical tastes—which often form a basis for youth cultures—and substance use tend to cluster (Vuolo, Uggen, & Lageson, 2013).

Many youth cultures place symbolic value on particular forms of drug use. In this regard, the use of specific substances can act as a social currency in certain subcultures. Additionally, consuming certain drugs in specific ways can shape social bonds within that scene, and influence social status. Marijuana use has been tied to many subcultures rooted in musical genres, including but not limited to reggae, rock, hip-hop, and electronic dance music scenes (Golub, Johnson, & Dunlap, 2006; Hunt, 2012; Pawson & Kelly, 2014; Perrone, 2009; Winders, 1983). Further, research has shown how specific social settings can influence the ways in which cannabis is consumed (Dunlap, Johnson, Benoit, & Sifaneck, 2005). In this regard, subcultural contexts can shape not only the patterns and practices of marijuana consumption, but also the ways in which these consumption practices unfold, as well as the meanings specific subcultures attribute to them.

Although jazz music is highly regarded as a well-established musical genre widely listened to by older adults, it was once itself the basis of a thriving youth culture, many decades ago. Descriptions of marijuana use among jazzmen form the basis of some of the earliest descriptions of drug use within subcultures. The early work of sociologist Howard Becker (Becker, 1953) highlighted the prominent role of marijuana smoking among young jazzmen, which established an early sociological examination of drug use by young people as a form of deviance. These early studies on marijuana-smoking jazzmen continue to have import for the study of drug use among young people. For the remainder of this chapter, we will focus on highlighting three current youth subcultures that prominently embrace the use of marijuana in the 21st century: the hip-hop scene, the jam band scene, and the rave scene. In doing so, we will describe the various ways in which the specific subcultural norms and values embodied within these different scenes influence and inform distinct marijuana consumption practices.

Similar to other youth subcultures, the hip-hop, jam band, and rave scenes revolve around particular tastes in music, as well as fashion. Youth cultures as nightlife scenes provide sources of entertainment and leisure activity for young adults, and substance use, whether legal or illegal, is relatively commonplace within these scenes. The use of marijuana holds particular symbolic value within hip-hop, jam band, and rave scenes, despite the fact that the subcultures surrounding these three nightlife scenes are quite distinct. The meanings underlying the shared practice of marijuana consumption can differ quite markedly across subcultures (Pawson & Kelly, 2014).

Below, we will describe the meanings and practice of cannabis use within each of these three subcultures. First, we will begin with a discussion of the historical and contextual significance of marijuana use within the hip-hop scene. We will also provide a descriptive account of the symbolic significance of smoking marijuana in the form of blunts within the hip-hop subculture. Next, we will turn to covering marijuana's entrenchment within the jam band subculture, as we highlight the key role that marijuana use plays in forming and sustaining a sense of community within the scene. Finally, we take a look at the rave scene (and the wider electronic dance music culture that grew out of it), and the ways in which marijuana is used along with popular club drugs like MDMA.

HIP-HOP

Hip-hop emerged as a new music genre during the 1970s, as deejays began to sample, mix, and scratch records to create extended breakbeats at dance parties in the South Bronx (Fricke & Ahearn, 2002). Emceeing soon

followed, as party promoters and hosts began to talk or rap over the records being played. Call and response chants were used to involve the audience in the music, and MC's would also showcase their rhyming abilities as they "battled" one another as a way to gain status within the scene (Chang, 2007).

Throughout the last several decades, hip-hop has become much more than just a new form of music, as those associated with it have developed their own unique subculture with particular forms of fashion, language, and activities being distinctly attributed to, and associated with, the hip-hop music scene (Blair, 1993; Chang, 2007; Fricke & Ahearn, 2002). Marijuana's pervasive presence within the hip-hop scene dates back several decades, and its use is considered to be a normalized aspect of the subculture.

As hip-hop music and culture flourished throughout the late 1980s and early 1990s, an inner city cultural backlash to the crack epidemic of the 1980s and heroin epidemics of the 1970s led to a decline in hard drug use, especially among young adults (Golub et al., 2006). As such, the denigration of hard drug use by inner city youth precipitated the increasing popularity of marijuana use, and its status as the drug of choice among members of the hip-hop scene (Allen, 1996; Golub et al., 2006). This affinity for smoking marijuana within the hip-hop scene is highlighted by the development of subculturally specific consumption practices and discourse. More specifically, the use of blunts, which is marijuana rolled up in cheap cigar shells, became entrenched within the cultural repertoire of the hip-hop subculture (Golub et al., 2006; Timberlake, 2009). It has come to be a defining feature of marijuana smoking in hip-hop scenes.

Blunts emerged as a nuanced form of marijuana consumption in the mid-1980s, and has its origins in the Jamaican spliff—marijuana rolled in large rolling papers or broad tobacco leaves commonly referred to as "fronto" leaves (Sifaneck, Kaplan, Dunlap, & Johnson, 2013). Using marijuana in the form of smoking blunts is an integral practice distinctly associated with the hip-hop subculture (Fig. e2.1). The blunt's overtly larger size, in comparison with joints, is commonly referenced as being aligned with hip-hop's core subcultural values of "phatness," or of "living large"—both connoting an outsized public presentation of success— and is of significant symbolic importance within the scene (Allen, 1996; Sifaneck, Johnson, & Dunlap, 2006). This particular style can also be seen in the hip-hop participant's affinity for consuming 40-ounce bottles of beer or malt liquor, as opposed to 12-ounce bottles, which not only are economical, but overtly emphasize substance consumption to onlookers.

Clothing and other apparel that either display the logos of commonly used cheap cigar brands like "Philly Blunts," or reference blunt smoking, are common symbols within hip-hop fashion (Sifaneck et al., 2013). Furthermore, the pervasive presence of blunt smoking within hip-hop media, such as, music videos, magazines, and movies legitimizes the consumption of marijuana in this form as a unique aspect of the hip-hop subculture (Sifaneck et al., 2006; Soller & Lee, 2010). Some researchers have noted that the presence of blunts in various media outlets eventually led to the expansion

FIGURE e2.1 **Blunts.**

and cooptation of smoking blunts beyond the hip-hop subculture, into broader popular culture (Sifaneck et al., 2013). Interestingly though, the use of blunts within other subcultures is believed to be relatively uncommon. For example, previous field research within the jam band scene reports a general absence of regular blunt smoking within this subculture, despite the pervasive presence of cannabis, which we will now turn to discuss (Pawson & Kelly, 2014).

JAM BAND

The jam band subculture, in many aspects, arose out of the remnants of the deadhead subculture that followed the rock band the Grateful Dead, as they toured around the United States. Throughout the 1990s, the subculture grew from a handful of bands to hundreds of bands showcasing a specific genre of music that mirrors the musical style of the Grateful Dead. Similar to deadheads, many fans of particular jam bands "tour" several different festivals, during the course of the summer (Hunt, 2008). As a result, some fans have, like the deadheads, been labeled based on the bands they follow (eg, "phishheads"). The term "head" more generally can also be used to refer to a member of this subculture, and, to some degree, may be considered a derivative of the "deadhead."

A core aspect of the jam band scene focuses on live performances involving extended musical improvisations, and the ritualistically occurring series of summertime festivals that feature various jam bands (Fig. e2.2). These festivals, similar in style to that of Woodstock, span several days in a row and, in many ways, function as the heart of the jam band subculture. These jam band festivals often involve outdoor camping, and are infused with "hippie" ideals promoting a shared sense of community, as well as embracing the recreational and spiritual use of psychedelic drugs (Epstein & Sardiello, 1990).

Furthermore, the parking lots of the venues where jam bands are performing frequently form a unique communal space, commonly referred to as shakedown streets, which is the name of a popular Grateful Dead song. Shakedown streets are essentially informal market places where scene participants who are following a particular jam band on their tour sell various wares in order to support themselves. Products like live recorded music, food, drugs, clothing, and jewelry are typically available for purchase, barter, or trade (Hunt, 2008; Sheptoski, 2000).

Both quantitative and qualitative studies have noted the ubiquitous drug use present at jam band shows and festivals (Morris, 2014; Vuolo et al., 2013). While jam band shows and festivals are often places described as being ideal for experimenting with psychedelic drugs, the scene's most pervasively used drug is marijuana, often cited as being the drug staple of the subculture (Hunt, 2008, 2010, 2012). This pervasive cannabis consumption traces back to the hippie culture in which its roots are based (Davis & Munoz, 1968; Zimmerman & Wieder, 1977). Jam band scene participants smoke mostly joints, and utilize pipes or "bowls" to smoke marijuana. Somewhat unique to the jam band scene are the presence of marijuana in various edible forms, although their use is growing elsewhere, with the rise of the medical marijuana industry. These edibles may be sold or bartered on shakedown streets, and have been referred to by some users as ganja goo balls (Fig. e2.3).

FIGURE e2.2 **Jam band performance.**

FIGURE e2.3 **Ganja goo ball.**

Furthermore, the scene's subcultural ethos of generosity can at times elevate marijuana to the status of being a pseudocommunal commodity, and its shared use within the scene illustrates participants' desire to connect with other participants. Several studies on the jam band scene have highlighted the central role and cultural importance of marijuana consumption within this context (Davis & Munoz, 1968; Hunt, 2012; Pawson & Kelly, 2014; Zimmerman & Wieder, 1977). One study in particular noted how the use of marijuana enabled a high level of sociality amongst participants, as the practice of sharing marijuana was found to function as a means to initiate interactions with others within the subculture (Pawson & Kelly, 2014). In this regard, marijuana has played a key role in forging and maintaining a sense of community and solidarity within the scene, as these publicly practiced routines enabled the reproduction of some of the key subcultural values of kindness and generosity within the scene.

RAVE CULTURE AND ELECTRONIC DANCE MUSIC

Electronic dance music (EDM) consists of a variety of musical genres that all share their historical roots in the advent of house music in the postdisco era (Reynolds, 1999). Although EDM existed prior to the development of raves, the musical form became more widely popularized among young people through rave culture. The emergence of raves as a cultural form for youth dates to the late 1980s. Raves gained a specific social currency in England during the late 1980s, with the development of the Acid House scene. The parties that showcased this particular form of house music, eventually known as raves, were typically held in desolate farm fields or abandoned factories and warehouses (Hill, 2003). These locations were typically illegally occupied, and the parties were grounded in a DIY mentality (Ingham, Purvis, & Clarke, 1999). The "Second Summer of Love" was the label applied to the birth of the rave scene, as some of the values of hippie youth culture from the late 1960s served as a foundation for the burgeoning rave scene; some of these values are articulated in the rave ideology of PLUR—short for Peace, Love, Unity, and Respect. The linkages of early rave culture to values shared with the youth cultures of the 1960s may have established a foundation for marijuana's acceptability within the rave scene (Fig. e2.4).

By the mid to late 1990s, the EDM subculture had evolved into a highly organized, commercialized, and global phenomenon. Raves themselves became more mainstream, and nightclubs routinely played EDM. As with many other incarnations of youth driven music that came before it, particular drug trends arose within this new music based subculture, as it gained popularity. MDMA, colloquially known as ecstasy, became a prominent component of the early rave scene, and was adopted in club culture more widely, as the music became more mainstream. In addition to MDMA, other substances like ketamine, GHB, cocaine, methamphetamine, and LSD have been associated with EDM scenes and, as such, have been referred to, as a group, under the category of "club drugs." The use of club drugs within the rave scene and EDM nightclubs has been the focus of many public health studies (Forsyth, 1996; Kelly, Parsons, & Wells, 2006; Lenton, Boys, & Norcross, 1997; Measham, Aldridge, & Parker, 2001), but the use of marijuana within these scenes has received less attention.

Although MDMA is the drug of choice often most readily attributed to the rave scene (and electronic dance music scenes more generally), the use of marijuana within the subculture is extraordinarily prevalent. Many studies note how rates of marijuana use are far higher than that of MDMA or other club drugs within the EDM subculture, with studies finding very high prevalence of participants reporting smoking marijuana within the last 6 months, as well as high prevalence of use within the last month (Barrett, Gross, Garand, & Pihl, 2005; Fernández-Calderón et al., 2011; McCaughan, Carlson, Falck, & Siegal, 2005). One study noted that marijuana was the most popular illicit drug used as participants leave a rave (Fernández-Calderón et al., 2011). Thus, marijuana is arguably the most commonly used illicit drug among young adults involved in these scenes, although its use within dance clubs has become complicated by

FIGURE e2.4 **Rave.**

the introduction of smoke-free air policies that prohibit smoking within bars and nightclubs.

The electronic dance music subculture is diverse, and can be divided into a number of subscenes with specific styles of electronic dance music. This has long been the case, as raves incorporated multiple genres of EDM, dating back to the 1990s. Yet, members of these subscenes often view themselves as participating in distinct scenes. The use of marijuana is highly associated with certain subgenres of EDM. For example, drum-n-bass music (also known as jungle) has been influenced by reggae and other breakbeat music, and members of this subculture also draw upon other symbolic markers from reggae and Jamaica, including marijuana. In this respect, cannabis use may be more common among members of the drum-n-bass scene than other subgenres of EDM, due to its strong symbolic representations within the scene, although cannabis use remains common across all EDM scenes. In many respects, marijuana has been widely incorporated into the drug consumption repertoires of participants in the EDM scene, although use in some subscenes remains more visible.

Commonly reported within the public health literature on EDM subcultures is the strategic use of marijuana within the scene (Boys, Lenton, & Norcross, 1997; Grov, Kelly, & Parsons, 2009; Hunt, Evans, Moloney, & Bailey, 2009; Jacinto, Duterte, Sales, & Murphy, 2008; McCaughan et al., 2005; Sterk, Theall, & Elifson, 2006); it has been reported as playing a prominent role in polydrug use repertoires for participants using club drugs (Grov et al., 2009). This notion of using particular drugs at particular times throughout the night is common to the EDM subculture, and functions as a way for participants to enhance pleasure and avoid the negative side effects of using club drugs (see the chapter by Herzig and Bachmann within this volume for more details). The role that marijuana plays with regards to modulating the effects and impacts of club drug use highlights how specific subcultural practices, such as drug sequencing, inform and influence patterns of marijuana consumption within a subcultural scene.

CONCLUSIONS

The use of marijuana continues to play a predominant role in various youth subcultures. From the overviews of the hip-hop, jam band, and EDM scenes, there are distinct symbolic values that each subculture places on the use of marijuana. More specifically, the subcultural norms and practices within these scenes inform and influence unique patterns of marijuana use. This is evident in the blunt smoking practices observed within the hip-hop scene, and its alignment with the norms and values of that subculture. Meanwhile, within the jam band scene, marijuana was used as a way in which to initiate interactions, and was a basis for experiencing notions of community within the subculture. Finally, when looking

at the EDM scene, much of the literature has focused on the specific role of marijuana use in modulating the effects or after effects of club drugs, such as MDMA, used by members of the subculture.

Youth cultures have a powerful influence on drug use practices among young people. Across all three of these music-based subcultures, these descriptions highlight not only how the use of marijuana is a normative aspect of drug consumption practices within these scenes, but also how the subcultural values embedded within these scenes inform and influence the ways in which the drug is used. Additionally, the meanings these patterns of use have for their respective participants vary across youth subcultures. These attributes of youth cultures are not merely sociological curiosities, but matter with respect to enhancing the health and well-being of adolescents and young adults. While young people are often lumped together in epidemiological studies and clinical practice, considerations of the nuances of the subcultures they participate in may provide meaningful insights for prevention and intervention efforts. Variations in these youth cultures create divergent meanings in cannabis use among youth, and thus generate different points of intervention. Strategies used in health promotion efforts may require a multifaceted approach, in order to maximize our reach to a wide-range of young people.

MINI-DICTIONARY

Blunt Marijuana rolled into the paper of a hollowed out cigar.
Club drugs Consisting of a diverse group of substances, from stimulants, depressants, and hallucinogens, the use of these substances has proliferated through the 1990s, due to a perceived association with rave and club culture. Primarily subsumed under this classification are: MDMA, ketamine, rohypnol, GHB, methamphetamine, cocaine, and LSD.
EDM Electronic dance music is a genre of music that is produced through electronic and digital means, rather than through traditional instruments. It is encapsulated by styles, such as, house, techno, trance, and drum-n-bass.
Fronto leaf Broad tobacco leaves used to roll marijuana, commonly referred to as spliffs by Jamaican and other Caribbean cultures. Spliffs rolled with fronto leaves are typically considered the predecessor of the modern blunt.
Hip-hop A genre of music most commonly including rapping over music produced by a DJ that was first generated by urban African-American youth.
Jam band A genre of rock music predicated on musical improvisation and long instrumental renditions.
MDMA The drug methylenedioxymethamphetamine, commonly known as ecstasy.

References

Allen, E. (1996). Making the strong survive: the contours and contradictions of message rap. In W. Perkins (Ed.), *Droppin' science. Critical essays on rap music and hip hop culture*. Philadelphia, PA: Temple University Press.

Barrett, S. P., Gross, S. R., Garand, I., & Pihl, R. O. (2005). Patterns of simultaneous polysubstance use in Canadian rave attendees. *Substance Use and Misuse*, *40*(9–10), 1525–1537.

Becker, H. S. (1953). Becoming a marihuana user. *American Journal of Sociology*, *59*(3), 235–242.

Blair, M. E. (1993). Commercialization of the rap music youth subculture. *Journal of Popular Culture*, *27*(3), 21–33.

Boys, A., Lenton, S., & Norcross, K. (1997). Polydrug use at raves by a Western Australian sample. *Drug and Alcohol Review*, *16*(3), 227–234.

Chang, J. (2007). *Can't stop won't stop. A history of the hip-hop generation*. New York, NY: Ebury Press.

Davis, F., & Munoz, L. (1968). Heads and freaks: patterns and meanings of drug use among hippies. *Journal of Health and Social Behavior*, 156–164.

Dunlap, E., Johnson, B. D., Benoit, E., & Sifaneck, S. (2005). Sessions, cyphers, and parties: settings for informal social controls of blunt smoking. *Journal of Ethnicity in Substance Abuse*, *4*(3–4), 43–79.

Epstein, J. S., & Sardiello, R. (1990). The wharf rats: a preliminary examination of alcoholics anonymous and the Grateful Dead head phenomena. *Deviant Behavior*, *11*(3), 245–257.

Fernández-Calderón, F., Lozano, O. M., Vidal, C., Ortega, J. G., Vergara, E., González-Sáiz, F., & Cid, F. (2011). Polysubstance use patterns in underground rave attenders.: a cluster analysis. *Journal of Drug Education*, *41*(2), 183–202.

Forsyth, A. J. (1996). Places and patterns of drug use in the Scottish dance scene. *Addiction*, *91*(4), 511–521.

Fricke, J., & Ahearn, C. (2002). *Yes yes y'all: the experience music project: oral istory of hip-hop's first decade*. Cambridge, MA: Da Capo Press.

Golub, A., Johnson, B. D., & Dunlap, E. (2006). The growth in marijuana use among American youths during the 1990s and the extent of blunt smoking. *Journal of Ethnicity in Substance Abuse*, *4*(3–4), 1–21.

Grov, C., Kelly, B. C., & Parsons, J. T. (2009). Polydrug use among club-going young adults recruited through time-space sampling. *Substance Use and Misuse*, *44*(6), 848–864.

Hathaway, A. D. (1997). Marijuana and lifestyle: exploring tolerable deviance. *Deviant Behavior*, *18*(3), 213–232.

Hill, A. (2003). Acid house and Thatcherism: contesting spaces in late 1980s Britain. *Space and Polity*, *7*(3), 219–232.

Hunt, G., Evans, K., Moloney, M., & Bailey, N. (2009). Combining different substances in the dance scene: enhancing pleasure, managing risk and timing effects. *Journal of Drug Issues*, *39*(3), 495–522.

Hunt, P. M. (2008). From Festies to Tourrats: Examining the relationship between Jamband subculture involvement and role meanings. *Social Psychology Quarterly*, *71*(4), 356–378.

Hunt, P. M. (2010). Are you kynd? Conformity and deviance within the jamband subculture. *Deviant Behavior*, *31*(6), 521–551.

Hunt, P. M. (2012). Examining the affective meanings of interaction settings in the jamband music subculture. *Journal of Public and Professional Sociology*, *4*(1), 5–25.

Ingham, J., Purvis, M., & Clarke, D. (1999). Hearing places, making spaces: sonorous geographies, ephemeral rhythms, and the Blackburn warehouse parties. *Environment and Planning D, Society and Space*, *17*, 283–306.

Jacinto, C., Duterte, M., Sales, P., & Murphy, S. (2008). Maximising the highs and minimising the lows: harm reduction guidance within ecstasy distribution networks. *International Journal of Drug Policy*, *19*(5), 393–400.

Kelly, B. C., Parsons, J. T., & Wells, B. E. (2006). Prevalence and predictors of club drug use among club-going young adults in New York City. *Journal of Urban Health*, *83*(5), 884–895.

Kelly, B. C., Trimarco, J., Pawson, M., LeClair, A., Parsons, J. T., & Golub, S. A. (2015). Symbolic boundaries, subcultural capital and prescription drug misuse across youth cultures. *Sociology of Health and Illness*, *37*, 325–339.

Lenton, S., Boys, A., & Norcross, K. (1997). Raves, drugs and experience: drug use by a sample of people who attend raves in Western Australia. *Addiction*, *92*(10), 1327–1337.

McCaughan, J. A., Carlson, R. G., Falck, R. S., & Siegal, H. A. (2005). From "Candy Kids" to "Chemi-Kids": a typology of young adults who attend raves in the midwestern United States. *Substance Use and Misuse*, *40*(9–10), 1503–1523.

Measham, F. C., Aldridge, J., & Parker, H. (2001). *Dancing on drugs: risk, health and hedonism in the British club scene*. London, UK: Free Association Books.

Morris, E. K. (2014). Destroying America: phish, music, and spaces of aesthetic and social exception. *International Review of the Aesthetics and Sociology of Music*, *45*, 167–181.

Parker, H., Aldridge, J., & Measham, F. (1999). *Illegal leisure: the normalization of adolescent recreational drug use*. London, UK, New York, NY: Routledge.

Pawson, M., & Kelly, B. C. (2014). Consumption and community: the subcultural contexts of disparate marijuana practices in jam band and hip-hop scenes. *Deviant Behavior*, *35*(5), 347–363.

Perrone, D. (2009). *The high life: club kids, harm and drug policy*. Boulder, CO: Lynne Rienner.

Reynolds, S. (1999). *Generation ecstasy: Into the world of techno and rave culture*. Psychology Press.

Sheptoski, M. (2000). Vending at Dead shows: the bizarre bazaar. In R. G. Adams & R. Sardiello (Eds.), *Deadhead social science: you ain't gonna learn what you don't want to know* (pp. 157–181). Walnut Creek, CA: Altamira Press.

Sifaneck, S. J., Johnson, B. D., & Dunlap, E. (2006). Cigars-for-blunts: choice of tobacco products by blunt smokers. *Journal of Ethnicity in Substance Abuse*, *4*(3–4), 23–42.

Sifaneck, S. J., Kaplan, C. D., Dunlap, E., & Johnson, B. D. (2013). Blunts and blowtjes: cannabis use practices in two cultural settings and their implications for secondary prevention. *Free Inquiry in Creative Sociology*, *31*(1), 3–14.

Soller, B., & Lee, J. P. (2010). Drug-intake methods and social identity: the use of marijuana in blunts among Southeast Asian adolescents and emerging adults. *Journal of Adolescent Research*, *25*(6), 783–806.

Sterk, C. E., Theall, K. P., & Elifson, K. W. (2006). Young adult ecstasy use patterns: quantities and combinations. *Journal of Drug Issues*, *36*(1), 201–228.

Timberlake, D. S. (2009). A comparison of drug use and dependence between blunt smokers and other cannabis users. *Substance Use and Misuse*, *44*(3), 401–415.

Vuolo, M., Uggen, C., & Lageson, S. (2013). Taste clusters of music and drugs: evidence from three analytic levels. *British Journal of Sociology*, *65*(3), 529–554.

Winders, J. A. (1983). Reggae, Rastafarians and revolution: rock music in the Third World. *Journal of Popular Culture*, *17*(1), 61–73.

Zimmerman, D. H., & Wieder, D. L. (1977). You can"t help but get stoned: notes on the social organization of marijuana smoking. *Social Problems*, *25*, 198–207.

PART III

CANNABIS, BEHAVIOR, PSYCHOPATHOLOGY AND NEUROPATHOLOGY

26 *Drug-related pictures, attentional bias, and cannabis use* 247
27 *Cannabis use and first-episode psychosis patients (FEP)* 257
28 *Cannabis, associative memory, fMRI, and the implicit association test* 267
29 *Stress response in cannabis users and psychosis* 278
30 *Motivation in chronic cannabis use* 288
31 *Cannabis use and its association to mental illness: a focus on mood and anxiety disorders* 298
32 *Cannabis use and well-being* 308
33 *Craving and cannabis: a potential paradox* 317
34 *Delta-9-tetrahydrocannabinol and catalepsy-like immobilization* 326
35 *The interactive nature of cannabis and schizophrenia risk genes* 335
36 *Neuroimaging findings in adolescent cannabis use and early phase psychosis* 345
37 *Cannabis smoking in adult schizophrenia: a cognitive and functional magnetic resonance imaging perspective* 357
38 *The long-lasting effects of cannabis use on movement and brain regions that control movement* 372
39 *Assessment of cannabis acute effects on driving skills: laboratory, simulator, and on-road studies* 379
40 *Chronic cannabis use and axonal fiber connectivity* 391
41 *Microglial activation and cannabis exposure* 401

42 *Cannabis and psychosis: correlation, causality, and consequences* *413*
43 *Cannabis use in bipolar disorder* *422*
44 *Cannabis use in epilepsy—risks and benefits* *431*
45 *Cannabis, cannabinoids, and visceral pain* *439*
46 *Cannabis and postoperative analgesia* *450*

CHAPTER

26

Drug-Related Pictures, Attentional Bias, and Cannabis Use

D. Asmaro

Department of Psychology, Simon Fraser University, Burnaby, BC, Canada

SUMMARY POINTS

- Presentations of drug cues to drug users have been shown to elicit both craving and neural responses, yet relatively little research has focused on cannabis use behavior.
- Using behavioral, electrophysiological, and functional neuroimaging studies, scientists have been able to characterize how cannabis users respond to cannabis cues.
- Behavioral research shows that reaction times to cannabis cues increase and that craving for the substance becomes amplified after cue exposure.
- Event-related potential research shows that early and late effects have been observed after cannabis cues are presented to cannabis users, therefore allowing scientists to better understanding the temporal processing of these cues within the brain.
- Functional magnetic resonance imaging (fMRI) research has shown that parts of the brain that are related to reward processing become activated by cannabis cues when users of the substance see images that have been conditioned to the effects of the drug.
- Understanding the behavioral and neural correlates of cannabis cue-reactivity as well as its association with increased attentional bias has important treatment implications.

KEY FACTS OF ELECTROENCEPHALOGRAM/EVENT-RELATED POTENTIALS

- An electroencephalogram (EEG) is a continuous recording of brain electrical activity that is acquired by using an array of electrodes.
- While brain electrical activity can be recorded directly from brain tissue most published findings are based on measurements that were obtained from the surface of the scalp as this is less invasive than the former approach.
- Event-related potentials are averaged electrophysiological responses that are extracted from the ongoing EEG. A researcher can choose to combine events that were flagged in the EEG signal and then extract the average waveform for that segment of the EEG across all events of a given type. For example, a researcher could look at all responses to neutral and target stimuli in the EEG, and then extract and average that data in order to create waveforms that reflect brain responses to these two types of stimuli.
- After signal averaging is completed for each participant, the researcher can create a grand-average where a group of participants' responses to different experimental stimuli are combined. These data can be visually inspected in order to determine where different responses occurred in time, and subsequent statistical analyses can be conducted to determine whether the amplitude or latency of these effects are significant.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00028-4
Copyright © 2017 Elsevier Inc. All rights reserved.

- EEG/ERPs have exquisite temporal resolution, and this can allow researchers to see how the brain processes stimuli on a millisecond by millisecond basis.
- However, the electrical signal generated by the brain is distorted by different tissues before it reaches the electrodes that are placed on a participant's scalp. Because of this, the technique has poor spatial resolution and therefore cannot be used to answer questions that involve determining where in the brain a particular signal is coming from.

LIST OF ABBREVIATIONS

BOLD response	Blood-oxygen level dependent response
DNA	Deoxyribonucleic acid
EAP	Early anterior positivity
EEG	Electroencephalogram
ERPs	Event-related potentials
fMRI	Functional magnetic resonance imaging
LPP	Late positive potential
mm	Millimeter
ms	Millisecond
MRI	Magnetic resonance imaging

INTRODUCTION

Human beings (as well as other animals) have been using psychoactive substances to alter conscious experience throughout the course of recorded history (Siegel, 1989). Cannabis is one such substance, and it has likely been used for thousands of years by people who inhabit a variety of different geographic regions around the world (Meyer & Quenzer, 2005). Despite the fact that use of the substance has lengthy historical roots, empirical investigations regarding its psychological effects have only been conducted in the more recent segment of human history. The advent of computers and neuroimaging methods have allowed scientists to address questions which are related to drug dependency and exploration into the effects of cannabis are certainly no exception. While some of these investigations have attempted to understand the acute effects of cannabis on human cognition and sensorimotor performance, other scientists have attempted to understand how frequent users of the drug respond to cannabis-related stimuli in their environments (Fig. 26.1). This chapter will explore the latter concept from the perspectives of behavioral and neuroscientific research, with an emphasis on how neural and behavioral effects can be measured and elucidated within laboratory environments. After a brief overview of some of the methodologies that are

FIGURE 26.1 **Cannabis cues in our everyday environments.** A store (also located on a major road) that sells cannabis-related products in downtown Vancouver, British Columbia. How might a regular cannabis user respond to a salient cue like this one?

currently being employed to study addiction processes in human beings, the chapter will provide a summary of findings that were obtained using a number of these techniques. Importantly, the research presented in this chapter will be centered around cannabis cue-reactivity and the effects of these cues on cannabis users. Research from multiple methodologies are also highlighted as the author believes that this will provide a more complete picture of how cannabis cue-reactivity influences the behavior and brain function of regular users as opposed to nonusers.

METHODS USED IN ADDICTION RESEARCH

Researchers have utilized a number of research methods to better understand how different drugs affect behavior, emotion, and other aspects of conscious experience. The use of computer-based methods has been extremely helpful in the assessment of motor and sensory effects, and the discovery of techniques that allow researchers to peer into the workings of a functioning human brain while experimental tasks are being completed have allowed for inference into the brain–behavior relationships that develop as substance use continues for prolonged periods of time. Although a variety of tasks have been used to understand the perceptual and cognitive experiences of drug users, the proceeding discussion will pertain to techniques that utilize *cue-reactivity paradigms* (where drug cues and other stimulus categories are presented to different groups of subjects). The following sections will introduce some of the of behavioral and brain-based methods that are currently being employed by those conducting research in this field.

Behavioral Methods

Reaction Times

One way that researchers have attempted to understand the effects of drug-cues on users of various substances is by asking them to respond as quickly as possible to target visual stimuli immediately after they appear on a computer monitor. Typically, experimental instructions will ask participants to respond to a colored square or other target that is superimposed on top of either a neutral, affective, or drug picture. Alternatively, instructions may specify that participants need to categorize these stimuli using different button presses. Programming tools that are used to create these computer-based tasks record the amount of time it takes for a button press response to be made and these responses can deemed correct or incorrect. For experiments that utilize these *reaction time* measurements it is usually standard practice to include only those trials that were deemed to be correct as this ensures that the participant was paying attention and was not distracted with anything that may have been happening in the testing environment. In studies, such as these, longer reaction times to drug stimuli (particularly for participants who were identified as having had past experience with the substance being investigated) may indicate that more attentional resources were allocated toward their detection and subsequent processing. This phenomenon has been termed *attentional bias* and it is frequently observed with substance users who meet criteria for substance abuse or substance dependence diagnoses (see Figs. 26.2 and 26.3).

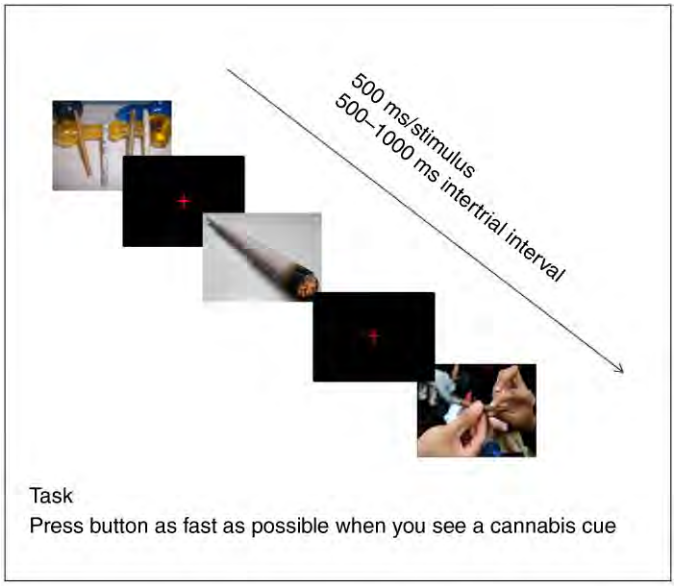


FIGURE 26.2 **Cannabis cue trial series.** A set of stimuli that might be presented in a typical cannabis cue-reactivity paradigm.

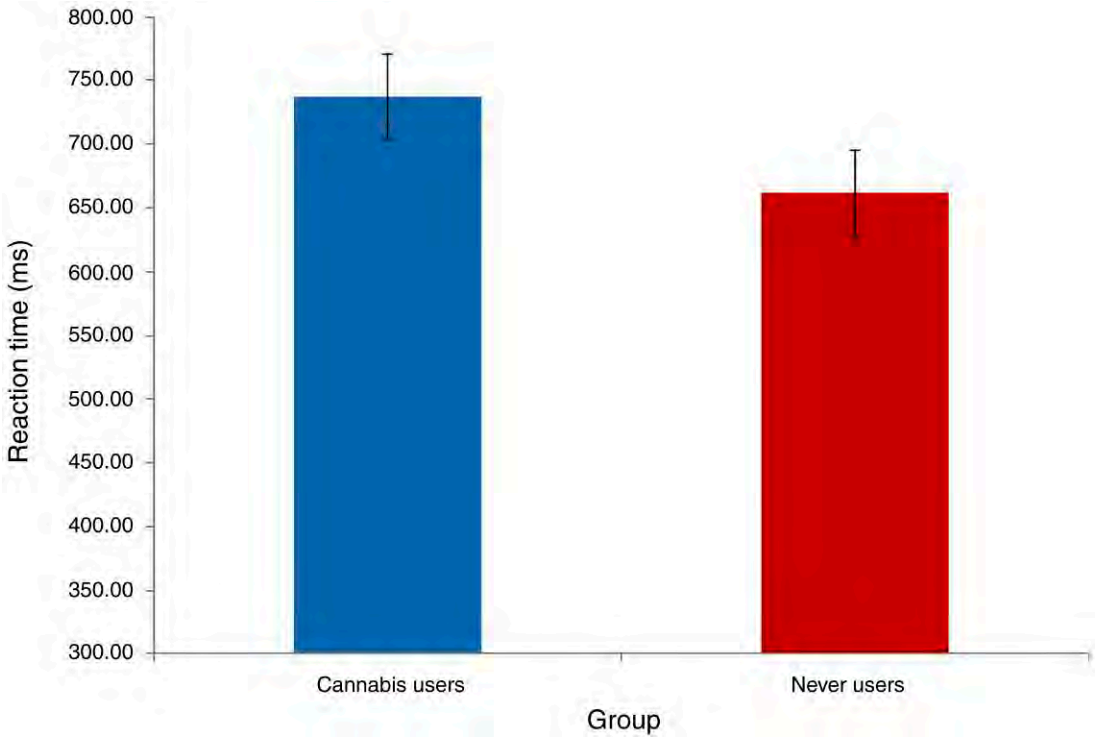


FIGURE 26.3 **Reaction times to cannabis cues.** Reaction times to cannabis-related cues for cannabis users and never users.

Cue-Induced Craving

Paradigms that present drug cues to participants typically include self-report measurements that have participants rate the degree of craving they were experiencing before, during, or after an experimental condition. These self-reports can take place before and at the very end of a task (where change in craving after viewing substance cues can be assessed by comparing pre- and postvalues), or they can be presented after every block of stimuli the participant views. A typical finding is that self-reported craving for a given drug (or even a food) is higher for those who are frequent consumers of the substance, yet these ratings remain low for those who belong to non-user/never a user groups. Furthermore, measurements of cue-induced craving may be used to assess the effects of effortful cognitive control, reappraisal, or satiety. Experimental manipulations of this kind may be especially useful for researchers who wish to understand the effects of different behavioral, cognitive, or pharmacotherapies in reducing cue-related craving as this is believed to play a significant role in relapse following serious attempts at abstinence.

Neuroimaging Methods

Event-Related Potentials

Event-related potentials (ERPs) allow researchers to measure brain responses to stimuli presented during experimental tasks by measuring voltage changes in brain electrical activity from the level of the scalp. ERPs refer to neural responses that are extracted from an ongoing electroencephalogram (EEG) and are obtained by attaching an array of electrodes to a participant's scalp. Events that are presented during an experimental task are called trials, and all trials that belong to the same category are eventually averaged together offline after the data collection session is completed. The process is repeated for each participant and averaged waveforms that depict scalp-recorded electrical activity are created using specialized computer software that allows researchers to remove artifacts and extract data from windows of time in the EEG epoch that are of interest to them. Later, a grand-average is created by averaging all participants' averaged brain responses to discrete stimulus types. Importantly, this technique has exquisite *temporal resolution* which can allow researchers to see how attention is allocated toward cues very early on, after stimulus presentation (ie, from 1 to 1000 ms after a cannabis cue was presented on screen), and also allows for other rapidly occurring cognitive effects to be detected and evaluated. Furthermore, the equipment is inexpensive relative to other technologies that are used to acquire brain data, and its maintenance is much more cost-effective compared to that seen with magnetic resonance imaging/functional magnetic resonance imaging (MRI/fMRI) technology. Despite its advantages, the electrical activity detected by the electrodes is distorted by different matter found in the head (eg, bone, cerebrospinal fluid) so detecting where brain activity occurred after drug or

FIGURE 26.4 **EEG recording equipment.** An electrode cap and some electrodes that would be attached to a subject and plugged into an amplifier for EEG recording.

neutral stimulus presentations is difficult to determine. Although some techniques have been developed to solve this problem, they currently offer a "best guess" solution and require validation using other techniques that are not subject to the same methodological constraints (eg, fMRI). See Luck (2005) for a useful discussion of the advantages and disadvantages of using the ERP technique relative to other imaging methods that are currently used by scientists (also see Fig. 26.4 for an example of recording equipment that might be found in a typical ERP laboratory).

Functional Magnetic Resonance Imaging

fMRI has been used extensively within the context of addiction research and is quite useful for determining which brain structures and neural networks are involved in the evaluation of drug cues versus other categories of stimuli. The MRI machine generates a strong magnetic field that effectively orients hydrogen atoms in our bodies to a certain orientation, and participants are asked to lie down in the scanner and remain very still during the experimental task. The MRI scan allows the researcher to take a picture of the brain after a radio pulse is transmitted through the magnetic field created by the scanner. This pulse disrupts the orientation of the hydrogen atoms, and the machine detects their realignment after the radio signal ceases (which allows for different tissue densities to be visualized). Following an MRI scan, an fMRI scan is conducted in order to map functional changes onto this previously created structural map. fMRI analyses rely on measuring the *blood-oxygen level dependent (BOLD) response* created by changes in the blood's oxygen levels. Importantly, when different regions of the brain are more active during a task more oxygen is used up by tissue in that region. When a radio wave is passed through the scanner during the presentation of different stimuli, it temporarily changes the orientation of hydrogen atoms within the blood, and blood that is deoxygenated has more magnetism that blood that is oxygenated. Deoxygenated blood will cause neighboring particles to spin more as they reorient after the radio wave pulse stops, and it is this spin–spin interaction that allows researchers to determine if a brain region was more or less active during the presentation of a given stimulus type (eg, a cannabis-related picture). This technique (when used in conjunction with a structural MRI scan) has excellent *spatial resolution*, meaning that it can detect the loci of brain activations in regions as small as 1 millimeter cube (mm^3). However, because its relies on measuring changes in blood-oxygen levels, the temporal resolution of this technique is sluggish and therefore not conducive to answering research questions that pertain to rapidly occurring cognitive/neural events (here, the ERP technique may be a more suitable apparatus). Furthermore, the equipment is quite expensive to acquire and maintain. However Despite its inherent limitations, many of the brain imaging studies that currently exist in the addiction literature have been conducted with the use of fMRI and invaluable information about the roles that different brain structures play in evaluating drug-related stimuli has been acquired by use of this method.

CANNABIS PICTURE PRESENTATIONS: BEHAVIORAL, ERP, AND fMRI FINDINGS

While a number of studies that look at drug-cue effects have been published in the addiction literature, only a small portion of this research has been dedicated to understanding the effects of cannabis cue presentations. The following sections will discuss some of these findings in detail.

Behavioral Studies

A number of studies have been conducted with the aim of delineating the effects of cannabis-related cues on craving, mood state, and attention. In one study, Lundahl and Johanson (2011) elicited cannabis cue-reactivity in their laboratory in the hope that they could accurately model what might also be seen in a real world setting. By having cannabis dependent users view video clips that depicted people smoking marijuana while also handling cannabis paraphernalia (eg, rolling papers, a pipe) several noteworthy effects were observed when these subjects were compared with cannabis-naive control participants. Within the cannabis user group, significant increases in cannabis craving were seen after these

multimodal cannabis cue presentations were delivered and this effect was not observed in the control group. Furthermore, the self-reported urge and desire to smoke marijuana was also found to be greater following cannabis versus neutral cue presentations. Wölfling, Flor, and Grüsser (2008) also used a cue-reactivity paradigm to study psychophysiological responses to cannabis, and although their study was primarily focused on cataloging ERP responses to pleasant, negative, neutral, alcohol, and cannabis cues, the behavioral component of the experiment found that cannabis dependent participants rated cannabis images as being more pleasant and more arousing than relative to control participants. Greater craving was also observed for the former group when self-report data obtained before and after cannabis cue exposure was compared. No differences in arousal or pleasantness ratings were found for other stimulus category contrasts. While the previously discussed studies have focused on cannabis cue-related responses in adults, similar effects also appear to be entrenched in adolescent cannabis users. Gray, LaRowe, and Upadhyaya (2008) presented 16–21 year old cannabis users with neutral and cannabis-related audio recordings, video clips, and in vivo stimuli (eg, marijuana cigarettes and pencils) to elicit craving and physiological responses. Craving ratings were found to be higher in the cannabis video and in vivo stimulus presentation conditions relative to their neutral counterparts. In vivo presentations of cannabis cues also evoked higher skin conductance and heart rate measurements relative to other modalities of cannabis cue presentations, suggesting that such presentations are particularly salient for cannabis users (also see Gray, LaRowe, Watson, & Carpenter, 2011 for additional evidence regarding the effects of in vivo cannabis cue exposure on craving, skin conductance, and heart rate in adolescents). Although research that looks specifically at cannabis cue-reactivity is sparse compared to the number of studies on other substances of abuse (eg, alcohol, tobacco, and cocaine), cue-induced craving does seem to be reliably observed in cannabis dependent users. The observation that presentations of cannabis cues can elicit this response in younger people also suggests that the human reward system is highly susceptible to the effects of drug-related *classical conditioning* and that this effect can take place relatively quickly. Lastly, cannabis users and nonusers have been found to differ from one another in terms of the degree of attentional bias they afford toward marijuana cues. In one study, participants were asked to view cannabis-related or neutral pictures that were presented on a computer screen while eye movements were recorded with a special set of goggles (Field, Eastwood, Bradley, & Mogg, 2006). They were also instructed to respond as quickly as possible to visual probes that replaced the different images from trial to trial. For cannabis users (but not control participants), eye gaze was directed toward cannabis cues for significantly longer periods of time relative to what was observed after neutral picture presentations. Cannabis-users also responded to the visual probes that replaced the cannabis pictures significantly faster than those that replaced the neutral pictures. Taken together, the reaction time and eye movement data again suggest that attentional bias toward cannabis cues may be present for those who use the substance regularly. Given that attentional bias is thought to play a role in the maintenance of substance addiction as well as relapse incidence following abstinence attempts, these findings may be particularly relevant for those who are trying to quit the habit. Importantly, attentional bias toward cannabis-related cues can also occur when stimuli that are less salient than vivid images are presented. For example, Field (2005) found that cannabis users took significantly more time to name the colors of visually presented cannabis-related words, therefore indicating that these words were able to elicit attentional bias despite being more subtle in nature.

Cannabis cue-reactivity paradigms have even been employed to better understand how genetic factors influence withdrawal trajectories in cannabis dependent subjects. Haughey, Marshall, Schacht, Louis, and Hutchison (2008) recruited a sample of daily cannabis users and measured cue-induced craving for cannabis before and after a 5-day abstinence period. Craving was induced by placing used bongs and pipes in a room while an audio tape instructed the participants to focus their attention on the items and imagine using them to self-administer the drug. Cheek cell and saliva samples were also collected for later DNA analysis during the initial session. After both testing sessions were completed it was found that cue-induced craving for cannabis significantly increased after 5 days of abstinence but only for cannabis users. Interestingly, cue-related craving was found to be higher for those users who had cannabis receptor CNR1 T/C gene expression (as opposed to those with a CNR1 T/T genotype), and these users were also found to be more dependent on cannabis relative to users with the latter homozygous allele pairing. When approached from a treatment perspective these findings are extremely informative given that they directly link a specific genetic makeup to heightened dependency and greater cue-related craving. Both of these factors are likely to influence treatment success, however, the researchers were careful to emphasize that additional follow-up research would need to be conducted before any firm conclusions could be asserted. Still, this research provides important ground work for future studies that aim to reduce relapse rates among cannabis users who are making abstinence attempts. In the future, these findings could also help clinicians tailor interventions to meet the needs of individual clients if they are identified as being at higher risk for future relapse.

Event-Related Potential Research

Researchers have combined cannabis cue-presentations with continuous EEG recordings in order to help understand how these cues are processed in the human brain and how neural events translate into cognition and observable behavior. One distinction that can be helpful for those trying to interpret ERP findings involves categorizing observed components as early (eg, between 100 and 300 ms after stimulus presentation) versus late occurring (eg, 300–2000 ms after stimulus presentation). This distinction is important as bottom-up (or stimulus driven) versus top-down (cognitively mediated) influences will be more or less likely depending on the window of time being examined. For instance, an ERP component that is elicited early on in stimulus processing may reflect an automatic orienting of attentional resources toward an environmental stimulus, and this may occur independently of higher-order cognitive influences or voluntary attention toward it. On the other hand, a component that is present between 400 and 700 ms may reflect more in-depth processing, where affective and cognitive appraisals will begin to exert some influence on the associated neural response. In the studies described in the following section, stimulus presentations have been delivered primarily through the visual modality.

One early occurring ERP component that is reported in the literature has been termed the EAP (early anterior positivity) effect, and it has been found over the frontal scalp in a window of time ranging between 200 and 350 ms. Although it was first reported in chocolate cravers when they viewed chocolate images (Asmaro et al., 2012), a subsequent study also found that this component was elicited after cannabis pictures were presented to participants who met criteria for cannabis dependence (Asmaro, Carolan, & Liotti, 2014). Importantly, this appeared to discriminate cannabis users from never users because the EAP effect was not observed in the latter group. This effect was thought to represent attentional bias being afforded toward the cannabis cues and may indicate that preconscious processing in the brain effectively identifies and directs attention toward craving inducing cues even before the user is aware that this is happening. Findings that pertain to neural correlates of attentional bias toward cannabis cues nicely complement findings that were presented in the previous section of this chapter, and they also provide evidence that earlier occuring attention-related processes can have a powerful influence on subjective craving.

Later occurring effects have also been observed when ERPs to cannabis cues were recorded during cue-reactivity paradigms. Of these effects, the late-positive potential (LPP) is one of the most frequently reported in the addiction literature (this is sometimes referred to as the P300/LPP complex), and has been observed in windows of time ranging from 300 ms after stimulus presentation and beyond. This component is believed to reflect a heightened arousal and greater attentional resource allocation toward affective stimuli when they are presented to participants (Polich & Criado, 2006), and cannabis dependent participants appear to have an LPP response after presentations of cannabis images (Wölfling et al., 2008; Henry, Kaye, Bryan, Hutchison, & Ito, 2014). One study has even demonstrated that the amplitude of the LPP (recorded in a window ranging between 300 and 600 ms) was found to be enhanced after cannabis dependent adolescents handled cannabis-related paraphernalia (Nickerson et al., 2011). In the latter study, LPPs elicited by cannabis cues were significantly larger those seen after presentations of other arousing cues, therefore suggesting that cannabis cues were subjectively more arousing than other stimulus types and perhaps more able to recruit attentional resources.

When information obtained from studies that reported early or late occurring ERP components is looked at holistically, one can begin to ascertain how cannabis stimulus processing occurs within the human brain at various points in the stream of perceptual processing. Early voltage enhancements to cannabis cues may alter downstream, later occurring processes that become elicited within cannabis users when they are presented with these stimuli and this may provide scientists with valuable information that ultimately helps to piece together how neural events give rise to observable behavior in the real world (Fig. 26.5). However, it is important to note that while ERPs can give us superb information about "when" different perceptual happen, they do not give reliable information about "where" in the brain these processes occur. In order to address questions related to "where," functional neuroimaging techniques need to be utilized in conjunction with cue-reactivity paradigms (Fig. 26.5).

fMRI Research

Research that utilizes the fMRI technique attempts to localize activations that are uniquely elicited by different stimuli or different tasks that participants are required to complete while they are being scanned. There have been a large number of studies within the addiction literature that report on results that were acquired with this technique, however, only a small subset of these has looked specifically at cannabis cue-reactivity. Understanding how brain structures facilitate the processing of these reward-related cues ultimately provides vital information about cannabis use behavior and the following paragraphs will discuss some of these current findings in more detail.

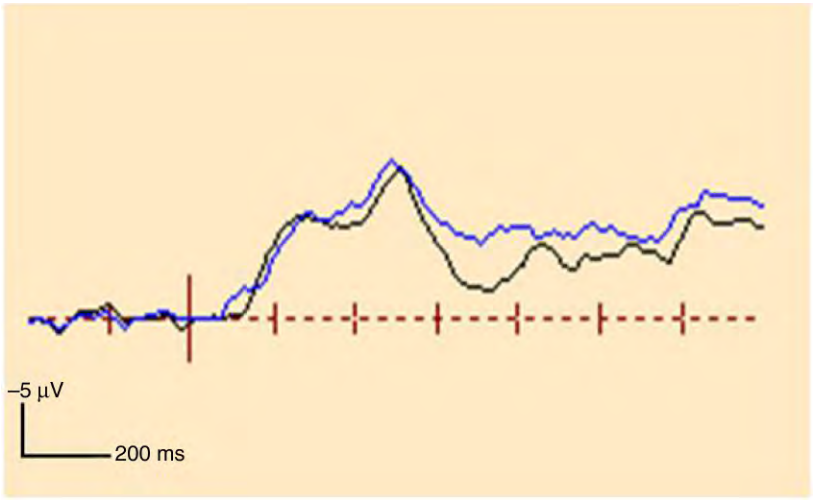


FIGURE 26.5 ERPs to cannabis and neutral cues: grand-averaged waveforms. ERPs (recorded at a frontal electrode channel) to cannabis-related *(black line)* and neutral cues *(blue line)* in a sample of cannabis users between 200 to 700 ms after visual stimulus presentation. Note the differences between the two responses and where they occur in time.

A number of fMRI studies have found that cannabis cues elicit activations in parts of the brain that are believed to play a fundamental role in allowing human beings to experience reward and process reward-related stimuli. Cousijn et al. (2013) found that frequent cannabis users and sporadic cannabis users (people who had used cannabis between 1 and 50 times during the course of their lives) showed increased activation in the medial frontal gyrus and orbitofrontal cortex (OFC) when they were presented with cannabis cues, yet this activation was not seen in nonusers. The OFC activation was particularly relevant, given that this structure is believed to play a role in triggering cue-related craving when drug users perceive drug stimuli (Koob & Volkow, 2010). Additionally, frequent users showed greater activation in a part of the brain called the ventral tegmental area (which is a part of the brain that releases a neurotransmitter called dopamine) when their cannabis cue elicited neural activations were contrasted with those of sporadic users. This is important, as the authors suggest that the processing of these reward-related cues may change as abuse of the substance becomes more frequent. Additional evidence that was acquired using fMRI also suggests that OFC activation to cannabis images becomes more pronounced as the number of years of cannabis abuse increases over time (Wetherill et al., 2014), and that greater activation in the nucleus accumbens (a dopaminergic neural region in the midbrain that projects to the ventral tegmental area) and OFC is also associated with higher frequencies of cannabis-related problems that interfere with everyday life (Filbey, Schacht, Myers, Chavez, & Hutchison, 2009) (see Fig. 26.6 for a representation of OFC location in brain space).

Lastly, fMRI research has also been used to link genetic composition to cannabis cue-reactivity. Similar to what was done in the previously discussed study by Haughey et al. (2008), Filbey, Schacht, Myers, Chavez, and Hutchison (2010) found that expression of the G allele for the CNR1 cannabis receptor and the C allele for the CAAH cannabis receptor impacted the degree of cannabis cue activation in parts of the brain that are associated with reward processing. Cannabis users who were CNR1 G allele heterozygotes had greater responses in the OFC, insula, inferior frontal gyrus, and in the dorsal anterior cingulate gyrus when cannabis cues were presented to them (as opposed to those who were A/A homozygotes). Additionally, cannabis users who were homozygotes for the FAAH C allele showed increased activation in the OFC, inferior frontal gyrus, striatum, and ventral tegmental area when cannabis versus control cue contrasts

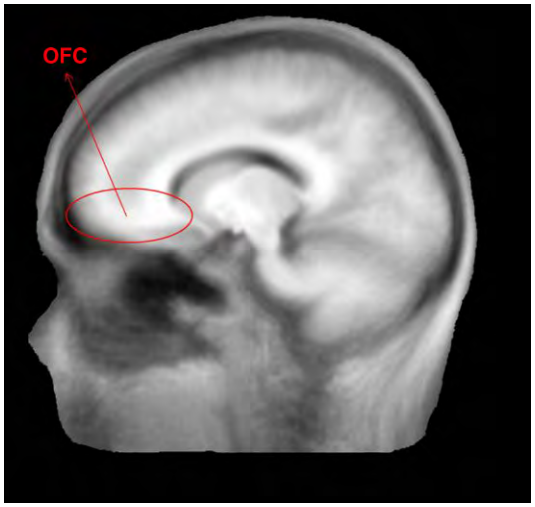


FIGURE 26.6 The orbitofrontal cortex in BESA brain space. A representation of where in the brain the OFC is located using an average brain model in BESA 5.3.

were examined. The researchers were also able to determine that cue-reactivity became more exaggerated as the number of risk alleles increased, therefore suggesting that there is a genetic basis for increased susceptibility to cannabis cue-reactivity. Findings, such as these support research that was conducted using behavioral techniques and could help focus prevention efforts that attempt to mitigate the development of cannabis abuse and dependence should such approaches be adopted in the future.

Taken together, it appears that cannabis cues can elicit activation in parts of the brain that are believed to be the neural substrates of reward processing and these activations could be used to determine genetic susceptibility for cannabis abuse/dependence. Importantly, parts of the brain that are activated by cannabis cues also appear to be activated in other paradigms that present substance users with cues related to a variety of abuse substances (which suggests that a common neural basis exists across substance abuse diagnoses).

CONCLUSIONS

Research on cannabis cue-reactivity has been conducted in a concerted effort to better understand the behavior of cannabis users and how exposure to cannabis-related cues uniquely impacts brain functioning. Using behavioral research techniques, researchers have found that greater craving and attentional bias is afforded toward cannabis cues by frequent users of the drug. Research that has employed electrophysiological techniques has found that early and late-occurring components provide information about the temporal processing of these cues by cannabis users and some of these findings appear to reflect attentional bias toward this specific kind of drug cue (which supports nicely findings that were obtained using behavioral techniques). Finally, fMRI research has been able to provide us with information about the brain regions that are involved in cannabis cue appraisal and have shown that such cues are able to activate parts of the brain that are involved in the processing of rewards. Cue-reactivity research has allowed us to better understand cannabis use and dependence and may facilitate effective treatment options in the future if such techniques are utilized within clinical settings to provide objective assessments of individual clients.

MINI-DICTIONARY

Attentional bias The exaggerated deployment of attentional resources to a salient stimulus that is present in a person's external environment. In addiction research this bias is seen in substance users and is related to increases in drug craving and longer drug stimulus fixations (which often result in increased reaction times).

Blood-oxygen level dependent response The measured changes in hemoglobin properties that are recorded during an fMRI scan. These responses characterize changes in blood oxygenation and the subsequent changes in the blood's magnetism that arise as a result.

Classical conditioning A process that takes place after a stimulus that initially had no reward value is repeatedly paired with the onset of a reward. Over time, the presentation of the stimulus (now called a conditioned stimulus) will elicit responses that are associated with the receipt of actual rewards.

Cue-reactivity paradigms Experimental paradigms that present stimuli to participants in order to elicit behavioral or neural responses to discrete categories of stimuli.

Dopamine A catecholamine class neurotransmitter that is primarily manufactured in the midbrain and in subcortical structures. Although it plays a vital role in many cognitive and motor functions it is also critically involved in signaling reward onsets.

Event-related potentials Electrophysiological responses that are observed at the level of the scalp after stimuli are presented to a participant. Although they can be derived after just one stimulus response is recorded, they are usually created by averaging all stimulus presentations of a given type together within single subjects and then across an entire sample.

Multimodal A term that denotes that a multiple sensory systems were involved in the processing of a stimulus (ie, vision and touch).

Orbitofrontal cortex A region in the brain's frontal lobe that is thought to be a core component of the human reward system. It is located at the anterior part of the brain just above where the eyes would be found.

Reaction time The amount of time it takes for a participant to respond to a stimulus that is presented to them. These are usually measured in milliseconds (ms).

Spatial resolution The ability to visualize locations in space in varying degrees of detail. A methodology that has higher spatial resolution will be able to extract more detail about a spatial location.

Temporal resolution The ability to record events that occur during varying lengths of time. A technique that has high temporal resolution will be able to discriminate events that occur very soon after each other (ie, from 1 ms to the next).

References

Asmaro, D., Carolan, P. L., & Liotti, M. (2014). Electrophysiological evidence of early attentional bias to drug-related pictures in chronic cannabis users. *Addictive Behaviors*, *39*, 114–121.

Asmaro, D., Jaspers-Fayer, F., Sramko, V., Taake, I., Carolan, P., & Liotti, M. (2012). Spatiotemporal dynamics of the hedonic processing of chocolate images in individuals with and without trait chocolate craving. *Appetite*, *58*, 790–799.

Cousijn, J., Goudriaan, A. E., Ridderinkhof, K. R., van den Brink, W., Veltman, D. J., & Wiers, R. W. (2013). Neural responses associated with cue-reactivity in frequent cannabis users. *Addiction Biology*, *18*, 570–580.

Field, M. (2005). Cannabis 'dependence' and attentional bias for cannabis-related words. *Behavioural Pharmacology*, *16*, 473–476.

Field, M., Eastwood, B., Bradley, B. P., & Mogg, K. (2006). Selective processing of cannabis cues in regular cannabis users. *Drug and Alcohol Dependence*, *85*, 75–82.

Filbey, F. M., Schacht, J. P., Myers, U. S., Chavez, R. S., & Hutchison, K. E. (2009). Marijuana craving in the brain. *Proceedings of the National Academy of Sciences*, *106*, 13016–13021.

Filbey, F. M., Schacht, J. P., Myers, U. S., Chavez, R. S., & Hutchison, K. E. (2010). Individual and additive effects of the CNR1 and FAAH genes on brain response to marijuana cues. *Neuropsychopharmacology*, *35*, 967–975.

Gray, K. M., LaRowe, S. D., & Upadhyaya, H. P. (2008). Cue-reactivity in young marijuana smokers: a preliminary investigation. *Psychology of Addictive Behaviors*, *22*, 582–586.

Gray, K. M., LaRowe, S. D., Watson, N. L., & Carpenter, M. J. (2011). Reactivity to in vivo marijuana cues among cannabis dependent adolescents. *Addictive Behaviors*, *36*, 140–143.

Haughey, H. M., Marshall, E., Schacht, J. P., Louis, A., & Hutchison, K. E. (2008). Marijuana withdrawal and craving: influence of the cannabinoid receptor 1 (CNR1) and fatty acid amide hydrolase (FAAH) genes. *Addiction*, *103*, 1678–1686.

Henry, E. A., Kaye, J. T., Bryan, A. D., Hutchison, K. E., & Ito, T. A. (2014). Cannabis cue reactivity and craving among never, infrequent and heavy cannabis users. *Neuropsychopharmacology*, *39*, 1214–1221.

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology*, *35*, 217–238.

Luck, S. (2005). *An introduction to the event-related potential technique*. Cambridge, MA: MIT Press.

Lundahl, L. H., & Johanson, C. E. (2011). Cue-induced craving for marijuana in cannabis-dependent adults. *Experimental and Clinical Psychopharmacology*, *19*, 224–230.

Meyer, J. S., & Quenzer, L. F. (2005). *Psychopharmacology: Drugs, the brain, and behavior*. Sunderland, MA: Sinauer Associates, Inc.

Nickerson, L. D., Ravichandran, C., Lundahl, L. H., Rodolico, J., Dunlap, S., Trksak, G. H., & Lukas, S. E. (2011). Cue reactivity in cannabis-dependent adolescents. *Psychology of Addictive Behaviors*, *25*, 168–173.

Polich, J., & Criado, J. R. (2006). Neuropsychology and neuropharmacology of P3a and P3b. *International Journal of Psychophysiology*, *60*, 172–185.

Siegel, R. K. (1989). *Intoxication: Life in pursuit of artificial paradise*. New York, NY: E.P. Dutton.

Wetherill, R. R., Childress, A. R., Jagannathan, K., Bender, J., Young, K. A., Suh, J. J., O'Brien, C. P., & Franklin, T. R. (2014). Neural responses to subliminally presented cannabis and other emotionally evocative cues in cannabis-dependent individuals. *Psychopharmacology*, *231*, 1397–1407.

Wölfling, K., Flor, H., & Grüsser, S. M. (2008). Psychophysiological responses to drug- associated stimuli in chronic heavy cannabis use. *European Journal of Neuroscience*, *27*, 976–983.

CHAPTER

27

Cannabis Use and First-Episode Psychosis Patients (FEP)

I. González-Ortega[*,**,†], *M. Martínez-Cengotitabengoa*[*,†], *A. González-Pinto*[*,**]

*Department of Psychiatry, University Hospital of Alava-Santiago, CIBERSAM, Vitoria, Spain
**University of the Basque Country, Vitoria, Spain
†National Distance Education University (UNED)-Centro Asociado de Vitoria, Vitoria, Spain

SUMMARY POINTS

- Cannabis use is a risk factor for psychosis, especially in vulnerable individuals.
- First-episode psychosis patients with cannabis abuse have a poorer clinical and functional outcome, compared with patients without cannabis abuse.
- Cannabis use is associated with cognitive deficits in first-episode psychosis (FEP) patients.
- Depressive symptoms are associated with cannabis abuse in FEP patients.
- Cannabis use is a target for the prevention and treatment of patients with first psychotic episodes.

KEY FACTS OF CANNABIS USE AND FIRST-EPISODE PSYCHOSIS PATIENTS

- Cannabis is the most widely used drug among patients with FEP.
- Cannabis use has become a target for the prevention and treatment of patients with first psychotic episodes because of its influence on the development and prognosis of the disease.

LIST OF ABBREVIATIONS

CUD	Cannabis use disorders
FEP	First-episode psychosis patients
5-HTTLPR	Short allele of the serotonin transporter gene-linked polymorphic region
THC	Δ^9-Tetrahydrocannabinol

INTRODUCTION

Cannabis is the most commonly abused substance in FEP (Koskinen, Lohonen, Koponen, Isohanni, & Miettunen, 2010). Cannabis use may play a role as a risk factor for psychosis by influencing the age at onset of psychosis (González-Pinto et al., 2008). Risk of developing psychosis has been associated with the frequency or duration of cannabis consumption, the dose of cannabis consumed, and an early age at onset of cannabis consumption (Arseneault, Cannon, Witton, & Murray, 2004), especially in vulnerable individuals (Dekker et al., 2008).

There is empirical evidence that prolonged cannabis use has a negative effect on the clinical and functional course of FEP patients. Cannabis use in FEP is associated with poor treatment adherence (Barbeito et al., 2013), more severe and chronic symptoms (Kovasznay et al., 1997), increased risk of relapse (Pencer, Addington, & Addington, 2005), and poorer long-term functioning. However, those patients who stopped using cannabis experience

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00030-2
Copyright © 2017 Elsevier Inc. All rights reserved.

a slow but steady improvement over time, both in functional outcome and negative symptoms (González-Pinto et al., 2011). Moreover, cannabis use is associated with cognitive deficits in patients with psychosis (Rais et al., 2008; Ayres et al., 2010; Meijer et al., 2012).

Because of the high rate of cannabis use among FEP patients, and its influence on the development and prognosis of psychosis, cannabis use has become a target for the prevention and treatment of patients with first psychotic episodes.

CANNABIS USE AS A RISK FACTOR FOR PSYCHOSIS

Substance misuse is a common comorbid problem in people presenting with a first episode of psychosis (Lambert et al., 2005). Cannabis is the most commonly abused substance in this population. The prevalence rate of cannabis use among patients with FEP ranges from 14% (Addington & Addington, 2007) to 66% (Wade, Harrigan, Harris, Edwards, & McGorry, 2006). The variation in reported prevalence rates may be due to the sample studied, and how cannabis use was defined, measured, or diagnosed (including prior, current or liftime use). In a recent metaanalysis examining the rate of cannabis use disorders (CUD) in schizophrenia and other psychotic disorders, the estimated median rate of CUD was higher in first-episode patients, than in long-term schizophrenia patients: 28.6% versus 22.0% for a current diagnosis of CUD, and 44.4% versus 12.2% for a lifetime diagnosis of CUD (Koskinen et al., 2010). These findings support the hypothesis that cannabis consumption may have a role in the etiology and evolution of psychosis. Further research has implicated cannabis use as an environmental factor associated with the development of psychotic illness (van Os, Kenis, & Rutten, 2010).

Although causal relationships are difficult to establish in psychiatry, there are important data that tip the balance in favor of a causal relationship between cannabis use and psychosis. Longitudinal studies indicate that cannabis use often precedes psychotic disorders (Kuepper et al., 2011; van Gastel et al., 2014). In a population-based cohort study in Germany, where almost two thousand people aged 14–24 years were followed up for 10 years, Kuepper et al. (2011) found that incident cannabis use increased the risk of incident psychosis 4 years later. They also found that continued cannabis use increased the risk of persistent psychotic symptoms. A major advantage of this study is that reverse causality (ie, premorbid subthreshold psychotic symptoms induce cannabis use) could be controlled for.

Other studies argue that cannabis use precipitates the onset of a psychotic episode in individuals with a pre-existing vulnerability to psychosis (Henquet et al., 2005). Research has indicated that genetic factors play a role in the development of psychotic disorders; for example, schizophrenia has an estimated heritability of 85%, and there is a 10-fold increased risk for psychotic disorders in siblings of patients with schizophrenia (Lewis et al., 2003). However, despite the research conducted in recent decades, there has been only limited progress in our understanding of the mechanisms involved in the disease. Neither genetic nor environmental variables alone appear to be sufficient or necessary to precipitate the disease (Rutten & Mill, 2009; van Os et al., 2010), making it important to study the gene–environment interaction.

It remains unclear whether the same genes that increase the risk of psychosis also increase the risk of cannabis use, or if other mechanisms are involved. Recently, investigators found preliminary evidence that some common genes are implicated in the association between psychosis and cannabis use (Power et al., 2014).

Cannabis use may play a role as a risk factor for psychosis by influencing the age at onset of psychosis. Risk of developing psychosis has been associated with the frequency or duration of cannabis consumption, the dose of cannabis consumed, and an early age at onset of cannabis consumption (Arseneault et al., 2004), especially in vulnerable individuals (Dekker et al., 2008). In a study of FEP patients, we found a strong and independent relationship between cannabis use and early age at onset of psychosis, such that the age at onset of psychosis decreased, as patients were classified as having cannabis use, abuse, and dependence versus cannabis nonusers (González-Pinto et al., 2008) (Fig. 27.1). This suggests that cannabis is a dangerous drug for young people at risk of developing psychosis to use. However, our findings are not consistent with the hypothesis that cannabis use and age at onset of psychosis are only associated because younger patients are more prone to using cannabis. The inverse dose relationship of cannabis use with age at onset of psychosis in this and other studies (Fergusson, Horwood, & Ridder, 2005) gives more strength to the hypothesis that the association between cannabis use and psychosis onset is causal, or that it is due to effect modification (ie, cannabis use is associated with the development of psychosis in people who are susceptible to developing psychotic symptoms), as discussed in previous studies of the general population (Fergusson, Poulter, Smith, & Boden, 2006). It is clear from studies that increasing use of cannabis is associated with an increasing risk of psychosis (Fergusson et al., 2006), whereas only heavy substance use (versus mild use or no use) is associated with worse symptomatic and functional outcomes in FEP patients (Wade, Harrigan, McGorry, Burgess, & Whelan, 2007).

Furthermore, although the association between cannabis use and age at onset of psychosis may be mediated or moderated by other factors, such as comorbid use of other drugs or sex (Veen et al., 2004), the results of our

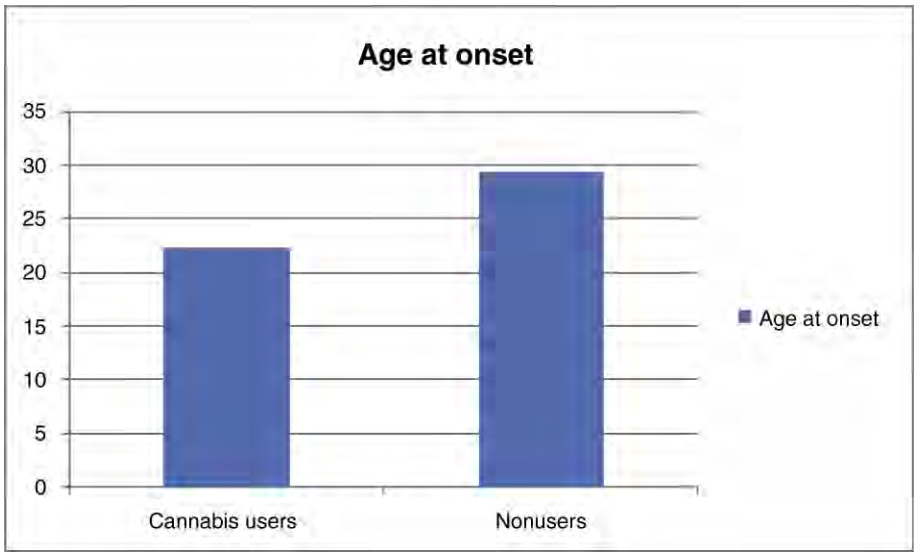


FIGURE 27.1 **Cannabis use and age at onset.** Relationship between age at onset and cannabis use in the long-term. *Source: From González-Pinto et al. (2008).*

study suggest that the effect of these variables is minimal or nonexistent (González-Pinto et al., 2008).

INFLUENCE OF CANNABIS USE ON CLINICAL AND FUNCTIONAL OUTCOMES, AND THE ROLE OF DEPRESSIVE SYMPTOMS IN FEP

There is empirical evidence that prolonged cannabis use has a negative effect on the clinical and functional course of FEP patients (Clausen et al., 2014; González-Pinto et al., 2011). Cannabis use may be a risk factor for nonadherence to treatment in FEP patients (Miller et al., 2009; Zammit et al., 2008). Longitudinal studies by our research group have shown that FEP patients who quit cannabis use during follow-up have better long-term adherence to medication (Barbeito et al., 2013), and improved clinical and functional outcomes (González-Pinto et al., 2011). Barbeito et al. (2013) demonstrated that the proportion of FEP patients using cannabis decreased from 52% at baseline to 29% at 8 years. Moreover, the majority (74%) of patients who continued to use cannabis over the 8-year follow-up period were classified as having bad adherence to medication, whereas only 17% of the patients who stopped using cannabis had bad adherence (Fig. 27.2). At the same time, patients with bad adherence at 8 years had worse functioning than those with good adherence (Fig. 27.3). There is, therefore, a relationship between long-term cannabis use, poor functionality and the pattern of adherence (Fig. 27.4). Moreover, involuntary first admission was associated with improvement in adherence during follow-up. These findings indicate that FEP patients with coexisting cannabis use are more likely to be nonadherent to medication, and suggest that interventions should aim to stop cannabis use and improve adherence.

Cannabis use is also associated with poorer outcomes in FEP patients. In our study of the influence of cannabis use on long-term outcomes, we compared FEP patients who stopped using cannabis with those who never used cannabis, and with patients who continued to use cannabis during 8 years of follow-up (González-Pinto et al., 2011). Results indicated that continued cannabis use had a deleterious effect on outcome. Specifically, patients who stopped using cannabis experienced a slow but steady improvement over time in their negative symptoms and functioning, whereas patients who continued using cannabis had a trend to increasing negative symptoms over time, and no improvement in functional outcome. All patients showed improvements in positive symptoms, over the long term.

Other studies have also demonstrated that FEP patients who use cannabis have a worse outcome than patients who do not use cannabis. In a systematic review that included seven follow-up studies of FEP patients, cannabis use was associated with worse outcomes (increased relapse and nonadherence) in the short- and medium-term, although differences in outcome between cannabis users and nonusers were more modest, when the studies controlled for use of other drugs, and baseline illness severity (Zammit et al., 2008). Similarly, other studies have shown that continued use of cannabis by FEP patients is associated with a worse functional outcome (Addington & Addington, 2007).

It is possible that the deleterious effect of continued cannabis use on symptoms and functional outcome is due, in part, to additive effects of psychosis and cannabis

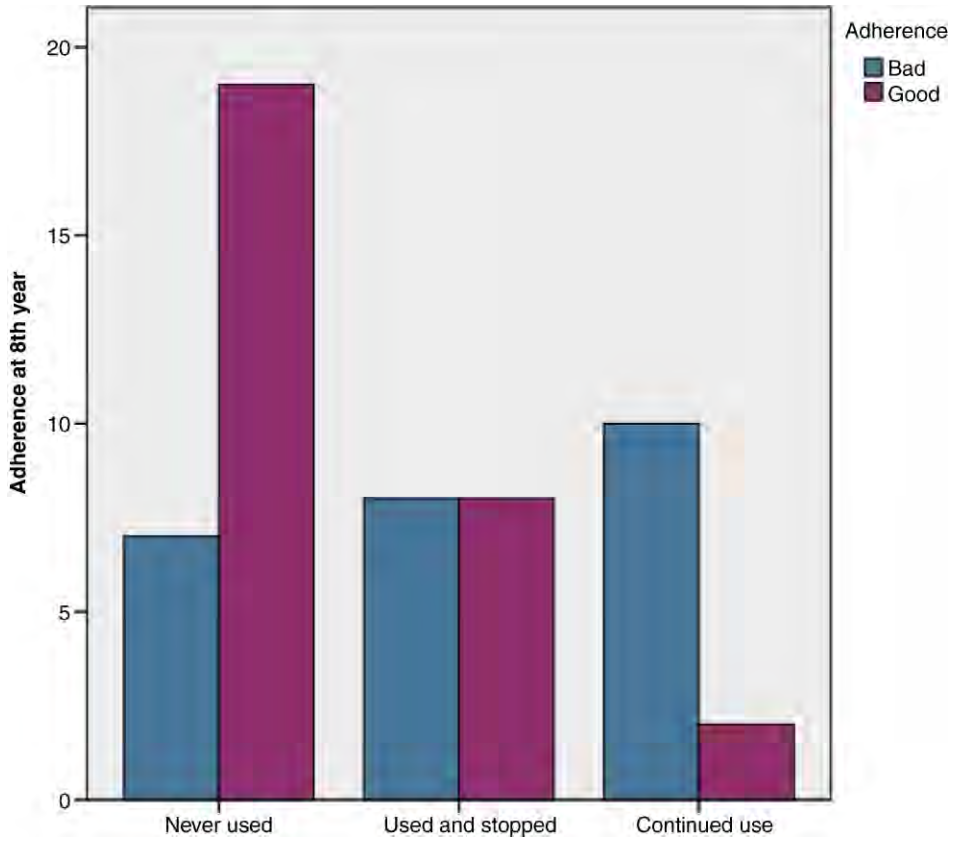


FIGURE 27.2 **Cannabis use and adherence.** Relationship between cannabis use and the pattern of adherence in the long-term. *Source: From Barbeito et al. (2013).*

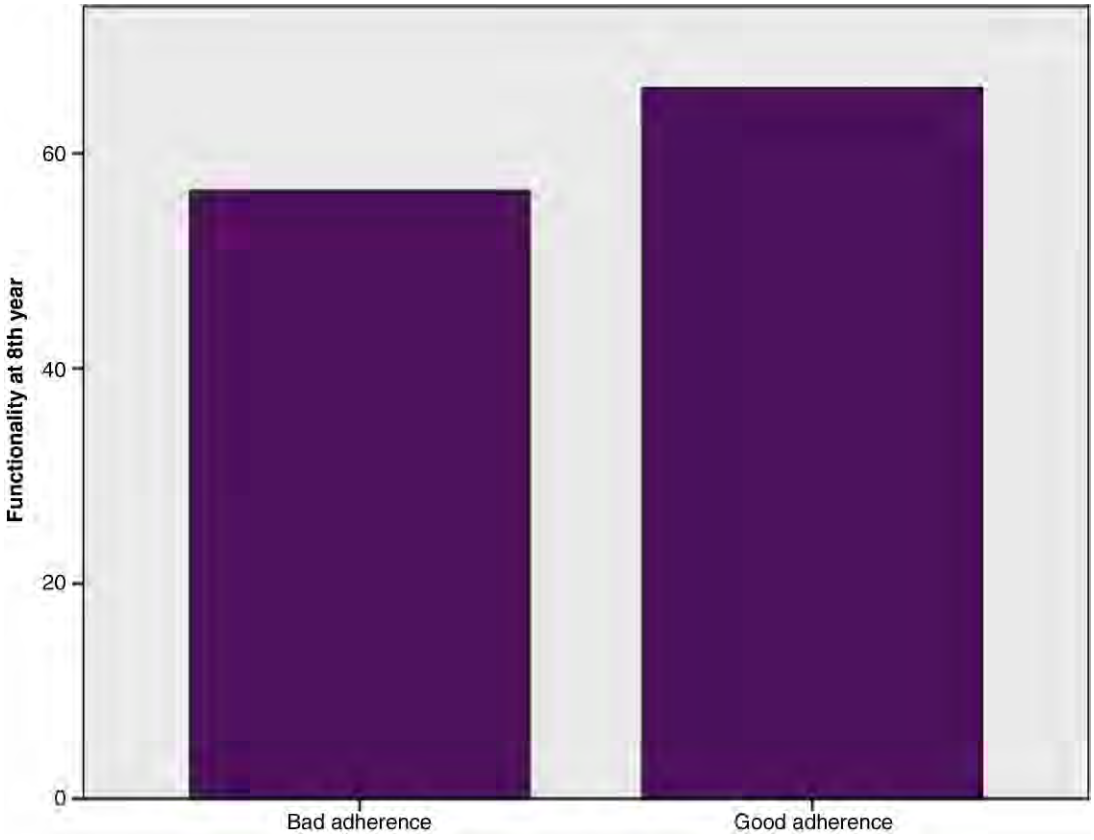


FIGURE 27.3 **Adherence and functionality.** Relationship between bad adherence and poor functionality in the long-term. *Source: From Barbeito et al. (2013).*

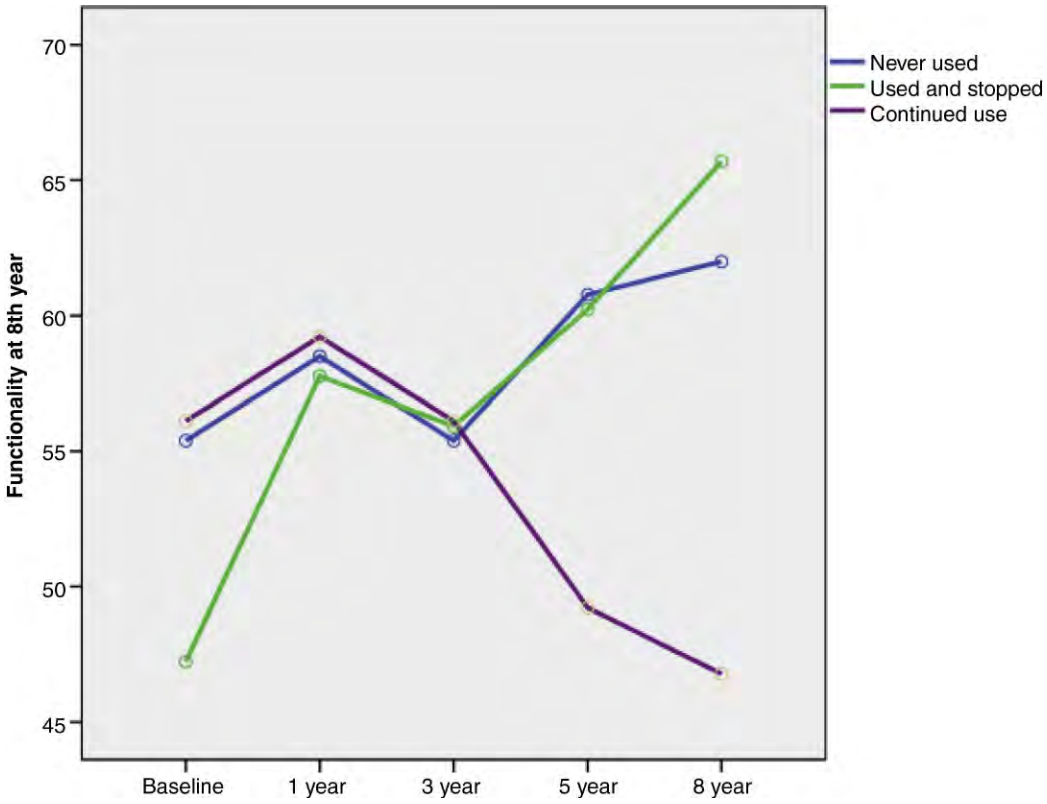


FIGURE 27.4 **Cannabis use and functionality.** Relationship between cannabis use and poor functionality long term. *Source: From Barbeito et al. (2013).*

use. Furthermore, use of other drugs, mainly stimulants, may contribute to the worsening of functional outcomes. From our study of the impact of cannabis use on long-term outcomes in FEP patients (González-Pinto et al., 2011), it is difficult to understand why those patients who were able to quit cannabis use during follow-up had a better long-term functional outcome than those who never used cannabis. It could be that, at initial hospitalization with a first psychotic episode, cannabis users have a phenotypic presentation that is as severe as non-users (although they could have a lower genetic loading), but that quitting cannabis reveals a more benign disease after some years of follow-up, at least for functional outcome and negative symptoms. If this is true, continued use of cannabis causes even more damage than is currently thought. The progressive improvement of symptoms and functioning during follow-up, in the group who stopped using cannabis, is not consistent with the theory that cannabis causes permanent and residual effects in the long term (González-Pinto et al., 2011). In a recent metaanalysis of studies that compared outcomes in patients with psychosis who stopped using substances with those who had never used substances, the authors concluded that prior cannabis use is not necessarily a poor prognostic factor for those who stop using it (Gupta, Mullin, Nielssen, Harris, & Large, 2013). However, we have shown that stopping cannabis use is clearly beneficial (González-Pinto et al., 2011).

Cannabis use is also associated with depressive symptoms, and FEP patients with depressive symptoms have poorer long-term outcomes. In the study conducted by Sönmez, Romm, Andreasssen, Melle, and Røssberg (2013), FEP patients with persistent depression had poorer functioning and more negative symptoms at 12 months follow-up than patients with no depression. Birchwood, Iqbal, and Upthegrove (2005) have suggested that persistent depression is an integral aspect of schizophrenia that is associated with disease severity and negative symptoms. This link between depression and negative symptoms is particularly important in patients with comorbid cannabis use, although the etiology of the association remains unclear (Hartley, Barrowclough, & Haddock, 2013).

Some studies have found an association between cannabis abuse and the presence of fewer or less severe depressive symptoms (Tosato et al., 2013), suggesting that cannabis may alleviate some of these symptoms. According to the self-medication hypothesis, patients with depression often show a poor repertoire of behaviors, with a lack of rewarding activities in their daily life, except for the use of addictive substances (Arendt et al., 2007); these facilitate the appearance of a depressed mood which, in turn, is associated with an increased risk of cannabis

consumption in an attempt by patients to relieve their negative emotional state (Schofield et al., 2006). Thus, one factor that may contribute to the difficulty in stopping cannabis use among patients with depressive symptoms is the anxiolytic effect of its two main constituents, Δ^9-tetrahydrocannabinol (THC) and cannabidiol (Campos, Moreira, Gomes, Del Bel, & Guimarães, 2012).

The presence of depressive symptoms has also been associated with the effects of chronic use, or subacute intoxication with cannabis; this is referred to as the "amotivational syndrome," and includes symptoms of anhedonia, apathy, passivity, lack of interest, or difficulties in functioning (Arendt et al., 2007). In fact, some studies have observed an increase in negative symptoms (D'Souza et al., 2005) or depressive symptoms (Lev-Ran et al., 2014), when THC is administered to patients with schizophrenia. On the other hand, recent findings suggest that the effect of cannabis use on depressive symptoms might be moderated by genetic factors, specifically the short allele of the serotonin transporter gene-linked polymorphic region (5-HTTLPR) genotype; that is, cannabis use increases the risk for a worsening of depressive symptoms in individuals with this genetic vulnerability (Otten & Engels, 2013). Therefore, the association found between depressive symptoms and cannabis use is inconsistent, which raises the question of whether cannabis use could be the result of depressive symptoms. Thus, there are at least three hypotheses that could explain the relationship between depressive symptoms and cannabis abuse: (1) depressive symptoms are a consequence of cannabis abuse (Lynskey et al., 2004); (2) the self-medication theory (Arendt et al., 2007; Degenhardt, Coffey, Moran, Carlin, & Patton, 2007); and (3) patients have a common genetic or environmental vulnerability for both disorders (Lynskey et al., 2004).

Depressive symptomatology may contribute to a greater difficulty in stopping cannabis use, and predict a worse clinical and functional outcome of psychosis. Depressive symptoms, therefore, should be a therapeutic target in patients with first psychotic episodes, especially those who use cannabis, with the aim of improving the clinical and functional course of the disease.

In conclusion, cannabis use in FEP patients is associated with poor treatment adherence (Barbeito et al., 2013), more severe and chronic symptoms (Kovasznay et al., 1997), increased risk of relapse (Pencer et al., 2005), and poorer long-term functioning (González-Pinto et al., 2011).

IMPACT OF CANNABIS USE ON COGNITIVE FUNCTIONING IN FEP

Cannabis use among healthy individuals has been associated with cognitive deficits in multiple domains, such as, memory, attention, inhibitory control, executive function, and decision making. Cognitive impairment has also been associated with the frequency, duration, and age at onset of cannabis use (Meier et al., 2012).

The association between cannabis use and cognitive deficits in patients with psychosis is less clear, and more difficult to demonstrate because cognitive deficits are also present in patients with psychosis who do not use cannabis. Some studies have shown cognitive impairment in FEP patients, compared with control subjects (Ayres et al., 2010), and cognitive deficits which decreases in gray matter volume, lateral ventricle enlargement, and other structural brain abnormalities (Minatogawa-Chang et al., 2009; Rosa et al., 2010; Schaufelberger et al., 2011; Trzesniak et al., 2012), in FEP cannabis users (Rais et al., 2008).

Moreover, D'Souza et al. (2005) found that acute administration of THC causes more deleterious cognitive effects in patients with psychosis than in healthy control subjects. A recent cross-sectional study has shown that current cannabis users have deficits in processing speed and verbal memory (Meijer et al., 2012). In contrast, other authors have reported that patients with psychosis, who use cannabis, have better cognitive functioning in some domains than patients who do not use cannabis (Leeson et al., 2009; Leeson, Harrison, Ron, Barnes, & Joyce, 2012; Loberg & Hugdahl, 2009). Moreover, Cunha et al. (2013) recently found that FEP patients with a history of cannabis use not only had less brain abnormalities (ie, gray matter and lateral ventricle volume was preserved), but also had less attentional and executive impairment, compared to patients who never used cannabis.

These conflicting findings are probably due to methodological limitations of the studies, and the effects of different moderators in the samples analyzed. Yücel et al. (2012) considered that there is probably a subgroup of cannabis users with psychosis who have better premorbid adjustment and cognitive performance. Similarly, Meijer et al. (2012) found that current cannabis use is associated with poorer cognitive performance, but that lifetime cannabis users may have a higher cognitive potential. This does not mean that cannabis has a beneficial effect on cognition; on the contrary, it worsens cognitive performance, although some of these individuals had better premorbid capacities. Accordingly, Winkel, van Beveren, and Simons (2011) found that the deleterious effect of cannabis use on cognition in patients with psychosis was moderated by the *AKT1* gene. They showed that cannabis-using patients who carry the CC variant in the *AKT1* gene performed worse on tests of attention than patients with this genotype who did not use cannabis, whereas cannabis users with the TT variant in *AKT1* performed as well as, or better, than cannabis nonusers (Winkel et al., 2011).

Other methodological limitations are that some studies included patients with a history of previous cannabis

use, and did not examine current cannabis use (de la Serna et al., 2010; DeRosse, Kaplan, Burdick, Lencz, & Malhotra, 2010). Stopping cannabis use has beneficial effects on outcome, as already discussed; this may include cognition, although this has not been well studied.

TREATMENT FOR FEP CANNABIS USERS

Research showing the involvement of environmental factors, such as cannabis use, in the development of psychosis (Rutten & Mill, 2009) will drive the search for new targets for prevention, early diagnosis, and intervention to reduce functional impairment of patients with psychotic disorders. People presenting with a first psychotic episode show reduced cognitive performance and functional impairment, compared with matched control subjects, and these deficits can lead to reduced social integration (Kravariti et al., 2009). Cannabis use has become a target for the prevention and treatment of patients with first psychotic episodes because of its influence on the development and prognosis of the disease. Unlike patients with chronic psychosis, individuals with a first psychotic episode show cannabis use of varying frequency and intensity, as well as greater motivation for change (Lambert et al., 2005). This highlights the need to intervene in the early stages of the disease, while the patient is young, and has a shorter history of substance abuse.

Early prevention, detection and treatment (pharmacological and psychological) of FEP patients is closely linked to better prognosis (Bertelsen et al., 2008). Early intervention may not only allow substance abuse in FEP patients to be detected, but may also enable it to be reduced. In a systematic review of studies examining the effectiveness of psychological intervention in FEP, Vega, González-Pinto, Ruiz de Azua, Barbeito, and Rico (2010) concluded that psychoeducation as an adjunct to medication is effective in improving negative symptoms and patient functionality. Despite the clinical consequences of cannabis use in early psychosis (Baeza et al., 2009), very few clinical trials have evaluated specific interventions aimed at reducing cannabis use in this patient population (Copeland, 2004). However, psychoeducation may be insufficient (Edwards et al., 2006). Furthermore, the effectiveness of the intervention and type of approach used were unclear, as well as the small sample size and the lack of appropriate control groups used in the studies, all this making it difficult to draw conclusions (Edwards et al., 2006).

There is an urgent need to develop specific interventions that effectively reduce cannabis consumption, as part of a comprehensive treatment program for patients with psychosis. An intervention focused on the management of cannabis use is needed to prevent the relapse and progressive impairment that often arises after the first psychotic episode (Lewis, Tarrier, & Drake, 2005). Some evidence suggests that cognitive behavioral therapy and motivational techniques might be effective in treating psychotic patients who are cannabis users (Edwards et al., 2006). Such psychological interventions are a promising therapeutic approach, not only for treating psychosis, but also for detecting and reducing drug abuse in patients experiencing a first psychotic episode. However, there is currently no evidence that any one specific type of psychosocial treatment is more effective than standard care at reducing substance use, or improving the course of illness in people with serious mental disorders. In a systematic review of 32 randomized controlled trials of the effectiveness of psychosocial interventions in people with both severe mental illness and substance misuse, the authors found that cognitive behavioral therapy, motivational interviewing, or motivational interviewing plus cognitive behavioral therapy had no advantage over usual treatment for reducing substance use, relapse, or global functioning (Hunt, Siegfried, Morley, Sitharthan, & Cleary, 2014).

Hunt et al. (2014) discussed some of the methodological limitations of existing studies, and concluded that there is a need for better quality trials that adhere to proper randomization methods; use clinically valuable, reliable, and validated measurement scales; and clearly report data, including retention in treatment, relapse, and abstinence rates. Finally, there is a need for interventions based on a more accurate understanding of the different patterns of cannabis consumption, and the factors that maintain or inhibit drug abuse in young people with first episode psychosis.

MINI-DICTIONARY

Attention Attention is the cognitive process that inlcudes the ability of selecting and inhibiting some information for further processing. There are different types of attention, such as: selective attention, focused attention, divided attention and/or sustained attention.

Canabidiol Active compound of cannabis that can counteract the effects of THC.

Cannabis abuse Continued use of cannabis despite negative consequences (health, work, school, family, or social relationships).

Cognitive functioning Cognitive functioning is the intellectual activity that includes mental processes, such as, attention, processing speed, learning and memory, executive function, verbal fluency, and working memory.

Executive function Cognitive process that involves reasoning, flexibility, and problem solving ability and cognitive skills to plan, organize, and execute tasks.

First episode psychosis (FEP) A first psychotic episode was defined as the first time a patient displayed positive psychotic symptoms of delusions and/or hallucinations.

Learning and memory Cognitive domain that includes the process of acquiring, processing, and consolidating knowledge.

Outcomes Outcomes of patients are the clinical and/or functional effects measured during follow-up, and associated with the course of disease.

Processing speed Ability to process information automatically and fluently. Processing speed requires visual-motor coordination, as well as motor and mental speed.

Psychosis Psychosis is a mental disorder characterized by loss of contact with reality. The affected organ is the brain, causing changes in thinking, perception, feelings, and behavior of the person. The most common diagnoses associated with psychosis are: schizophrenia, schizophreniform disorder, schizoaffective disorder, brief psychotic disorder, schizotypal disorder, bipolar disorder, and major depressive disorder with psychotic symptoms.

Δ^9-Tetrahydrocannabinol (THC) Main psychoactive substance of cannabis.

Verbal fluency Ability to form and express words that includes phonological and semantic fluency tasks.

Working memory Ability to manipulate and maintain information over short-term.

References

Addington, J., & Addington, D. (2007). Patterns, predictors and impact of substance use in early psychosis: a longitudinal study. *Acta Psychiatrica Scandinavica*, *115*, 304–309.

Arendt, M., Rosenberg, R., Fjordback, L., Brandholdt, J., Foldager, L., Sher, L., & Munk-Jørgensen, P. (2007). Testing the self-medication hypothesis of depression and aggression in cannabis-dependent subjects. *Psychological Medicine*, *37*, 935–945.

Arseneault, L., Cannon, M., Witton, J., & Murray, R. M. (2004). Causal association between cannabis and psychosis: examination of the evidence. *British Journal of Psychiatry*, *184*, 110–117.

Ayres, A.M., Scazufca, M., Menezes, P.R., Nakano, E.Y., Regina, A.C., Schaufelberger, M.S., …, Busatto, G.F. (2010). Cognitive functioning in subjects with recent-onset psychosis from a low–middle-income environment: multiple-domain deficits and longitudinal evaluation. *Psychiatry Research*, *179*, 157–164.

Baeza, I., Graell, M., Moreno, D., Castro-Fornieles, J., Parellada, M., González-Pinto, A., …, Arango, C. (2009). Cannabis use in children and adolescents with first episode psychosis: influence on psychopathology and short-term outcome (CAFEPS study). *Schizophrenia Research*, *113*, 129–137.

Barbeito, S., Vega, P., Ruiz de Azúa, S., Saenz, M., Martinez-Cengotitabengoa, M., González-Ortega, I., …, González-Pinto, A. (2013). Cannabis use and involuntary admission may mediate long-term adherence in first-episode psychosis patients: a prospective longitudinal study. *BMC Psychiatry*, *13*, 326.

Bertelsen, M., Jeppesen, P., Petersen, L., Thorup, A., Ohlenschlaeger, J., Le Quach, P., …, Nordentoft, M. (2008). Five-year follow-up of a randomized multicenter trial of intensive early intervention vs standard treatment for patients with a first episode of psychotic illness: the OPUS trial. *Archives of General Psychiatry*, *65*, 762–771.

Birchwood, M., Iqbal, Z., & Upthegrove, R. (2005). Psychological pathways to depression in schizophrenia: studies in acute psychosis, post psychotic depression and auditory hallucinations. *European Archives of Psychiatry and Clinical Neurosciences*, *255*, 202–212.

Bossong, M. G., van Hell, H. H., Jager, G., Kahn, R. S., Ramsey, N. F., & Jansma, J. M. (2013). The endocannabinoid system and emotional processing: a pharmacological fMRI study with Δ9-tetrahydrocannabinol. *European Neuropsychopharmacology*, *23*, 1687–1697.

Campos, A. C., Moreira, F. A., Gomes, F. V., Del Bel, E. A., & Guimarães, F. S. (2012). Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences*, *367*, 3364–3378.

Clausen, L., Hjorthøj, C. R., Thorup, A., Jeppesen, P., Petersen, L., Bertelsen, M., & Nordentoft, M. (2014). Change in cannabis use, clinical symptoms and social functioning among patients with first-episode psychosis: a 5-year follow-up study of patients in the OPUS trial. *Psychological Medicine*, *44*, 117–126.

Copeland, J. (2004). Developments in the treatment of cannabis use disorder. *Current Opinion in Psychiatry*, *17*, 161–167.

Cunha, P.J., Rosa, P.G., Ayres, A de Mello., Duran, F.L., Santos, L.C., Scazufca, M., …, Schaufelberger, M.S. (2013). Cannabis use, cognition and brain structure in first-episode psychosis. *Schizophrenia Research*, *147*, 209–215.

De la Serna, E., Mayoral, M., Baeza, I., Arango, C., Andrés, P., Bombin, I., …, Castro-Fornieles, J. (2010). Cognitive functioning in children and adolescents in their first episode of psychosis: differences between previous cannabis users and nonusers. *Journal of Nervous and Mental Disease*, *198*, 159–162.

Degenhardt, L., Coffey, C., Moran, P., Carlin, J. B., & Patton, G. C. (2007). The predictors and consequences of adolescent amphetamine use: findings from the Victoria Adolescent Health Cohort Study. *Addiction*, *102*, 1076–1084.

Dekker, N., de Hann, L., van den Berg, S., de Gier, M., Becker, H., & Linzen, D. H. (2008). Cessation of cannabis use by patients with recent-onset schizophrenia and related disorders. *Psychopharmacology Bulletin*, *41*, 142–153.

DeRosse, P., Kaplan, A., Burdick, K. E., Lencz, T., & Malhotra, A. K. (2010). Cannabis use disorders in schizophrenia: effects on cognition and symptoms. *Schizophrenia Research*, *120*, 95–100.

D'Souza, D.C., Abi-Saab, W.M., Madonick, S., Forselius-Bielen, K., Doersch, A., Braley, G., …, Krystal, J.H. (2005). Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biological Psychiatry*, *57*, 594–608.

Edwards, J., Elkins, K., Hinton, M., Harrigan, S. M., Donovan, K., Athanasopoulos, O., & McGorry, P. D. (2006). Randomized controlled trial of a cannabis-focused intervention for young people with first-episode psychosis. *Acta Psychiatrica Scandinavica*, *114*, 109–117.

Fergusson, D. M., Horwood, L. J., & Ridder, E. M. (2005). Tests of causal linkages between cannabis use and psychotic symptoms. *Addiction*, *100*, 354–366.

Fergusson, D. M., Poulton, R., Smith, P. F., & Boden, J. M. (2006). Cannabis and psychosis. *British Medical Journal*, *332*, 172–175.

González-Pinto, A., Vega, P., Ibañez, B., Mosquera, F., Barbeito, S., Gutierrez, M., …, Vieta, E. (2008). Impact of cannabis and other drugs on age at onset of psychosis. *Journal of Clinical Psychiatry*, *69*, 1210–1216.

González-Pinto, A., Alberich, S., Barbeito, S., Gutierrez, M., Vega, P., Ibáñez, B., …, Arango, C. (2011). Cannabis and first-episode psychosis: different long-term outcomes depending on continued or discontinued use. *Schizophrenia Bulletin*, *37*, 631–639.

Gupta, P., Mullin, K., Nielssen, O., Harris, A., & Large, M. (2013). Do former substance users with psychosis differ in their symptoms or function from non-substance users? A systematic meta-analysis. *Australian and New Zealand Journal of Psychiatry*, *47*, 524–537.

Hartley, S., Barrowclough, C., & Haddock, G. (2013). Anxiety and depression in psychosis: a systematic review of associations with positive psychotic symptoms. *Acta Psychiatrica Scandinavica*, *128*, 327–346.

Henquet, C., Krabbendam, L., Spauwen, J., Kaplan, C., Lieb, R., Wittchen, H. U., & van Os, J. (2005). Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. *British Medical Journal*, *330*, 11–15.

Hunt, G. E., Siegfried, N., Morley, K., Sitharthan, T., & Cleary, M. (2014). Psychosocial interventions for people with both severe mental illness and substance misuse. *Schizophrenia Bulletin*, *40*, 18–20.

Koskinen, J., Löhönen, J., Koponen, H., Isohanni, M., & Miettunen, J. (2010). Rate of cannabis use disorders in clinical samples of patients

with schizophrenia: a meta-analysis. *Schizophrenia Bulletin*, *36*, 1115–1130.

Kovasznay, B., Fleischer, J., Tanenberg-Karant, M., Jandorf, L., Miller, A. D., & Bromet, E. (1997). Substance use disorders and the early course of illness in schizophrenia and affective psychosis. *Schizophrenia Bulletin*, *23*, 195–201.

Kravariti, E., Morgan, K., Fearon, P., Zanelli, J.W., Lappin, J.M., Dazzan, P., …, Reichenberg, A. (2009). Neuropsychological functioning in first-episode schizophrenia. *The British Journal of Psychiatry*, *195*, 336–345.

Kuepper, R., van Os, J., Lieb, R., Wittchen, H. U., Höfler, M., & Henquet, C. (2011). Continued cannabis use and risk of incidence and persistence of psychotic symptoms: 10 year follow-up cohort study. *British Medical Journal*, *1*, 342, d738.

Lambert, M., Conus, P., Lubman, D.I., Wade, D., Yuen, H., Moritz, S., …, Schimmelmann, B.G. (2005). The impact of substance use disorders on clinical outcome in 643 patients with first-episode psychosis. *Acta Psychiatrica Scandinavica*, *112*, 141–148.

Leeson, V. C., Sharma, P., Harrison, M., Ron, M. A., Barnes, T. R., & Joyce, E. M. (2009). IQ trajectory, cognitive reserve, and clinical outcome following a first-episode of psychosis: a 3-year longitudinal study. *Schizophrenia Bulletin*, *37*, 768–777.

Leeson, V. C., Harrison, I., Ron, M. A., Barnes, T. R. E., & Joyce, E. M. (2012). The effect of cannabis use and cognitive reserve on age at onset and psychosis outcomes in first-episode schizophrenia. *Schizophrenia Bulletin*, *38*, 873–880.

Lev-Ran, S., Roerecke, M., Le Foll, B., George, T. P., Mckenzie, K., & Rehm, J. (2014). The association between cannabis use and depression: a systematic review and meta-analysis of longitudinal studies. *Psychological Medicine*, *44*, 797–810.

Lewis, C.M., Levinson, D.F., Wise, L.H., DeLisi, L.E., Straub, R.E., Hovatta I., …, Helgason, T. (2003). Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. *American Journal of Human Genetics*, *73*, 34–48.

Lewis, S., Tarrier, N., & Drake, R. (2005). Integrating non-drug treatments in early schizophrenia. *British Journal of Psychiatry*, *Suppl. 48*, S65–S71.

Loberg, E., & Hugdahl, K. (2009). Cannabis use and cognition in schizophrenia. *Frontiers in Human Neuroscience*, *3*, 1–8.

Lynskey, M.T., Glowinski, A.L., Todorov, A.A., Bucholz, K.K., Madden, P.A., Nelson, E.C., …, Heath, A.C. (2004). Major depressive disorder, suicidal ideation, and suicide attempt in twins discordant for cannabis dependence and early-onset cannabis use. *Archives of General Psychiatry*, 61, 1026–1032.

Meier, M.H., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R.S.E., …, Moffitt, T.E. (2012). Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proceedings of the National Academy of Sciences of the United States of America*, 109(40), E2657–E2664.

Meijer, J. H., Dekker, N., Koeter, M. W., Quee, P. J., van Beveren, N. J., Meijer, C. J., et al. (2012). Cannabis and cognitive performance in psychosis: a cross-sectional study in patients with non-affective psychotic illness and their unaffected siblings. *Psychological Medicine*, *42*, 705–716.

Miller, R., Ream, G., McCormack, J., Gunduz-Bruce, H., Sevy, S., & Robinson, D. A. (2009). A prospective study of cannabis use as a risk factor for non-adherence and treatment dropout in first-episode schizophrenia. *Schizophrenia Research*, *113*, 138–144.

Minatogawa-Chang, T.M., Schaufelberger, M.S., Ayres, A.M., Duran, F.L., Gutt, E.K., Murray, R.M., …, Busatto, G.F. (2009). Cognitive performance is related to cortical grey matter volumes in early stages of schizophrenia: a population-based study of first-episode psychosis. *Schizophrenia Research*, *113*, 200–209.

Otten, R., & Engels, R. C. (2013). Testing bidirectional effects between cannabis use and depressive symptoms: moderation by the serotonin transporter gene. *Addiction Biology*, *18*, 826–835.

Pencer, A., Addington, J., & Addington, D. (2005). Outcome of a first episode of psychosis in adolescence: a 2-year follow-up. *Psychiatry Research*, *133*, 35–43.

Power, R.A., Verweij, K.J., Zuhair, M., Montgomery, G.W., Henders, A.K., Heath, A.C, …, Martin, N.G. (2014). Genetic predisposition to schizophrenia associated with increased use of cannabis. *Molecular Psychiatry*, *19*, 1201–1204.

Rais, M., Cahn, W., Van Haren, N., Schnack, H., Caspers, E., Hulshoff Pol, H., & Kahn, R. (2008). Excessive brain volume loss over time in cannabis-using first-episode schizophrenia patients. *American Journal of Psychiatry*, *165*, 490–496.

Rosa, P.G., Schaufelberger, M.S., Uchida, R.R., Duran, F.L., Lappin, J.M., Menezes, P.R., …, Busatto, G.F. (2010). Lateral ventricle differences between first-episode schizophrenia and first-episode psychotic bipolar disorder: a population-based morphometric MRI study. *World Journal of Biological Psychiatry*, *11*, 873–887.

Rutten, B., & Mill, J. (2009). Epigenetic mediation of environmental influences in major psychotic disorders. *Schizophrenia Bulletin*, *35*, 1045–1056.

Schaufelberger, M.S., Lappin, J.M., Duran, F.L., Rosa, P.G., Uchida, R.R., Santos, L.C., …, Busatto, G.F. (2011). Lack of progression of brain abnormalities in first-episode psychosis: a longitudinal magnetic resonance imaging study. *Psychological Medicine*, *41*, 1677–1689.

Schofield, D., Tennant, C., Nash, L., Degenhardt, L., Cornish, A., Hobbs, C., & Brennan, G. (2006). Reasons for cannabis use in psychosis. *Australian and New Zealnd Journal of Psychiatry*, *40*, 570–574.

Sönmez, N., Romm, K. L., Andreasssen, O. A., Melle, I., & Røssberg, J. I. (2013). Depressive symptoms in first episode psychosis: a one-year follow-up study. *BMC Psychiatry*, *13*, 106.

Tosato, S., Lasalvia, A., Bonetto, C., Mazzoncini R., Cristofalo, D., De Santi, K., …, PICOS-VENETO Group. (2013). The impact of cannabis use on age of onset and clinical characteristics in first-episode psychotic patients. Data from the Psychosis Incident Cohort Outcome Study (PICOS). *Journal of Psychiatric Research*, *47*, 438–444.

Trzesniak, C., Schaufelberger, M.S., Duran, F.L., Santos, L.C., Rosa, P.G., McGuire, P.K., …, Busatto, G.F. (2012). Longitudinal follow-up of cavum septum pellucidum and adhesio interthalamica alterations in first-episode psychosis: a population-based MRI study. *Psychological Medicine*, *42*, 2523–2534.

van Gastel, W. A., Vreeker, A., Schubart, C. D., MacCabe, J. H., Kahn, R. S., & Boks, M. P. M. (2014). Change in cannabis use in the general population: a longitudinal study on the impact on psychotic experiences. *Schizophrenia Research*, *157*, 266–270.

van Os, J., Kenis, G., & Rutten, B. P. F. (2010). The environment and schizophrenia. *Nature*, *11*, 203–212, 468.

Veen, N. D., Selten, J. P., van der Tweel, I., Feller, W. G., Hoek, H. W., & Kahn, R. S. (2004). Cannabis use and age at onset of schizophrenia. *American Journal of Psychiatry*, *161*, 501–506.

Vega, P., Gonzaléz-Pinto, A., Ruiz de Azúa, S., Barbeito, S., & Rico, R. (2010). Evaluación de la efectividad de la intervención psicológica en los primeros episodios psicóticos mediante tratamiento multidisciplinar. Plan de Calidad para el Sistema Nacional de Salud del Ministerio de Sanidad y Politica Social. Agencia de Evaluación de Tecnologías Sanitarias del País Vasco; Informes de Evaluación de Tecnologías Sanitarias: OSTEBA.

Wade, D., Harrigan, S., Harris, M. G., Edwards, J., & McGorry, P. D. (2006). Pattern and correlates of inpatient admission during the initial acute phase of first-episode psychosis. *Australian and New Zeland Journal of Psychiatry*, *40*, 429–436.

Wade, D., Harrigan, S., McGorry, P. D., Burgess, P. M., & Whelan, G. (2007). Impact of severity of substance use disorder on symptomatic and functional outcome in young individuals with first-episode psychosis. *Journal of Clinical Psychiatry*, *68*, 767–774.

Winkel, R., van Beveren, N. J. M., & Simons, C. Genetic Risk and Outcome of Psychosis (GROUP) Investigators. (2011). AKT1 Moderation of Cannabis-Induced Cognitive Alterations in Psychotic Disorder. *Neuropsychopharmacology*, *36*, 2529–2537.

Yücel, M., Bora, E., Lubman, D.I., Solowij, N., Brewer, W.J., Cotton, S.M., …, Pantelis, C. (2012). The impact of cannabis use on cognitive functioning in patients with schizophrenia: a meta-analysis of existing findings and new data in a first-episode sample. *Schizophrenia Bulletin*, *38*, 316–330.

Zammit, S., Moore, T. H., Lingford-Hughes, A., Barnes, T. R., Jones, P. B., Burke, M., & Lewis, G. (2008). Effects of cannabis use on outcomes of psychotic disorders: systematic review. *British Journal of Psychiatry*, *193*, 357–363.

CHAPTER

28

Cannabis, Associative Memory, fMRI, and the Implicit Association Test

S.L. Ames, A.W. Stacy

School of Community and Global Health, Claremont Graduate University, Claremont, CA, United States

SUMMARY POINTS

- Imaging neurobiological processes of cannabis users with paradigms that tap into associative memory advances the understanding of habit-based processes.
- Associative memory principles provide a neurobiologically plausible explanation for why cannabis use can become self-perpetuating.
- Implicit association test (IAT) is one method used to examine associative memory structures.
- Neural activity generated by IAT compatible and incompatible trials allow for the detection of potential associative/habit-based learning, since they reflect neural response to *more* or *less* effortful processing of concepts.
- Marijuana users showed greater neural activity in striatal brain regions during compatible trials of a marijuana-IAT.
- Nonusers showed greater neural activity in regions requiring more effortful processing during incompatible trials.
- Marijuana IAT imaging findings suggest that different networks mediate (1) implicit associations underlying habit, namely circuitry dependent on the striatum, and (2) effortful/control processes that are more dependent on prefrontal and frontal cortical regions.

KEY FACTS OF A DUAL PROCESS DISTINCTION IN BRAIN FUNCTION RELEVANT TO HABITUAL BEHAVIORS

- A complex interaction between cortical and subcortical neural systems is thought to underlie the growth of habitual behaviors.
- A key subcortical neural system implicated in implicit associative/habit-type processes is the amygdala-striatal system.
- The striatum is further divided into regions; the dorsal region includes the caudate and putamen, and ventral region consists of the nucleus accumbens.
- The amygdala-striatal system is critical for the incentive motivational effects of a variety of nonnatural rewards (Robbins et al., 1989).
- The insula is a subcortical region emerging as a substrate involved in this habit-based circuitry.
- Key cortical circuits supporting executive functions (eg, inhibitory control, attentional processes, working memory capacity, and reflective/deliberative processes) involve several brain structures of the prefrontal and frontal cortex.
- These include (but are not limited to) the dorsolateral prefrontal cortex, lateral regions of the orbitofrontal cortex, and adjacent ventrolateral region (inferior frontal gyrus; eg, Aron, Robbins, & Poldrack, 2004), and anterior cingulate cortex (Botvinick, 2007).

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00032-6
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

ACC	Anterior cingulate cortex
A-O	Action outcome
CB1	Cannabinoid 1
CB1-R	Cannabinoid 1 receptors
DLPFC	Dorsolateral prefrontal cortex
fMRI	Functional magnetic resonance imaging
IAT	Implicit association test
RIFG	Right inferior frontal gyrus
S-R	Stimulus-response

INTRODUCTION

Cannabis is one of the most prevalent drugs used around the world. The imaging of neurobiological processes of cannabis users with task-based paradigms that tap into associative memory processes can help us understand habit-based processes that perpetuate its use. Drug use habits, as well as some other appetitive behaviors (ie, rewarding behaviors that are often dopamine dependent), may be conceptualized as a potent form of reinforced associative learning (Yin & Knowlton, 2006a). Several researchers have proposed that associative learning processes and memories that involve reward or positive outcomes mediate, and can explain, appetitive behaviors (DiChiara, 2002; Schultz, 2004). Animal studies have provided evidence that exposure to cues previously associated with a reward can trigger a neurobiological anticipatory response that occurs quite rapidly (within a few milliseconds) at the synaptic level (Phillips, Stuber, Heien, Wightman, & Carelli, 2003). Through repetitive experiences, neutral cues strongly associated with rewarding behaviors can come to represent the behavior, and elicit an "automatic" pattern of activation in memory, which, in turn, promotes the behavior (Stacy, Ames, & Knowlton, 2004; White, 1996). These automatic associative effects have implications for cannabis use and other habits highly sensitive to prior learning experiences and predictive cues.

Although considerable research has focused on neuroadaptive effects of drug use (Bechara, 2005; Everitt, Dickinson, & Robbins, 2001; Robinson & Berridge, 2003; Volkow, Fowler, Wang, & Goldstein, 2002), nuances of neural processes and functional changes underlying drug and other habit formation continue to be revealed. Use of new and emerging imaging methodologies has contributed to increased understanding of the brain mechanisms through which continued drug use influences cognitions, memory, emotional states, and behavior itself. These imaging methodologies in concert with refined cognitive paradigms allow researchers to observe neural populations associated with drug use-related phenomena. This chapter addresses one, such paradigm—IAT—in the study of cannabis use, and elaborates on associative memory processes believed to account for IAT-related neural effects. Associative memory principles provide a neurobiologically plausible explanation for why cannabis and other appetitive behaviors can become self-perpetuating.

OVERVIEW OF ASSOCIATIVE MEMORY

Associations in memory develop through experience, with associative learning processes likely enhanced through the reinforcing effects of a behavior. Associative memories may involve any linked information, and can operate nonreflectively and spontaneously when associations are sufficiently strong. Strong, spontaneously-acting associations can be revealed when an individual applies previously acquired knowledge without recalling a previous event, or without being prompted for deliberation. Paradigms used to study the activation of associated concepts in memory have been around for decades, and have contributed to many theories from basic research that are diverse in structural and computational architectures but, nonetheless, converge on the fundamental importance of associations or connections in memory (for examples of applicable theories, see Stacy & Wiers, 2006).

Consistent with theories of memory that propose parallel processes, automatic associative or relational memory effects operate well beyond the traditional associations studied in conditioning literatures, extending to perceptual, affective, and more general multimodal/conceptual distributed forms of memory that can jointly affect the prevailing pattern of activation in memory. Central associations may involve key habit associations [stimulus–response (S-R) associations], action outcome associations (A–O associations), as well as conceptual, perceptual, or affective associations in which an event or situation triggers a chain of activations across distributed memory systems, triangulating on a strongly associated behavior, rather than less-strongly associated behavioral options. A biasing effect of automatic memory processes on behavior is consistent with a history of work in social psychology revealing, such effects (for summary, see Payne & Gawronski, 2010), and with our application of these processes to appetitive health behaviors (Stacy, Ames, Wiers, & Krank, 2010). In research at the neural level, some researchers have pointed out that, at some point, A–O associations may not be relevant because habit has become so strong that S-R associations prevail (Yin & Knowlton, 2006a). Thus, habits may prevail paradoxically, despite negative outcomes. Further, in the present analysis, the "S" in S-R can be usefully defined as not only a single instigating stimulus or setting linked to behavior, but also as a constellation of cues that together can automatically promote a

distributed pattern of activation in memory that reflexively triggers, or at least biases a behavioral response, more than alternatives.

NEUROSCIENCE OF DUAL SYSTEMS AND ASSOCIATIVE MEMORY PROCESSES IN HABIT FORMATION

Research on impaired memory function and imaging of brain activation has helped define the influence of associative processes on behavior, and provided evidence for the neural basis of distinct memory/cognitive systems (Gabrieli, 1998; Knowlton, Mangels, & Squire, 1996; Rolls, 2000; White, 1996). A distinction between spontaneous associative memory and controlled process systems has been reinforced through several independent lines of research in neuroscience (Bechara, Noel, & Crone, 2006; Chein & Schneider, 2005; Levy, Stark, & Squire, 2004; Yin & Knowlton, 2006b).

With respect to drug use, a dual process distinction proposed by several researchers describes appetitive behaviors as a dynamic interplay between (1) a relatively implicit or automatic associative system (or set of systems), and (2) an executive control/inhibitory system (Bechara, 2005; Stacy et al., 2004; Strack & Deutsch, 2004; Wiers et al., 2007). As habit-type appetitive behaviors develop, an imbalance can occur between executive control and automatic associative systems. The general idea is that associative processes that are reinforced and likely enhanced by appetitive behaviors (such as drug use) result in neurobiological consequences that include the strengthening of motivationally relevant associations that affect subsequent behavior. As associative processes become stronger, the involvement of neural regions implicated in control processes is weakened in effects on behavior. That is, behavior can come to be increasingly under cue control, and less under voluntary control (Bechara et al., 2006; Stacy et al., 2004; White, 1996; Wiers et al., 2007). Cues can then trigger an essentially "automatic" pattern of activation in memory that can be described in a variety of neural network models (Botvinick & Plaut, 2004; Bowers, 2009). Across neural systems, this evolution may be represented by the "transition from prefrontal cortical control systems to subcortical striatal control and within the striatum from ventral to dorsal domains of the striatum" (p. 1741; Vollstädt-Klein et al., 2010) as habitual behaviors develop (Everitt & Robbins, 2005; Robbins, Ersche, & Everitt, 2008; Schneider & Chein, 2003). In the compatible decision theory of Kahneman (2003), the associative system (labeled System 1 in his taxonomy) governs the majority of human decision-making, and is more or less the "default" system that guides behavior, unless modified by control processes (System 2).

LINKING ASSOCIATIVE EFFECTS TO CANNABIS-RELATED NEURAL SUBSTRATES

Although drugs have specific receptor sites in the brain, most act directly or indirectly on brain reward (ie, dopamine) systems. Cannabis indirectly activates dopamine systems by stimulating neurons that modulate the release of dopamine through direct effects on cannabinoid receptors (Baler & Volkow, 2006). Animal models have shown endocannabinoid signaling through cannabinoid 1 receptors (CB1-R) to play a key role in reward and habit development, with the striatum implicated as a critical neural substrate for CB1 signaling (Hilario, Clouse, Yin, & Costa, 2007). Dopaminergic activity in the striatum reinforces the repetition of certain behaviors, and the encoding and processing of proximal stimuli associated with rewarding events (eg, Everitt & Robbins, 2005; Cardinal & Everitt, 2004). As learned associations in memory are strengthened, they may come to represent and subsequently cue behavior spontaneously, with little involvement of deliberative or control processes. Yin and Knowlton (2006a) reviewed multiple lines of evidence mapping cue-habit associations to the dorsolateral striatum (putamen), action–outcome associations to the dorsomedial striatum (caudate), and cue and affective outcome associations with the basolateral amygdala. These types of associations and implicated brain regions involve complex neural circuitry with clear implications for cannabis use.

ASSOCIATIVE MEMORY ASSESSMENTS, THE IMPLICIT ASSOCIATION TEST, AND CANNABIS USE

Consistent findings in behavioral research document the value of associative process effects in the prediction of drug use across a range of populations (for reviews, Rooke, Hine, & Thorsteinsson, 2008; Wiers & Stacy, 2006). Prospective studies have confirmed these predictive effects with the use of varied indirect tests of association (Grenard, Dent, & Stacy, 2013; Kelly, Haynes, & Marlatt, 2008; Kelly, Masterman, & Marlatt, 2005; Thush et al., 2007). Associative memory assessments of drug use tap into and activate preexisting associations in memory (Wiers & Stacy, 2006), with some aspects of memory expressed by facilitated performance (reaction time) on indirect tests of association. In general, these tests of association do not require conscious or intentional recognition of past experiences, and are relatively free from demand characteristics (Roediger, 1990).

The IAT (Greenwald, McGhee, & Schwartz, 1998) is one of the most common methods used to examine attitude and behavior-related associative memory structures.

Hundreds of studies have been published using the IAT to evaluate a variety of associations, for example, toward gender, age, race, alcohol, and other drugs, among others (for review, Greenwald, Poehlman, Uhlmann, & Banaji, 2009). With respect to cannabis, in a behavioral study comparing marijuana users to nonusers, Field, Mogg, and Bradley (2004) found significantly more negative implicit associations toward marijuana-related words in the group of nonusers. In another behavioral study, Ames et al. (2007) used an adapted unipolar marijuana-IAT in a population of at-risk youth and found positive reinforcement associations (ie, excitement) to be predictive of marijuana use, controlling for gender, ethnicity, explicit cognitions, sensation seeking, and working memory capacity.

The IAT is a categorization task that provides a relative measure of associative strength between concepts. On the task, participants categorize stimuli rapidly (making as few mistakes as possible) into categories, by pressing a left or right response key. Reaction time to the task is faster when the categories are highly associated in memory, and when associated categories require the same key press. Mapping of key to category is switched in different within-subject conditions to assess relative association strength. The basic assumption is that past experiences can be represented by the ease of information processing of associated concepts, and this is assessed by reaction time, or rate of processing of information (see Greenwald et al., 1998). The behavioral IAT effect (D600) can be calculated as the mean reaction time in incompatible task blocks (concepts unlikely to be associated), minus the mean reaction time in compatible task blocks (concepts likely to be associated), divided by the pooled standard deviation for the blocks (Greenwald, Nosek, & Banaji, 2003).

IMAGING THE IMPLICIT ASSOCIATION TEST

Human functional imaging techniques provide a way to correlate cognitive processes engaged during a task with patterns of activation in neural populations. For example, the processing of drug-relevant associations on a task can be correlated with neural activity in specific brain regions. Although functional magnetic resonance imaging (fMRI) provides insight into neural activation of cognitive processes associated with tasks, findings are correlational, and other variables (ie, confounders) may affect findings attributed to information processing. Nevertheless, measuring task-related neural responses can, at times, be a more sensitive measure than some behavioral measures, providing key information about relevant neural processes.

Several studies have observed the neural correlates of IAT performance in the scanner. It is important to note that, although the IAT is a test of the relative strength of associations of specific concepts, when evaluating brain function, the task involves dual cognitive processes. That is, IAT performance recruits both effortful/control and automatic associative processes. The neural activity comparisons generated by the task conditions (ie, compatible and incompatible trials) allow for the detection of potential associative/habit-based learning, since they reflect neural response to *more* or *less effortful/controlled processing* of concepts.

The neural correlates of nonsubstance-related IATs have been observed in several imaging studies (eg, racial preference, Beer et al., 2008; flowers/insect pleasantness, Chee, Sriram, Soon, & Lee, 2000; politics, Knutson, Wood, Spampinato, & Grafman, 2006; gender and race-related, Knutson, Mah, Manly, & Grafman, 2007; morality, Luo et al., 2006). The task elicits neural populations involved in automatic associative processing during the task's compatible trials and effortful/controlled processing on incompatible trials. In general, performance on incompatible trials has been found to elicit greater frontal activity, relative to the compatible trials [eg, increased activity in the ventrolateral, dorsolateral prefrontal cortex (DLPFC), and anterior cingulate (ACC): Chee et al., 2000; Luo et al., 2006; left inferior frontal gyrus: Knutson et al., 2006; middle frontal gyrus: Knutson et al., 2007]. In a study by Beer et al. (2008) on a racial preference IAT, activity in the caudate, insular cortex, and lateral orbital frontal cortex were observed during compatible association trials. Both the caudate and the insula have been implicated in habit-based circuitry (Noël, Brevers, & Bechara, 2013).

In a substance-related imaging study, Ames et al. (2014) assessed activity in neural substrates of an alcohol-IAT focused on positive outcomes of alcohol use, in heavy and light drinkers. Behaviorally, heavy drinkers showed significantly stronger positive implicit associations toward alcohol use than light drinkers. In terms of neural response, heavy drinkers showed greater activity during compatible trials (relative to incompatible trials) in habit-based regions, namely the left putamen, insula, and amygdala. Alternatively, no significant difference in activity between conditions was observed in light drinkers. During both compatible and incompatible trials, light drinkers showed more activity in the left orbital frontal cortex (relative to heavy drinkers), and both groups showed significantly greater activity in the DLPFC during incompatible trials (relative to compatible trials). Findings from this imaging study suggest that different networks mediate associative and effortful/control processes, and activation of these networks vary by level of experience with alcohol.

IMAGING THE MARIJUANA-IAT

In another substance-related IAT imaging study, Ames and colleagues (2013) evaluated differences in neural correlates of 13 marijuana-users and 15 nonusing

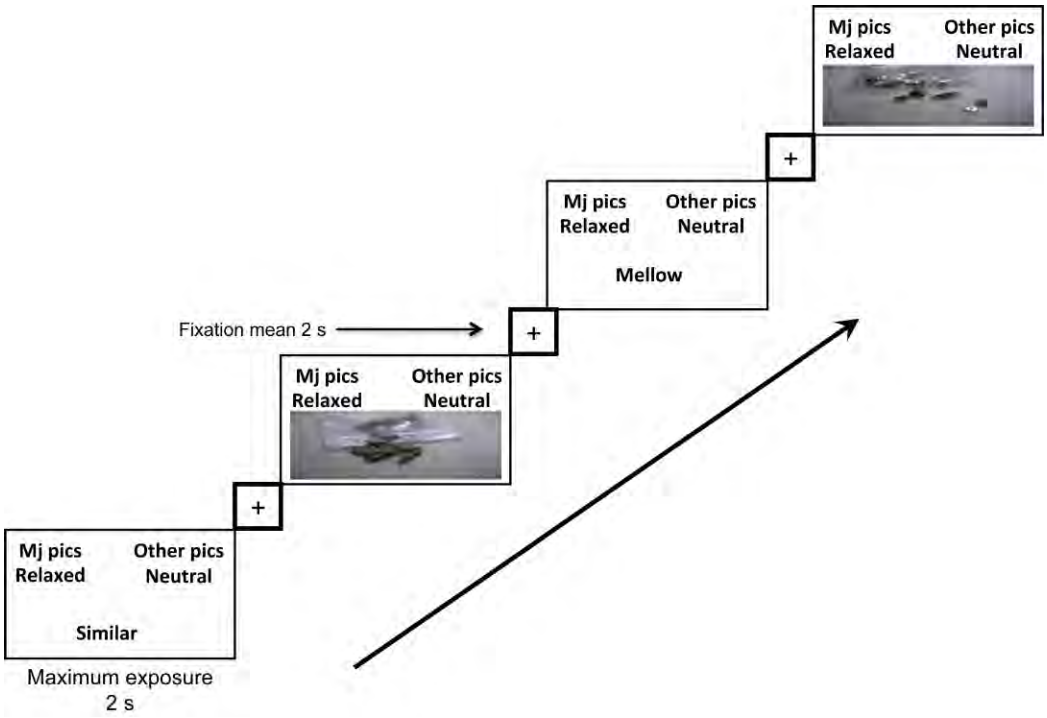


FIGURE 28.1 Temporal layout of 40 compatible trials or implicit associations toward marijuana use. Individuals react faster when categorizing *strongly associated* concepts that share a response key. This should be fairly easy to do for someone with past experience of marijuana use. Temporal jitter was used in the presentation of the fixation with onset timing ranging from 1 to 4.5 s, with a mean exposure of 2 s, followed by stimuli presentation. Maximum exposure of test stimuli was for 2 s. After a participant pressed a response key, the screen would go blank for the remainder of the 2 s. Total trial time ranged from 3 to 6.5 s. *Source: Reprinted from Ames et al. (2013), with permission from Elsevier; see http://dx.doi.org/10.1016/j.bbr.2013.09.013.*

controls, while performing a marijuana-IAT in the scanner. The majority of the nonusing controls had never used marijuana, with only two having used only a few times in their lifetimes. The marijuana users reported using marijuana more than 500 times in the past 3 years, and current daily use.

There were no significant differences in mean age between groups [marijuana users, mean age 21.15 (1.9); controls mean age 20.27 (2.3)]. However, 18% of the marijuana users were female, and 67% of the nonusers were female ($p < .01$). All participants were right-handed, native English speakers, and void of a history of psychiatric or neurological disorders. Further, participants were matched on ethnicity, and past 3-year alcohol, tobacco, and other drug use, with minimal lifetime use of drugs other than marijuana. There were no significant differences between groups in working memory capacity (a proxy measure for general fluid intelligence; see Kane & Engle, 2002).

Imaging Paradigm

The marijuana-IAT consisted of five different words and five different images that were randomly presented (Ames et al., 2007). The target categories were "Marijuana Pictures" and "Other Pictures." Images were closely matched in size and shape, and consisted of a joint, rolling papers, pipe, dried marijuana buds, and dried marijuana in a plastic baggie. The matched "Other Pictures" were images of a ballpoint pen, small memo pad, small flashlight, loose thumbtacks, and thumbtacks in a clear plastic box. The affective categories paired with the images consisted of a "Relaxed" category for positive reinforcement, and a "Neutral" category. Relaxed category words were chill, calm, cool, mellow, and comfortable. The matched Neutral category words were count, square, common, similar, and historical. The task was a standard IAT (Nosek, Greenwald, & Banaji, 2005), and included seven blocks of trials inclusive of both practice and test trials. The order of IAT incompatible and compatible blocks of trials was counterbalanced across participants. The headers for compatible association trials were "marijuana pictures + relaxed words," and "other pictures + neutral words." The headers for incompatible trials were "marijuana pictures + neutral words," and "other pictures + relaxed words" (Figs. 28.1, 28.2).

In the scanner, participants were instructed: "*Press the #1 key for items that fit into a category on the top left. Press the #4 key for items that fit into a category on the top right. The categories change from time to time. You will not receive any instructions during the task. Go as fast as you*

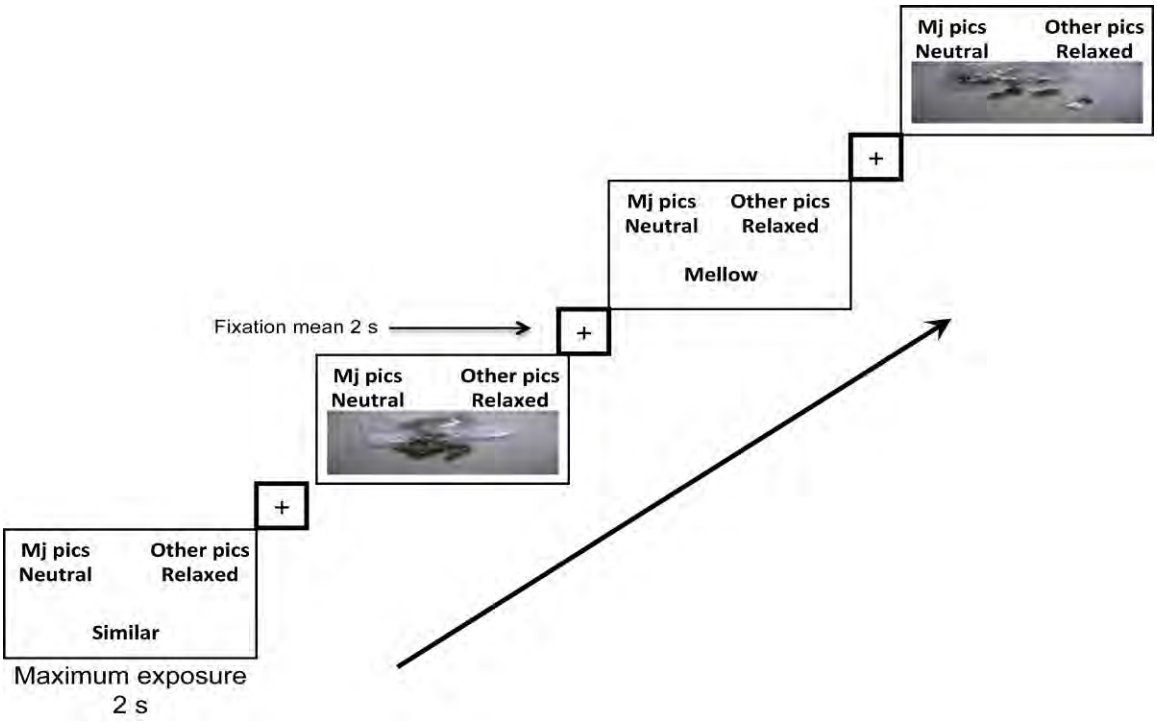


FIGURE 28.2 **Temporal layout of 40 incompatible trials.** In this figure, the attribute categories are switched. Individuals react slower when categorizing concepts *not typically associated* that share a response key. These trials require more effortful processing across all subjects. *Source: Reprinted from Ames et al. (2013), with permission from Elsevier; see http://dx.doi.org/10.1016/j.bbr.2013.09.013.*

can without making mistakes. Please wait for the task to start automatically."

Based on previous imaging studies, it was expected that the marijuana users (compared to nonusing controls) would show greater neural activity in regions critical for associative memory processes underlying habit, during the compatible trials. Individuals with positive past experiences with marijuana should be able to perform these trials with little effort, and faster than individuals with little to no experience with the drug. The neural regions expected to be more active among the marijuana users on the compatible trials were the dorsal striatum (caudate/putamen), and ventral striatum (nucleus accumbens).

The incompatible trials were expected to elicit more activity among all participants in regions requiring more controlled processing of information, when compared to the compatible trials. The idea was that, during incompatible trials, participants are categorizing concepts that require more deliberative/effortful processing, since the concepts are not generally associated with each other. Further, it was predicted that the degree of increased activity in these regions would be less in the marijuana-using group, perhaps reflecting poorer controlled processing due to chronic effects from use. The regions implicated were lateral regions of the orbitofrontal cortex, dorsolateral and adjacent ventrolateral, and inferior frontal gyrus, all regions associated with control processing (Aron, Robbins, & Poldrack, 2004).

For imaging, the study used a 3 Tesla magnetic resonance scanner, fitted with head coil arrays for parallel imaging to minimize signal loss and image distortion. An MRI-compatible response box with a fiber-optics response pad and four buttons that accepts a trigger from the scanner was used to collect participants' responses. Participants lay supine on the scanner bed while visual stimuli were back-projected onto a screen and viewed through a mirror attached onto the head coil. Functional images were acquired using a z-shim gradient, single-shot T2*-weighted echo-planar imaging sequence with prospective acquisition correction. The scanning parameters were as follows: TR = 2000 ms (whole brain); TE = 25 ms; flip angle = 90 degree; 64 × 64 matrix size with resolution 3 × 3 mm^2; bandwidth: 1906 Hz/pixel. The image data underwent the standard preprocessing to improve signal detection and sensitivity of statistical analyses. The fMRI whole brain analyses used a standard two-level general linear modeling approach (Friston et al., 1995). F tests were used to detect the main effects of condition and group and their interaction. The significance threshold was set at an exploratory uncorrected $p < .005$, with clusters larger than 30 voxels.

Behaviorally, no significant difference between the marijuana users and nonusing comparison group was found on the D600 measure of the IAT. However, the mean response latencies on the compatible trials were less than the mean for the incompatible trials across all

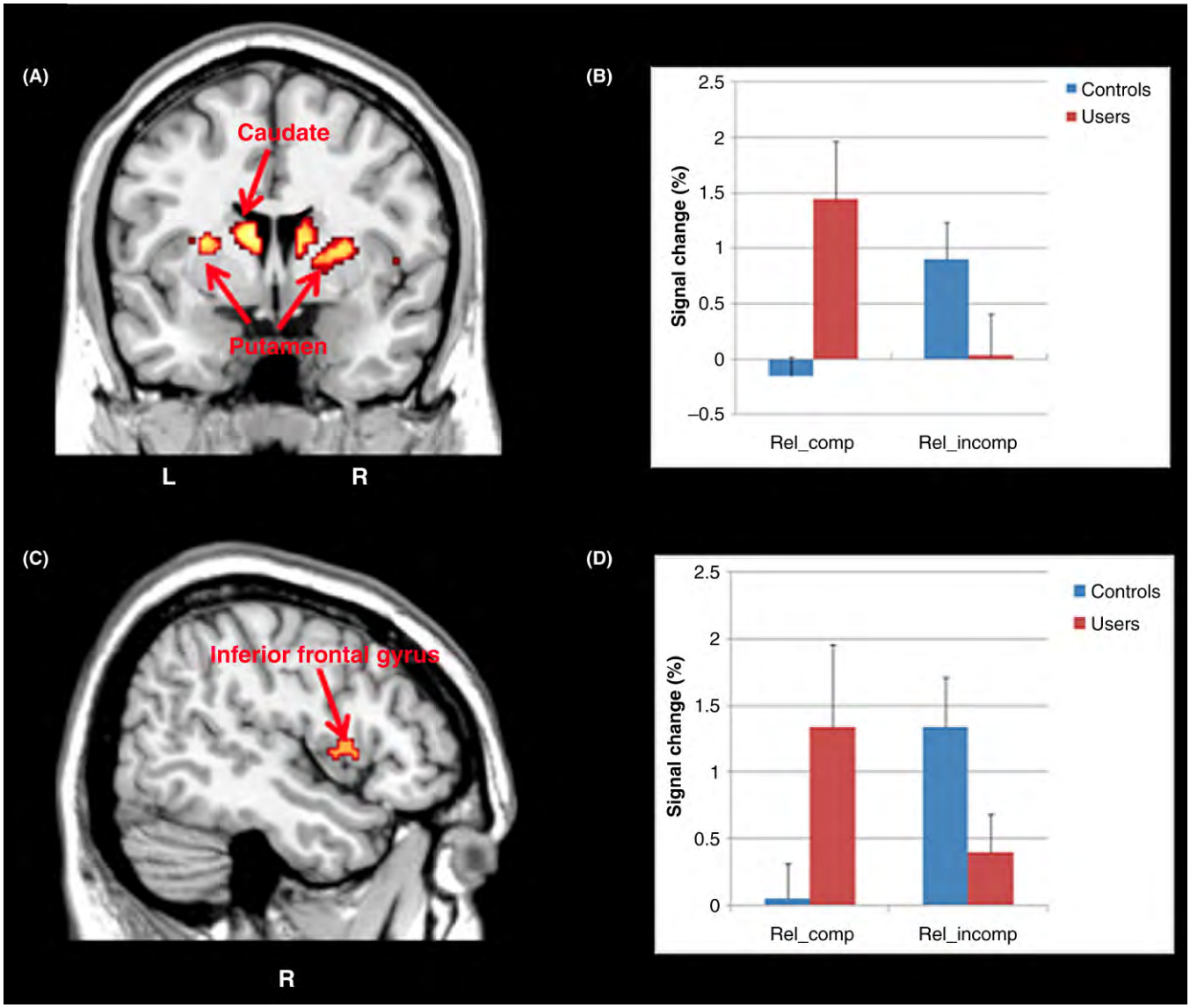


FIGURE 28.3 **Interaction effect during compatible and incompatible trials by user and control groups in subcortical and cortical regions.** (A) Coronal view of activity in the caudate and putamen; (B) signal change (%) for both caudate and putamen. Error bars denote within-subject error; (C) sagittal view of the activity in the right inferior frontal gyrus; (D) signal change (%) for the right inferior frontal gyrus. Error bars denote within-subject error. Significance set at $p < .005$, uncorrected, voxels > 30. *Source: Reprinted from Ames et al. (2013), with permission from Elsevier; see http://dx.doi.org/10.1016/j.bbr.2013.09.013.*

participants, and fewer errors were made during compatible trials than incompatible trials. It is possible that no group effect was found because the stimuli presented were too culturally common and a limited set to behaviorally differentiate users from nonusers. The categories and stimuli used are important determinants of behavioral IAT effects (Nosek, Greenwald, & Banaji, 2007).

Although the behavioral measure did not detect group differences, the data clearly showed differences in neural response, confirming a distinctive advantage of using fMRI in detecting differences in functioning that otherwise might not be detected with conventional behavioral assessments. Imaging data showed a clear interaction between the two groups and task conditions. Findings revealed a difference between marijuana users and nonusers in regions of the brain implicated in habit-based (associative) learning (Robbins et al., 2008). Increased bilateral activity in the caudate and putamen (striatal regions) were observed in the marijuana users during the compatible trials. These are the trials in which the strength of positive implicit associations toward marijuana, among the users, should be detected as a result of repetitive use experiences. This pattern of activity observed in the marijuana users is consistent with animal research regarding CB1 activity in the dorsal striatum, and the role of endocannabinoids in habit formation and habitual responding (Hilario et al., 2007) (Fig. 28.3).

TABLE 28.1 Significant Regions of Activity Within Groups During Compatible and Incompatible Trials

	Hemisphere	Region	*x*	*y*	*z*	Voxels in cluster	T statistic
Compatible association trials							
Marijuana users	Left	Inferior frontal gyrus	−46	28	24	2223	6.16
	Right	Posterior cingulate cortex	6	−40	6	85	4.85
	Left	Inferior frontal gyrus operculum	−54	12	0	85	4.24
	Right	**Putamen**	**30**	**−16**	**0**	**63**	**4.18**
	Right	**Caudate**	**20**	**24**	**8**	**223**	**3.99**
	Left	**Putamen**	**−24**	**6**	**12**	**100**	**3.74**
	Left	**Caudate**	**−18**	**−4**	**20**	**49**	**3.73**
	Right	Hippocampus	22	−28	−6	30	3.65
Incompatible association trials							
Nonusing controls	Left	Frontal inferior gyrus operculum	−58	12	2	165	5.70
	Right	Superior frontal gyrus	30	24	50	2179	5.70
	Left	Hippocampus	−28	−30	−4	95	4.85
	Left	Middle frontal gyrus	−42	54	10	750	4.59
	Left	Inferior frontal gyrus	−48	30	24	261	4.30
	Right	Caudate	18	10	22	252	4.02

Note: All peak activity based on whole brain analyses. Results are for single group/condition results relative to fixation. Significance $p < .005$, uncorrected, voxels > 30. Bolded regions represent predicted regions.
Reprinted from Ames et al. (2013), with permission from Elsevier; see http://dx.doi.org/10.1016/j.bbr.2013.09.013.

Further, among the marijuana users (relative to nonusers), some activity in the right inferior frontal gyrus (RIFG) was observed during compatible trials. This finding may have been the result of a need for inhibitory control over intensified striatal activity. That is, the marijuana users may have been working harder to inhibit habit-based tendencies, or suppressing well-learned associations requiring the engagement of frontal regions. The RIFG has been implicated in a more general role of executive processing, including detection of salient cues during task performance (Hampshire, Chamberlain, Monti, Duncan, & Owen, 2010), as well as inhibitory control (Aron et al., 2004).

Interestingly, little activity was observed during task performance in the ventral striatum (accumbens), a region traditionally implicated in motivation and reward. The lack of significant activity in this region is consistent with theories arguing that what may originate as motivational (supported by the ventral striatum) in essence transfers control to the dorsal striatum, the region supporting more habitual responding, following repetition of rewarding behavior, and without need for involvement of the ventral striatum (Robbins et al., 2008; Yin & Knowlton, 2006b). The marijuana users were quite habitual users, suggesting that transfer of control was plausible. It is also conceivable that merely observing marijuana stimuli was simply not sufficient to activate reward processing.

Finally, on incompatible trials, the nonusing group showed more activity in some frontal regions (although not the predicted regions) relative to fixation. Again, these trials require more effortful processing of information since the concepts to be categorized are not typically associated. Alternatively, marijuana users showed no significant activity on these trials in the study (see Tables 28.1 and 28.2). This finding partially supported the prediction that these trials would elicit more activity in frontal regions among all participants, but less activity in marijuana users perhaps as a result of chronic effects from current daily use. This finding is similar to other research that has shown reduced frontal activity in substance-using populations (Koob & Volkow, 2010).

In sum, the marijuana users showed greater activity in regions of the brain implicated in habit-based associative learning during compatible trials relative to fixation and relative to nonusers, while nonusers showed greater activity in regions of the brain requiring more effortful/controlled processing during incompatible trials relative to fixation. These findings are consistent with habit-related changes that occur in neural systems, and the transition or switch from predominantly effortful or control-related processing to more automatic (or implicit)

TABLE 28.2 Significant Regions of Activity Associated With the Group by Condition Interaction

Hemisphere	Region	*x*	*y*	*z*	Voxels in cluster	F statistic
Left	Caudate	−8	12	12	322	15.34
Right	Caudate	10	8	16	283	14.79
Right	Putamen	30	−14	0	46	14.07
Left	Putamen	−28	0	12	64	12.20
Right	Inferior frontal cortex	44	16	4	48	10.74

Note: All Fs, $p < .005$, uncorrected, voxels > 30.
Reprinted from Ames et al. (2013), with permission from Elsevier; see http://dx.doi.org/10.1016/j.bbr.2013.09.013.

processing with experience (Chein & Schneider, 2005; Schneider & Chein, 2003). Findings from this work provide support for a framework of appetitive behaviors proposing that (1) implicit associations underlying habit are mediated through neural circuitry dependent on the striatum, and (2) deliberative/controlled behaviors are mediated through circuitry more dependent on the prefrontal and frontal cortex.

CONCLUDING REMARKS

The imaging of neurobiological processes of cannabis users with task-based paradigms that engage associations in memory, such as the IAT, contribute insight into the neural substrates of marijuana-related associative memory processes (habit-based learning). Eliciting activation of associative drug-relevant memories during performance on substance-related IATs advances knowledge about neural mediators of a repeated or habitual behavior. Finally, neuroimaging research extends findings derived through behavioral research, demonstrating convergent, consistent predictive effects of associative memory processes in drug use. Such processes have implications for interventions, as outlined in previous work by the authors, and others working in this intriguing area.

MINI-DICTIONARY

Appetitive behaviors Behaviors that are rewarding and tend to be, in part, dopamine dependent.
Associative memory Memory for any linked information or interrelated concepts; memory revealed on indirect tests of association that tap into preexisting associations generally without awareness.
Dopamine Primary neurotransmitter involved in brain reward pathways; typically associated with arousal, reward, motivation and pleasure.
Functional magnetic resonance imaging (fMRI) Indirect measure of neural activity utilizing different magnetic properties of oxygenated and deoxygenated hemoglobin. Blood oxygenation level-dependent (BOLD) fMRI detects the hemodynamic response associated with neural activation during cognitive tasks and is used to observe relative changes in regional blood flow.
Implicit association test (IAT) An indirect test of association; a categorization task that provides a relative measure of associative strength between concepts assessed through rate of processing.
TE Echo time, or the time between consecutive 90 degree radio frequency pulse.
TR Repetition time or time between pulse sequences.

References

Ames, S. L., Grenard, J. L., Thush, C., Sussman, S., Wiers, R. W., & Stacy, A. W. (2007). Comparison of indirect assessments of association as predictors of marijuana use among at-risk adolescents. *Experimental and Clinical Psychopharmacology*, *15*, 204–218.

Ames, S. L., Grenard, J. L., Stacy, A. W., Xiao, L., He, Q., Wong, S., Xue, G., Wiers, R. W., & Bechara, A. (2013). Functional imaging of implicit marijuana associations during performance on an Implicit Association Test (IAT). *Behavioural Brain Research*, *256*, 494–502.

Ames, S. L., Grenard, J. L., He, Q., Stacy, A. W., Wong, S., Xiao, L., Xie, G., & Bechara, A. (2014). Functional imaging of an alcohol-Implicit Association Test (IAT). *Addiction Biology*, *19*, 467–481.

Aron, A. R., Robbins, T. W., & Poldrack, R. A. (2004). Inhibition and the right inferior frontal cortex. *Trends in Cognitive Sciences*, *8*, 70–177.

Baler, R. D., & Volkow, N. D. (2006). Drug addiction: the neurobiology of disrupted self-control. *Trends in Molecular Medicine*, *12*(12), 559–566.

Bechara, A. (2005). Decision making, impulse control and loss of willpower to resist drugs: a neurocognitive perspective. *Nature Neuroscience*, *8*(11), 1458–1463.

Bechara, A., Noel, X., & Crone, E. A. (2006). Loss of willpower: Abnormal neural mechanisms of impulse control and decision-making in addiction. In R. W. Wiers, & A. W. Stacy (Eds.), *Handbook of implicit cognition and addiction* (pp. 215–243). Thousand Oaks, CA: SAGE Publications.

Beer, J. S., Stallen, M., Lombardo, M. W., Gonsalkorale, K., Cunningham, W. A., & Sherman, J. W. (2008). The quadruple process model approach to examining the neural underpinnings of prejudice. *Neuroimage*, *43*, 775–783.

Botvinick, M. M. (2007). Conflict monitoring and decision making: reconciling two perspectives on anterior cingulated function. *Cognitive, Affective and Behavioral Neuroscience*, *7*(4), 356–366.

Botvinick, M., & Plaut, D. C. (2004). Doing without schema hierarchies: a recurrent connectionist approach to normal and impaired routine sequential action. *Psychological Review*, *111*(2), 395–429.

Bowers, J. S. (2009). On the biological plausibility of grandmother cells: Implications for neural network theories in psychology and neuroscience. *Psychological Review*, *116*(1), 220–251.

Cardinal, R. N., & Everitt, B. J. (2004). Neural and psychological mechanisms underlying appetitive learning: links to drug addiction. *Current Opinion in Neurobiology*, *14*(2), 156–162.

Chee, M. W. L., Sriram, N., Soon, C. S., & Lee, K. M. (2000). Dorsolateral prefrontal cortex and the implicit association of concepts and attributes. *NeuroReport*, *11*(1), 135–140.

Chein, J. M., & Schneider, W. (2005). Neuroimaging studies of practice-related change: fMRI and meta analytic evidence of a domain-general control network for learning. *Cognitive Brain Research*, *25*, 607–623.

DiChiara, G. (2002). Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. *Behavioural Brain Research*, *137*, 75–114.

Everitt, B. J., & Robbins, T. W. (2005). Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nature Neuroscience*, *8*, 1481–1489.

Everitt, B. J., Dickinsonm, A., & Robbins, T. W. (2001). The neuropsychological basis of addictive behaviour. *Brain Research*, *36*, 129–138.

Field, M., Mogg, K., & Bradley, B. P. (2004). Cognitive bias and drug craving in recreational cannabis users. *Drug and Alcohol Dependence*, *74*(1), 105–111.

Friston, K. J., Holmes, A. P., Worsley, K. J., Poline, J. B., Frith, C. D., & Frackowiak, R. S. J. (1995). Statistical parametric maps in functional imaging: a general linear approach. *Human Brain Mapping*, *2*, 189–210.

Gabrieli, J. D. E. (1998). Cognitive neuroscience of human memory. *Annual Review of Psychology*, *49*, 87–115.

Greenwald, A. G., McGhee, D. E., & Schwartz, J. L. K. (1998). Measuring individual differences in implicit cognition: the implicit association test. *Journal of Personality and Social Psychology*, *74*(6), 1464–1480.

Greenwald, A. G., Nosek, B. A., & Banaji, M. R. (2003). Understanding and using the Implicit Association Test: I. An improved scoring algorithm. *Journal of Personality and Social Psychology*, *85*(2), 197–216.

Greenwald, A. G., Poehlman, T. A., Uhlmann, E., & Banaji, M. R. (2009). Understanding and using the Implicit Association Test: III. Meta-analysis of predictive validity. *Journal of Personality and Social Psychology*, *97*, 17–41.

Grenard, J. L., Dent, C. W., & Stacy, A. W. (2013). Longitudinal effects of exposure to alcohol advertisements on teenage alcohol-related problems. *Pediatrics*, *131*(2), e369–e379.

Hampshire, A., Chamberlain, S. R., Monti, M. M., Duncan, J., & Owen, A. M. (2010). The role of the right inferior frontal gyrus: inhibition and attentional control. *Neuroimage*, *50*, 1313–1319.

Hilario, M. R. F., Clouse, E., Yin, H. H., & Costa, R. M. (2007). Endocannabinoid signaling is critical for habit formation. *Frontiers in Integrative Neuroscience*, *1*, 1–12.

Kahneman, D. (2003). A perspective on judgment and choice: mapping bounded rationality. *American Psychologist*, *58*(9), 697–720.

Kane, M. J., & Engle, R. W. (2002). The role of prefrontal cortex in working-memory capacity, executive attention, and general fluid intelligence: an individual-differences perspective. *Psychonomic Bulletin and Review*, *9*(4), 637–671.

Kelly, A. B., Masterman, P. W., & Marlatt, G. A. (2005). Alcohol related associative strength and drinking behaviours: concurrent and prospective relationships. *Drug and Alcohol Review*, *24*(6), 489–498.

Kelly, A. B., Haynes, M. A., & Marlatt, G. A. (2008). The impact of adolescent tobacco-related associative memory on smoking trajectory: an application of negative binomial regression to highly skewed longitudinal data. *Addictive Behaviors*, *33*(5), 640–650.

Knowlton, B. J., Mangels, J. A., & Squire, L. R. (1996). A neostriatal habit learning system in humans. *Science*, *273*(5280), 1399–1402.

Knutson, K. M., Wood, J. N., Spampinato, M. V., & Grafman, J. (2006). Politics on the brain: an fMRI investigation. *Social Neuroscience*, *1*, 25–40.

Knutson, K. M., Mah, L., Manly, C. F., & Grafman, J. (2007). Neural correlates of automatic beliefs about gender and race. *Human Brain Mapping*(28), 915–930.

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology Reviews*, *35*, 217–238.

Levy, D. A., Stark, C. E. L., & Squire, L. R. (2004). Intact conceptual priming in the absence of declarative memory. *Psychological Science*, *15*(10), 680–686.

Luo, Q., Nakic, M., Wheatley, T., Richell, R., Martin, A., & Blair, R. J. (2006). The neural basis of implicit moral attitude--an IAT study using event-related fMRI. *NeuroImage*, *30*(4), 1449–1457.

Noël, X., Brevers, D., & Bechara, A. (2013). A neurocognitive approach to understanding the neurobiology of addiction. *Current Opinion in Neurobiology*, *23*, 632–638.

Nosek, B. A., Greenwald, A. G., & Banaji, M. R. (2005). Understanding and using the Implicit Association Test: II. Method variables and construct validity. *Personality and Social Psychological Bulletin*, *31*(2), 166–180.

Nosek, B. A., Greenwald, A. G., & Banaji, M. R. (2007). The implicit association test at age 7: a methodological and conceptual review. In J. A. Bargh (Ed.), *Automatic processes in social thinking and behavior*. New York, NY: Psychology Press.

Payne, B. K., & Gawronski, B. (2010). A History of Implicit Social Cognition: Where is it coming from? Where is it now? Where is it going? In B. Gawronski, & B. K. Payne (Eds.), *Handbook of implicit social cognition: measurement, theory, and applications* (pp. 1–15). New York, NY: The Guilford Press.

Phillips, P. E. M., Stuber, G. D., Heien, M. L. A. V., Wightman, R. M., & Carelli, R. M. (2003). Subsecond dopamine release promotes cocaine seeking. *Nature*, *422*, 614–618.

Robbins, T. W., et al. (1989). Limbic-striatal interactions in reward-related processes. *Neuroscience and Biobehavioral Reviews*, *13*(2–3), 155–162.

Robbins, T. W., Ersche, K. D., & Everitt, B. J. (2008). Drug addiction and the memory systems of the brain. *Annals of the New York Academy of Sciences*, *1141*, 1–21.

Robinson, T. E., & Berridge, K. C. (2003). Addiction. *Annual Review of Psychology*, *54*, 25–53.

Roediger, H. L. (1990). Implicit memory: retention without remembering. *American Psychologist*, *45*, 1043–1056.

Rolls, E. T. (2000). Memory systems in the brain. *Annual Review of Psychology*, *51*, 599–630.

Rooke, S. E., Hine, D. W., & Thorsteinsson, E. B. (2008). Implicit cognition and substance use: a meta-analysis. *Addictive Behaviors*, *33*(10), 1314–1328.

Schneider, W., & Chein, J. M. (2003). Controlled & automatic processing: behavior, theory, and biological mechanisms. *Cognitive Science*, *27*, 525–559.

Schultz, W. (2004). Neural coding of basic reward terms of animal learning theory, game theory, microeconomics and behavioural ecology. *Current Opinion in Neurobiology*, *14*, 139–147.

Stacy, A. W., & Wiers, R. W. (2006). An implicit cognition, associative memory framework for addiction. In M. R. Munafo, & I. P. Albery (Eds.), *Cognition and addiction* (pp. 31–71). New York, NY: Oxford University Press.

Stacy, A. W., Ames, S. L., & Knowlton, B. (2004). Neurologically plausible distinctions in cognition relevant to drug abuse etiology and prevention. *Substance Use and Misuse*, *39*, 1571–1623.

Stacy, A. W., Ames, S. L., Wiers, R. W., & Krank, M. (2010). Associative memory in appetitive behavior: framework and relevance to epidemiology and prevention. In L. M. Scheier (Ed.), *Handbook of drug use etiology* (pp. 165–182). Washington, DC: APA Books.

Strack, F., & Deutsch, R. (2004). Reflective and impulsive determinants of social behavior. *Personality and Social Psychology Review*, *8*(3), 220–247.

Thush, C., Wiers, R. W., Ames, S. L., Grenard, J. L., Sussman, S., & Stacy, A. W. (2007). Apples and oranges? Comparing implicit measures of alcohol-related cognition predicting alcohol use in at-risk adolescents. *Psychology of Addictive Behaviors*, *21*(4), 587–591.

Volkow, N. D., Fowler, J. S., Wang, G., & Goldstein, R. Z. (2002). Role of dopamine, the frontal cortex and memory circuits in drug addiction: insights from imaging studies. *Neurobiology of Learning and Memory*, *78*(3), 610–624.

Vollstädt-Klein, S., Wichert, S., Rabinstein, J., Bühler, M., Klein, O., Ende, G., Hermann, D., & Mann, K. (2010). Initial, habitual and compulsive alcohol use is characterized b a shift of cue processing from ventral to dorsal striatum. *Addiction*, *105*, 1741–1749.

White, N. M. (1996). Addictive drugs as reinforcers: multiple partial actions on memory systems. *Addiction*, *91*(7), 921–949.

Wiers, R. W., & Stacy, A. W. (2006). *Handbook of implicit cognition and addiction*. Thousand Oaks, CA: SAGE Publications.

Wiers, R. W., Bartholow, B. D., van den Wildenberg, E., Thush, C., Engels, R., Sher, K., Grenard, J., Ames, S., & Stacy, A. W. (2007). Automatic and controlled processes and the development of addictive behavior in adolescents: a review and a model. *Pharmacology, Biochemistry and Behavior*, *86*(2), 263–283.

Yin, H. H., & Knowlton, B. J. (2006a). Addiction and learning in the brain. In R. W. Wiers, & A. W. Stacy (Eds.), *Handbook of implicit cognition and addiction* (pp. 167–184). Thousand Oaks, CA: SAGE publications.

Yin, H. H., & Knowlton, B. J. (2006b). The role of the basal ganglia in habit formation. *Nature Review Neuroscience*, *7*, 464–476.

CHAPTER

29

Stress Response in Cannabis Users and Psychosis

M. Bioque[*], *H.-H. Tseng*[**,†], *R. Mizrahi*[**]

[*]Barcelona Clínic Schizophrenia Unit, Hospital Clínic de Barcelona, Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM), Barcelona, Spain; [**]Centre for Addiction and Mental Health (CAMH), Research Imaging Centre, Toronto, ON, Canada; [†]Department of Psychosis Studies, Institute of Psychiatry, King's College London, London, United Kingdom

SUMMARY POINTS

- Several neuropsychiatric disorders are related to stress, and stress exposure modifies the onset and evolution of these conditions. Stress activates the hypothalamic–pituitary–adrenal axis (HPA) and the sympathetic nervous system (SNS). This activation results in a compensatory, physiological change or adaptation.
- The dysregulation of the stress response, including dopamine release, is a potential etiological factor in the development and relapse of dopamine-related human disorders, including addictions, drug-induced psychosis, and schizophrenia.
- Dopamine is one of the main actors in stress response, specially related to addictions, drug-induced psychosis, and schizophrenia.
- Exogenous cannabinoids alter the dopaminergic balance, most frequently causing alterations of dopamine release at downstream forebrain territories.
- Putative physiological and psychological effects of cannabis could be potentiated in individuals experiencing adverse environmental stress.
- The exposure to certain environmental factors interacting with genetic factors can alter dopaminergic system function, in response to stress.
- The endocannabinoid system—proposed as a main homeostatic system implicated in the regulation of the complex neuroimmune interactions in diverse neuropathological scenarios, reducing both neurodegenerative and inflammatory damage—may play a role as a possible link between stress, continuous cannabis use, and psychosis.
- Several studies have related endocannabinoid system alterations with psychotic disorders, while frequent cannabis exposure, especially in adolescence, may increase the risk of psychosis.
- Knowing the prominent role that the endocannabinoid system has in stress recovery, future research in this filed will have to clarify its relation to dopamine response to stress, in psychotic disorders, and how it is altered for exogenous cannabis use.

KEY FACTS OF PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC)

- Peripheral blood mononuclear cells (PBMC) are any blood cell having round nuclei, including lymphocytes, monocytes, and macrophage.
- As a main actor of the immune system, PBMC are critical to fight infection and foreign intruders in the body.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00033-8
Copyright © 2017 Elsevier Inc. All rights reserved.

- PBMCs are widely used in research and clinical applications in the fields of immunology (including autoimmune disorders), infectious disease, hematological malignancies, cancer, vaccine development, and transplants.
- PBMCs are also vital to cellular therapy, a process that introduces new blood cells into damaged tissue to treat a particular disease. This type of therapy often focuses on hereditary diseases, and may include gene therapy, stem cell treatments, and cell replacement therapy.
- There are currently emerging new biomarkers (inflammatory, metabolic, vitamin...) to neuropsychiatric diseases in PBMC.
- These cells can be extracted from whole blood using ficoll, a reagent that separates layers of blood that will separate the blood into a top layer of plasma, followed by a layer of PBMCs, and a bottom fraction of polymorphonuclear cells (such as neutrophils and eosinophils) and erythrocytes.

KEY FACTS OF MONTREAL IMAGING STRESS TASK (MIST)

- The Montreal Imaging Stress Task (MIST) is a computerized task derived from the Trier Mental Challenge Test, and adapted for imaging applications, consisting of a computerized mental arithmetic challenge and social evaluative threat component.
- The social evaluative threat involves built-in visual and auditory cues, as well as pretask priming and external feedback from the investigator.
- The MIST can be used in functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) research, with either a block or an event-related design.
- The MIST has two test conditions (control and experimental). In both conditions, participants are asked to mentally solve arithmetic problems displayed on the computer screen, while positioned in the scanner.
- The feedback (correct/incorrect/time-out) and an assessment of overall performance in comparison to the population average is displayed on the screen.
- The experimental condition provides a time-limit associated with visual and auditory cues for each question, with the available time per question adjusted to keep the individual's performance below the adequate level.
- In the experimental condition, the investigator presents negative performance evaluations to further increase the social evaluative threat.

LIST OF ABBREVIATIONS

2AG	2-Arachidonoylglycerol
AEA	Anandamide
CB1	Cannabinoid receptor 1
CB2	Cannabinoid receptor 2
DA	Dopamine
DAGL	Diacylglycerol lipase
ECS	Endocannabinoid system
FAAH	Fatty acid amide hydrolase (FAAH)
FEP	First episode of psychosis
fMRI	Functional magnetic resonance imaging
HPA	Hypothalamic–pituitary–adrenal axis
MAGL	Monoacylglycerol lipase
MIST	Montreal Imaging Stress Task
mRNA	Messenger ribonucleic acid
NAPE	*N*-Acyl phosphatidylethanolamine phospholipase
PBMC	Peripheral blood mononuclear cells
PET	Positron emission tomography
SNS	Sympathetic nervous system
VTA	Ventral tegmental area
WB	Western blot

STRESS AND MENTAL DISORDERS

The physiologist Hans Selye defined for the first time the physiological responses to stressors (Selye, 1950). He adapted the term to its current use in the medical vocabulary from physics and engineering, and was the first to use the terms "stress" and "stress response" (Selye, 1950). The stress response, consisting of a three-phase process, and defined by Selye as "general adaptation syndrome," results from the adaptation necessary to overcome situations in which an organism has to fight or escape to survive (eg, higher blood pressure, a faster cardiac rhythm, suppression of the digestive processes, redirection of blood to muscles) (Selye, 1950).

Stress begins with a stimulus of external or internal origin to the organism that activates the hypothalamic–pituitary–adrenal axis (HPA) and the sympathetic nervous system (SNS). This activation results in a compensatory, physiological change or adaptation, so that the organism can deal with the threat. In response to those environmental or psychological stressors, the initial stress response comprises the generation of cerebral cortical neuronal activity, extending to the limbic system, where it activates the cascading physiological responses (summarized in Fig. 29.1), such as, the release of catecholamine (including dopamine—DA), glucocorticoids, and excitatory amino acids. Available data has been summarized in reviews of Borges, Gayer-Anderson, and Mondelli (2013) and Garcia-Bueno, Caso, and Leza (2008).

Several neuropsychiatric disorders, including schizophrenia and drugs use disorders, are related to stress, and deregulation of the stress response is a potential etiological factor in the development and relapse of these DA-related mental illnesses (see Table 29.1). For

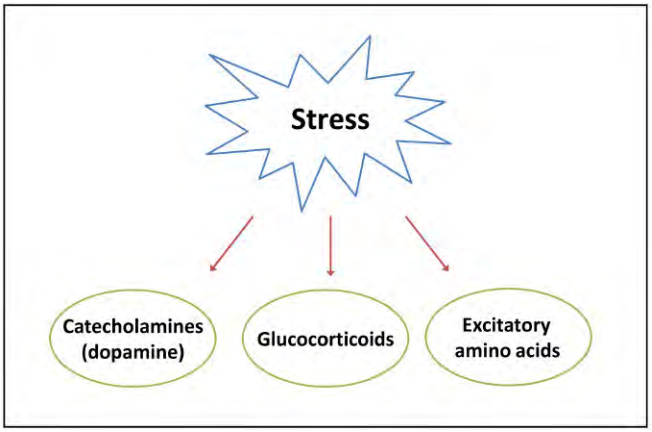


FIGURE 29.1 **Brain response induced by stress.**

example, stress-related HPA activity is often elevated in first-episode and nonmedicated psychosis, and may represent one of the biomarkers of vulnerability to psychosis (Walker et al., 2010).

In this chapter, the discussions will be focused on the dopaminergic response to stress, given the particular relevance of the dopaminergic systems for psychosis illnesses. The research method of dopaminergic response to stress will be briefly introduced, and dopaminergic response to stress in chronic cannabis users and in psychosis will be discussed accordingly, followed by a discussion of the potential link between stress, continuous cannabis use, and the development of psychosis.

STRESS STUDY, INDUCTION, AND EVALUATION

The widespread use of animal models is leading to the progressive unraveling of the basic mechanisms responsible for the consequences of stress on mammalian physiology. In rodents, those tasks or designs characterized by exposure to an unavoidable physical stress are the most extensively used: immobilization, exposure to extreme temperatures, fasting, immersion or electric shocks, etc. Models of physical stress, such as fasting or forced exercise, are more suitable for the study of metabolic responses, whereas models of social or emotional stress, such as crowding or social isolation, have translational value for psychiatric disorders, although precise and robust interpretations are more challenging. Further information can been found in reviews of Garcia-Bueno et al. (2008) and Blanchard, McKittrick, and Blanchard (2001).

In humans, measurement of the cortisol-awakening response (Pruessner, Hellhammer, & Kirschbaum, 1999) and stressful interviewing protocols, including free speech and mental arithmetic tasks (Kirschbaum, Pirke, & Hellhammer, 1993), are the most used stress protocols for accessing basal activity and reactivity of HPA axis. For further discussions, see review of Kudielka and Wust (2010).

Montreal Imaging Stress Task (MIST)

One of these stressful interview tasks based in mental arithmetic task questions is the Montreal Imaging Stress Task (MIST) (Dedovic et al., 2005), which has a special interest for the studies developed in this chapter. Together with neuroimaging techniques, this task has shown to be appropriate to investigate the effects of perceiving and processing psychosocial stress in the human brain.

Briefly, during the MIST subjects perform mental arithmetic tasks on a computer screen that also displays information about the performance level (numbers of errors, and time spent), and performance feedback (correct, incorrect, timeout) for each problem. During the stress condition, subjects complete six 6-min block segments of arithmetic tasks. The time constraint is adjusted to be slightly beyond each individual's abilities. Because of the manipulation of the difficulty level, the average performance was set at 20–30% correct answers. In addition, subjects are given negative verbal feedback by the investigator between each block, telling them that they need to improve their performance to reach minimum performance requirements. Before the stress task, subjects are required to perform a nonstress sensory motor control session, with similar arithmetic tasks, but without time constraints or negative verbal feedback.

TABLE 29.1 Main Dopaminergic Pathways in the Brain

Dopaminergic pathway	Description	Disorders related
Mesolimbic	Transmits dopamine (DA) from the ventral tegmental area (VTA) to the limbic system via the nucleus accumbens	Addictions to drugs of abuse
Mesocortical	Transmits DA from the VTA to the frontal cortex	Schizophrenia
Nigrostriatal	Transmits DA from the substantia nigra to the striatum	Motor control and movement disorders
Tuberoinfundibular	Transmits dopamine from the hypothalamus to the pituitary gland. This pathway influences the secretion of certain hormones, including prolactin	Hyperprolactinemia due to antipsychotics

Different neuroimaging techniques, including functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), have been successfully used with the MIST in order to determine the cortical activation and neurochemical response to stress. These studies have been done especially with cannabis users and subjects with psychotic spectrum disorders in different phases (Lederbogen et al., 2011; Mizrahi et al., 2012, 2013a,b; Lataster et al., 2014). Other physiological measures, such as saliva-derived cortisol immunoassay, have also been used to assess the stress response to induction combined to the MIST (Dedovic et al., 2005; Mizrahi et al., 2013a).

DOPAMINE RESPONSE TO PSYCHOSOCIAL STRESS IN CHRONIC CANNABIS USERS

Numerous animal studies show that exogenous cannabinoids alter the excitatory/inhibitory balance reaching dopamine cells, most frequently causing alterations of dopamine release at downstream forebrain territories. However, the attempts to establish whether the psychomimetic effects of Δ^9-tetrahydrocannabinol (Δ^9-THC, the primary psychoactive compound of cannabis) in humans are mediated via excessive DA release in the striatum have produced mixed results. For further details, see review of Murray, Mehta, & Di Forti (2014).

Although many individuals admit recreational use of cannabis, fewer users become dependent (about 9% of users), compared to most of the use of other illicit drugs (see Table 29.2).

However, the percentage in cannabis users increases among those who started at a younger age (about 17%) and among the daily users (25–50%; NIDA, 2014). Among cannabis users, psychosocial stress is a potent trigger of relapse, and an important risk factor for chronic use. See review of Hyman and Sinha (2009).

Potentially addictive drugs share the ability to produce long-lasting adaptations in neural systems (Gruber & Pope, 2002). Low striatal DA receptor (D2/3) availability, and low amphetamine-induced DA release in the ventral striatum have been observed in several substance-use disorders, including alcoholism, heroin, cocaine, and methamphetamine use (Volkow et al., 1993, 1994, 2001; Martinez et al., 2012), but less is known about the changes in DA transmission in chronic cannabis use.

By using the MIST task, it has been demonstrated that chronic cannabis users, during early abstinence, did not show difference in stress-induced DA release (Mizrahi et al., 2013b). This work supports previous observations by Urban et al. (2012), who found no difference between cannabis users and healthy controls in DA release following an acute amphetamine challenge, and pointing to the fact that chronic cannabis use does not seem to increase DA release. Consistent with these findings, cocaine dependence has been shown to reduce amphetamine-induced DA release (Martinez et al., 2007).

This different dopaminergic response can partially be explained by the fact that cannabinoids produce behavioral, as well as neurochemical, changes dependent on the environmental conditions under which they are administered. For example, classical animal studies showed that cannabinoids administered to rats housed in stressful conditions altered striatal DA uptake and metabolism, in contrast to the absence of effect on rats housed in normal conditions (Littleton, Maclean, & Brownlee, 1976). Furthermore, cross-sensitization between Δ^9-THC and stress reported in animal models (Suplita Ii, Eisenstein, Neely, Moise, & Hohmann, 2008) suggest that cannabis effects could be potentiated in individuals experiencing adverse environmental stress.

STRESS-INDUCED DOPAMINE RELEASE IN PSYCHOSIS

The research in recent decades demonstrates the heterogeneity of the etiology of psychotic disorders, where environmental factors play a key role as genetic factors (van Os & Kapur, 2009) (see Fig. 29.2). These environmental factors can be summarized as biological stress (eg, prenatal stress, high paternal age, malnutrition,

TABLE 29.2 Percentage of Dependence Development Between Drugs of Abuse Users

Drug of abuse	Dependence between users (%)
Alcohol	30.4 (men) 16.0 (women)
Tobacco	22
Cannabis	9
Cocaine	15
Heroin	23

Note: None of the tables or figures are copyrighted. They have all been elaborated by the authors.
National Institute of Drug Abuse web page (NIDA, 2014).

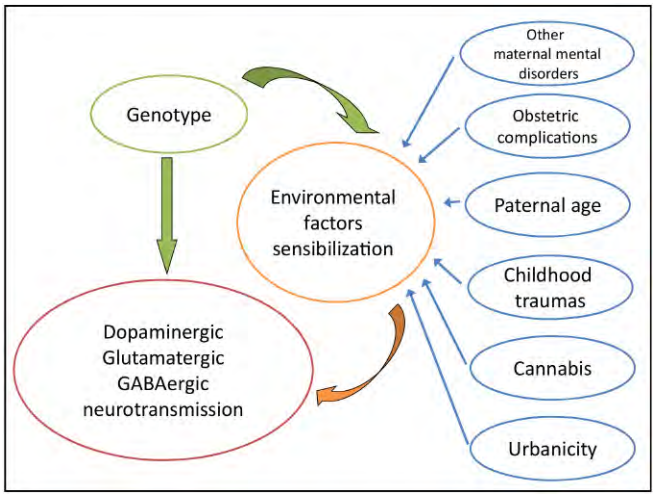


FIGURE 29.2 **Gene–environment interaction in psychotic disorders.**

infections during pregnancy, perinatal hypoxia), psychosocial stress (eg, prenatal stress, presence of traumatic events, membership of a minority ethnic groups), or drug exposure (Matheson, Shepherd, Laurens, & Carr, 2011).

The exposure to certain environmental factors interacting with genetic factors can alter dopaminergic transmission, neuroendocrine and cognitive functioning, patterns of interpersonal interaction, and affective processing, and may lead to a psychopathology worsening (see review of van Os & Kapur, 2009). An exaggerated response with increased DA release under biological stressor (stimulant) has also been observed in schizophrenia (Laruelle & Abi-Dargham, 1999; Yuii, Suzuki, & Kurachi, 2007). These findings are in line with the vulnerability-stress model, suggesting a crucial role of the interaction between vulnerability and environmental stressors in the development of psychotic disorders (Walker & Diforio, 1997).

One of the proposed underlying neurobiological mechanisms that result in the exaggerated response to stress is DA sensitization. Repeated exposure to life stressors or DA drugs sensitizes neurochemical response of the mesolimbic/mesocortical DA system progressively, which in turn increases stress-associated neurochemical activation of HPA axis and stress-associated DA release (Laruelle & Abi-Dargham, 1999; Yuii et al., 2007). Consequently, the dopaminergic system responds to mundane environmental stimuli with excessive DA release, precipitating illness in vulnerable individuals, and relapse in those with schizophrenia.

Converging evidences links dysregulated dopamine function with disrupted salience processing, and the development of psychotic symptoms (Kapur, 2003). Salience processing refers to "…a process whereby events and thoughts come to grab attention, drive action, and influence goal-directed behavior…" (Kapur, 2003). Animal studies show that midbrain dopamine neurons are activated by unexpected salient events. Functional imaging studies have located altered salience response in prefrontal and striatal dopaminergic projection fields in psychotic patients. Together with the evidence that increased subcortical striatal DA synthesis and DA release and transmission in psychosis (Howes & Kapur, 2009), the functional dysregulation of dopamine system in the striatum are a core feature of psychosis (Grimm, Heinz, & Walter, 2014), and may lead to aberrant salience. With repeated exposure to life stressors or DA drugs, the DA system may have been tune into a sensitized, hyperresponsive state, in which daily-life events are responded as unexpected salient events. This may account for the altered sense of meaning and significance that predates the onset of psychosis. For a comprehensive review, see Winton-Brown, Fusar-Poli, Ungless, and Howes (2013).

So far, few studies have examined the association between stress and psychotic-like experiences in vulnerable individuals. Vulnerable persons with genetic high risk for psychosis (ie, first-degree relatives) show increased homovanillic acid (a DA metabolite) following a metabolic stress challenge which correlates with increased psychotic-like experiences (Myin-Germeys, Marcelis, Krabbendam, Delespaul, & van Os, 2005; Holtzman, Shapiro, Trotman, & Walker, 2012; Holtzman et al., 2013). These findings are in line with those in patients with schizophrenia (both antipsychotic-free and treated), who demonstrate a significant homovanillic acid elevation, relative to matched healthy controls, in response to a physiologic stress test (Breier, Davis, Buchanan, Monde, & Munson, 1993), suggesting increased DA release in response to stress, in both subjects at higher risk, and with overt psychosis.

By using the MIST task, Mizrahi et al. (2012) report increased stress-induced DA release in both clinical high-risk group and schizophrenia patients (Mizrahi et al., 2012). This DA sensitization in schizophrenia-related disorders is consistent with previous studies in amphetamine-induced DA release in patients with schizophrenia (Laruelle & Abi-Dargham, 1999; Yuii et al., 2007). These results again highlight the role of DA dysregulation in psychosis, and its possible link to stress.

ENDOCANNABINOID SYSTEM: A POSSIBLE LINK BETWEEN STRESS, CONTINUOUS CANNABIS USE, AND PSYCHOSIS

The endocannabinoid signaling system (ECS) is a widespread neuromodulatory system that modulates neurotransmitter release in brain and in the periphery (Salzet, Breton, Bisogno, & Di Marzo, 2000; Hillard, Weinlander, & Stuhr, 2012). Considerable data support the notion that

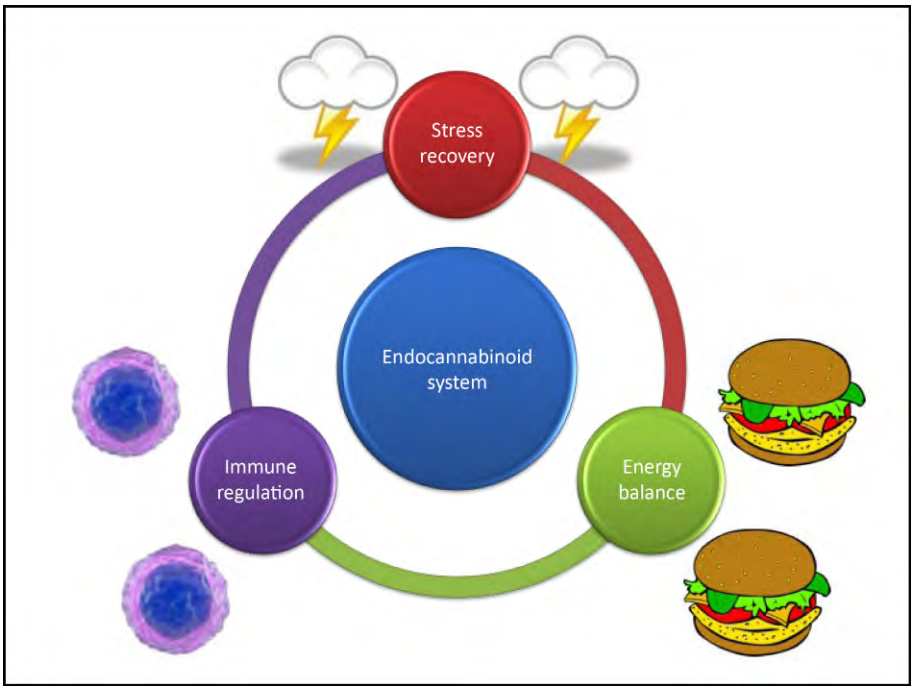


FIGURE 29.3 **Main endocannabinoid system functions in mammals.**

endocannabinoid signaling has three broad and overlapping functions in mammals (summarized in Fig. 29.3) (Klein & Cabral, 2006; Di Marzo, Ligresti, & Cristino, 2009; Hill et al., 2010). In particular, the ECS is present in stress-responsive neural and peripheral circuits, reducing both neurodegenerative and inflammatory damage (see review of Wolf, Tauber, & Ullrich, 2008).

In short, the ECS comprises the arachidonate-based lipids anandamide (AEA) and 2-arachidonoylglycerol (2AG); their cannabinoid G protein-coupled receptors, CB1 and CB2; enzymes involved in cannabinoid synthesis, for example, N-acyl phosphatidylethanolamine phospholipase (NAPE) and Diacylglycerol lipase (DAGL); and the enzymes that are responsible for their degradation or reuptake, fatty acid amide hydrolase (FAAH) and Monoacylglycerol lipase (MAGL) (summarized in Fig. 29.4) (Di Marzo et al., 1994; Stella, Schweitzer, & Piomelli, 1997).

CANNABIS USE, ENDOCANNABINOID SYSTEM, AND PSYCHOSIS

Cannabis use is one of the most important and studied environmental risk factors related to psychosis (van Os & Kapur, 2009). Epidemiological study shows a higher incidence of psychosis with early cannabis use, which has been linked to a significant increased risk of developing psychosis, with an estimated odds ratio of 2.10–2.93 (Moore et al., 2007). Around 25–50% of subjects who suffer a first episode of psychosis use cannabis (see review of Volkow, 2009). The cessation of cannabis use after a first episode, both in adults and adolescents, is associated with clinical and functional improvement (Gonzalez-Pinto et al., 2011).

Several lines of research have related the ECS with psychotic disorders, such as, a dramatic elevations of AEA in cerebrospinal fluid (CSF) in patients with schizophrenia (Leweke et al., 2007); a reduced peripheral expression of endocannabinoid synthesis enzymes, and increased expression of degradative enzymes in first-episode psychosis (Bioque et al., 2013). In terms of central cannabinoid CB1 receptors, there is an association between CB1 receptors polymorphism and schizophrenia (Ujike et al., 2002). An increased CB1 binding is reported in postmortem autoradiography and in vivo PET studies in patients with schizophrenia (Zavitsanou, Garrick, & Huang, 2004; Wong et al., 2010). In terms of peripheral CB2 receptors, the patient subgroup with a history of heavy cannabis use showed a lower CB2 receptor expression (Bioque et al., 2013), and an association has also been reported between a diminished CB2 function and an increased susceptibility to schizophrenia, together with other risk factors (Ishiguro et al., 2010).

ENDOCANNABINOID SYSTEM, STRESS REGULATION, AND PSYCHOSIS RISK

The role of ECS in stress regulation and recovery postulate the potential link between ECS and DA system responding to stress. It has been proposed that ECS

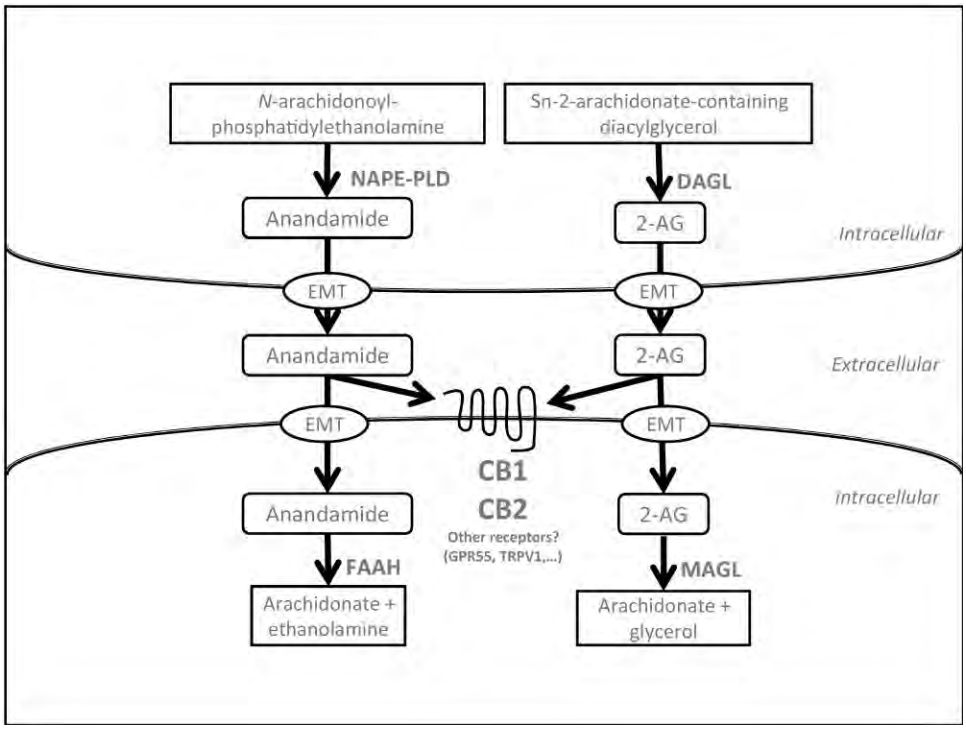


FIGURE 29.4 **Main endocannabinoid system components.** *2-AG*, 2-arachidonoylglycerol diacylglycerol; *CB2*, cannabinoid receptor 2; *DAGL*, diacylglycerol; *EMT*, endocannabinoid membrane transporter; *FAAH*, fatty acid amide hydrolase; *GPR55*, G-protein coupled receptor 55; *MAGL*, monoacylglycerol lipase; *NAPE-PLD*, *N*-acyl-phosphatidylethanolamine-selective phosphodiesterase; *PBMC*, peripheral blood mononuclear cell; *TRPV1*, transient receptor potential vanilloid type 1.

activity mediates HPA function via regulating the intensity and duration of the neurohormonal response to stress. The presence of CB1 receptors within the corticolimbic circuits, and robust evidence from animal studies implicating AEA, plays a regulatory role in HPA (Hill et al., 2005, 2009).

Notably, the human cortical ECS undergoes dramatic changes in early life and adolescence (Cass et al., 2014), with increased metabolism until early adulthood (Long, Lind, Webster, & Weickert, 2012). These early-life changes in the ECS could potentially increase the sensitivity to cannabis use, and potentiate their susceptibility to environmental and social stressors, which may, in turn, increase the risk for psychosis. The observation that cannabis use before the age of 15 leads to increased risk for psychosis (Arseneault et al., 2002), while later use does not have the same effect on brain neurochemistry, supports this speculation. In addition, risk of psychosis has been shown to increase with childhood trauma, and cannabis use through a synergistic interaction (Harley et al., 2010), which would be consistent with the aforementioned data related to the role of the ECS in stress regulation, and supports the notion.

Exogenous cannabinoids stimulate CB1 receptors, and suppress the excessive HPA response to stress. In this sense, cannabis use in psychosis and in at-risk youths could represent an attempt to regulate an existing abnormal stress response. However, frequent cannabis exposure may downregulate AEA signaling in the CNS, and potentially dampen ECS, which has only been seen in patients with schizophrenia (Leweke et al., 2007). Nevertheless, the neurobiological mechanisms underlying this increased psychosis susceptibility in cannabis users remain poorly understood (D'Souza, Sewell, & Ranganathan, 2009).

Although existing evidence cannot explain why cannabis use itself may lead to psychosis in at-risk populations, an intermingled relationship between cannabis use and stress response in DA system in this population has been observed. A recent study shows that in those at risk for psychosis with concomitant cannabis use, despite aggravated psychotic symptoms in response to stress, the DA system does not show significant response (increase in DA release) to the psychosocial stress task (MIST) (Mizrahi et al., 2013a), suggesting that minute increases in DA release may already contribute to the aggravation of psychotic-like experiences.

CONCLUSIONS

Repeated environmental and social stress, and substance use in earlier years, contributes to sensitization of DA systems, and the risk of developing psychosis,

wherein the alteration of ECS may play a modulatory role. Genetic traits, on the other hand, represent a vulnerability that interacts with these environmental factors, may further increase the susceptibility to upcoming social stressors and continuous drug use, and ultimately leads to neurochemical and other functional changes underlying the development of psychosis.

In this regard, early interventions with pharmacological and/or psychosocial approaches in adolescence or early adulthood are extremely important, and establishing reliable biomarkers which may capture the change during the "critical window" represent the stepping stone for initiating these early interventions in time. The sophisticated interplay between dopamine system and ECS implicates that novel biomarkers should aim to depict changes in both systems. Future research should focus on the modulation of stress-induced DA release, clarifying the interaction between DA stress response and ECS, and potential alterations of ECS modulatory effects on stress recovery, particularly in those individuals who have higher risk to develop psychosis. This may, in turn, shed light on novel ways to detect, intervene and, perhaps, even prevent the impact of developing psychosis.

MINI-DICTIONARY

Dopamine Hormone and neurotransmitter of the catecholamine and phenethylamine families that plays a number of important roles in the human brain and body, including a major role in reward-motivated behavior, motor control, and in controlling the release of other hormones.

Drug dependence Compulsive use of a substance, despite its negative or dangerous effects, that leads to a state in which the person needs a drug to function normally.

Endocannabinoid system Group of neuromodulatory lipids and their receptors in the brain that are involved in a variety of physiological processes, including appetite, pain sensation, mood, and memory; it mediates the psychoactive effects of cannabis.

Functional magnetic resonance imaging (fMRI) A functional neuroimaging procedure that measures brain activity by detecting associated changes in blood flow, relying on the fact that cerebral blood flow and neuronal activation are coupled.

Hypothalamic–pituitary–adrenal (HPA) axis The common mechanism for interactions among glands, hormones, and parts of the midbrain, a major part of the neuroendocrine system that controls reactions to stress and regulates many body processes, including digestion, the immune system, mood and emotions, sexuality, and energy storage and expenditure.

Montreal Imaging Stress Task (MIST) A series of computerized mental arithmetic challenges, along with social evaluative threat components that are built into the program, or presented by the investigator, developed to induce moderate psychologic stress in a functional imaging setting, and evaluate the effects of stress on physiology and brain activation.

Physiological or biological stress An organism's response to an agent, such as an environmental condition or a stimulus.

Positron emission tomography (PET) A nuclear medicine, functional imaging technique that produces a three-dimensional image of functional processes in the body, based on the detection of pairs of gamma rays emitted indirectly by a positron-emitting radionuclide (tracer), which is introduced into the body on a biologically active molecule.

Psychosis Refers to an abnormal condition of the mind, generally described as a mental health state involving a loss of contact with reality.

Sensitization Nonassociative learning process in which repeated administrations of a stimulus results in the progressive amplification of a response. It is often characterized by an enhancement of response to a whole class of stimuli, in addition to the one that is repeated.

References

Arseneault, L., Cannon, M., Poulton, R., Murray, R., Caspi, A., & Moffitt, T. E. (2002). Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. *British Medical Journal*, *325*(7374), 1212–1213.

Bioque, M., Garcia-Bueno, B., Macdowell, K. S., Meseguer, A., Saiz, P. A., Parellada, M., Gonzalez-Pinto, A., Rodriguez-Jimenez, R., Lobo, A., Leza, J. C., & Bernardo, M. CIBERSAM. (2013). Peripheral endocannabinoid system dysregulation in first-episode psychosis. *Neuropsychopharmacology*, *38*(13), 2568–2577.

Blanchard, R. J., McKittrick, C. R., & Blanchard, D. C. (2001). Animal models of social stress: effects on behavior and brain neurochemical systems. *Physiology Behavior*, *73*(3), 261–271.

Borges, S., Gayer-Anderson, C., & Mondelli, V. (2013). A systematic review of the activity of the hypothalamic-pituitary-adrenal axis in first episode psychosis. *Psychoneuroendocrinology*, *38*(5), 603–611.

Breier, A., Davis, O. R., Buchanan, R. W., Monde, L. A., & Munson, R. C. (1993). Effects of metabolic perturbation on plasma homovanillic acid in schizophrenia relationship to prefrontal cortex volume. *Archives of General Psychiatry*, *50*(7), 541–550.

Cass, D. K., Flores-Barrera, E., Thomases, D. R., Vital, W. F., Caballero, A., & Tseng, K. Y. (2014). CB1 cannabinoid receptor stimulation during adolescence impairs the maturation of GABA function in the adult rat prefrontal cortex. *Molecular Psychiatry*, *19*(5), 536–543.

Dedovic, K., Renwick, R., Mahani, N. K., Engert, V., Lupien, S. J., & Pruessner, J. C. (2005). The Montreal Imaging Stress Task: using functional imaging to investigate the effects of perceiving and processing psychosocial stress in the human brain. *Journal of Psychiatry and Neuroscience*, *30*(5), 319–325.

Di Marzo, V., Fontana, A., Cadas, H., Schinelli, S., Cimino, G., Schwartz, J. C., & Piomelli, D. (1994). Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature*, *372*(6507), 686–691.

Di Marzo, V., Ligresti, A., & Cristino, L. (2009). The endocannabinoid system as a link between homoeostatic and hedonic pathways involved in energy balance regulation. *International Journal of Obesity*, *33*(Suppl. 2), S18–S24.

D'Souza, D. C., Sewell, R. A., & Ranganathan, M. (2009). Cannabis and psychosis/schizophrenia: human studies. *European Archives of Psychiatry and Clinical Neuroscience*, *259*(7), 413–431.

Garcia-Bueno, B., Caso, J. R., & Leza, J. C. (2008). Stress as a neuroinflammatory condition in brain: damaging and protective mechanisms. *Neuroscience and Biobehavioral Reviews*, *32*(6), 1136–1151.

Gonzalez-Pinto, A., Alberich, S., Barbeito, S., Gutierrez, M., Vega, P., Ibanez, B., Haidar, M. K., Vieta, E., & Arango, C. (2011). Cannabis and First-Episode Psychosis: different long-term outcomes depending on continued or discontinued use. *Schizophrenia Bulletin*, *37*(3), 631–639.

Grimm, O., Heinz, A., Walter, H., et al. (2014). Striatal response to reward anticipation: evidence for a systems-level intermediate phenotype for schizophrenia. *JAMA Psychiatry*, *71*(5), 531–539.

Gruber, A. J., & Pope, H. G., Jr. (2002). Marijuana use among adolescents. *Pediatric Clinics of North America*, *49*(2), 389–413.

Harley, M., Kelleher, I., Clarke, M., Lynch, F., Arseneault, L., Connor, D., Fitzpatrick, C., & Cannon, M. (2010). Cannabis use and childhood trauma interact additively to increase the risk of psychotic symptoms in adolescence. *Psychological Medicine*, *40*(10), 1627–1634.

Hill, M. N., Patel, S., Carrier, E. J., Rademacher, D. J., Ormerod, B. K., Hillard, C. J., & Gorzalka, B. B. (2005). Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. *Neuropsychopharmacology*, *30*(3), 508–515.

Hill, M. N., McLaughlin, R. J., Morrish, A. C., Viau, V., Floresco, S. B., Hillard, C. J., & Gorzalka, B. B. (2009). Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic-pituitary-adrenal axis. *Neuropsychopharmacology*, *34*(13), 2733–2745.

Hill, M. N., McLaughlin, R. J., Bingham, B., Shrestha, L., Lee, T. T., Gray, J. M., Hillard, C. J., Gorzalka, B. B., & Viau, V. (2010). Endogenous cannabinoid signaling is essential for stress adaptation. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(20), 9406–9411.

Hillard, C. J., Weinlander, K. M., & Stuhr, K. L. (2012). Contributions of endocannabinoid signaling to psychiatric disorders in humans: genetic and biochemical evidence. *Neuroscience*, *204*, 207–229.

Holtzman, C. W., Shapiro, D. I., Trotman, H. D., & Walker, E. F. (2012). Stress and the prodromal phase of psychosis. *Current Pharmaceutical Design*, *18*(4), 527–533.

Holtzman, C. W., Trotman, H. D., Goulding, S. M., Ryan, A. T., Macdonald, A. N., Shapiro, D. I., Brasfield, J. L., & Walker, E. F. (2013). Stress and neurodevelopmental processes in the emergence of psychosis. *Neuroscience*, *249*, 172–191.

Howes, O. D., & Kapur, S. (2009). The dopamine hypothesis of schizophrenia: version III—the final common pathway. *Schizophrenia Bulletin*, *35*(3), 549–562.

Hyman, S. M., & Sinha, R. (2009). Stress-related factors in cannabis use and misuse: implications for prevention and treatment. *Journal of Substance Abuse Treatment*, *36*(4), 400–413.

Ishiguro, H., Horiuchi, Y., Ishikawa, M., Koga, M., Imai, K., Suzuki, Y., Morikawa, M., Inada, T., Watanabe, Y., Takahashi, M., Someya, T., Ujike, H., Iwata, N., Ozaki, N., Onaivi, E. S., Kunugi, H., Sasaki, T., Itokawa, M., Arai, M., Niizato, K., Iritani, S., Naka, I., Ohashi, J., Kakita, A., Takahashi, H., Nawa, H., & Arinami, T. (2010). Brain cannabinoid CB2 receptor in schizophrenia. *Biological Psychiatry*, *67*(10), 974–982.

Kapur, S. (2003). Psychosis as a state of aberrant salience: a framework linking biology, phenomenology, and pharmacology in schizophrenia. *American Journal of Psychiatry*, *160*(1), 13–23.

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, *28*(1–2), 76–81.

Klein, T. W., & Cabral, G. A. (2006). Cannabinoid-induced immune suppression and modulation of antigen-presenting cells. *Journal of Neuroimmune Pharmacology*, *1*(1), 50–64.

Kudielka, B. M., & Wust, S. (2010). Human models in acute and chronic stress: assessing determinants of individual hypothalamus-pituitary-adrenal axis activity and reactivity. *Stress*, *13*(1), 1–14.

Laruelle, M., & Abi-Dargham, A. (1999). Dopamine as the wind of the psychotic fire: new evidence from brain imaging studies. *Journal of Psychopharmacology*, *13*(4), 358–371.

Lataster, J., Collip, D., Ceccarini, J., Hernaus, D., Haas, D., Booij, L., van Os, J., Pruessner, J., Van Laere, K., & Myin-Germeys, I. (2014). Familial liability to psychosis is associated with attenuated dopamine stress signaling in ventromedial prefrontal cortex. *Schizophrenia Bulletin*, *40*(1), 66–77.

Lederbogen, F., Kirsch, P., Haddad, L., Streit, F., Tost, H., Schuch, P., Wust, S., Pruessner, J. C., Rietschel, M., Deuschle, M., & Meyer-Lindenberg, A. (2011). City living and urban upbringing affect neural social stress processing in humans. *Nature*, *474*(7352), 498–501.

Leweke, F. M., Giuffrida, A., Koethe, D., Schreiber, D., Nolden, B. M., Kranaster, L., Neatby, M. A., Schneider, M., Gerth, C. W., Hellmich, M., Klosterkotter, J., & Piomelli, D. (2007). Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: impact of cannabis use. *Schizophrenia Research*, *94*(1–3), 29–36.

Littleton, J. M., Maclean, K. I., & Brownlee, G. (1976). Proceedings: Alterations in dopamine uptake in rat corpus striatum induced by combinations of stress and delta8-tetrahydrocannabinol (delta8-THC). *British Journal of Pharmacology*, *56*(3), 370P.

Long, L. E., Lind, J., Webster, M., & Weickert, C. S. (2012). Developmental trajectory of the endocannabinoid system in human dorsolateral prefrontal cortex. *BMC Neuroscience*, *13*, 87.

Martinez, D., Narendran, R., Foltin, R. W., Slifstein, M., Hwang, D. R., Broft, A., Huang, Y., Cooper, T. B., Fischman, M. W., Kleber, H. D., & Laruelle, M. (2007). Amphetamine-induced dopamine release: markedly blunted in cocaine dependence and predictive of the choice to self-administer cocaine. *American Journal of Psychiatry*, *164*(4), 622–629.

Martinez, D., Saccone, P. A., Liu, F., Slifstein, M., Orlowska, D., Grassetti, A., Cook, S., Broft, A., Van Heertum, R., & Comer, S. D. (2012). Deficits in dopamine D(2) receptors and presynaptic dopamine in heroin dependence: commonalities and differences with other types of addiction. *Biological Psychiatry*, *71*(3), 192–198.

Matheson, S. L., Shepherd, A. M., Laurens, K. R., & Carr, V. J. (2011). A systematic meta-review grading the evidence for non-genetic risk factors and putative antecedents of schizophrenia. *Schizophrenia Research*, *133*(1–3), 133–142.

Mizrahi, R., Addington, J., Rusjan, P. M., Suridjan, I., Ng, A., Boileau, I., Pruessner, J. C., Remington, G., Houle, S., & Wilson, A. A. (2012). Increased stress-induced dopamine release in psychosis. *Biological Psychiatry*, *71*(6), 561–567.

Mizrahi, R., Kenk, M., Suridjan, I., Boileau, I., George, T. P., McKenzie, K., Wilson, A. A., Houle, S., & Rusjan, P. (2013a). Stress-induced dopamine response in subjects at clinical high risk for schizophrenia with and without concurrent cannabis use. *Neuropsychopharmacology*, *39*(6), 1479–1489.

Mizrahi, R., Suridjan, I., Kenk, M., George, T. P., Wilson, A., Houle, S., & Rusjan, P. (2013b). Dopamine response to psychosocial stress in chronic cannabis users: a PET study with [11C]-+-PHNO. *Neuropsychopharmacology*, *38*(4), 673–682.

Moore, T. H., Zammit, S., Lingford-Hughes, A., Barnes, T. R., Jones, P. B., Burke, M., & Lewis, G. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet*, *370*(9584), 319–328.

Murray, R. M., Mehta, M., & Di Forti, M. (2014). Different dopaminergic abnormalities underlie cannabis dependence and cannabis-induced psychosis. *Biological Psychiatry*, *75*(6), 430–431.

Myin-Germeys, I., Marcelis, M., Krabbendam, L., Delespaul, P., & van Os, J. (2005). Subtle fluctuations in psychotic phenomena as functional states of abnormal dopamine reactivity in individuals at risk. *Biological Psychiatry*, *58*(2), 105–110.

NIDA. (2014). *DrugFacts*. Bethesda, MD: National Institute of Drug Abuse, May. From http://www.drugabuse.gov/publications/term/160/DrugFacts

Pruessner, J. C., Hellhammer, D. H., & Kirschbaum, C. (1999). Burnout, perceived stress, and cortisol responses to awakening. *Psychosomatic Medicine*, *61*(2), 197–204.

Salzet, M., Breton, C., Bisogno, T., & Di Marzo, V. (2000). Comparative biology of the endocannabinoid system possible role in the immune response. *European Journal of Biochemistry*, *267*(16), 4917–4927.

Selye, H. (1950). Stress and the general adaptation syndrome. *British Medical Journal*, *1*(4667), 1383–1392.

Stella, N., Schweitzer, P., & Piomelli, D. (1997). A second endogenous cannabinoid that modulates long-term potentiation. *Nature*, *388*(6644), 773–778.

Suplita Ii, R. L., Eisenstein, S. A., Neely, M. H., Moise, A. M., & Hohmann, A. G. (2008). Cross-sensitization and cross-tolerance between exogenous cannabinoid antinociception and endocannabinoid-mediated stress-induced analgesia. *Neuropharmacology*, *54*(1), 161–171.

Ujike, H., Takaki, M., Nakata, K., Tanaka, Y., Takeda, T., Kodama, M., Fujiwara, Y., Sakai, A., & Kuroda, S. (2002). CNR1, central cannabinoid receptor gene, associated with susceptibility to hebephrenic schizophrenia. *Molecular Psychiatry*, *7*(5), 515–518.

Urban, N. B. L., Slifstein, M., Thompson, J. L., Xu, X., Girgis, R. R., Raheja, S., Haney, M., & Abi-Dargham, A. (2012). Dopamine release in chronic cannabis users: a [11C]raclopride positron emission tomography study. *Biological Psychiatry*, *71*(8), 677–683.

van Os, J., & Kapur, S. (2009). Schizophrenia. *Lancet*, *374*(9690), 635–645.

Volkow, N. D. (2009). Substance use disorders in schizophrenia-clinical implications of comorbidity. *Schizophrenia Bulletin*, *35*(3), 469–472.

Volkow, N. D., Fowler, J. S., Wang, G. J., Hitzemann, R., Logan, J., Schlyer, D. J., Dewey, S. L., & Wolf, A. P. (1993). Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. *Synapse*, *14*(2), 169–177.

Volkow, N. D., Fowler, J. S., Wang, G. J., Logan, J., Schlyer, D., MacGregor, R., Hitzemann, R., & Wolf, A. P. (1994). Decreased dopamine transporters with age in health human subjects. *Annals of Neurology*, *36*(2), 237–239.

Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Franceschi, D., Sedler, M., Gatley, S. J., Miller, E., Hitzemann, R., Ding, Y. S., & Logan, J. (2001). Loss of dopamine transporters in methamphetamine abusers recovers with protracted abstinence. *Journal of Neuroscience*, *21*(23), 9414–9418.

Walker, E. F., & Diforio, D. (1997). Schizophrenia: a neural diathesis-stress model. *Psychological Review*, *104*(4), 667–685.

Walker, E. F., Brennan, P. A., Esterberg, M., Brasfield, J., Pearce, B., & Compton, M. T. (2010). Longitudinal changes in cortisol secretion and conversion to psychosis in at-risk youth. *Journal of Abnormal Psychology*, *119*(2), 401–408.

Winton-Brown, T. T., Fusar-Poli, P., Ungless, M. A., & Howes, O. D. (2013). Dopaminergic basis of salience dysregulation in psychosis. *Trends in Neurosciences*, *37*(2), 85–94.

Wolf, S. A., Tauber, S., & Ullrich, O. (2008). CNS immune surveillance and neuroinflammation: endocannabinoids keep control. *Current Pharmaceutical Design*, *14*(23), 2266–2278.

Wong, D. F., Kuwabara, H., Horti, A. G., Raymont, V., Brasic, J., Guevara, M., Ye, W., Dannals, R. F., Ravert, H. T., Nandi, A., Rahmim, A., Ming, J. E., Grachev, I., Roy, C., & Cascella, N. (2010). Quantification of cerebral cannabinoid receptors subtype 1 (CB1) in healthy subjects and schizophrenia by the novel PET radioligand [11C]OMAR. *Neuroimage*, *52*(4), 1505–1513.

Yuii, K., Suzuki, M., & Kurachi, M. (2007). Stress sensitization in schizophrenia. *Annals of the New York Academy of Science*, *1113*, 276–290.

Zavitsanou, K., Garrick, T., & Huang, X. F. (2004). Selective antagonist [3H]SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulate cortex in schizophrenia. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *28*(2), 355–360.

CHAPTER

30

Motivation in Chronic Cannabis Use

R. Hirst, L. Sodos, S. Gade, L. Rathke

Palo Alto University, Palo Alto, CA, United States

SUMMARY POINTS

- Empirical evidence for the "amotivational syndrome" in cannabis users is contradictory, with many studies finding no support.
- It is likely that other confounding factors, such as, socioeconomic status, family conflict, and anticonventional behaviors contribute to the negative correlation between cannabis use and motivation for educational and occupational achievement.
- Laboratory measures of motivation yield mixed results, though most evidence suggests that chronic cannabis users interpret monetary reinforcers differently than nonusers.
- A distinction must be made between research examining the acute effects of cannabis use (which is not well supported), and the long-term effects on those who use regularly but are not currently intoxicated.
- It is critical to measure motivation in cannabis users within the research design to ensure validity in the assessment of cognitive performance and other characteristics.
- The authors suggest a unique method for enhancing the motivation of chronic cannabis users during research participation, referencing the contribution that will be made to research that could contribute to legislation on marijuana policy.

KEY FACTS OF CHRONIC CANNABIS USE

- The motivational effects of cannabis are difficult to isolate, as many users also use other legal and illicit substances.
- "Motivation" is difficult to define, and cannabis may affect various aspects of motivation differently.
- Research suggests that the "amotivational syndrome" only exists in a small proportion of chronic cannabis users.
- 40% of the variance in apathy in chronic cannabis users may be due to the reduction in striatal dopamine synthesis that may underlie reduced reward sensitivity and amotivation.
- Positron Emission Tomography (PET) imaging suggests that cannabis users exhibit differential activation of the ventral medial prefrontal cortex, an important component of the dopaminergic reward pathway, during decision-making and motivational tasks.
- Despite the proposed link between motivation and chronic cannabis use, tests of effort (to ensure participants are putting forth adequate effort for valid results) are rarely used in research on cannabis users.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00034-X
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

CVLT-II California Verbal Learning Test-II
IGT Iowa Gambling Task
PET Positron emission tomography
TOMM Test of memory malingering

INTRODUCTION

One of the most commonly associated stereotypes of chronic cannabis users is a lack of motivation, sometimes referred to as the "amotivational syndrome." This notion gained widespread publicity in the 1970s, when marijuana use was increasing, and concern about its effects grew. Suggested symptoms included apathy, lethargy, lack of motivation, and difficulty concentrating (Kolansky & Moore, 1972; Smith, 1968; Soueif, 1976). As many of these variables overlap with aspects of cognitive functioning, it has garnered much debate, with many studies finding little evidence for support (eg, Halikas, Weller, Morse, & Shapiro, 1982). In fact, the original proponent later stated that the syndrome exists "only in a very small and susceptible segment of adolescent marijuana users" (Smith & Seymour, 1982, p. 69). The difficulty in defining and operationalizing motivation has also contributed to the confusion. Some researchers suggested that the syndrome characteristics actually represented symptoms of preexisting mood or anxiety disorders (eg, Musty & Kaback, 1995). Others hypothesized that some cannabis users simply experience a reorientation in attentional style, meaning, and values, which does not follow the norm, and is thus characterized as pathological (eg, Nelson, 1994-5). Neurobiological evidence suggests that many substances of abuse, including cannabis, affect reward mechanisms in the mesolimbic dopamine system of the brain (for review, see Martin-Soelch et al., 2001). Thus, it is possible that cannabis users may process reinforcers differently if changes have occurred within the dopamine system, thereby impacting their motivation to work for that reinforcer. A thorough review of the physiological mechanisms is beyond the scope of this chapter, but several neuroimaging studies examine motivation in cannabis users through the dopamine system, and the results of these studies will be described.

In this chapter, we examine motivation in chronic cannabis users through studies assessing self-report, educational and occupational achievement, and laboratory measures of motivation. Methodological variability likely contributes to the conflicting evidence demonstrated, and we present limitations to the current studies. Further, a distinction is made between research on the acute effects of cannabis intoxication and the long-term effects on regular users. Finally, we describe the importance of measuring motivation in cannabis users within all research designs, and suggest a unique method for enhancing the motivation of chronic cannabis users during research participation.

MOTIVATION: ACHIEVEMENT AND SELF-REPORT

Motivation Self-Report

Initial studies suggesting the "amotivational syndrome" in chronic cannabis users primarily consisted of subjective self-reports from the participants themselves, describing their affective states and employment histories, as well as their behavior while under the acute effects of the drug (for review, see Kolansky & Moore, 1972). However, researchers gathered these patients from psychiatric clinical practice, and the samples are representative of patients suffering from cannabis dependence with other mental health difficulties and problems in functioning, rather than the typical cannabis user.

More recent analyses of nonpsychiatric samples have yielded mixed results when analyzing self-report of motivation in chronic cannabis users, which is likely due, in part, to differences in how motivation is operationalized. Face valid self-report motivational measures demonstrate a low incidence of amotivation (about 5%) in chronic cannabis users (Halikas et al., 1982). On another self-report measure, the apathy evaluation scale, daily cannabis users did not differ from nonusers (Fig. 30.1; Barnwell, Earleywine, & Wilcox, 2006). Further, low motivation may simply be misattributed to cannabis use—one study found that 5% of all undergraduates reported symptoms of amotivation, regardless of cannabis use status (Duncan, 1987).

When defining motivation as personally salient life goals, researchers found a negative relationship with cannabis use. University students who attribute strong meaning toward life goals were less likely to use cannabis (Wright & Palfai, 2012). Further, participants who rated academic achievement as a meaningful goal were less likely to use cannabis, while those who rated socialization as meaningful were more likely to use. However, Simons, Christopher, Oliver, and Stanage (2006) assessed 15–24 year olds and found that cannabis use had no significant relationship to achievement goals in one's life. Thus, evidence for a relationship between cannabis use and life goals appears mixed.

Motivation has also been examined through self-reported life satisfaction and subjective well-being. One study found higher levels of life satisfaction in heavy cannabis users, relative to light users, suggesting that while life goals may differ, heavy users do not have a passive view of achievement (Kouri, Pope, Yurgelun-Todd, & Gruber, 1995). Cannabis users may simply derive life

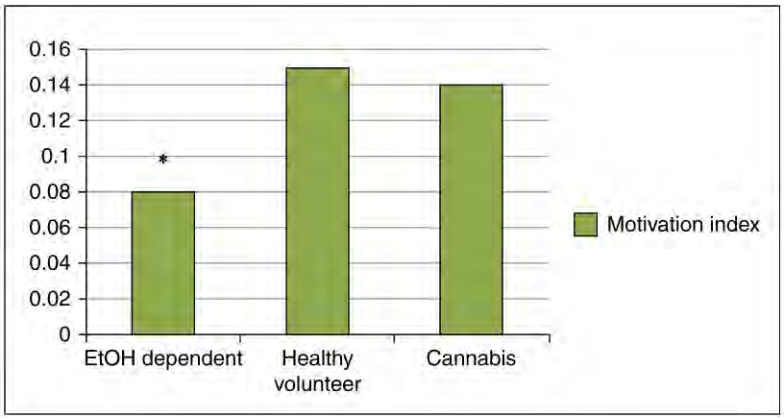


FIGURE 30.1 Motivation scores on the apathy evaluation scale. A comparison of motivation scores among cannabis users and nonusers yielded no significant group differences. *Source: Barnwell et al. (2006): Open Access article distributed under terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), permitting unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

satisfaction from nontraditional routes, as a subculture with different values than the norm (Earleywine, 2003). Others found that, although users reported lower subjective well-being than nonusers, removing medical cannabis users from the analyses eliminated this difference (Barnwell et al., 2006). This suggests that medical illness may be responsible for lower subjective well-being in users, as opposed to cannabis use itself.

Another recent study examined self-reported motivation in cannabis users through an examination of dopamine and found that perceived apathy negatively correlated with dopamine synthesis capacity (Bloomfield, Morgan, Kapur, Curran, & Howes, 2014). Thus, subjective apathy reported by cannabis users could be due to decreased capacity for dopamine production, though a longitudinal study would be necessary to determine if cannabis use directly led to decreased dopamine production.

The limitations of these correlational analyses should be kept in mind, as none of these studies evaluated cannabis users prior to initiation, thus reducing the possibility of causal inferences. Further, in early research, researchers rarely controlled for other confounding factors (eg, low socioeconomic status, mood disorder diagnoses, or preexisting personality traits), which may explain the conflicting findings.

Motivation for Achievement: High School

Another definition of motivation toward life goals is achievement in academics. As cannabis use is most often initiated during high school, the impact of use on educational attainment is of particular interest. Some cross-sectional studies have identified a negative impact of chronic marijuana use on grade point average and academic performance (Resnick et al., 1997), but not on negative attitudes toward school (Jones & Heaven, 1998). Cannabis use has also been associated with lower school satisfaction, poor school performance, absenteeism, and higher rates of suspension or expulsion from school (for review, see Fergusson, Horwood, & Beautrais, 2003). The link with high school dropout has been mixed, with some studies finding an association (eg, Ensminger, Lamkin, & Jacobson, 1996), and others finding none (eg, Ellickson, Bui, Bell, & McGuigan, 1998). Based upon retrospective report, college graduates reported significantly lower use of cannabis during adolescence, relative to high school-only graduates, even after controlling for socioeconomic status, academic ability, self-esteem, and delinquency (Mensch & Kandel, 1988). Another study linked low academic motivation and substance use (including cannabis use specifically) in high school students, as well as a relationship between perceived negative school attitudes of friends, and increased cannabis use (Fig. 30.2; Bryant & Zimmerman, 2002). Thus, most evidence does support a link between lower academic achievement and cannabis use.

It is important to note that these conclusions are limited by cross-sectional and correlational research designs, which make it impossible to draw causal inferences related to cannabis. One study employed a longitudinal design to examine motivation for educational achievement in high school students. Researchers followed 1182 New York students over a 5-year period (Brook, Balka, & Whiteman, 1999). Those who used cannabis once per month or more were more likely to leave school before completing 12th grade, despite controlling for a range of factors at age 14. They were also more likely to report delinquency, drug-related problems, and sexual risk-taking. Other longitudinal studies linked low academic motivation to youth cannabis use (Brown, Schulenberg, Bachman, O'Malley, & Johnston, 2001; Ellickson, Martino, & Collins, 2004); however, neither of these studies found that increased cannabis use over time was associated with decreased motivation. Therefore, it remains unclear

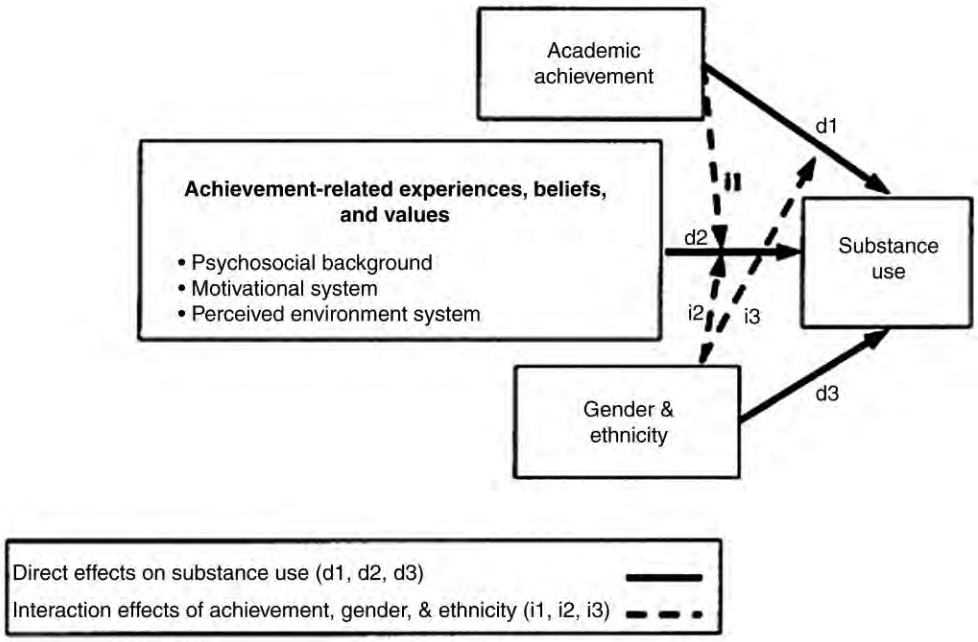


FIGURE 30.2 **Conceptual model of achievement and achievement-related effects on substance use during adolescence.** Many factors influence cannabis use in adolescents, including low academic motivation and perceived negative school attitudes of friends. *Source: Bryant and Zimmerman (2002): APA article; permission is not required for figures from a journal article.*

whether cannabis truly demonstrated a causal influence on academic achievement, or whether students who exhibited a vulnerability to substance use also exhibit a tendency toward low school achievement.

Motivation for Achievement: University and Employment

Longitudinal studies have also examined motivation through university and employment status. A 25-year longitudinal study in New Zealand revealed that increased marijuana use was associated with school dropout, failure to enter college, and failure to obtain a university degree (Fergusson et al., 2003). While some studies show that young cannabis users are at greater risk for unemployment (eg, Brook, Adams, Balka, & Johnson, 2002), others demonstrate that many frequent cannabis users earn more than non-users (eg, Kaestner, 1994). Further, one study showed that university cannabis users were more likely to pursue graduate study (Hochman & Brill, 1973). Thus, there is some evidence that cannabis use is distinct from motivation toward higher education and employment achievement. It is possible that there are subsets of cannabis users who are driven to achieve success, both academically and occupationally, regardless of cannabis use.

The relationship between cannabis use and educational and occupational achievements remains unclear, with conflicting findings. It is likely that variability in research methodology contributes to this confusion. Researchers have proposed several hypotheses for the link between motivation for achievement and chronic cannabis use.

Theories of Motivation for Achievement

Cannabis Use Leads to Educational Underachievement

One theory is that cannabis use leads to educational underachievement, either through cognitive or motivational deficits (Solowij, 1998), or through the encouragement of anticonventional behaviors (eg, Kandel, Davies, Karus, & Yamaguchi, 1986). Support for this theory is weak, because those who use cannabis also often experience other disadvantages, such as, low socioeconomic status (SES), childhood adversity, early-onset behavioral difficulties, and negative peer influences. Many studies suggest that, with increasing age and cannabis use, the effects on achievement actually decrease, rather than increase (eg, Fergusson et al., 2003). Although controlling for these factors reduced the relationship between cannabis use and early school leaving, a significant association remained for those who initiated use early in life (Fergusson et al., 2003). Therefore, while experimentation with cannabis use may be normative during high school, earlier initiation may be suggestive of other behavioral problems (Wills, McNamara, Vaccaro, & Hirky, 1996).

Furthermore, metaanalyses of the effect of chronic cannabis use on cognition found only a small effect on two cognitive domains: learning, and forgetting/retrieval (Grant, Gonzalez, Carey, Natarajan, & Wolfson, 2003).

Other cognitive domains (eg, attention, reaction time, executive functioning) demonstrated effect sizes with confidence intervals overlapping zero, suggesting little, if any, effect of cannabis use (Grant et al., 2003). In addition, these studies included participants who used more frequently (at least 4 days per week) and for longer periods of time (about 5 years) than most adolescents included in the studies on educational achievement. Thus, there is no strong evidence to suggest that cannabis use leads to such significant impairments that adolescents are no longer capable of completing school.

Educational Underachievement Leads to Cannabis Use

A second theory explaining the link between motivation for achievement and cannabis suggests that the experience of educational underachievement encourages cannabis use, similar to the social development model of Hawkins and Weis (1985) where school failure leads to education demotivation and, subsequently, association with deviant peers and substance use. Evidence for this theory is weak; when allowances are made for confounding factors, educational failure was not associated with increased risk of later cannabis use (eg, Schulenberg, Bachman, O'Malley, & Johnston, 1994). Later cannabis use was not associated with earlier academic deficits, but rather with socially disadvantaged backgrounds, childhood adversity, parents with prior criminality or substance use, early conduct problems, novelty-seeking behavior, and affiliations with delinquent or substance-abusing peers—suggesting later cannabis use was associated with a pattern of many factors related to the development of externalizing behaviors, including substance use. Cannabis use had the greatest impact on young users, with impacts declining with increasing age (Schulenberg et al., 1994). In fact, illicit drug use in young adulthood was unrelated to academic motivation. Therefore, most evidence does not support the theory that school failure leads to cannabis use.

Propensity for Problem Behaviors Leads to Both Cannabis Use and Educational Underachievement

The third and final theory explaining the link between low motivation for academic achievement and chronic cannabis use is that a tendency toward anticonventional behaviors, and association with peer groups engaging in these behaviors, leads to lower prioritization of education and occupation and the selection of an alternative lifestyle. Jessor and Jessor (1977) first proposed this problem behavior theory, stating that adolescents exhibit one common syndrome representing a penchant for a range of problem behaviors, including relationships with deviant peers. Donovan and Jessor (1985) suggested that this represents general unconventionality, assumed to stem from both personal and environmental influences that increase problem behavior in general. In other words, individuals with this syndrome exhibit many problematic behaviors that may or may not include cannabis use.

Although this theory is vulnerable to the risk of circular logic, it is generally supported, and suggests that the effects of early cannabis use are more strongly related to the social context of cannabis use, rather than true physiological effects of the drug (eg, Fergusson & Horwood, 1997; for review, see Lynskey & Hall, 2000).

Limitations of Research on Motivation

As mentioned previously, cross-sectional methodologies make causality nearly impossible to determine and are vulnerable to overinterpretation. For example, Brown et al. (2001) analyzed cross-sectional data over a 20-year period and found variability in the relationship between low academic motivation and cannabis use, as this link was identified during some time periods but not during others. Thus, cross-sectional studies reporting this relationship (eg, Wright & Palfai, 2012) may not reveal the full complexity of the interaction of factors, as it is unclear which variable presented first in the individual. Further, many longitudinal studies first assess subjects in their teens; in many cases, cannabis initiation may have already occurred.

Another difficulty in drawing conclusions from the research to date is the variability in defining "chronic" users: definitions vary from any "current" users of cannabis, to those who use 4 or more days per week, to those who use daily. Many cannabis users also use other substances (such as alcohol or other illicit drugs), making it difficult to determine the effects of cannabis alone (Hall, Solowij, & Lemon, 1994). Researchers may also group multiple forms of substance use into one variable (eg, Duncan, Duncan, Biglan, & Ary, 1998), despite evidence that substances differ in their impact on educational or occupational attainment, such as, by gender (Mensch & Kandel, 1988) and by race (Ellickson et al., 1998). Cultural differences also exist; in regions where cannabis use is widespread, such as Jamaica and Costa Rica, those with steady jobs smoked more cannabis than those who were unemployed or had an unstable work history (Carter, Coggins, & Doughty, 1980). Thus, results of broad, longitudinal studies with diverse populations do not necessarily generalize to all cannabis users.

Finally, and perhaps most significantly, numerous confounds impact the relationship between cannabis use and motivation for achievement. These include factors, such as, parental age and education, socioeconomic status, changes in family environment, childhood verbal, physical, or sexual abuse, parental attachment, parental drug use, childhood cognitive and educational ability, behavioral problems, and other illicit substance use.

Early studies made little attempt to take these into account, and while more recent studies do control for some of these confounding factors, there is variability in the precision of measurement. In addition, other confounds may be present and, as yet, unidentified by researchers.

MOTIVATION: LABORATORY MEASURES

Despite the many studies examining chronic cannabis users' motivation through self-report and educational and occupational achievement, comparatively few attempted to measure motivation in a controlled, laboratory setting. The results of these studies are generally mixed, again, perhaps due to the difficulty in operationalizing such a construct. Motivation is often operationalized as the quality or completeness of task performance in order to gain some reinforcer, usually money. Yet there is variability in the extent to which monetary rewards are motivating for participants, due to differences in socioeconomic status or in the value of, or desire for, money. This variability makes it quite difficult to design a study where the reinforcer motivates all participants to the same degree. Furthermore, a distinction should be made between assessing cannabis users' motivation while under the acute effects of the drug and assessing whether a more persistent impact on motivation exists in long-term chronic cannabis users.

Laboratory Measures of Motivation: Acute Effects

To examine the hypothesis that cannabis intoxication impacts motivation, Cherek, Lane, and Dougherty (2002) designed a two-option experimental task. The "work" option required participants to systematically increase output to earn a monetary reward, while the "nonwork" alternative did not require any output to earn money. Participants under the influence of cannabis switched out of the "work" option and into the "nonwork" option earlier than in a placebo condition, despite being told that the nonwork option would result in less money earned. While the implications of the results are limited by a small sample size ($N = 5$), this may be interpreted as revealing lower motivation in the user group, particularly given that increasing the monetary reinforcement resulted in staying in the "work" option longer.

Many other studies, however, find no evidence for decreased motivation in users under the influence of cannabis. For example, although researchers found that both monetary compensation and verbal encouragement were insufficient to improve performance on cognitive tasks (Creason & Goldman, 1981; Pihl & Sigal, 1978), users performed as well as nonusers on the tasks, suggesting that they were already putting forth full effort. Kagel et al. (1980) analyzed the effect of cannabis intoxication on work output in a residential environment, and found no difference in total output between cannabis and placebo conditions. Although work output decreased immediately after cannabis administration, participants increased productivity after the acute effects of the drug had subsided. Cohen (1976) found no differences between users and nonusers in motivation, productivity, or clarity in work tasks during a 64-day intoxication period of smoking at least one joint per day. Another study demonstrated that, in a residential environment, administration of cannabis actually resulted in an increase in work activities (a nonsense-word alphabetizing task; Foltin et al., 1990). All six participants showed increases in work activities while under the effects of cannabis, relative to a placebo condition. Overall, there is little laboratory evidence for decreased motivation when under the acute effects of cannabis.

Laboratory Measures of Motivation: Effects of Chronic Use

Recently, laboratory studies of motivation in cannabis users have focused on the effects of chronic cannabis use, rather than intoxication. Lane, Cherek, Pietras, and Steinberg (2005) assessed the performance of adolescent daily cannabis users who were not currently under the influence of cannabis, using the two-option experimental motivational measure by Cherek et al. (2002) described previously. Relative to nonusers, cannabis users switched to the "nonwork" option earlier in the experiment and earned more total money from that option than from the "work" option, which the authors interpreted as suggestive of lower motivation (Lane et al., 2005). However, total money earned was not reported, so it was unclear whether the switch was actually detrimental for the cannabis users in amount earned (Fig. 30.3).

Researchers have also examined motivational inhibitory control by assessing risk-taking during gambling-type tasks. Most studies identify poorer performance on gambling tasks in chronic cannabis users, relative to nonusers (eg, Fridberg et al., 2010). One study found that users took greater risks on a gambling task measuring motivational inhibitory control, despite no group differences in cognitive inhibitory control (inhibiting a response in reaction to a stimulus; Griffith-Lendering, Huijbregts, Vollebergh, & Swaab, 2012). However, it is interesting that users and nonusers did not differ in the amount of "points" won or lost on the task, suggesting that greater risk-taking did not have negative consequences for the participants.

Positron Emission Tomography (PET) imaging also suggests differences in brain activity between chronic cannabis users and nonusers while completing

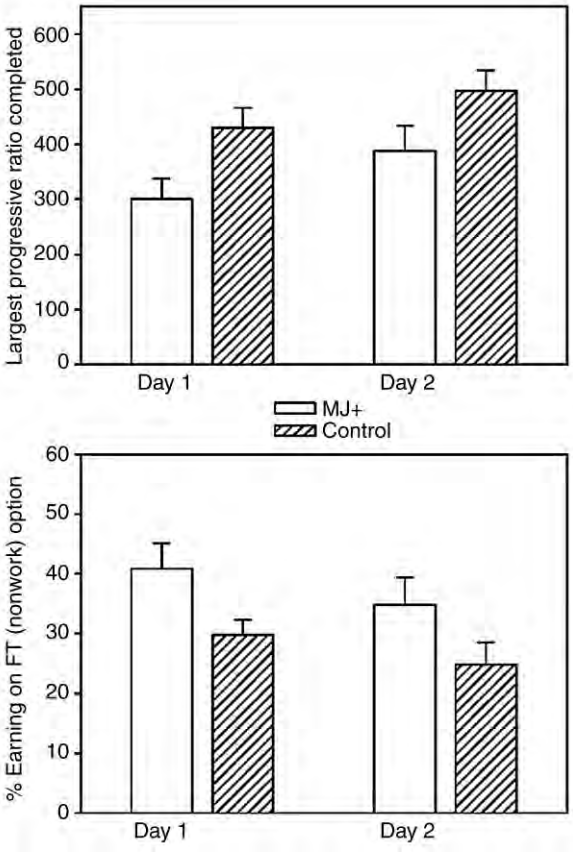


FIGURE 30.3 Performance on a motivational task in marijuana users and controls. Marijuana users completed a smaller ratio on the "work" option of the task relative to controls on both days. Marijuana users also obtained a greater percent of their total earnings from the "nonwork" option of the task on both days. *Source: Lane, Cherek, Pietras, & Steinberg (2005). Permission is not required; license obtained.*

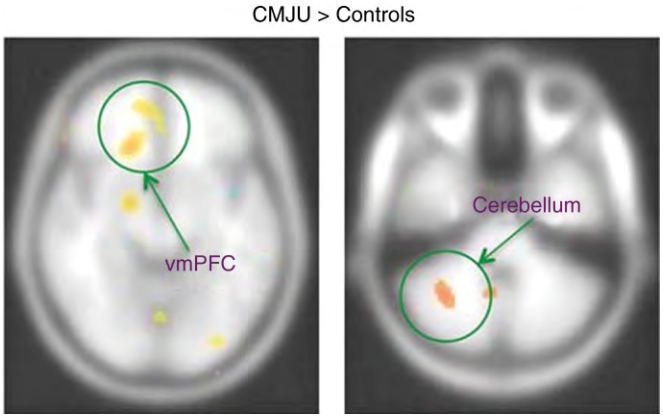


FIGURE 30.4 Differences in PET activation between chronic marijuana users and controls during the Iowa Gambling Task (IGT). Results shown indicate that chronic marijuana users showed greater activity in the vmPFC and in the cerebellum, compared with control subjects. *Source: Vaidya et al. (2012). Open Access article; permission not required.*

gambling tasks. On the Iowa Gambling Task (IGT; Bechara, Damasio, Damasio, & Anderson, 1994), duration of cannabis use correlated with greater activity in the ventromedial prefrontal cortex, an area involved in the mesolimbic dopamine reward system described earlier (Fig. 30.4), despite cannabis users performing equally well as nonusers on the standard version of the task (Fig. 30.5; Vaidya et al., 2012). This suggests that prolonged exposure to cannabis could affect neural reward circuitry, though not necessarily in a way that impacts performance on a motivation task. However, participants also reported other drug use, both legal and illicit, making it difficult to determine whether the effects were due to cannabis or other substances. Ultimately, this evidence suggests that cannabis users demonstrate increased sensitivity for immediate rewards, which could weaken motivational inhibitory control and increase the propensity toward future risk-taking or reward-seeking behavior (Nestor, Hester, & Garavan, 2010). This may explain why users sometimes exhibit greater risk-taking performance. However, longitudinal studies are necessary to determine whether this is a preexisting vulnerability or a deficit that develops subsequent to cannabis use.

Nevertheless, not all laboratory evidence supports the presence of motivational deficits in chronic cannabis users. Using a spatial recognition task with monetary reinforcement, researchers demonstrated that cannabis users performed equally well relative to nonusers and tobacco smokers, with performance improving similarly when monetary reinforcers were provided to enhance motivation (Martin-Soelch et al., 2009). Further, there were no group differences in the amount of money earned performing the task. In addition, a comparison of cannabis users and nonusers during performance on a reaction time task assessing motivation for monetary reward revealed no group differences (Voon et al., 2014), although,

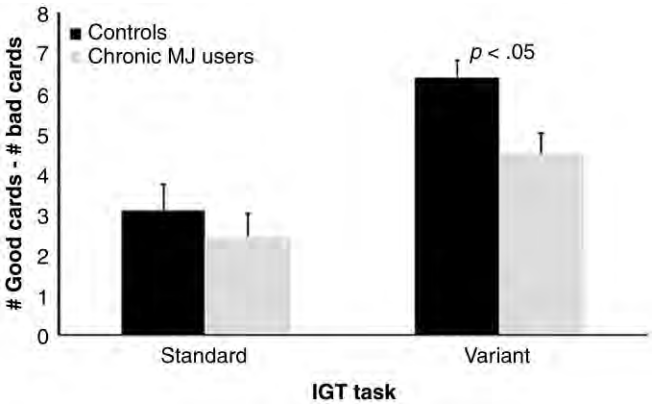


FIGURE 30.5 Performance differences between chronic marijuana users and controls on the Iowa Gambling Task (IGT). There were no group differences in performance on the standard version of the IGT; however, chronic marijuana users performed worse than controls on the more challenging variant version. *Source: Vaidya et al. (2012). Open Access article; permission is not required.*

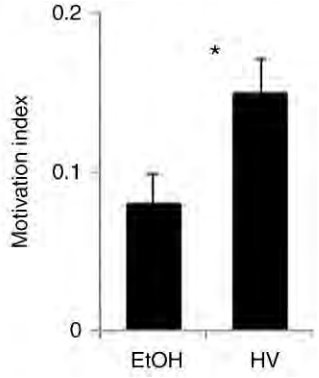


FIGURE 30.6 Motivational index scores in chronic alcohol users (EtOH) relative to healthy volunteers (HV). Alcohol-dependent participants demonstrated decreased motivation to reward feedback than both cannabis-dependent participants and healthy volunteers. There were no significant differences in motivation between cannabis-dependent participants and healthy volunteers. *Source: Voon et al. (2014). Open Access article; permission is not required.*

interestingly, alcohol-dependent participants did exhibit decreased motivation (Fig. 30.6).

Given the variability in cannabis users' response to monetary rewards, Macher and Earleywine (2012) selected a unique method for enhancing motivation. Prior to administering a battery of cognitive tests, the researchers informed one group of chronic users "it is important that you try your very best on these tasks, because this research will be used to support legislation on marijuana policy." Interestingly, those users did significantly better (by nearly one standard deviation) on the California Verbal Learning Test-II, a neuropsychological test of learning and memory, relative to users who were not given that statement (Fig. 30.7). Further, their cognitive abilities were comparable to nonusers, suggesting that frequent cannabis users may not demonstrate learning and memory deficits provided they are inherently motivated to perform well on the assessment.

Despite the widespread notion of a link between motivation and cannabis use, few research studies on cognition in cannabis users include performance validity tests, or effort tests (tests which ensure that participants are motivated, and putting forth sufficient effort on assessments to obtain valid results). In fact, the authors were able to find only one study examining cognitive functioning in chronic cannabis users that used a measure of effort. Rabin, Zakzanis, Daskalakis, and George (2013) used the Test of Memory Malingering (TOMM; Tombaugh, 1997) "as a proxy for motivation and adequacy of effort."

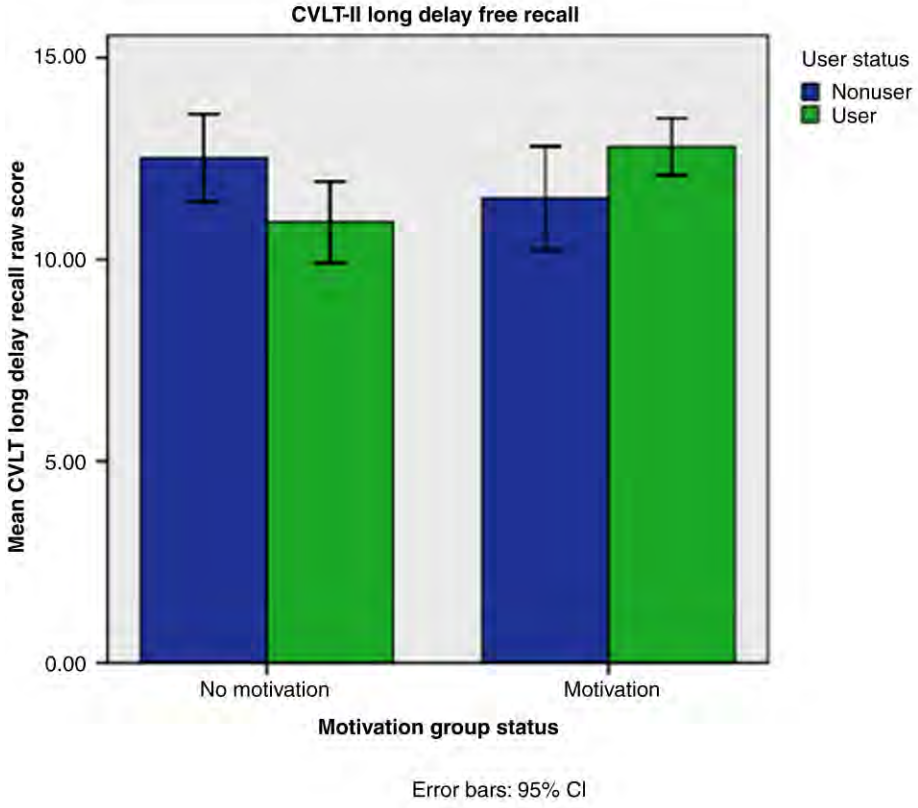


FIGURE 30.7 Learning and memory performance on the California Verbal Learning Test-II (CVLT-II) in chronic cannabis users and nonusers, in motivated and nonmotivated conditions. Cannabis users given a motivational statement demonstrated better learning and memory performance than those not given the statement, and their performance was consistent with nonusers. *Source: Macher and Earleywine (2012). Permission obtained from Routledge Taylor & Francis.*

However, the authors did not clarify whether any participants failed this measure of effort, and if they did, whether their results were excluded from the study. All participants in the study by Macher and Earleywine (2012) passed the three effort tests administered; however, the lower performance of those who were not given the motivational statement suggests that they may not have put forth their absolute best effort. Further research examining the effect of this motivational statement is necessary, and effort tests to ensure adequate motivation should be included in all studies, before drawing conclusions about the effects of chronic cannabis use on cognition.

CONCLUDING REMARKS

The relationship between chronic cannabis use and motivation remains unclear, but research has revealed some patterns. Most evidence suggests that chronic cannabis use does not lead to an "amotivational syndrome," resulting in school dropout or poor occupational success. Many contributing factors exist, and it is most likely that individuals with multiple risk factors are drawn to various problem behaviors, of which cannabis use is often only one. However, laboratory motivational measures provide mixed evidence, some in support and some in opposition. Results indicate that cannabis users do exhibit enhanced motivation when rewards are inherently motivating for them. However, most evidence suggests that users are often differentially motivated to work toward monetary reinforcers, relative to nonusers. Further research utilizing laboratory tasks with the supplement of neuroimaging will be critical in understanding the dopamine reward system in chronic cannabis users and its impact on motivation.

MINI-DICTIONARY

"Amotivational syndrome" A proposed syndrome of chronic cannabis users, including symptoms, such as, apathy, lethargy, lack of motivation, and difficulty concentrating. Most research findings do not support this syndrome.
Cognitive inhibitory control The ability to inhibit a response in reaction to a stimulus.
Effort tests Measures used during neuropsychological assessment to ensure that examinees are giving adequate effort for valid test results.
Hawkins and Weis's social development model (1985) A hypothesis that school failure leads to education demotivation, and, subsequently, association with deviant peers and substance use (including cannabis use)
Iowa Gambling Task (IGT) An empirically-validated measure of complex monetary decision-making.
Jessor and Jessor's problem behavior theory (1977) A theory that adolescents exhibit one common syndrome representing a penchant for a range of problem behaviors that may or may not include cannabis use.
Mesolimbic dopamine system A system of the brain involved in motivation, reinforcement, and reward circuitry.
Motivational inhibitory control The control over one's feeling of inherent motivation.
Positron emission tomography (PET) A functional imaging technique producing a three-dimensional image of metabolic activity in the brain.
Test of memory malingering (TOMM) An empirically-validated measure of effort used in neuropsychological assessment.

References

Barnwell, S. S., Earleywine, M., & Wilcox, R. (2006). Cannabis, motivation, and life satisfaction in an internet sample. *Substance Abuse Treatment, Prevention and Policy*, *1*, 2–9.

Bechara, A., Damasio, A. R., Damasio, H., & Anderson, S. W. (1994). Insensitivity to future consequences following damage to human prefrontal cortex. *Cognition*, *50*, 7–15.

Bloomfield, M. A. P., Morgan, C. J. A., Kapur, S., Curran, H. V., & Howes, O. D. (2014). The link between dopamine function and apathy in cannabis users: an [^{18}F]-Dopa PET imaging study. *Psychopharmacology*, *231*, 2251–2259.

Brook, J. S., Balka, E. B., & Whiteman, M. (1999). The risks for late adolescence of early adolescent marijuana use. *American Journal of Public Health*, *89*, 1549–1554.

Brook, J. S., Adams, R. E., Balka, E. B., & Johnson, E. (2002). Early marijuana use: risks for the transition to young adulthood. *Psychological Medicine*, *32*, 79–91.

Brown, T. N., Schulenberg, J., Bachman, J. G., O'Malley, P. M., & Johnston, L. D. (2001). Are risk and protective factors for substance use consistent across historical time? National data from the high school classes of 1976 through 1997. *Prevention Science*, *2*(1), 29–43.

Bryant, A. L., & Zimmerman, M. A. (2002). Examining the effects of academic beliefs and behaviors on changes in substance use among urban adolescents. *Journal of Educational Psychology*, *94*(3), 621–637.

Carter, W. E., Coggins, W., & Doughty, P. L. (1980). *Cannabis in Costa Rica: a study of chronic marijuana use*. Philadelphia: Institute for the Study of Human Issues.

Cherek, D. R., Lane, S. D., & Dougherty, D. M. (2002). Possible amotivational effects following marijuana smoking under laboratory conditions. *Experimental and Clinical Psychopharmacology*, *10*(1), 26–38.

Cohen, S. (1976). The 94-day cannabis study. *Annals of the New York Academy of Sciences*, *282*, 211–220.

Creason, C. R., & Goldman, M. (1981). Varying levels of marijuana use by adolescents and the amotivational syndrome. *Psychological Reports*, *48*, 447–454.

Donovan, J. E., & Jessor, R. (1985). Structure of problem behavior in adolescence and young adulthood. *Journal of Consulting and Clinical Psychology*, *53*, 890–904.

Duncan, D. F. (1987). Lifetime prevalence of "amotivational syndrome" among users and non-users of hashish. *Psychology of Addictive Behaviors*, *1*, 114–119.

Duncan, S. C., Duncan, T. E., Biglan, A., & Ary, D. (1998). Contributions of the social context to the development of adolescent substance use: a multivariate latent growth modeling approach. *Drug and Alcohol Dependence*, *50*, 57–71.

Earleywine, M. (2003). *Understanding Marijuana*. New York, NY: Oxford University Press.

Ellickson, P., Bui, K., Bell, R., & McGuigan, K. A. (1998). Does early drug use increase the risk of dropping out of high school? *Journal of Drug Issues*, *28*(2), 357–380.

Ellickson, P. L., Martino, S. C., & Collins, R. L. (2004). Marijuana use from adolescence to young adulthood: multiple developmental trajectories and their associated outcomes. *Health Psychology*, *23*(3), 299–307.

Ensminger, M. E., Lamkin, R. P., & Jacobson, N. (1996). School leaving: a longitudinal perspective including neighborhood effects. *Child Development*, *67*, 2400–2416.

Fergusson, D. M., & Horwood, L. J. (1997). Early onset cannabis use and psychosocial adjustment in young adults. *Addiction*, *92*, 279–296.

Fergusson, D. M., Horwood, L. J., & Beautrais, A. L. (2003). Cannabis and educational achievement. *Addiction*, *98*, 1681–1692.

Foltin, R. W., Fischman, M. W., Brady, J. V., Bernstein, D. J., Capriotti, R. M., Nellis, M. J., & Kelly, T. H. (1990). Motivational effects of smoked marijuana: behavioral contingencies and low-probability activities. *Journal of the Experimental Analysis of Behavior*, *53*, 5–19.

Fridberg, D. J., Queller, S., Ahn, W. Y., Kim, W., Bishara, A. J., Busemeyer, J. R., et al. (2010). Cognitive mechanisms underlying risky decision-making in chronic cannabis users. *Journal of Mathematical Psychology*, *54*(1), 28–38.

Grant, I., Gonzalez, R., Carey, C. L., Natarajan, L., & Wolfson, T. (2003). Non-acute (residual) neurocognitive effects of cannabis use: a meta-analytic study. *Journal of the International Neuropsychological Society*, *9*, 679–689.

Griffith-Lendering, M. F. H., Huijbregts, S. C. J., Vollebergh, W. A. M., & Swaab, H. (2012). Motivational and cognitive inhibitory control in recreational cannabis users. *Journal of Clinical and Experimental Neuropsychology*, *34*(7), 688–697.

Halikas, J. A., Weller, R. A., Morse, C., & Shapiro, T. (1982). Incidence and characteristics of amotivational syndrome, including associated findings, among chronic marijuana users. In National Institute on Drug Abuse (Ed.), *Marijuana and youth: Clinical observations on motivation and learning* (pp. 11–23). Rockville, MD: National Institute on Drug Abuse.

Hall, W., Solowij, N., & Lemon, J. (1994). *The health and psychological consequences of cannabis use*. Canberra, ACT: Commonwealth Department of Human Services and Health.

Hawkins, J. D., & Weis, J. G. (1985). The social development model: an integrated approach to delinquency prevention. *Journal of Primary Prevention*, *6*(2), 73–97.

Hochman, J. S., & Brill, N. Q. (1973). Chronic marijuana use and psychosocial adaptation. *American Journal of Psychiatry*, *130*, 132–139.

Jessor, R., & Jessor, S. L. (1977). *Problem behavior and psychosocial development: a longitudinal study of youth*. New York, NY: Academic Press.

Jones, S. P., & Heaven, P. C. L. (1998). Psychosocial correlates of adolescent drug-taking behavior. *Journal of Adolescence*, *21*, 127–134.

Kaestner, R. (1994). The effects of illicit drug use on the labor supply of young adults. *Journal of Human Resources*, *29*, 123–126.

Kagel, J. H., Battalio, R. C., & Miles, C. G. (1980). Marihuana and work performance: results from an experiment. *Journal of Human Resources*, *15*(3), 373–395.

Kandel, D. B., Davies, M., Karus, D., & Yamaguchi, K. (1986). The consequences in young adulthood of adolescent drug involvement. *Archives of General Psychiatry*, *43*, 746–754.

Kolansky, H., & Moore, W. T. (1972). Toxic effects of chronic marihuana use. *Journal of the American Medical Association*, *222*(1), 35–41.

Kouri, E., Pope, H. G., Yurgelun-Todd, D., & Gruber, S. (1995). Attributes of heavy vs occasional marijuana smokers in a college population. *Biological Psychiatry*, *38*, 475–481.

Lane, S. D., Cherek, D. R., Pietras, C. J., & Steinberg, J. L. (2005). Performance of heavy marijuana-smoking adolescents on a laboratory measure of motivation. *Addictive Behaviors*, *30*, 815–828.

Lynskey, M., & Hall, W. (2000). The effect of adolescent cannabis use on educational attainment: a review. *Addiction*, *95*(11), 1621–1630.

Macher, R. B., & Earleywine, M. (2012). Enhancing neuropsychological performance in chronic cannabis users: the role of motivation. *Journal of Clinical and Experimental Neuropsychology*, *34*(4), 405–415.

Martin-Soelch, C., Leenders, K. L., Chevalley, A. -F., Missimer, J., Kunig, G., Magyar, S., Mino, A., et al. (2001). Reward mechanisms in the brain and their role in dependence: evidence from neurophysiological and neuroimaging studies. *Brain Research Reviews*, *36*, 139–149.

Martin-Soelch, C., Kobel, M., Stoecklin, M., Michael, T., Weber, S., Krebs, B., & Opwis, K. (2009). Reduced response to reward in smokers and cannabis users. *Neuropsychobiology*, *60*, 94–103.

Mensch, B. S., & Kandel, D. B. (1988). Dropping out of high school and drug involvement. *Sociology of Education*, *61*, 95–113.

Musty, R. E., & Kaback, L. (1995). Relationships between motivation and depression in chronic marijuana users. *Life Sciences*, *56*, 2151–2158.

Nelson, P. L. (1994-5). Cannabis amotivational syndrome and personality trait absorption: a review and reconceptualization. *Imagination, Cognition and Personality*, *14*(1), 43–58.

Nestor, L., Hester, R., & Garavan, H. (2010). Increased ventral striatal BOLD activity during non-drug reward anticipation in cannabis users. *Neuroimage*, *49*(1), 1133–1143.

Pihl, R. O., & Sigal, H. (1978). Motivation levels and the marihuana high. *Journal of Abnormal Psychology*, *87*(2), 280–285.

Rabin, R. A., Zakzanis, K. K., Daskalakis, Z. J., & George, T. P. (2013). Effects of cannabis use status on cognitive function, in males with schizophrenia. *Psychiatry Research*, *206*, 158–165.

Resnick, M. D., Bearman, P. S., Blum, R. W., Bauman, K. E., Harris, K. M., Jones, J., et al. (1997). Protecting adolescents from harm: findings from the National Longitudinal Study on Adolescent Health. *Journal of the American Medical Association*, *278*, 823–832.

Schulenberg, J., Bachman, J. G., O'Malley, P. M., & Johnston, L. D. (1994). High school educational success and subsequent substance use: a panel analysis following adolescents into young adulthood. *Journal of Health and Social Behavior*, *35*, 45–62.

Simons, J. S., Christopher, M. S., Oliver, M. N. I., & Stanage, E. J. (2006). A content analysis of personal strivings: associations with substance use. *Addictive Behaviors*, *31*, 1224–1230.

Smith, D. E. (1968). The acute and chronic toxicity of marijuana. *Journal of Psychedelic Drugs*, *2*(1), 37–48.

Smith, D. E. & Seymour, R. B. (1982). Clinical perspectives on the toxicity of marijuana: 1967–1981. In Marijuana and youth: Clinical observations on motivation and learning (DHHS Publication No. ADM 82-1186). Washington, D.C.: U.S. Government Printing Office.

Solowij, N. (1998). *Cannabis and cognitive functioning*. Cambridge, UK: Cambridge University Press.

Soueif, M. I. (1976). Differential association between chronic cannabis use and brain function deficits. In R. L. Dornbush, A. M. Freedman, & M. Finx (Eds.), *Annals of the New York Academy of Sciences: Vol. 282. Chronic cannabis use* (pp. 323–343). New York, NY: New York Academy of Sciences.

Tombaugh, T. N. (1997). The Test of Memory Malingering (TOMM): normative data from cognitively intact and cognitively impaired individuals. *Psychological Assessment*, *9*, 260–268.

Vaidya, J. G., Block, R. I., O'Leary, D. S., Ponto, L. B., Ghoneim, M. M., & Bechara, A. (2012). Effects of chronic marijuana use on brain activity during monetary decision-making. *Neuropsychopharmacology*, *37*, 618–629.

Voon, V., Irvine, M. A., Derbyshire, K., Worbe, Y., Lange, I., Abbott, S., Morein-Zamie, S., et al. (2014). Measuring "waiting" impulsivity in substance addictions and binge eating disorder in a novel analogue of rodent serial reaction time task. *Biological Psychiatry*, *75*, 148–155.

Wills, T. A., McNamara, G., Vaccaro, D., & Hirky, A. E. (1996). Escalated substance use: a longitudinal grouping analysis from early to middle adolescence. *Journal of Abnormal Psychology*, *105*(2), 166–180.

Wright, L. L., & Palfai, T. P. (2012). Life goal appraisal and marijuana use among college students. *Addictive Behavior*, *37*(7), 797–802.

CHAPTER

31

Cannabis Use and Its Association to Mental Illness: A Focus on Mood and Anxiety Disorders

S. Lev-Ran[*,**], *D. Feingold*[*,†]

*Department of Psychiatry, Sheba Medical Center, Tel Hashomer, Israel
**Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
†Ariel University, Ariel, Israel

SUMMARY POINTS

- This chapter reviews the association between cannabis use and mental illness, focusing on mood and anxiety disorders.
- There is strong evidence suggesting that cannabis use and mental illness highly cooccur; that is, that mental illness is common among cannabis users (compared to nonusers), and that cannabis use is common among individuals with mental illness (compared to those without mental illness).
- Notwithstanding, evidence indicates that the majority of cannabis users do not suffer from concurrent mental illness and vice versa.
- Longitudinal studies reveal conflicting results regarding the association between cannabis use and future incidence of mood and anxiety disorders, and suffer from methodological challenges.
- The inverse notion that mood and anxiety disorders are associated with future initiation of mood and anxiety disorders does not receive sufficient support in longitudinal studies.
- Causal association between cannabis use and mood and anxiety disorders is yet well established. Cross-sectional association between the two may be attributed to cooccurring sociodemographic and clinical variables.
- Cannabis use may have a negative effect on the course of mood and anxiety disorders, and on treatment efficacy for these disorders.

KEY FACTS ON MENTAL ILLNESS

- Mental illness is generally associated with disturbances in mood, thought, and/or behavior.
- Mental illness affects about 46% of the population in the Western world over their lifetime course, and about 30% of the population over a 1-year course.
- Each year, mental illness accounts for about 25% of all health loss due to disability worldwide, and approximately 7% of the life-years lost.
- People with mental illness are more prone than people without mental illness to use addictive substances like alcohol, cannabis, and other drugs.
- There are various means for treating metal illness, including psychiatric medication, psychotherapy, and vocational interventions.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00035-1
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

AOR	Adjusted odds ratio
BPD	Bipolar disorder
CUD	Cannabis use disorders
GAD	Generalized anxiety disorder
MDD	Major depressive disorder
NESARC	National epidemiologic survey on alcohol and related conditions
OR	Odds ratio
THC	Tetrahydrocannabinol

INTRODUCTION

Cannabis is the most commonly used illicit drug, with estimated worldwide 12-month prevalence of 2.8–4.5% of the population (Degenhardt et al., 2011; UNODC, 2012), or roughly between 125 to 203 million individuals (Degenhardt & Hall, 2012). Mental illness steadily affects about 30% of the population in high-income countries each year (Kessler et al., 2005), accounting for about one quarter of all health loss due to disability (World Health Organization, 2002) and approximately 7% of the global loss of life-years worldwide (Whiteford et al., 2013). There is strong scientific evidence indicating an association between cannabis use and mental illness. This chapter will review this association, addressing both cross-sectional data on the prevalence of mental illness among cannabis users and vice versa, as well as longitudinal data exploring potential causal association between cannabis use and mental illness. As the association between cannabis use and psychotic disorders is covered elsewhere in this book, we will focus on longitudinal data exploring the association between cannabis use and two major groups of psychiatric disorders: mood disorders, and anxiety disorders.

Several methodological challenges should be recognized a priori when addressing the association between cannabis use and mental illness. One of the most evident is the lack of consistency with regard to the measures used for both cannabis use and mental illness. For example, while some studies define exposure to cannabis as the mere use of cannabis, or in some cases according to the frequency of cannabis use (monthly/weekly/daily), other studies refer exclusively to disorders associated with pathological cannabis use, that is, cannabis abuse or cannabis dependence, collectively termed "cannabis use disorder" (CUD). Moreover, there is no standard measure for cannabis—not only are there multiple methods of ingestion, cannabis strains differ substantially in their potency (as measured by tetrahydrocannabinol (THC) concentrations), and cannabis "joints" may include substantially different amounts of cannabis (Mariani, Brooks, Haney, & Levin, 2011). Similar discrepancies can be found in the definition of mental illness used. Though mental illness is usually defined using criteria-based diagnosis, as defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM) (American Psychiatric Association, 2013) or the International Classification of Diseases (ICD) (World Health Organization, 1992), many studies exploring the association between cannabis use and mental illness define mental illness as presence of symptoms associated with the illness, rather than a full diagnosis. Furthermore, studies vary in terms of disorders included in the general term "any mental illness," and the particular disorders included when dealing with "mood" or "anxiety disorders." Accordingly, presenting standardized and comparable data regarding the association between cannabis use and mental illness is challenging. Nevertheless, it seems like many of the limitations mentioned above will be part of any report exploring the association between cannabis use and mental illness, and the aim of this chapter is to represent the current state of knowledge on this association.

COOCCURRENCE OF CANNABIS USE AND MENTAL ILLNESS

Cannabis Use Among Individuals With Mental Illness

Substantial data regarding the cooccurrence of cannabis use and mental illness is derived from large-scale population-based samples focusing on substance use and mental illness in the general population. In the United States, three major cohorts have sought to investigate the cooccurrence of substance use, including cannabis use, and mental illness: the Epidemiological Catchment Area (ECA) conducted in the early 1980s (Regier et al., 1984), the National Comorbidity Survey (NCS) conducted in the early 1990s (Kessler et al., 1994), and the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC), conducted in the early 2000s (Grant & Kaplan, 2005). Similar large-scale projects were carried out elsewhere worldwide, including Australia, (Degenhardt, Hall, & Lynskey, 2000) and Europe (Hibell et al., 2012).

The NESARC data presents detailed information on cannabis use and mental illness (Fig. 31.1), reporting that among individuals with any mental illness prevalence of past-year cannabis use was 9.8%, compared to 1.7% among individuals without mental illness. Though rates of cannabis use in this sample are reported to be lower than those in other population-based samples (Grucza, Abbacchi, Przybeck, & Gfroerer, 2007), these data nevertheless emphasize the increased rates of cannabis use among individuals with mental illness. Individuals with mental illness were almost 10 times as likely to use cannabis weekly and more than 5 times as likely to use cannabis less than weekly, compared to individuals without mental illness. Furthermore, it is suggested that 83% of

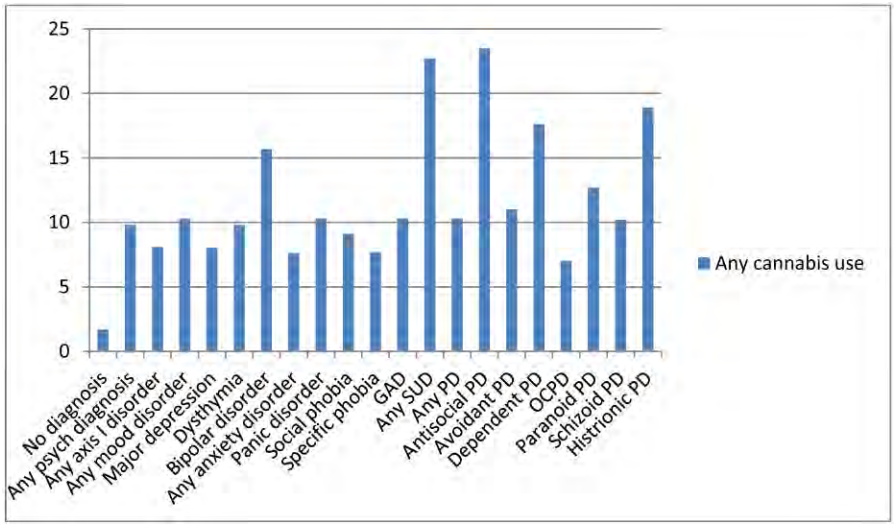


FIGURE 31.1 **Prevalence of cannabis use according to specific mental illness.** This graph describes the prevalence of past-year cannabis use among individuals suffering from various mental illnesses. For comparison, the bar on the left indicates the prevalence of cannabis use among individuals with no mental illness (Lev-Ran et al., 2013b).

all cannabis use in the United States is attributed to individuals who had a diagnosis of past-year mental illness (Lev-Ran, Le Foll, McKenzie, George, & Rehm, 2013b). Among individuals with mental illness, prevalence of cannabis use increases with the increase in the number of psychiatric diagnoses (Fig. 31.2). This data is important in light of finding indicating that the majority of individuals diagnosed with mental illness have an additional comorbid disorder (Gadermann, Alonso, Vilagut, Zaslavsky, & Kessler, 2012; Regier et al., 1990).

The growing body of cross-sectional evidence on the association of cannabis use and mood disorders led Degenhardt et al. (2003) to conclude that "there is increasing evidence that regular cannabis use and depression occur together more often than we might expect by chance" (Degenhardt et al. 2003, p. 1497). Lifetime prevalence of cannabis use among individuals with major depressive disorder (MDD) was 60% (Chen, Wagner, & Anthony, 2002), and results from the National Survey on Drug Use and Health show that 25% of the teenagers with past-year depression also reported they used cannabis in the past year, compared to 12% who did not have depression (SAMHSA, 2007). A 10-year longitudinal study conducted in Europe revealed that prevalence of lifetime cannabis use was significantly higher among teenagers with lifetime depression [OR = 2.7 (1.6–4.4)], dysthymia [OR = 6.0 (2.6–13.8)] and hypomania/mania [OR = 4.7 (2.2–10.0)], compared to those without a specific diagnosis (Wittchen et al., 2007).

Prevalence of cannabis use was found to be significantly higher among individuals with bipolar disorder (BPD) compared to the general population (Etain et al., 2012). Prevalence of lifetime CUDs among individuals with bipolar I disorder was reported to be as high

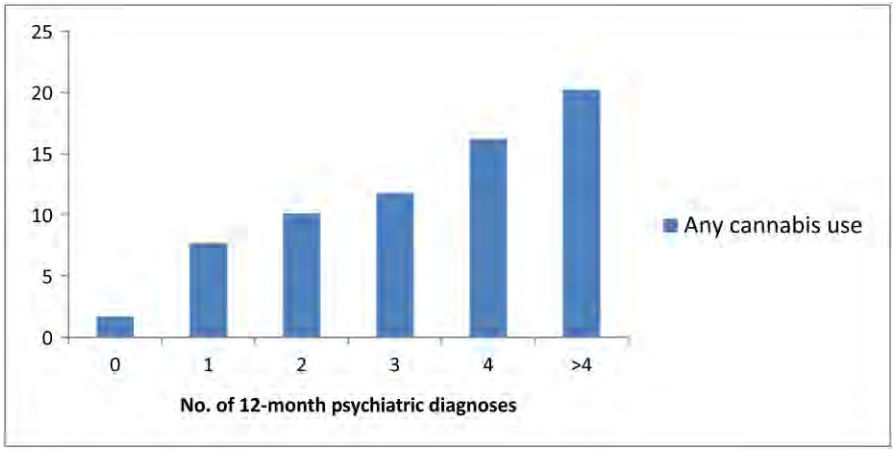


FIGURE 31.2 **Prevalence of cannabis use according to the number of 12-month mental illnesses.** This graph describes the prevalence of cannabis use according to the number of psychiatric diagnoses individuals had in the last 12 months (Lev-Ran et al., 2013b).

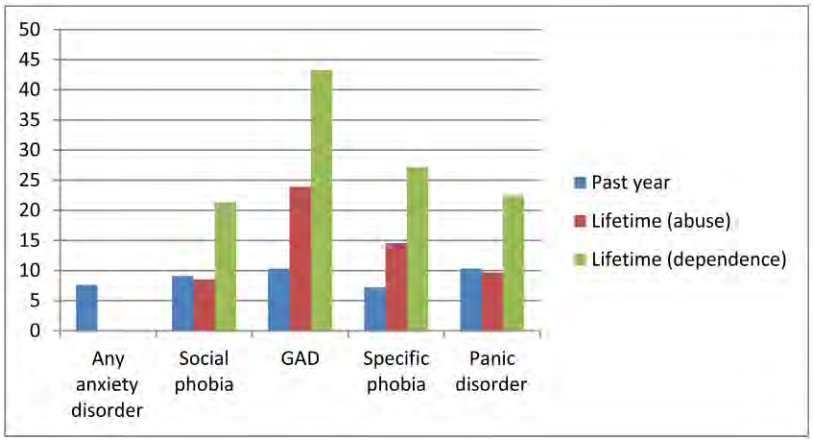


FIGURE 31.3 **Prevalence of past-year cannabis use, and lifetime abuse and dependence according to specific anxiety disorders.** This graph describes the prevalence of cannabis use in the past year, as well as lifetime cannabis abuse and dependence, among individuals with various anxiety disorders (Buckner et al., 2012b; Lev-Ran et al., 2013b).

as 46% (Cerullo & Strakowski, 2007), and prevalence of 12-month CUDs among individuals with bipolar I and II disorder was reported to be 7.2%, 6 times higher than that the general population in the same sample (Lev-Ran, Le Foll, McKenzie, George, & Rehm, 2013a). Reports based on the NESARC sample show that individuals with anxiety disorders are more prone to use cannabis and suffer from a CUD, compared to individuals without mental illness (Fig. 31.3).

Mental Illness Among Cannabis Users

Evidence suggest that rates of mental illness among cannabis users are related to the pattern of cannabis use, as this cooccurrence is more common among individuals diagnosed with a CUD, compared to occasional users (Troisi, Pasini, Saracco, & Spalletta, 1998). Data from the Epidemiologic Catchment Area study suggests that more than 50% of the individuals with a lifetime CUD had a concurrent diagnosis of mental illness (Regier et al., 1990), and that individuals with a CUD were 2 times more prone to receive such diagnoses, compared to those without a CUD. Anxiety disorders were the most common class of disorders among individuals with a CUD; 26.4% of individuals with a CUD had an anxiety disorder, compared to 7.3% in the general population. Troisi et al. (1998) reported that 29% of the individuals who used cannabis in the past year also received a concurrent psychiatric diagnosis.

The National Survey on Mental Health and Wellbeing (NSMHWB) revealed that past-year prevalence of mood and anxiety disorders among cannabis users without a history of a CUD was 12.1% and 8%, respectively, more than twice as high as nonusers (Degenhardt, Hall, & Lynskey, 2001). In Canada, Cheung et al. (2010) reported that prevalence of mood or anxiety disorders among heavy cannabis users (defined as almost-daily or daily users) was 18.1%, more than twice the rate of these disorders among abstainers (8.4%).

Results from several epidemiological surveys conducted in Australia and in the United States suggest that the past-year prevalence of any anxiety disorder is 17–29% among individuals with past-year cannabis dependence (Degenhardt et al., 2001; Stinson, Ruan, Pickering, & Grant, 2006), compared to 5–7% among individuals who did not use cannabis (Degenhardt et al., 2001; Regier et al., 1990). Analysis revealed that cannabis users [OR = 1.78 (1.31–2.41)] and individuals with cannabis dependence [OR = 4.3 (2.88–6.4)] were significantly more likely to receive a past-year diagnosis of an anxiety disorder, compared to nonusers (Degenhardt et al., 2001).

Possible Mechanisms Underlying Concurrent Cannabis Use and Mental Illness

As presented above, there is strong evidence suggesting that cannabis use and mental illness cooccur more than one would expect by chance. Several explanations were given to the underlying mechanisms that account for this strong association. Notably, it was argued that the association between cannabis use and mental illness is attributed to various common factors that may elevate the risk for developing mental illness and initiating cannabis use. For example, it was suggested that stressful life events (de Graaf, Bijl, Ten Have, Beekman, & Vollebergh, 2004), use and misuse of other substances rather than cannabis (van Laar, van Dorsselaer, Monshouwer, & de Graaf, 2007), and a neurological predisposition related to the influence of the human endocannabinoid system on mental health (Hill & Patel, 2013) may crucially attribute to the association between cannabis use and mental illness. Conversely, some writers argue for a direct causal association between the two

phenomena, suggesting that cannabis use may lead individuals to develop mental illness, or that mental illness may lead individuals to initiate cannabis use, or to use it more frequently. This notion will be discussed in the following sections of this chapter.

Significance and Limitations of Cross-Sectional Data

Cross-sectional data on the cooccurrence of cannabis use and mental illness is of high importance, as it holds valuable clinical implications for both general psychiatric services and substance-related treatment services. Evidence regarding the high prevalence of cooccurring cannabis use and mental illness in the population imply that a complete clinical evaluation of both mental illness and patterns of substance use is required in both settings, in order to allow for more accurate diagnosis and efficient treatment, as Johns (2001) concludes: "for any psychiatric patient, risk-management and care-planning is incomplete without a thorough assessment of substance misuse" (Johns, 2001, p. 121). However, cross-sectional data is limited in terms of exploring the causal association between cannabis use and mental illness (Degenhardt, Hall, & Lynskey, 2003). We will now review available longitudinal research pertaining to the association between cannabis use and mood and anxiety disorders.

CANNABIS AND MOOD DISORDERS

The term "mood disorders" relates to a group of mental illnesses associated with dysregulation in affective states, accompanied by changes in levels of activity and motivation, with disturbed patterns of sleep and appetite (Hammen, 2003). MDD and BPD are considered the most severe mood disorders, accounting for 60% of the general burden of disease of mental illness, and nearly 4% of the burden of disease of any medical condition worldwide (Whiteford et al., 2013). With high mortality rate and substantial functional disability caused directly by these two conditions, extensive research has focused on identifying clinical correlates associated with MDD and BPD, including substantial work on the association with substance use and misuse (Moore et al., 2007; Whiteford et al., 2013). Fig. 31.4 presents the 3-year incidence of mood and anxiety disorders, by category, among lifetime cannabis users, compared to nonusers (van Laar et al., 2007).

Longitudinal studies have enabled further investigation of the cross-sectional association between cannabis use and mood disorders by exploring two alternative temporal hypotheses. The majority of the studies focused on the association between cannabis use at baseline and the incidence of mood disorder at follow-up, revealing conflicting findings. Several studies showed that cannabis use at baseline was significantly associated with future occurrence of MDD (Bovasso, 2001; Fergusson & Horwood, 1997) and BPD (Tijssen et al., 2010), while other studies reported that cannabis users, even heavy users, were not significantly more prone than nonusers to develop MDD or BPD at follow-up (Degenhardt et al., 2013; Georgiades & Boyle, 2007). A metaanalysis exploring the longitudinal association between cannabis use and depression conducted by Lev-Ran et al. (2014) concluded that cannabis use, and particularly heavy cannabis use, may be associated with an increased risk for developing depression [OR = 1.17 (1.05–1.30)] for any cannabis

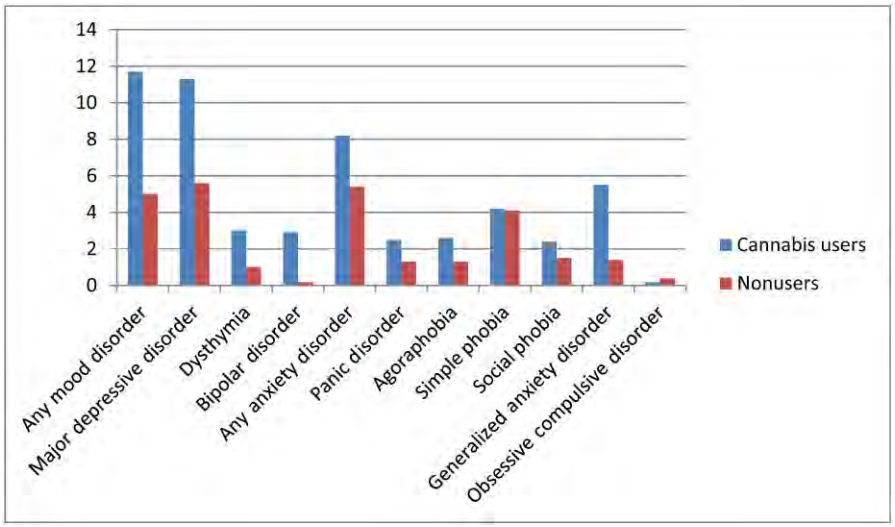


FIGURE 31.4 Three-year incidence of mood and anxiety disorders, by category, among lifetime cannabis users compared to nonusers. This graph shows the percentage of cannabis users and nonusers that developed various mood and anxiety disorders over the course of 3 years (van Laar et al., 2007).

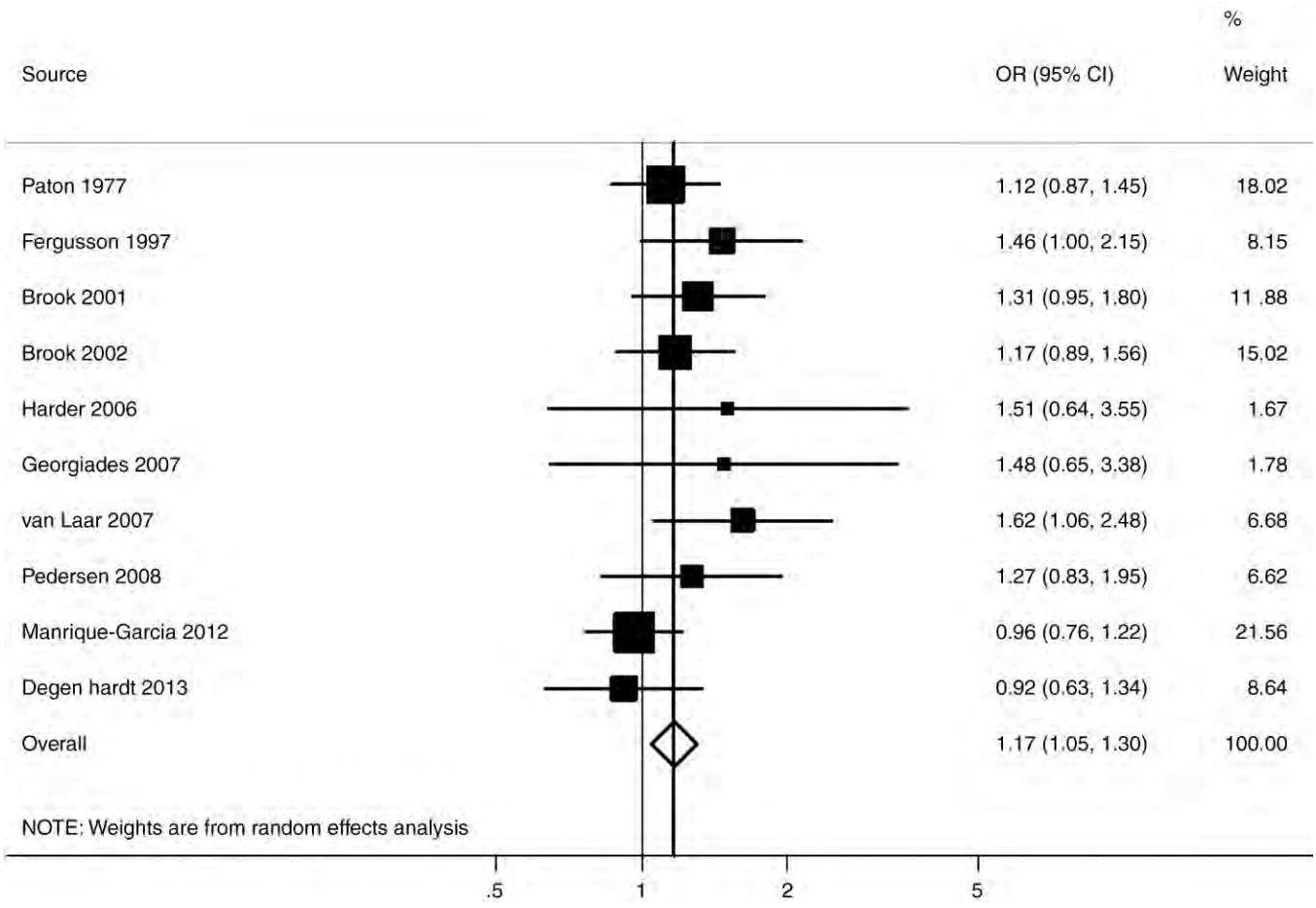


FIGURE 31.5 Forest plot showing adjusted odds ratios (AORs) and 95% confidence intervals (CIs) for any depressive outcome, according to cannabis use in individual studies (random effects). This graph describes the odds ratio (adjusted) for developing a depressive disorder among cannabis users, compared to nonusers (Lev-Ran et al., 2014).

use, and OR = 1.62 (1.21–2.16) for heavy cannabis use (Figs. 31.5 and 31.6). Though results of this metaanalysis were in line with an integrative data analysis published by Horwood et al. (2012), based on four Australian cohorts, the authors concluded that results pertaining to increased risk for depression among cannabis users should be regarded with caution, given modest pooled results and heterogeneity between studies, in terms of adjustment for confounders. Data regarding causal association between cannabis use and BPD is sparse. Though individual studies point to a possible longitudinal association between the two, some of these do not control for baseline manic and hypomanic symptoms, confounding conclusions (van Rossum, Boomsma, Tenback, Reed, & van Os, 2009). Hence, available data do not suffice to clearly indicate a longitudinal association between cannabis use and BPD.

An inverse notion, claiming that mood disorders at baseline may be associated with an increased risk for future cannabis use, has been reported in several clinical observations (Khantzian & Albanese, 2008), exploratory studies (Ogborne, Smart, Weber, & Birchmore-Timney, 2000), and retrospective studies (Gruber, Pope, & Oliva, 1997). Furthermore, a longitudinal association between MDD and initiation of cannabis use has been reported (Feingold, Weiser, Rehm & Lev-Ran, 2015). The term "self-medication" was elaborated by Khantzian (1985), suggesting that substance use is primarily a means of subduing psychological distress. According to Bolton, Robinson, and Sareen (2009) up to 24% of individuals with mood disorders who use alcohol, drugs, or misused prescription medication report they did so in order to relieve depressive or manic symptoms. Yet, based on large-scale longitudinal studies, there is yet no sufficient data based on multiple studies suggesting a causal relationship between MDD or BPD and the initiation of cannabis use, or increased frequency of use (Bolton et al., 2009; Degenhardt et al., 2003).

Effects of Cannabis Use on the Course of Mood Disorders

There is some evidence linking cannabis use with changes in the course of illness among individuals with mood disorders. Cannabis use was significantly associated with elevated sense of dysphoria among individuals

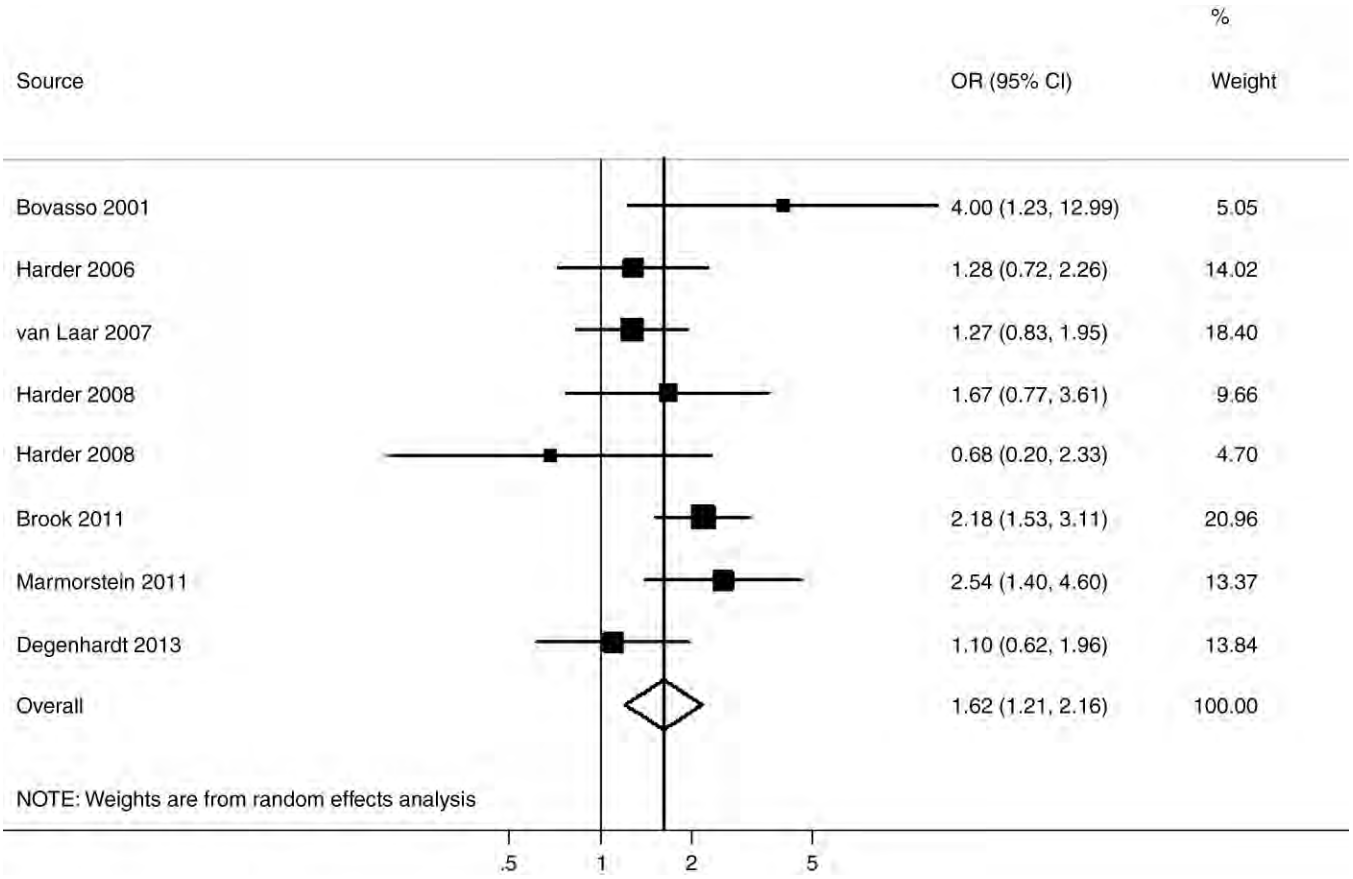


FIGURE 31.6 Forest plot showing adjusted odds ratios (AORs) and 95% confidence intervals (CIs) for any depressive outcome, according to heavy cannabis use (defined as a cannabis use disorder or at least weekly use) in individual studies (random effects). This graph describes the odds ratio (adjusted) for developing a depressive disorder, among heavy cannabis users, defined as individuals using cannabis at least weekly, or suffering from a cannabis use disorder (Lev-Ran et al., 2014).

with MDD (Ablon & Goodwin, 1974), and an incline in depressive symptoms among adolescents, over a 4-year course (Otten & Engels, 2013). There is some evidence that cannabis use, and frequent cannabis in particular, may moderate the efficiency of pharmacological treatment for depressive symptoms in clinical population (Bricker et al., 2007). Individuals with BPD and cooccurring cannabis problem were more prone to have earlier onset of the disorder (Etain et al., 2012; Lev-Ran, Le Foll, McKenzie, George, & Rehm, 2013a), more depressive and manic/hypomanic episodes per year (Lev-Ran et al., 2013a), longer duration of mixed and manic episodes (Strakowski et al., 2007), and poorer life functioning (Agrawal, Nurnberger, & Lynskey, 2011).

CANNABIS AND ANXIETY DISORDERS

There is evidence that cannabis use, particularly consumption of cannabis with high-concentration THC, can induce anxiety-like symptoms that are usually residue as the effect of THC subsides (D'Souza et al., 2004; Fusar-Poli et al., 2009). Evidence regarding longitudinal association between cannabis use and anxiety disorders is conflicting, and suffers from scarcity of substantial research on the subject. Several retrospective studies suggested that cannabis use may precede the development of panic attacks (Zvolensky et al., 2006) and generalized anxiety disorder (GAD) (Kessler et al., 2002). Furthermore, evidence from a longitudinal cohort study conducted in New Zealand revealed that past-year cannabis use at the age of 16 predicted significantly higher prevalence of any anxiety disorder in a 2-year follow-up (Fergusson & Horwood, 1997). However, these associations were not maintained after controlling for sociodemographic and various clinical variables. A prospective study conducted in the Netherlands (van Laar et al., 2007) revealed similar results; after controlling possible sociodemographic and clinical confounding variables, no significant association was found between cannabis use at baseline and occurrence of anxiety disorders in general [AOR = 1.18 (0.71–1.97)], as well as for individual anxiety disorders: panic disorder [AOR = 1.29 (0.61–2.71)], agoraphobia

[AOR = 1.14 (0.53–2.46)], specific phobias [AOR = 0.91 (0.52–1.58)], social phobia [AOR = 0.80 (0.36–1.79)], GAD [AOR = 1.83 (0.97–3.44)], and obsessive compulsive disorder [AOR = 0.12 (0.01–1.28)].

An inverse notion states that specific anxiety disorders may serve as risk factors for future initiation of cannabis use and incidence of CUD, possibly as means of self-medicating anxiety symptoms (Buckner, Crosby, Wonderlich, & Schmidt, 2012a). This notion is supported by evidence suggesting that one of the most common reasons for using cannabis in the general population is relief from stress (Hathaway, 2003). Accordingly, several prospective and longitudinal studies controlling for confounding psychiatric and substance use disorders at baseline suggested that preliminary social anxiety and GAD were associated with increased risk for future incidence of cannabis use and CUD (Buckner et al., 2008; Marmorstein, White, Loeber, & Stouthamer-Loeber, 2010).

Effects of Cannabis Use on the Course of Anxiety Disorders

Individuals who used cannabis were more prone to have earlier onset of panic attacks (Zvolensky et al., 2006), elevated intensity of anxiety symptoms during panic attacks (Szuster, Pontius, & Campos, 1988), and increased sensitivity to anxiety symptoms (Buckner, Leen-Feldner, Zvolensky, & Schmidt, 2009). Whether cannabis use reduces the efficacy of treatment for anxiety disorders is yet unclear. Bricker et al. (2007) reported that use of cannabis at least monthly did not affect the efficiency of a combined medical and psychological treatment for panic disorder and social phobia, compared to less than monthly use, yet additional research is needed to understand the impact of cannabis use, especially frequent use, on treatment of anxiety disorders.

SUMMARY

Repeated studies show substantial bilateral cross-sectional associations between cannabis use and mental illness: individuals with mental illness consume more cannabis, and individuals who use cannabis have increased prevalence of mental illness. Nevertheless, available research indicates that the majority of cannabis users, particularly occasional users, do not suffer from concurrent mental illness. Moreover, the available cross-sectional data does not suffice to point to a causal relationship between cannabis and mental illness. Longitudinal research indicates that cannabis use, particularly heavy use, may be associated with increased incidence of mood disorders (MDD and BPD), though individual studies suffer from various methodological drawbacks that confound clear conclusions. There is no clear evidence for a longitudinal association between cannabis use and incidence of anxiety disorders, and though cannabis users may be more prone to develop anxiety disorders, this is probably attributed to additional sociodemographic and clinical factors, and not to a direct causal relationship. In both cases of mood and anxiety disorders, it seems that concurrent cannabis use has a negative effect on the course of illness, and possibly on treatment.

Additional research pertaining to the association between cannabis use and mental illness is required. This includes longitudinal research controlling for baseline mental illness, as well as multiple potential covariates, focusing both on incidence as well as course of mental illness among cannabis users. In addition, improving methods for quantifying cannabis consumption is important for determining possible dose-dependent effects on mental illness. As rates of cannabis use continue to be high worldwide, further elucidating possible associations between cannabis use and mental health holds great implication for public health.

MINI-DICTIONARY

Adjusted odds ratio (AOR) A statistical quantification of the strength of association between an exposure variable and an outcome variable, after controlling for possible contributions of other factors in the model.

Anxiety disorders A group of psychiatric disorders associated with excessive feelings of anxiety (about the future) and fear (from present events), usually manifesting in physical, cognitive, and emotional symptoms.

Bipolar disorder (BPD) A psychiatric disorder characterized by episodes of mania or hypomania (excessively elated or irritable mood causing functional difficulties), usually alternating with episodes of depression.

Cannabis use disorders A collective term indicating cannabis abuse or dependence. In the updated DSM-5, previous cannabis abuse and dependence are combined into one diagnosis called "cannabis use disorder."

Major depressive disorder (MDD) A psychiatric disorder characterized by a pervasive low mood or loss of interest or pleasure in normally enjoyable activities. Additional symptoms may include sleep and appetite disturbances, difficulties in concentration, excessive guilt or worthlessness and suicidal thoughts and intent.

Mental illness A collective term indicating psychiatric disorders, generally characterized by dysregulation of mood, thought, and/or behavior, as recognized by the Diagnostic and Statistical Manual (DSM) or International Classification of Diseases (ICD).

Mood disorders A group of psychiatric disorders associated with dysregulation in affective states, levels of activity, motivation and patterns of sleep and appetite.

Odds ratio (OR) A statistical quantification of the strength of association between an exposure variable and an outcome variable. When the odds ratio is larger than 1, the exposure variable is considered to significantly increase the risk of the outcome variable.

References

Ablon, S. L., & Goodwin, F. K. (1974). High frequency of dysphoric reactions to tetrahydrocannabinol among depressed patients. *American Journal of Psychiatry*, *131*, 448–453.

Agrawal, A., Nurnberger, J. I., Jr., & Lynskey, M. T. (2011). Cannabis involvement in individuals with bipolar disorder. *Psychiatry Research*, *185*, 459–461.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Arlington, VA: American Psychiatric Publishing.

Bolton, J. M., Robinson, J., & Sareen, J. (2009). Self-medication of mood disorders with alcohol and drugs in the National Epidemiologic Survey on Alcohol and Related Conditions. *Journal of Affective Disorders*, *115*, 367–375.

Bovasso, G. B. (2001). Cannabis abuse as a risk factor for depressive symptoms. *American Journal of Psychiatry*, *158*, 2033–2037.

Bricker, J. B., Russo, J., Stein, M. B., Sherbourne, C., Craske, M., Schraufnagel, T. J., & Roy-Byrne, P. (2007). Does occasional cannabis use impact anxiety and depression treatment outcomes? Results from a randomized effectiveness trial. *Depression and Anxiety*, *24*, 392–398.

Buckner, J. D., Crosby, R. D., Wonderlich, S. A., & Schmidt, N. B. (2012a). Social anxiety and cannabis use: an analysis from ecological momentary assessment. *Journal of Anxiety Disorders*, *26*, 297–304.

Buckner, J. D., Heimberg, R. G., Schneier, F. R., Liu, S. M., Wang, S., & Blanco, C. (2012b). The relationship between cannabis use disorders and social anxiety disorder in the National Epidemiological Study of Alcohol and Related Conditions (NESARC). *Drug and Alcohol Dependence*, *124*, 128–134.

Buckner, J. D., Leen-Feldner, E. W., Zvolensky, M. J., & Schmidt, N. B. (2009). The interactive effect of anxiety sensitivity and frequency of marijuana use in terms of anxious responding to bodily sensations among youth. *Psychiatry Research*, *166*, 238–246.

Buckner, J. D., Schmidt, N. B., Lang, A. R., Small, J. W., Schlauch, R. C., & Lewinsohn, P. M. (2008). Specificity of social anxiety disorder as a risk factor for alcohol and cannabis dependence. *Journal of Psychiatric Research*, *42*, 230–239.

Cerullo, M. A., & Strakowski, S. M. (2007). The prevalence and significance of substance use disorders in bipolar type I and II disorder. *Substance Abuse Treatment and Prevention Policy*, *2*, 29.

Chen, C. Y., Wagner, F. A., & Anthony, J. C. (2002). Marijuana use and the risk of Major Depressive Episode. Epidemiological evidence from the United States National Comorbidity Survey. *Social Psychiatry and Psychiatric Epidemiology*, *37*, 199–206.

Cheung, J. T., Mann, R. E., Ialomiteanu, A., Stoduto, G., Chan, V., Ala-Leppilampi, K., & Rehm, J. (2010). Anxiety and mood disorders and cannabis use. *The American Journal of Drug and Alcohol Abuse*, *36*, 118–122.

D'Souza, D. C., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y. T., Braley, G., Gueorguieva, R., & Krystal, J. H. (2004). The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology*, *29*, 1558–1572.

de Graaf, R., Bijl, R. V., Ten Have, M., Beekman, A. T., & Vollebergh, W. A. (2004). Pathways to comorbidity: the transition of pure mood, anxiety and substance use disorders into comorbid conditions in a longitudinal population-based study. *Journal of Affective Disorders*, *82*, 461–467.

Degenhardt, L., Bucello, C., Calabria, B., Nelson, P., Roberts, A., Hall, W., Lynskey, M., Wiessing, L., Mora, M. E., Clark, N., Thomas, J., Briegleb, C., & McLaren, J. (2011). What data are available on the extent of illicit drug use and dependence globally? Results of four systematic reviews. *Drug and Alcohol Dependence*, *117*, 85–101.

Degenhardt, L., Coffey, C., Romaniuk, H., Swift, W., Carlin, J. B., Hall, W. D., & Patton, G. C. (2013). The persistence of the association between adolescent cannabis use and common mental disorders into young adulthood. *Addiction*, *108*, 124–133.

Degenhardt, L., & Hall, W. (2012). Extent of illicit drug use and dependence, and their contribution to the global burden of disease. *Lancet*, *379*, 55–70.

Degenhardt, L., Hall, W., & Lynskey, M. (2000). Cannabis use and mental health among Australian adults: Findings from the National Survey of Mental Health and Well-Being g (NDARC Technical Report 98). Sydney: National Drug and Alcohol Research Centre.

Degenhardt, L., Hall, W., & Lynskey, M. (2001). The relationship between cannabis use, depression and anxiety among Australian adults: findings from the National Survey of Mental Health and Well-Being. *Social Psychiatry and Psychiatric Epidemiology*, *36*, 219–227.

Degenhardt, L., Hall, W., & Lynskey, M. (2003). Exploring the association between cannabis use and depression. *Addiction*, *98*, 1493–1504.

Etain, B., Lajnef, M., Bellivier, F., Mathieu, F., Raust, A., Cochet, B., Gard, S., M'Bailara, K., Kahn, J. P., Elgrabli, O., Cohen, R., Jamain, S., Vieta, E., Leboyer, M., & Henry, C. (2012). Clinical expression of bipolar disorder type I as a function of age and polarity at onset: convergent findings in samples from France and the United States. *Journal of Clinical Psychiatry*, *73*, e561–e566.

Feingold, D., Weiser, M., Rehm, J., & Lev-Ran, S. (2015). The association between cannabis use and mood disorders: a longitudinal study. *Journal of Affective Disorders*, *172*, 211–218.

Fergusson, D. M., & Horwood, L. J. (1997). Early onset cannabis use and psychosocial adjustment in young adults. *Addiction*, *92*, 279–296.

Fusar-Poli, P., Crippa, J. A., Bhattacharyya, S., Borgwardt, S. J., Allen, P., Martin-Santos, R., Seal, M., Surguladze, S. A., O'Carrol, C., Atakan, Z., Zuardi, A. W., & McGuire, P. K. (2009). Distinct effects of {delta}9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Archives of General Psychiatry*, *66*, 95–105.

Gadermann, A. M., Alonso, J., Vilagut, G., Zaslavsky, A. M., & Kessler, R. C. (2012). Comorbidity and disease burden in the National Comorbidity Survey Replication (NCS-R). *Depression and Anxiety*, *29*, 797–806.

Georgiades, K., & Boyle, M. H. (2007). Adolescent tobacco and cannabis use: young adult outcomes from the Ontario Child Health Study. *Journal of Child Psychology and Psychiatry*, *48*, 724–731.

Grant, B.F., & Kaplan, K. (2005). Source and accuracy statement for the Wave 2 National Epidemiologic Survey on Alcohol and Related Conditions (NESARC). Rockville, MD: National Institute on Alcohol Abuse and Alcoholism.

Gruber, A. J., Pope, H. G., Jr., & Oliva, P. (1997). Very long-term users of marijuana in the United States: a pilot study. *Substance Use and Misuse*, *32*, 249–264.

Grucza, R. A., Abbacchi, A. M., Przybeck, T. R., & Gfroerer, J. C. (2007). Discrepancies in estimates of prevalence and correlates of substance use and disorders between two national surveys. *Addiction*, *102*, 623–629.

Hammen, C. (2003). Mood Disorders. In G. Stricker, T. A. Widiger, & I. B. Weiner (Eds.), *Handbook of psychology, Vol. 8: clinical psychology* (2nd ed., pp. 121–146). Hoboken, NJ: John Wiley & Sons Inc.

Hathaway, A. D. (2003). Cannabis effects and dependency concerns in long-term frequent users: a missing piece of the public health puzzle. *Addiction Research Theory*, *11*, 441–458.

Hibell, B., Guttormsson, U., Ahlström, S., Balakireva, O., Bjarnason, T., Kokkevi, A., & Kraus, L. (2012). The 2011 ESPAD report: substance use among students in 36 European countries. Stockholm, Sweden: Swedish Council for Information on Alcohol and Other Drugs.

Hill, M. N., & Patel, S. (2013). Translational evidence for the involvement of the endocannabinoid system in stress-related psychiatric illnesses. *Biology of Mood and Anxiety Disorder*, *3*, 19.

Horwood, L. J., Fergusson, D. M., Coffey, C., Patton, G. C., Tait, R., Smart, D., Letcher, P., Silins, E., & Hutchinson, D. M. (2012). Cannabis and depression: an integrative data analysis of four Australasian cohorts. *Drug and Alcohol Dependence*, *126*, 369–378.

Johns, A. (2001). Psychiatric effects of cannabis. *British Journal of Psychiatry*, *178*, 116–122.

Kessler, R. C., Andrade, L. H., Bijl, R. V., Offord, D. R., Demler, O. V., & Stein, D. J. (2002). The effects of co-morbidity on the onset and

persistence of generalized anxiety disorder in the ICPE surveys. *Psychological Medicine*, *32*, 1213–1225.

Kessler, R. C., Demler, O., Frank, R. G., Olfson, M., Pincus, H. A., Walters, E. E., Wang, P., Wells, K. B., & Zaslavsky, A. M. (2005). Prevalence and treatment of mental disorders, 1990 to 2003. *New England Journal of Medicine*, *352*, 2515–2523.

Kessler, R. C., McGonagle, K. A., Zhao, S., Nelson, C. B., Hughes, M., Eshleman, S., Wittchen, H. U., & Kendler, K. S. (1994). Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Archives of General Psychiatry*, *51*, 8–19.

Khantzian, E. J. (1985). The self-medication hypothesis of addictive disorders: focus on heroin and cocaine dependence. *American Journal of Psychiatry*, *142*, 1259–1264.

Khantzian, E. J., & Albanese, M. J. (2008). *Understanding addiction as self medication: finding hope behind the pain*. New York, NY: Rowman & Littlefield Publishers, Inc.

Lev-Ran, S., Le Foll, B., McKenzie, K., George, T. P., & Rehm, J. (2013a). Bipolar disorder and co-occurring cannabis use disorders: characteristics, co-morbidities and clinical correlates. *Psychiatry Research*, *209*, 459–465.

Lev-Ran, S., Le Foll, B., McKenzie, K., George, T. P., & Rehm, J. (2013b). Cannabis use and cannabis use disorders among individuals with mental illness. *Comprehensive Psychiatry*, *54*, 589–598.

Lev-Ran, S., Roerecke, M., Le Foll, B., George, T. P., McKenzie, K., & Rehm, J. (2014). The association between cannabis use and depression: a systematic review and meta-analysis of longitudinal studies. *Psychological Medicine*, *44*, 797–810.

Mariani, J. J., Brooks, D., Haney, M., & Levin, F. R. (2011). Quantification and comparison of marijuana smoking practices: blunts, joints, and pipes. *Drug and Alcohol Dependence*, *113*, 249–251.

Marmorstein, N. R., White, H. R., Loeber, R., & Stouthamer-Loeber, M. (2010). Anxiety as a predictor of age at first use of substances and progression to substance use problems among boys. *Journal of Abnormal Child Psychology*, *38*, 211–224.

Moore, T. H., Zammit, S., Lingford-Hughes, A., Barnes, T. R., Jones, P. B., Burke, M., & Lewis, G. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet*, *370*, 319–328.

Ogborne, A. C., Smart, R. G., Weber, T., & Birchmore-Timney, C. (2000). Who is using cannabis as a medicine and why: an exploratory study. *Journal of Psychoactive Drugs*, *32*, 435–443.

Otten, R., & Engels, R. C. (2013). Testing bidirectional effects between cannabis use and depressive symptoms: moderation by the serotonin transporter gene. *Addiction Biology*, *18*, 826–835.

Regier, D. A., Farmer, M. E., Rae, D. S., Locke, B. Z., Keith, S. J., Judd, L. L., & Goodwin, F. K. (1990). Comorbidity of mental disorders with alcohol and other drug abuse. Results from the Epidemiologic Catchment Area (ECA) Study. *JAMA*, *264*, 2511–2518.

Regier, D. A., Myers, J. K., Kramer, M., Robins, L. N., Blazer, D. G., Hough, R. L., Eaton, W. W., & Locke, B. Z. (1984). The NIMH Epidemiologic Catchment Area program. Historical context, major objectives, and study population characteristics. *Archives of General Psychiatry*, *41*, 934–941.

Substance Abuse and Mental Health Services Administration. (2007). *Results from the 2006 National Survey on Drug Use and Health: National Findings*. Rockville, MD: Office of Applied Studies, NSDUH Series H-32, DHHS Publication No. SMA 07-4293.

Stinson, F. S., Ruan, W. J., Pickering, R., & Grant, B. F. (2006). Cannabis use disorders in the USA: prevalence, correlates and co-morbidity. *Psychological Medicine*, *36*, 1447–1460.

Strakowski, S. M., DelBello, M. P., Fleck, D. E., Adler, C. M., Anthenelli, R. M., Keck, P. E., Jr., Arnold, L. M., & Amicone, J. (2007). Effects of co-occurring cannabis use disorders on the course of bipolar disorder after a first hospitalization for mania. *Archives of General Psychiatry*, *64*, 57–64.

Szuster, R. R., Pontius, E. B., & Campos, P. E. (1988). Marijuana sensitivity and panic anxiety. *Journal of Clinical Psychiatry*, *49*, 427–429.

Tijssen, M. J., van Os, J., Wittchen, H. U., Lieb, R., Beesdo, K., & Wichers, M. (2010). Risk factors predicting onset and persistence of subthreshold expression of bipolar psychopathology among youth from the community. *Acta Psychiatrica Scandinavica*, *122*, 255–266.

Troisi, A., Pasini, A., Saracco, M., & Spalletta, G. (1998). Psychiatric symptoms in male cannabis users not using other illicit drugs. *Addiction*, *93*, 487–492.

United Nations Office on Drugs and Crime. (2012). *World Drug Report 2012*. New York: United Nations publication, Sales No. E.12.XI.1.

van Laar, M., van Dorsselaer, S., Monshouwer, K., & de Graaf, R. (2007). Does cannabis use predict the first incidence of mood and anxiety disorders in the adult population? *Addiction*, *102*, 1251–1260.

van Rossum, I., Boomsma, M., Tenback, D., Reed, C., & van Os, J. (2009). Does cannabis use affect treatment outcome in bipolar disorder? A longitudinal analysis. *Journal of Nervous and Mental Diseases*, *197*, 35–40.

Whiteford, H. A., Degenhardt, L., Rehm, J., Baxter, A. J., Ferrari, A. J., Erskine, H. E., Charlson, F. J., Norman, R. E., Flaxman, A. D., Johns, N., Burstein, R., Murray, C. J., & Vos, T. (2013). Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *Lancet*, *382*, 1575–1586.

Wittchen, H. U., Frohlich, C., Behrendt, S., Gunther, A., Rehm, J., Zimmermann, P., Lieb, R., & Perkonigg, A. (2007). Cannabis use and cannabis use disorders and their relationship to mental disorders: a 10-year prospective-longitudinal community study in adolescents. *Drug and Alcohol Dependence*, *88*(Suppl. 1), S60–70.

World Health Organization. (1992). *ICD 10 classifications of mental and behavioural disorder: clinical descriptions and diagnostic guidelines*. Geneva, Switzerland: World Health Organization.

World Health Organization. (2002). *The World Health Report 2002*. Geneva: World Health Organization.

Zvolensky, M. J., Bernstein, A., Sachs-Ericsson, N., Schmidt, N. B., Buckner, J. D., & Bonn-Miller, M. O. (2006). Lifetime associations between cannabis, use, abuse, and dependence and panic attacks in a representative sample. *Journal of Psychiatric Research*, *40*, 477–486.

CHAPTER

32

Cannabis Use and Well-Being

J. Allen, M.D. Holder, Z. Walsh

Psychology Department, IKBSAS, University of British Columbia, Kelowna, BC, Canada

SUMMARY POINTS

- The majority of research on the relationship between cannabis and well-being is concentrated on depression, anxiety, and psychopathology.
- The primary ways of studying the relationship between cannabis and well-being are causation, reverse causation, and the third factor model.
- Early onset (before the age of 17) initiation of cannabis use and gender have been identified as risk factors in the development of depressive and anxious symptoms.
- Some studies find cannabis to be associated with increased well-being.
- Although many studies fail to do so, after controlling for confounding factors, such as personality, demographics, and polydrug use, the relationship between cannabis and well-being often becomes nonexistent.
- Some individuals may benefit from cannabis use, through reduction of depressive and anxious symptoms, and facilitation of social relationships.
- Individuals who use cannabis for therapeutic purposes (CTP) may benefit psychologically through increased confidence in their ability to self-manage their illness, reduction in pain-related anxiety, increased positive, and decreased negative experiences.

KEY FACTS OF WELL-BEING

- Well-being includes the balance of the combination of positive and negative components (ie, depression, anxiety, happiness, life satisfaction, etc.).
- The positive component of well-being includes a combination of affective (happiness) and subjective (life satisfaction) dimensions.
- The negative component of well-being includes low, but appropriate, levels of depressive and anxious symptoms.
- Well-being and its components can be assessed with self-report measures (eg, Positive and Negative Affect Schedule and the Satisfaction with Life Scale).

KEY FACTS OF POSITIVE PSYCHOLOGY

- In contrast to traditional psychology, positive psychology is the scientific study of variables that allow individuals and communities to thrive, and how these can be promoted.
- Although referred to earlier, positive psychology formally originated in 1998, when Martin Seligman became president of the American Psychological Association, and proposed it as his theme.
- Positive psychology is intended to supplement traditional psychology, and provide a more complete version of the human condition.
- Goals of positive psychology include: families and schools that allow children to flourish, workplaces that foster satisfaction and high productivity, communities that encourage civic engagement, therapists who identify and nurture their patients' strengths, the teaching of Positive Psychology, and the dissemination of Positive Psychology interventions in schools, corporations, government, hospitals, and communities.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00036-3
Copyright © 2017 Elsevier Inc. All rights reserved.

KEY FACTS OF CANNABIS FOR THERAPEUTIC PURPOSES

- The use of CTP can be traced to antiquity, and cannabis-based medicines played a prominent role in the western pharmacopeia from the late 19th century until the 1920s.
- Cannabis-based medicines were marginalized beginning in the 1930s due to technological changes and social stigma.
- Cannabis-based medicines have experienced a renaissance in the 21st century due to patient advocacy, and increased understanding of the endocannabinoid system.
- Human research with cannabis has been impeded by barriers related to the war on drugs.
- There is empirical support for the effectiveness of cannabis for the treatment of symptoms related to HIV, cancer, multiple sclerosis, and chronic pain conditions.
- Anecdotal and preclinical evidence suggests that cannabis-based medicines may have broad applications for physical and mental health.

LIST OF ABBREVIATIONS

AIDS Acquired immunodeficiency syndrome
CTP Cannabis for therapeutic purposes
HIV Human immunodeficiency disease

INTRODUCTION

Cannabis is the most commonly used illicit substance worldwide, with 147 million people using it annually (WHO, 2013). The effective use of cannabis for medicinal, spiritual, and recreational purposes can be traced to antiquity (Russo, 2007). However, diverse sociocultural factors led to the modern stigmatization and marginalization of cannabis (Boyd, 2004), and by the 1940s, mainstream characterizations focused almost exclusively on the putative negative impacts of cannabis use on human health. The 21st century has witnessed a more balanced and nuanced evaluation of the impact of cannabis use on well-being, with public discourse characterized by increased consideration of both potential positive and negative influences.

Traditionally, mental health fields, including psychology and medicine, have primarily focused on dysfunctions, deficits, disorders, and diseases (Holder, 2012). Consistent with this tradition, recent research on cannabis and well-being focuses on negative associations, such as those involving depression, anxiety, and psychopathology (Moore et al., 2007). However, historical descriptions of cannabis use often focused on the euphoric and salutary effects. Most notably, Moreau, in the 1840s, introduced Europeans to the potential of cannabis as an antidepressant, with the potential for "effectively combatting the fixed ideas of depressives, disrupting the chain of their ideas, of unfocusing their attention on such and such a subject." (Moreau, 1973, p. 211). Despite the focus of mental health fields on the negative associations between cannabis and well-being, some research does address the possible role cannabis may play in positive well-being.

CANNABIS AND POSITIVE WELL-BEING

In contrast with the traditional focus of mental healthcare on the identification and treatment of maladaptive behaviors and emotional states, positive psychology focuses on strengths and factors that enhance well-being. Positive psychology aims to identify variables that promote positive well-being, and to understand how these variables can be used to encourage well-being, rather than exclusively focusing on dysfunctions, deficits, disorders, and diseases (Holder, 2012). Although it is important to consider ill-being, the relevance of positive well-being should not be underestimated. For instance, happier individuals have been shown to be healthier, more productive members of society, experience more satisfaction and success in their careers, and have more successful relationships (Holder, 2012). Additionally, an absence of positive mental health predicts a 62% increased likelihood of mortality, after controlling for other variables, including depression and anxiety (Keyes & Simoes, 2012).

Studies of cannabis are often limited as they only consider ill-being, and studies that examine the effects of cannabis on positive well-being are generally limited to examining only one dimension of well-being, such as happiness or life satisfaction. Although happiness and life satisfaction are both related and components of well-being, they are separable. Happiness refers to an individual's subjective evaluation of the positive affective components of their life, and includes both hedonic and eudaimonic components. Predictors of happiness include marriage, job satisfaction, involvement in leisure activities, and social relationships (Argyle, 2001). Life satisfaction refers to an individual's cognitive evaluation of their quality of life as a whole. Together, happiness and life satisfaction contribute to one's overall well-being. Well-being refers to an individual's evaluation of various aspects of their lives, including satisfaction with their health, relationships, recreation, work, meaning, and purpose, as well as their affective reactions to life events and level of interest and engagement. These evaluations can be positive or negative, and, taken together, are encompassed under the label of subjective well-being.

Research on cannabis use and positive well-being has yielded inconsistent results. Although mood often improves while under the influence of cannabis (Green, Kavanagh, & Young, 2003), it is unclear whether this endures once the person is no longer experiencing cannabis intoxication. A longitudinal study showed that adolescent cannabis use precedes lower life satisfaction, and an increased incidence of major depressive disorder (Georgiades & Boyle, 2007), and both current and former heavy cannabis use is associated with reduced life satisfaction and happiness (Gruber, Pope, Hudson, & Yurgelun-Todd, 2003). Additionally, those who use cannabis less than 100 times do not differ from abstainers in their level of life satisfaction, although increased use is associated with decreased life satisfaction (Fergusson & Boden, 2008). There is also evidence linking cannabis use and increased well-being. For instance, cannabis users have higher levels of subjective well-being than nonusers (Barnwell, Earleywine, & Wilcox, 2006), and heavy users have higher life satisfaction in comparison to occasional users (Kouri, Pope, Yurgelun-Todd, Gruber, & 1995). One of the few studies to explore the relationship between cannabis use and well-being assessed frequency of use (past year, past month, and amount of cannabis consumed per session). No direct relationship was found between frequency of cannabis use and well-being, although there was a relationship between the negative consequences of drug use and well-being (Allen & Holder, 2013). Users who experienced negative consequences, such as getting into a fight or missing a test, reported more depression symptoms and negative affect. These results suggest it may not be simply that cannabis use affects well-being, but whether people experience negative consequences resulting from drug use. Additionally, participants low in the personality traits of Agreeableness and Conscientiousness were more likely to use cannabis, as well as experience negative consequences. Personality traits account for a large percentage of the variance in happiness, and they are strong predictors of happiness; individuals who exhibit stable levels of happiness also typically display high levels of Extraversion, and low levels of Neuroticism (Steel, Schmidt, & Shultz, 2008). Therefore, personality likely plays a large role in mediating the relationship between cannabis use and well-being (Table 32.1).

TABLE 32.1 Medical Cannabis and Well-Being: Direct and Indirect Effects

Direct	Indirect
Analgesia	Reduced symptom-related anxiety
Sedation	Increased social support from other users
Appetite	Increased positive affect
Other symptom reduction	Improved self-efficacy (via self-cultivation)

MOTIVATIONS FOR CANNABIS USE

Cannabis users have identified the importance of both hedonistic and therapeutic motives for use (Nail, Gunderson, & Kolb, 1974). Among the hedonistic motives, the sole desire to "feel happy" was identified by 17% of light users and 36% of heavy users. Among the therapeutic motives, the desires to be in a social group, increase understanding of others, and having closer relationships were significant to both occasional and heavy users. Social motives, such as bonding with people and hanging out with friends, are identified by 25% of adolescent cannabis users, with 9% citing it as their primary motive (Lee, Neighbors, & Woods, 2007). Social motives for cannabis use, such as enjoying a party or fitting in with a group, have also been identified in adults (Buckner, Zvolensky, & Schmidt, 2012). Social relations are critical to well-being; indeed, social affiliation is the primary strategy used by undergraduates to promote happiness (Tkach & Lyubomirsky, 2006). Additionally, an investigation of the friendship components which are the strongest predictors of happiness found "companionship" and "self-validation" to be significant (Demir & Weitekamp, 2007). Companionship is an important contributor in both initiation and continuation of cannabis use (Shrier, Walls, Kendall, & Blood, 2012). The combination of social inclusion, companionship, and self-validation provided by social cannabis use is likely a contributor to the continuation of use, particularly among groups where social motivation is important, such as adolescents and postsecondary students (Table 32.2).

TABLE 32.2 Primary Motives for Cannabis Use: Recreational and CTP

Recreational	CTP
Social motivation (eg, fit into a group, fun, companionship, self-validation)	Symptom reduction (eg, appetite stimulation, antiemetic effects, and analgesia for chronic pain)
General anxiety reduction (can be counter-effective)	Anxiety reduction associated with mental health issues, chronic pain, and illness
	Aid in sleep
	Escape from reality and relax
	Coping with mental health disorders (clinical and personality)

Note: CTP, cannabis use for therapeutic purposes.

CANNABIS AND ILL-BEING

Recent research has focused primarily on negative associations between cannabis and well-being, and has been conceptualized in three primary ways. The first way is causal, suggesting that cannabis use leads to an increase in negative affect (Bovasso, 2001; Johns, 2001). The second way suggests that individuals with preexisting mood and psychotic disturbances self-medicate using cannabis (Hooshmand, Willoughby, & Good, 2012). The third way is the third factor model, in which both cannabis use and its negative associations are caused by independent factors, such as personality, environment, neurobiology, or social influence (Crippa et al., 2009).

CANNABIS AND DEPRESSION

Research on cannabis use and depression is equivocal, and much research on this topic has been unable to control for diverse factors, including physical and emotional development. Adolescence is a period of heightened risk for both the initiation of substance use and the development of psychological and behavioral dysfunction. Interest in the identification of potential interactions between cannabis and the developing brain has led to substantial research on the effects of cannabis use on adolescents (Hayatbakhsh et al., 2007). Cross-sectional studies of adolescents have identified associations between cannabis use, depression, and suicide (Fergusson, Horwood, & Swain-Campbell, 2002; Rey, Sawyer, Raphael, Patton, & Lynskey, 2002). Longitudinal studies have shown that cannabis use during adolescence is associated with depression and depressive symptoms in adulthood, with some studies reporting stronger associations among those whose use began in early adolescence (Hayatbakhsh et al., 2007; de Graaf et al., 2010). However, results of recent studies regarding the associations between cannabis use and adolescent depression are equivocal; a study assessing early onset and adult onset cannabis users found both groups equally at risk for experiencing a depressive episode (Fairman & Anthony, 2012). Additionally, cannabis use during adolescence may be unrelated to depression 10 years later (Degenhardt et al., 2010), and although cannabis worsens depressive symptoms in young women predisposed to depression and anxiety, it does not directly cause depression (Marmorstein et al., 2010). The effects of cannabis use on depression may be attenuated when cannabis use begins in adulthood, after a critical period of brain development (van Laar, van Dorsselaer, Monshouwer, & de Graaf, 2007). Cannabis use and dependence are only weakly associated with increased risk of depression in adults (Chen, Wagner, & Anthony, 2002), and heavy and infrequent cannabis users demonstrate a similar prevalence of mood disorders (Cheung et al., 2010).

Gender may moderate the association between cannabis use and the development of depression, with female cannabis users more likely to exhibit depressive symptoms than males. For example, adult marijuana abuse and dependence were associated with lifetime incidence of major depressive disorder in females, but not in males (Durdle, Lundahl, Johanson, & Tancer, 2008). Similarly, studies of adolescents report stronger associations between cannabis use and depression among females than males (Poulin, Hand, Boudreau, & Santor, 2005; Patton et al., 2002). These findings indicate the need for further examination of sex and gender-related variations in the functioning of the cannabinoid system, and add to broader calls for the routine incorporation of sex and gender analyses in studies of mental health and addictions (Johnson, Greaves, & Repta, 2007). Though some research has reported a positive association between cannabis use and depression, other cross-sectional and longitudinal studies have failed to find these associations (Pedersen, 2008; Ranney, 2013), and a large online survey found that frequent cannabis users reported less depressed mood, and more positive affect than abstainers (Denson & Earleywine, 2006).

CANNABIS AND ANXIETY

Cannabis has also been associated with anxiety, and, as is the case with depression, the empirical literature remains equivocal, with reports of both anxiolytic and anxiogenic effects. Positive associations have been reported between cannabis use and anxiety (Bonn-Miller, Zvolenksy, Leen-Feldner, Feldner, & Yartz, 2005), and higher prevalence of anxiety disorders have been identified among heavier cannabis users (Cheung et al., 2010). Consistent with these findings of anxiogenic effects are common reports of panic-like responses to cannabis intoxication (Crippa et al., 2009), and longitudinal studies indicating that cannabis use by adolescents predicts young adult anxiety disorders (Degenhardt et al., 2013; Hayatbakhsh et al., 2007). In contrast, anxiolytic effects are among the primary motives for recreational cannabis use (Simons, Correia, Carey, & Borsari, 1998), and a diagnosis of mood or anxiety disorders in adolescence predicted cannabis use 10 years later (Wittchen et al., 2007). A literature review (Crippa et al., 2009) concluded that, although cannabis use and anxiety disorders are positively correlated, the directionality of the relationship remains unclear.

CONFOUNDING FACTORS

In sum, research remains equivocal regarding the association between cannabis and depression and anxiety. Variability in findings across studies may be

attributable to diverse factors, including sample selection (ie, treatment vs community), and assessment of cannabis use (ie, diagnosis vs frequency of use). Additionally, many studies lack controls for potential confounds, such as personality or polydrug use. Some studies fail to observe a relationship between cannabis use and depression, after controlling for confounding factors, such as personality, demographics, and polydrug use (Degenhardt, Hall, & Lynskey, 2001), and adjusting for between-group differences and risk factors (Harder, Morral, & Arkes, 2006). Cannabis use is unrelated to anxiety, after controlling for variables including personality, demographics, polydrug use, and baseline mental health (Degenhardt et al., 2001; van Laar et al., 2007), and covariation among rates of cannabis use, depression, and anxiety may be attributed to personality characteristics underlying all three variables. Therefore, interpretation of studies that did not assess important mediators of the relationship between cannabis and well-being should be cautious.

DOSE–RESPONSE THEORY

Quantity and frequency of use may play an important role in moderating the association between cannabis use and mental health. A review examined the extent to which increased consumption of cannabis leads to greater incidence of psychiatric symptoms (Moore et al., 2007). This review found some support for a dose–response relationship for depression, but not for psychosis or anxiety. Other studies found more consistent support for a dose–response relationship between cannabis and well-being, such that risk of mood disorders appears to be elevated in weekly and daily cannabis users, but not for less frequent cannabis users (van Laar et al., 2007; Poulin et al., 2005). Heavy cannabis use may increase the risk for anxiety (Cheung et al., 2010), with frequent users showing more severe levels of anxiety (Bonn-Miller, Zvolensky, Bernstein, & Timothy, 2008). Additionally, weekly adolescent users were twice as likely to develop anxiety in adulthood, and daily users 5 times more likely (Patton et al., 2002).

SELF-MEDICATION THEORY

The directionality of the associations between cannabis use and ill-being is difficult to infer from extant studies. Some studies suggest that their findings can be explained through the "self-medication hypothesis" (Poulin et al., 2005), and it has been hypothesized that mentally ill individuals self-medicate, using drugs and alcohol, to gain relief from psychiatric issues and negative emotional states (Khantzian, 1985). Studies on the self-medication hypothesis in relation to cannabis use, depression, and anxiety have produced mixed results (Hooshmand et al., 2012; Martins, 2011). Consistent with the self-medication perspective, some adolescents reported that they did not use cannabis for recreational purposes, but rather to alleviate feelings of anxiety, stress, and depression (Bottorff, Johnson, Moffat, & Mulvogue, 2009). In addition, cannabis self-medication may vary according to gender (Poulin et al., 2005). Furthermore, other studies failed to identify a predictive association of anxiety for subsequent cannabis use (Chabrol, Ducangé, Casas, Roura, & Carey, 2005), or have reported that cannabis produced neither anxiolytic or anxiogenic effects in participants (Tournier, Sorbara, Gindre, Swendsen, & Verdoux, 2003). Indeed, a study of frequent cannabis users found only moderate elevations in anxiety preceding use, and no decrease in anxiety subsequent to use (Buckner, Crosby, Silgado, Wonderlich, & Schmidt, 2012). Furthermore, depressed individuals provided rationale similar to nondepressed participants (eg, "to get high," "to increase pleasure," "to become more outgoing") for using cannabis (Arendt et al., 2007).

CANNABIS USE FOR THERAPEUTIC PURPOSES

The relationship between cannabis and well-being is complicated among those who use CTP. Interest in the therapeutic potential of cannabis has enjoyed a renaissance associated with the identification of the endocannabinoid system in the 1990s. Over the past two decades, some governments have instituted policies and laws, which enable their citizens to access CTP. Although motives for recreational and therapeutic use sometimes overlap, there are unique considerations when it comes to CTP. Currently, cannabis and its preparations are utilized for diverse physiological effects, including their appetite-stimulating and antiemetic properties in the treatment of HIV/AIDS, and cancer-related chemotherapy, symptom relief treatment in neurodegenerative diseases, such as Parkinson's and multiple sclerosis, as well as their analgesic effect on chronic pain.

In addition to reducing physical symptoms associated with illness, there may be psychological benefits to CTP. Although not well researched, there are situations in which cannabis may be a unique and effective contributor to psychological well-being. For instance, many people applying for medical cannabis licenses have exhausted other treatment options, including prescription drugs, therapy, and surgery (Reinarman, Nunberg, Lanthier, & Heddleston, 2011), and some are doubtful of traditional medicines' utility in managing their condition, and their ability to self-manage their medications.

A summary of the research on the biopsychosocial model in chronic pain concluded people's appraisals of their symptoms and their ability to self-manage pain and related problems were associated with more positive treatment outcomes (Turk & Okifuji, 2002). Although some medical conditions are intractable, the option to access CTP may increase people's confidence in their ability to take a proactive approach to symptom management through alternative treatment options.

Chronic illness is associated with anxiety related to current and anticipated pain (Turk & Okifuji, 2002). Cannabis users with chronic pain may expect cannabis to alleviate their negative mood and somatosensory states (Zvolensky et al., 2011). Furthermore, across medical conditions, those who use high levels of CTP report that they use cannabis to address anxiety and depression (Walsh et al., 2013). Chronic physical conditions are associated with mood and anxiety disorders (Scott et al., 2007), and health-related quality of life is often diminished by this relationship (Sherbourne et al., 2000). Medical cannabis users report feelings of relaxation and happiness while under the influence (Harris et al., 2000), and motives for cannabis use include enjoyment of the effects and feelings, forgetting problems and worries, and helpfulness in sleeping (Zvolensky et al., 2007). In this context, cannabis removes negative emotions through symptom reduction, and also facilitates positive emotions by allowing users a period of escape from illness, and the opportunity to relax and enjoy themselves. The Broaden and Build Theory of Positive Emotions states that positive emotions allow people to build on their physical and psychological resources by broadening their thought-action repertoires (Fredrickson, 2004). These resources fuel resiliency in times of struggle, and can be drawn on later for coping and survival (Fredrickson, 2004). Although positive emotions are momentary, they may have enduring consequences for positive well-being by removing lingering negative emotions, and providing people with resources to deal with stress. People may become more successful at dealing with stress by utilizing the resources they acquired through positive emotions experienced while using cannabis. Medicinal cannabis users tend to be more depressed than the general population (Korthuis et al., 2008), and chronic illness is strongly related to depression (Moussavi et al., 2007). Users of CTP report more depressed mood and increased somatic complaints, in comparison to recreational users, suggesting that factors related to illness contribute to well-being, independent of cannabis use (Denson & Earleywine, 2006). In sum, cannabis use may increase well-being among CTP users by increasing confidence in their ability to self-manage their illness, reducing pain-related anxiety, increasing positive and decreasing negative experiences while under the influence, and facilitating the use of coping resources acquired during those experiences.

LIMITATIONS AND FUTURE DIRECTIONS

The extant research on cannabis and well-being is limited. For instance, although some studies attempt to determine causality through the use of longitudinal methodology (Hayatbakhsh et al., 2007), the majority of studies are cross-sectional (Rey et al., 2002). Drug use and well-being may vary greatly over time, and although experimental research is not plausible, longitudinal design should be utilized in an attempt to determine causality. Also, many studies do not employ multiple measures, or base their results on a few questions. Measuring a variety of psychological dimensions (eg, happiness, life satisfaction, and depression) is beneficial in ensuring a more complete assessment of well-being, and ensuring that the findings are not dependent on any single measure. Future research on the impact of cannabis use on well-being would benefit from the incorporation of a positive psychology perspective, particularly with regard to CTP. The majority of research on the relationship between cannabis and well-being is concentrated on ill-being (Moore et al., 2007). The purpose of positive psychology is not to replace the common research focus on dysfunction and disorder, but rather to supplement it with a more complete version of the human condition. Positive psychology emphasizes goals, well-being, satisfaction, happiness, and interpersonal skills (Holder, 2012). If there is a possible role for cannabis to play in promoting positive well-being, it would be important to identify what it is, and the best way to encourage it. Additionally, there are gaps in the current research on the role of cannabis in medicinal users. For instance, the relationship between cannabis and well-being appears to be distinct when the primary motive is medicinal, rather than recreational. Although recreational and medicinal users identify some of the same motives (eg, relaxation), medicinal cannabis users may experience additional benefit through increased confidence in their ability to self-manage their illness, reduction of pain-related anxiety, increased positive and decreased negative experiences while under the influence, and facilitation of coping resources acquired during those experiences. Future research should concentrate on the unique differences in motivation between recreational and medicinal users, and determine the extent of cannabis' efficacy in satisfying these motives. By examining the relationship between cannabis and well-being through the lens of positive psychology, insight may be gained into the potential unique benefits of cannabis for both recreational and medicinal users (Table 32.3).

TABLE 32.3 Future Research Directions: Recreational and CTP Users

- Identification of unique and shared motivations for cannabis use among recreational and CTP users
- Further incorporation of the positive psychology perspective to understand the relationship between cannabis use and well-being, particularly concentrated on social inclusion, companionship, and self-validation
- Elucidating the possible roles of positive psychology in CTP: increased confidence in ability to self-manage illness, reduced pain-related anxiety, increased positive and decreased negative experiences while under the influence, and enhanced coping during those experiences
- The extent to which recreational and CTP users' motivations are satisfied

Note: CTP, cannabis for therapeutic purposes.

MINI-DICTIONARY

Anxiogenic Producing anxiety.
Anxiolytic Reducing anxiety.
Biopsychosocial model The view of illness as a dynamic and reciprocal interaction between biological, psychological, and sociocultural variables that shapes a person's response to pain.
Broaden and Build Theory of Positive Emotions The theory that positive emotions allow people to build on their physical and psychological resources by broadening their thought-action repertoires, that these resources fuel resiliency in times of struggle, and can be drawn on later for coping and survival.
CTP (cannabis for therapeutic purposes) The use of cannabis to treat medical or psychological ailments.
Dose–response hypothesis The hypothesis that increased cannabis use is associated with an increased development of psychological dysfunction.
Happiness An affective component of well-being, conceptualized as an individual's subjective state of mind, which is subject to changes in mood in a similar way as emotions.
Life satisfaction An individual's cognitive evaluation of their quality of life as a whole.
Positive psychology The scientific study of positive human functioning, and flourishing on multiple levels that include the biological, personal, relational, institutional, cultural, and global dimensions of life.
Self-medication hypothesis The hypothesis that substances of abuse relieve human psychological suffering in susceptible individuals, and there is a considerable degree of psychopharmacologic specificity in an individual's preferred drug.
Well-being An individual's evaluation of aspects of their lives, including satisfaction with their health, relationships, recreation, work, meaning, and purpose, and also their affective reactions to life events, and level of interest and engagement.

References

Allen, J., & Holder, M. D. (2013). Marijuana use and well-being in university students. *Journal of Happiness Studies*, *15*(2), 301–321.

Arendt, M., Rosenberg, R., Fjordback, L., Brandthold, J., Foldager, L., Sher, L., & Munk-Jorgensen, P. (2007). Testing the self-medication hypothesis of depression and aggression in cannabis dependent subjects. *Psychological Medicine*, *37*(7), 935–945.

Argyle, M. (2001). *The psychology of happiness* (2nd ed.). East Sussex, England: Routledge.

Barnwell, S. S., Earleywine, M., & Wilcox, R. (2006). Cannabis, motivation, and life satisfaction in an internet sample. *Substance Abuse Treatment, Prevention, and Policy*, *1*, 1–9.

Bonn-Miller, M. O., Zvolensky, M. J., Leen-Feldner, E. W., Feldner, M. T., & Yartz, A. R. (2005). Marijuana use among daily tobacco smokers: relationship to anxiety-related factors. *Journal of Psychopathology and Behavioral Assessment*, *27*(4), 279–289.

Bonn-Miller, M. O., Zvolensky, M. J., Bernstein, A. M., & Timothy, R. (2008). Marijuana coping motives interact with marijuana use frequency to predict anxious arousal, panic related catastrophic thinking and worry among current marijuana use among current marijuana users. *Depression and Anxiety*, *25*(10), 862–873.

Bottorff, J. L., Johnson, J. L., Moffat, B. M., & Mulvogue, T. (2009). Relief-oriented use of marijuana by teens. *Substance Abuse, Treatment, Prevention, and Policy*, *4*(7), 1–11.

Bovasso, G. B. (2001). Cannabis abuse as a risk factor for depressive symptoms. *The American Journal of Psychiatry*, *158*(12), 2033–2037.

Boyd, A. J. (2004). Medical marijuana and personal autonomy. *The John Marshall Law Review*, *37*(4), 1253–1288.

Buckner, J. D., Crosby, R. D., Silgado, J., Wonderlich, S. A., & Schmidt, N. B. (2012a). Immediate antecedents of marijuana use: an analysis from ecological momentary assessment. *Journal of Behavior Therapy and Experimental Psychiatry*, *43*(1), 647–655.

Buckner, J. D., Zvolensky, M. J., & Schmidt, N. B. (2012b). Cannabis-related impairment and social anxiety: the roles of gender and cannabis use motives. *Addictive Behaviors*, *37*(11), 1294–1297.

Chabrol, H., Ducongé, E., Casas, C., Roura, C., & Carey, K. B. (2005). Relations between cannabis use and dependence, motives for cannabis use and anxious, depressive and borderline symptomatology. *Addictive Behaviors*, *30*(4), 829–840.

Chen, C. Y., Wagner, F. A., & Anthony, J. C. (2002). Marijuana use and the risk of major depressive episode: epidemiological evidence from the United States National Comorbidity Survey. *Social Psychiatry and Psychiatric Epidemiology*, *37*(5), 199–206.

Cheung, J. T. W., Mann, R. A., Ialomiteanu, A., Stoduto, G., Chan, V., Ala-Leppilampi, K., & Rehm, J. (2010). Anxiety and mood disorders and cannabis use. *American Journal of Drug and Alcohol Abuse*, *36*, 118–122.

Crippa, J. A., Zuardi, A. W., Martin-Santos, R., Bhattacharyya, S., Atakan, Z., … Fusar-Poli, P. (2009). Cannabis and anxiety: a critical review of the evidence. *Human Psychopharmacology: Clinical and Experimental*, *24*(7), 515–523.

de Graaf, R., Radovanovic, M., van Laar, M., Fairman, B., Degenhardt, L., … Anthony, J. C. (2010). Early cannabis use and estimated risk of later onset of depression spells: epidemiologic evidence from the population-based World Health Organization World Mental Health Survey Initiative. *American Journal of Epidemiology*, *172*(2), 149–159.

Degenhardt, L., Hall, W., & Lynskey, M. (2001). Alcohol, cannabis, and tobacco use among Australians: a comparison of their associations with other drug use and use disorders, affective and anxiety disorders, and psychosis. *Addiction*, *96*(11), 1603–1614.

Degenhardt, L., Coffey, C., Carlin, J. B., Swift, W., Moore, E., & Patton, G. C. (2010). Outcomes of occasional cannabis use in adolescence: 10-year follow-up study in Victoria, Australia. *British Journal of Psychiatry*, *196*(4), 290–295.

Degenhardt, L., Coffey, C., Romaniuk, H., Swift, W., Carlin, J. B., Hall, W. D., & Patton, G. C. (2013). The persistence of the association between adolescent cannabis use and common mental disorders into young adulthood. *Addiction*, *108*(1), 124–133.

Demir, M., & Weitekamp, L. E. (2007). I am so happy cause today I found my friend: friendship and personality as predictors of happiness. *Journal of Happiness Studies*, *8*(2), 181–211.

Denson, T. F., & Earleywine, M. (2006). Decreased depression in marijuana users. *Addictive Behaviors*, *31*(4), 738–742.

Durdle, H., Lundahl, L. H., Johanson, C. E., & Tancer, M. (2008). Major depression: the relative contribution of gender, MDMA, and cannabis use. *Depression and Anxiety*, *25*(3), 241–247.

Fairman, B. J., & Anthony, J. C. (2012). Are early-onset cannabis smokers at an increased risk of depression spells? *Journal of Affective Disorders*, *138*(1), 154–162.

Fergusson, D. M., & Boden, J. M. (2008). Cannabis use and later life outomes. *Addiction*, *103*(6), 969–976.

Fergusson, D. M., Horwood, J. L., & Swain-Campbell, N. (2002). Cannabis use and psychosocial adjustment in adolescence and young adulthood. *Addiction*, *97*(9), 1123–1135.

Fredrickson, B. L. (2004). The broaden-and-build theory of positive emotions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *359*(1449), 1367–1378.

Georgiades, K., & Boyle, M. H. (2007). Adolescent tobacco and cannabis use: young adult outcomes from the Ontario child health study. *Journal of Child Psychology and Psychiatry*, *48*(7), 724–731.

Green, B., Kavanagh, D., & Young, R. (2003). Being stoned: a review of self-reported cannabis effects. *Drug and Alcohol Review*, *22*(4), 453–460.

Gruber, A. J., Pope, H. G., Hudson, J. I., & Yurgelun-Todd, D. (2003). Attributes of long-term heavy cannabis users: a case-control study. *Psychological Medicine*, *33*(8), 1415–1422.

Harder, V. S., Morral, A. R., & Arkes, J. (2006). Marijuana use and depression among adults: testing for causal associations. *Addiction*, *101*(10), 1463–1472.

Harris, D., Jones, R. T., Shank, R., Fernandez, E., Goldstein, K., & Mendelson, J. (2000). Self reported marijuana effects and characteristics of 100 San Francisco medical marijuana club members. *Advances in Alcohol and Substance Use*, *19*(3), 89–103.

Hayatbakhsh, M. R., Najman, J. M., Jamrozik, K., Mamon, A. A., Alati, R., & Bor, W. (2007). Cannabis and anxiety and depression in young adults: a large prospective study. *Journal of the American Academy of Child and Adolescent Psychiatry*, *46*(3), 408–417.

Holder, M. D. (2012). *Happiness in children: Measurement, correlates and enhancement of positive subjective well-being*. Dordrecht, The Netherlands: Springer.

Hooshmand, S., Willoughby, T., & Good, M. (2012). Does the direction of effects in the association between depressive symptoms and health-risk behaviors differ by behavior? A longitudinal study across the high school years. *Journal of Adolescent Health*, *50*(2), 140–147.

Johns, A. (2001). Psychiatric effects of cannabis. *British Journal of Psychiatry*, *178*, 116–122.

Johnson, J. L., Greaves, L., & Repta, R. (2007). *Better science with sex and gender: A primer for health research*. Vancouver, BC: Women's Health Research Network.

Keyes, C. L. M., & Simoes, E. J. (2012). To flourish or not: positive mental health and all-cause mortality. *American Journal of Public Health*, *102*(11), 2164–2172.

Khantzian, E. J. (1985). The self-medication hypothesis of addictive disorders: focus on heroin and cocaine dependence. *American Journal of Psychiatry*, *142*, 1259–1264.

Korthuis, T., Zephyrin, L. C., Fleishman, J. A., Saha, S., Josephs, J. S., Mcgrath, M. M., … Gebo, K. A. (2008). Health-related quality of life in HIV-infected patients: the role of substance use. *AIDS Patient Care and STDs*, *22*(11), 859–867.

Kouri, E., Pope, H. G., Yurgelun-Todd, D., & Gruber, S. (1995). Attributes of heavy vs. occasional marijuana smokers in a college population. *Biological Psychiatry*, *38*(7), 475–481.

Lee, C. M., Neighbors, C., & Woods, B. A. (2007). Marijuana motives: young adults' reasons for using marijuana. *Addictive Behaviors*, *32*(7), 1384–1394.

Marmorstein, N. R., White, H., Chung, T., Hipwell, A., Stouthamer-Louber, M., & Loeber, R. (2010). Associations between first use of substances and change in internalizing symptoms among girls: differences by symptom trajectory and substance use type. *Journal of Clinical Child and Adolescent Psychology*, *39*(4), 545–558.

Martins, S. S. (2011). Conditional substance abuse and dependence by diagnosis of mood or anxiety disorder or schizophrenia in the U.S. Population. *Drug and Alcohol Dependence*, *119*(1–2), 28–36.

Moore, T. H. N., Zammit, S., Lingford-Hughes, A., Barnes, T. R. E., Jones, P. B., … Lewis, G. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *The Lancet*, *370*(9584), 312–328.

Moreau, J. J. (1973). *Hashish and mental illness*. New York, NY: Raven Press.

Moussavi, S., Chatterji, S., Verdes, E., Tandon, A., Patel, V., & Ustun, B. (2007). Depression, chronic diseases, and decrements in health: results from the World Health Surveys. *The Lancet*, *370*(9590), 851–858.

Nail, R., Gunderson, E. K., & Kolb, D. (1974). Motives for drug use among light and heavy users. *Journal of Nervous and Mental Disease*, *159*(2), 131–136.

Patton, G. C., Coffey, C., Carlin, J. B., Degenhardt, L., Lynskey, M., & Hall, W. (2002). Cannabis use and mental health in young people: cohort study. *British Medical Journal*, *325*, 1195–1198.

Pedersen, W. (2008). Does cannabis use lead to depression and suicidal behaviors? A population-based longitudinal study. *Acta Psychiatrica Scandinavica*, *118*(5), 395–403.

Poulin, C., Hand, D., Boudreau, B., & Santor, D. (2005). Gender differences in the association between substance use and elevated depressive symptoms in a general adolescent population. *Addiction*, *100*(4), 525–535.

Ranney, M. L. (2013). Correlates of depressive symptoms among at-risk youth presenting to the emergency department. *General Hospital Psychiatry*, *35*(5), 537–544.

Reinarman, C., Nunberg, H., Lanthier, F., & Heddleston, T. (2011). Who are medical marijuana patients? Population characteristics from nine California assessment clinics. *Journal of Psychoactive Drugs*, *43*(2), 128–135.

Rey, J. M., Sawyer, M. G., Raphael, B., Patton, G. C., & Lynskey, M. (2002). Mental health of teenagers who use cannabis: results of an Australian survey. *British Journal of Psychiatry*, *180*(3), 216–221.

Russo, E. B. (2007). History of cannabis and its preparations in saga, science, and sobriquet. *Chemistry and Biodiversity*, *4*(8), 1614–1648.

Scott, K. M., Bruffaerts, R., Tsnag, A., Ormel, J., Alonso, J., … Von Korff, M. (2007). Depression-anxiety relationships with chronic physical conditions: results from the World Mental Health surveys. *Journal of Affective Disorders*, *103*(1–3), 113–120.

Sherbourne, C. D., Hays, R. D., Fleishman, J. A., Vitiello, B., Magruder, K. M., Bing, E. G., … Shapiro, M. F. (2000). Impact of psychiatric conditions on health-related quality of life in persons with HIV infection. *The American Journal of Psychiatry*, *157*(2), 248–254.

Shrier, L. A., Walls, C. E., Kendall, A. D., & Blood, E. A. (2012). The context of desire to use marijuana: momentary assessment of young people who frequently use marijuana. *Psychology of Addictive Behaviors*, *26*(4), 821–829.

Simons, J., Correia, C. J., Carey, K. B., & Borsari, B. E. (1998). Validating a five-factor marijuana motives measure: relations with use, problems, and alcohol motives. *Journal of Counseling Psychology*, *45*(3), 265–273.

Steel, P., Schmidt, J., & Shultz, J. (2008). Refining the relationship between personality and subjective well-being. *Psychological Bulletin*, *134*(1), 138–161.

Tkach, C., & Lyubomirsky, S. (2006). How do people pursue happiness? Increasing strategies and well-being. *Journal of Happiness Studies*, *7*(2), 183–225.

Tournier, M., Sorbara, F., Gindre, C., Swendsen, J. D., & Verdoux, H. (2003). Cannabis use and anxiety in daily life: a naturalistic investigation in a non-clinical population. *Psychiatry Research*, *118*(1), 1–8.

Turk, D. C., & Okifuji, A. (2002). Psychological factors in chronic pain: evolution and revolution. *Journal of Consulting and Clinical Psychology*, *70*(3), 678–690.

van Laar, M., van Dorsselaer, S., Monshouwer, K., & de Graaf, R. (2007). Does cannabis use predict the first incidence of mood and anxiety disorders in the adult population? *Addiction*, *102*(8), 1251–1260.

Walsh, Z., Callaway, R., Belle-Isle, L., Capler, R., Kay, R., Lucas, P., & Holtzman, S. (2013). Cannabis for therapeutic purposes: patient characteristics, access, and reasons for use. *International Journal of Drug Policy*, *24*(6), 511–516.

WHO. (2013). *World health statistics 2013*. Available from http://apps.who.int/iris/bitstream/10665/82058/1/WHO_HIS_HSI_13.1_eng.pdf?ua=1

Wittchen, H. U., Frolich, C., Behrendt, S., Günther, A., Rehm, J., Zimmerman, P., … Perkonigg, A. (2007). Cannabis use and cannabis use disorders and their relationship to mental disorders: a 10-year prospective-longitudinal community study in adolescents. *Drug and Alcohol Dependence*, *88*, S60–S70.

Zvolensky, M. J., Vujanovic, A. A., Bernstein, A., Bonn-Miller, M. O., Marshall, E. C., & Leyro, T. M. (2007). Marijuana use motives: a confirmatory test and evaluation among young adult marijuana users. *Addictive Behaviors*, *32*(12), 3122–3130.

Zvolensky, M. J., Cougle, J. R., Bonn-Miller, M. O., Norberg, M. M., Johnson, K., Kosiba, J., & Asmundson, G. J. (2011). Chronic pain and marijuana use among a nationally representative sample of adults. *The American Journal on Addictions*, *20*(6), 538–542.

CHAPTER

33

Craving and Cannabis: A Potential Paradox

M.J. Loflin, M. Earleywine

Department of Psychology, School of Arts and Sciences, University at Albany, State University of New York, Albany, NY, United States

SUMMARY POINTS

- Cannabis is the second most common reason for entering treatment for substance abuse and dependency in the United States, and the most commonly used illicit drug.
- Current models of cannabis use include craving as a potential indicator of problematic use, and a major reason for relapse following abstinence.
- While a substantial literature exists, supporting craving's role in substance abuse generally, less basic science exists for cannabis craving.
- Given cannabis' pharmacological properties, craving for the plant might not develop the same as other drugs of abuse.
- Despite inconsistencies with theoretical etiology, a significant proportion of cannabis users in treatment report craving for the plant.

KEY FACTS

- The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) is most the commonly used classification manual of mental disorders in the United States. ICD-10 more commonly used outside the United States.
- Majority of research on substance abuse conducted using DSM criterion schema.
- Changes to classification of substance use disorders in Fifth edition of manual controversial in the research community.
- DSM-5 added criterion of craving for diagnosis of cannabis use disorder and lowered overall criterion threshold.

LIST OF ABBREVIATIONS

ANS	Autonomic nervous system
CBI	Cannabinoid receptor
DSM	Diagnostic and Statistical Manual of Mental Disorders
IRT	Item response theory
THC	Tetrahydrocannabinol
VTA	Ventral tegmental area

INTRODUCTION

Marijuana (cannabis) is the second most commonly cited reason for seeking drug treatment. Marijuana users represent 17.5% of all those who are in treatment for substance-related problems in the United States (SAMHSA, 2012). Identifying what differentiates problematic from nonproblematic use of the plant has been a major focus of cannabis research to date, and is vital as cannabis regulations loosen across the United States and globally. Research on drug abuse more generally suggests that craving might be an important factor of interest. However, it is unclear whether craving can result as a natural development of ingestion of the cannabis

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00037-5
Copyright © 2017 Elsevier Inc. All rights reserved.

plant, and whether or not it is a distinguishing indicator of problematic use. Evidence exists that both supports and refutes the assumption that problematic cannabis users crave. While basic science would suggest that cannabinoid intoxication should not result in craving, many problematic cannabis users do report "craving." These users cite craving as a major reason for relapse, following abstinence and an obstacle in treatment (Budney, Vandrey, Hughes, Thostenson, & Bursac, 2008; McRae, Hedden, Malcolm, Carter, & Brady, 2007; Coffey et al., 2002). In addition, applied research on cannabis craving uses similar cue-based craving paradigms to manipulate craving as those used for other drugs (Singleton, Trotman, Zavahir, Taylor, & Heishman, 2002; Wolfling, Flor, & Grusser, 2008). This assumes that cannabis craving functions the same as other substances of abuse. There is an apparent paradox in the literature between the known etiology of craving and problematic cannabis users' experience.

FUNCTION OF CRAVING IN DRUG ABUSE

The role of craving in drug abuse has received increased attention in the research literature due to the new inclusion of craving as a diagnostic criterion for substance-related disorders in the DSM 5 (American Psychiatric Association, 2014). From the bench to the bedside, experimental and applied research suggests that drug users' experience of craving is associated with maintenance of use, an increased risk of dependency, and an increased risk of relapse following abstinence from use of the drug (Flannery et al., 2006; Robinson & Berridge, 2003; Everitt, 1997; Pickens & Johanson, 1992; Ludwig, Wikler, & Stark, 1974). Though no universally accepted definition of drug craving exists, the experience of craving is broadly defined by its motivational state, where the individual experiences strong desire to use a drug (Tiffany & Wray, 2012). Craving differs from thoughts of enjoyment, or liking, of the effects of a drug in both degree and kind (West, 1987). Craving is certainly a more severe form of liking or wanting (Kozlowski, Mann, Wilkinson, & Poulos, 1989); however, dependency and other problems related to use do not predict differences across users in how well one likes or enjoys the effects of a drug (Volkow et al., 1993). Craving represents a shift away from mere enjoyment of the rewarding aspects of use of the drug (pleasurable effects) toward a motivational drive to obtain the drug (Robinson & Berridge, 1993; Wise, 2004).

Despite a substantial body of literature supporting cravings role in drug seeking behavior (for review see Tiffany & Wray, 2012), researchers and clinicians disagree on how best to conceptualize and measure craving (Drobes & Thomas, 1999). Moreover, theoretical inconsistency across measures and assumptions of drug similarity can lead to problems, when attempting to cross-apply work on craving from one substance to another. The rationale provided by the American Psychiatric Association's work group responsible for the addition of craving in the newest DSM cited extensive literature on the role of craving in problematic alcohol and cocaine users (Casteel & Valora, 2010). No work was cited that investigated the role of craving for other drug classes. Clinicians are meant to assume that what is good for the goose is good for the gander when diagnosing drug-related problems. Studies that rely on an assumption of similar nomothetic principles of drug abuse across substances tend to cite early work demonstrating that, across drug users, we see similar reports of problems (Koston, Rounsaville, Babor, Spitzer, & Williams, 1987; Feingold & Rounsaville, 1995). This assumption, however, also assumes that those individual symptoms and problems are also similar in both degree and kind across substances, and equally indicate problematic use. This assumption is not supported by the literature; individual symptom criteria perform differently across substances in item response theory (IRT) analysis (Gillespie, Neale, Prescott, Aggen, & Kendler, 2007). If craving is understood as a more severe form of liking, or enjoyment of a drug's effects, and drug's effects differ across classes, then endorsement of "craving" cannot be assumed to be analogous when cross-comparing drug users. To explore whether we have grounds to cross-apply this literature to cannabis we will first turn to the literature on craving, more generally.

THEORETICAL ETIOLOGY

Craving is generally thought to develop through a process of behavioral learning and neurobiological stimulation from ingestion of the psychoactive substance (Skinner & Aubin, 2010). When a behavior is followed by reward, it is more likely that the behavior will be repeated in the future (Skinner, 1938). Using a substance with pleasurable effects results in reward via the subjective effect of the drug (Berridge & Robinson, 2003). Through a process of instrumental conditioning, regular use of the rewarding substance will increase motivation for future use of the drug. Moreover, tenants of learning theory can also explain how environmental cues can take on incentive value, increasing one's motivational state to use through a process of Pavlovian conditioning. When environmental variables related to drug use are repeatedly paired with the pleasurable/rewarding effects of use of that drug, those variables (drug cues) will begin to act as conditioned stimuli that can invoke expectation of the anticipated response (Fig. 33.1). When the pleasurable response (eg, intoxication, euphoria) that would typically follow presentation of drug cues is denied (as one

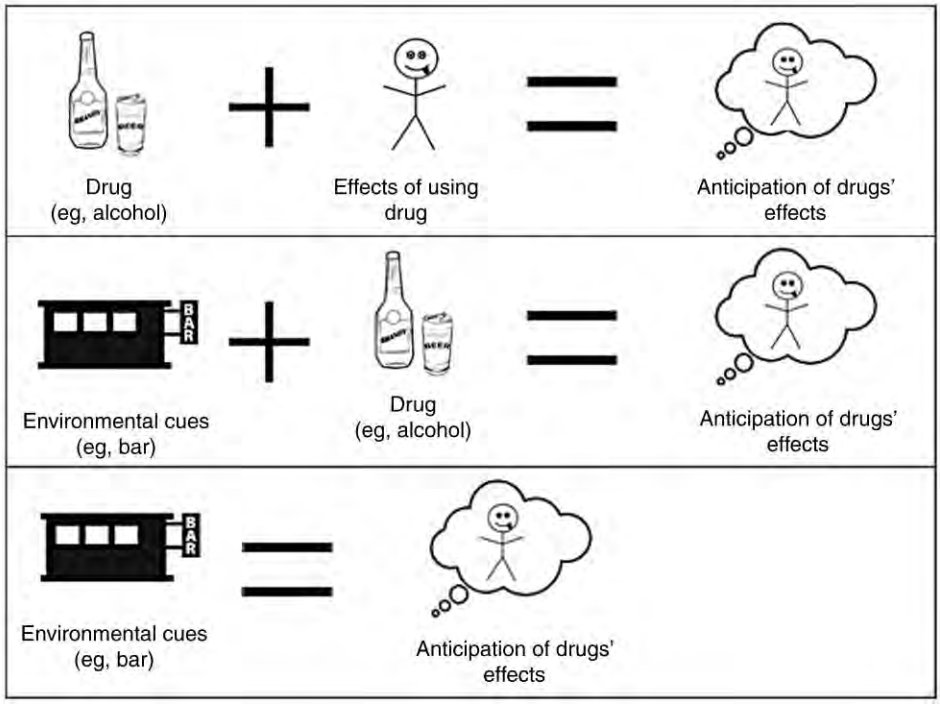


FIGURE 33.1 **How stimuli in the environment can cue craving.** Drug related stimuli in the environment can eventually take on incentive value through repeated pairings with the drug, and start to act as conditioned stimuli to elicit craving.

would see if cues are presented and use is restrained), the body goes into an anticipatory appetitive response. The anticipatory response is functionally analogous to an acute state of withdrawal (Verheul, van den Brink, & Geerlings, 1999). Environmental cues associated with use of the drug then motivate future use of the drug through both positive and negative forms of reinforcement.

While enjoyment of the effects of the drug can certainly act as a reinforcer, pleasure and reward are distinct phenomena (Smith, Berridge, & Aldridge, 2011). Not all behaviors that are subjectively pleasurable are craveable in a pathological sense. Early research in addiction attributed the craveable nature of drugs to activation of dopamine pathways in the ventral tegmental area (VTA), colloquially referred to as the "pleasure pathway" (Fig. 33.2). Studies suggested that the subjective pleasurable experience of drug use was due to activation of the dopaminergic system (Wise, 1980; Volkow et al., 1999). Dopamine, however, likely plays a more complicated role in development of motivation to use a drug than simply being the neurotransmitter associated with pleasurable experience. Craving is more than mere liking. More recent work in addiction suggests that mesolimbic dopamine release is associated with increases in attention (Berridge, 2007; Schultz, 2013). This new role of dopamine would explain why pathological craving is present in substance abuse, gambling, and binge eating (Potenza, 2008; Blumenthal & Gold, 2010), but to a lesser degree with other naturally enjoyable, but less abused, activities. Activation of

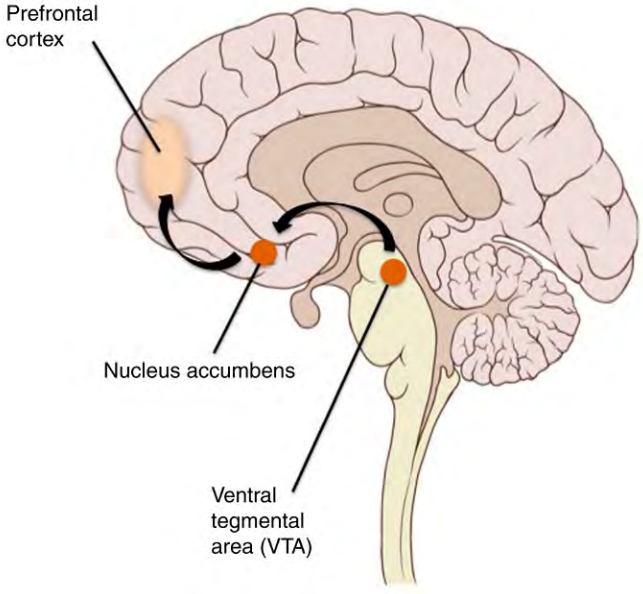


FIGURE 33.2 **The "pleasure pathway."** The pleasurable effects of drugs are largely attributable to neural communication between the ventral tegmental area *(VTA)*, nucleus accumbens, and the prefrontal cortex. *Source: Modification from open content image created by Patrick J. Lynch, medical illustrator; C. Carl Jaffe, MD, cardiologist. http://creativecommons.org/licenses/by/2.5/. Image is available for replication without prior permissions, with no usage restrictions, except to preserve creative credits as noted earlier.*

dopaminergic pathways in the VTA increase attention, thereby increasing attention to environmental stimuli being presented at the time of drug use. By increasing the salience of stimuli, dopamine acts to reduce the proximal distance between cues, use of the drug, and the pleasurable effects. Learning, therefore, occurs more rapidly. Dopaminergic activation, by means of increasing attention to cues, facilitates more effective reinforcement.

CRAVING AND CUE-REACTIVITY

When stimuli in the environment become associated with drug reward through Pavlovian conditioning, the stimuli can then elicit the conditioned response. Expectation of the drug's effects can be induced even in the absence of use of the drug itself. This state of expectation that motivates interest in use of the drug is referred to as cue-elicited craving (Drummond, 2000). Presenting environmentally relevant cues and examining whether or not they induce drug-incentive expectation can determine whether or not craving for a drug is present. If cues associated with the use of a drug lead to an increase in interest for taking the drug, there is support that classical conditioning has occurred. Studies using cue-based paradigms to test for craving appear frequently in the literature. Support for the use of these paradigms comes from neuroimaging studies (a visual of neuroimaging technology appears in Fig. 33.3), which find that drug-related stimuli are able to induce dopamine release in the mesolimbic VTA, even before the experience of pleasure (Duvauchelle, Ikegami, & Castaneda, 2000). The conditioned stimulus can invoke a conditioned response.

Testing for the presentation of craving using cue-eliciting stimuli rests on the assumption that all forms of drug craving are functionally and etiologically similar. The paradigm assumes that craving develops through a process of classical and instrumental conditioning, and that the readiness of the user to learn these associations is increased through the pharmacological effects of the drug on VTA dopamine release. If we assume this general etiological framework for craving, then the paradigm makes specific predictions about what will occur following the presentation of drug-related cues.

In addition to overt thoughts about wanting to use the drug, we would also expect to see changes in attention, cognitive functioning, and autonomic arousal as the user enters into an anticipatory state where the user is awaiting pleasure, and experiencing acute withdrawal. The body is expecting the pharmacological and subjective effects of drug use when shown cues of the drug. As the body prepares for the drug's anticipated effects, dopamine is being released and increasing selective attention to reward-sensitive stimuli (Duvauchelle et al., 2000). We would expect users who have been cued to overly attend to drug-relevant stimuli. Moreover, this state of increased selective attention to drug cues should also make it more difficult to attend to stimuli that are not relevant to use of the drug, and interfere in completion of cognitive tasks that rely on switching of attention. Indeed, studies on alcohol and cocaine abuse find that attention to drug-related stimuli tends to increase, and cognitive flexibility that requires attentional switching tends to decrease (increased perseverance) following the presentation of drug cues (Dunning et al., 2011; Cox, Fadardi, & Pothos, 2006; Ehrman et al., 2002; Franken, Kroon, & Hendriks, 2000).

Finally, cues should also increase physiological arousal. Autonomic nervous system (ANS) activity is a major component of emotion (for review see Kreibig, 2010). Craving is an affective state driven by an appetitive response (Orford, 2001). When primed to anticipate the effects of a drug, which is rewarding, the user is thinking

FIGURE 33.3 **Neuroimaging.** Researcher examining data output of one neural imaging technique, functional magnetic resonance imaging (fMRI). *Source: From National Institute of Mental Health. Appears in the public domain. No permissions are needed to replicate as "NIMH publications are in the public domain and may be reproduced or copied without permission from the National Institute of Mental Health."*

TABLE 33.1 Withdrawal Symptoms Most Often Reported by Newly Abstinent Drug Users

List of common withdrawal symptoms
Cold shakes
Chills and sweating
Fever-like symptoms
Mood swings
Anxiety and depression
Bone pain
Vomiting
Insomnia
Diarrhea

about experiencing pleasure. Imagining exposure leads to the affective (emotional) experience of anticipatory pleasure (Marlatt, 1987). Engaging with emotionally evocative materials that signal anticipatory pleasure activates ANS activity. The experience of anticipatory pleasure is associated with increases in overall galvanic skin-conductance response (Bernat, Patrick, Benning, & Tellegan, 2006; Codispoti, Surcinelli, & Baldaro, 2008) and decreases in heart rate (Bernat et al., 2006; Codispoti et al., 2008; Ritz, Thons, Fahrenkrug, & Dahme, 2005). Some studies suggest, however, that when anticipatory pleasure is cued through cognitive processes (eg, imaginally invoked), heart rate instead decreases (Van Diest et al., 2001).

In addition, neuroadaptation to the effects of the drug establish a threshold of pleasure/homeostasis that the brain anticipates when in a given state (in the presence of drug cues; DiFranza & Wellman, 2005). When anticipated effects are not reached, the body experiences mild withdrawal symptoms, such as irritability/anxiety (Jasova, Bob, & Fedor-Freybergh, 2007; Table 33.1). The body invokes a mild stress response. Therefore, autonomic arousal would also be expected following the presentation of drug-cues as a product of frustration from anticipating, but not receiving reward. Autonomic activation during states of anxiety is primarily a sympathetic response. Anxiety increases heart rate (Adsett, Schottsteadt, & Wolf, 1962) and increases galvanic skin response (Chan & Lovibond, 1996). Drug cues that can invoke an emotional experience of anticipatory pleasure and/or acute withdrawal-related stress, therefore, should modify heart rate and overall galvanic skin response.

CUE-REACTIVITY PARADIGM

Specific predictions that stem from theories of craving have been tested using the cue-reactivity paradigm. Cue-elicited craving manipulations with alcohol, nicotine, cocaine, and heroin all consistently find that when presented with drug-relevant cues users demonstrate increases in self-reported craving (Hone-Blanchet, Wensign, & Fecteau, 2014; Carter & Tiffany, 1999; Cepeda-Benito & Tiffany, 1996; Cortese et al., 2014). These studies also find postcue changes in attention and increases in autonomic arousal (Carter & Tiffany, 1999; Cepeda-Benito & Tiffany, 1996).

THEORETICAL ISSUES WITH CANNABIS CRAVING

Craving of cannabis is assumed to develop and function the same as other drugs of abuse. Interventions focused on reducing craving to all drugs, including cannabis, rely on this assumption of similarity in etiology and function. Nevertheless, the dependency risk associated with cannabis is less understood than with other substances of abuse. The assumption that cannabis craving develops and functions similar to other drugs of abuse might not be supported.

The addictive potential of most drugs of abuse is thought to result from properties of the substance acting on the brain's mesolimbic dopamine system (Wise, 2004; Hyman, Malenka, & Nestler, 2006). This system projects from the VTA to the ventral striatum, which is composed of the nucleus accumbens. The generally accepted theory of the rewarding, and thereby addictive, nature of drugs of abuse is that these substances enhance synaptic dopamine levels in the ventral striatum. As discussed previously, the enhancement of dopamine in the VTA is functionally related to both reward/reinforcement, and increased selective attention. Increased synaptic dopamine levels in the VTA have been found following administration of many drugs of abuse, including amphetamine (Breier et al., 1997), cocaine (Schlaepfer, Pearlson, Wong, Marenco, & Dannals, 1997), alcohol (Boileau et al., 2003), and nicotine (Brody et al., 2006).

Some work does suggest that cannabis can stimulate striatal dopamine neurotransmission (Martin-Santos et al., 2010; Bossong et al., 2009). However, the amount of increased dopaminergic availability following administration of psychoactive components of cannabis differs markedly from other drugs of abuse. Whereas amphetamine, cocaine, alcohol, and nicotine cause 10–30% reductions in dopamine D2/D3 receptor availability, human administration of tetrahydrocannabinol (THC) is only related to decreases of 3.4–3.9% (Bossong et al., 2009). Reductions in receptor availability lead to increases in striatal dopamine levels, suggesting that the amount of increase in striatal dopamine after ingestion of THC is less than the increases seen for amphetamine, cocaine, alcohol, and nicotine. Moreover, while high doses of THC can induce small increases in dopamine release in the VTA, the effect likely occurs indirectly

through CBI receptors on glutamate and GABA neurons (Schlicker & Kathmann, 2001; Lupica, Riegel, & Hoffman, 2004). Drugs with higher dependency indices tend to have stronger and more direct effects on the VTA dopamine pathway (Hyman et al., 2006). Whether or not the increase in dopamine following use of cannabis is sufficient to lead to craving in its classic sense has not been determined.

Even if use of cannabis does work on the dopaminergic pleasure pathway, the effects of cannabis intoxication undermine a purely biobehavioral etiology for craving of cannabis. Dopamine's role in craving development is in increasing attention to cues and reward for the purposes of increasing reinforcement of drug use. Reinforcement leads to craving by means of classical and instrumental conditioning. Two important effects of cannabis' psychoactive compound Δ^9-THC are decreased sustained and transient attention (Crean, Crane, & Mason, 2011; Ilan, Smith, & Gevins, 2004) and acute impairment of several forms of memory (for review see Ranganathan & D'Souza, 2006). Right dorsolateral prefrontal cortex, (para)hippocampal, and regions of the cerebellum show evidence of decreased activation during acute intoxication of cannabis, major pathways for associative learning. Basic and applied studies demonstrate that intoxication leads to deficits in associative learning (Nestor, Roberts, Garavan, & Hester, 2007; Skosnik et al., 2008). Contextual encoding is also disrupted during cannabis intoxication (Ranganathan & D'Souza, 2006). If craving develops in part through behavioral reinforcement, then associative learning is critical for being able to learn the pairing of stimulus and response. If associative learning is disrupted, then reinforcement value can't be learned, and cues should not lead to an appetitive response (Fig. 33.4).

Known neurological intoxication effects of cannabis use are inconsistent with current theory of the etiology of drug craving. Cannabis users might not "crave" in the same way that other drug users experience craving. Nevertheless, many of those in treatment for cannabis use do report experiencing craving.

CUE-REACTIVITY STUDIES WITH CANNABIS

Cannabis craving is typically studied using the same cue-reactivity paradigms as other drugs of abuse. Nickerson et al. (2011) looked at cue-reactance of cannabis by showing pictures of flower marijuana to cannabis-dependent adolescents. They found that those who were presented with the cue reported higher craving for marijuana than those who were not. Comparable results of increased self-reported craving appear in other studies that employ primarily visual cues (Lundahl & Johanson, 2011; Wolfling et al., 2008). The study by Wolfling et al. (2008) examined physiological responses to visual material associated with cannabis (ie, pictures of marijuana) among heavy long-term cannabis users. Again, the results showed that visual cues increased self-reported craving, but also found that the cues increased arousal, specifically skin-conductance, among the cannabis users. This result relating cues to arousal did not replicate, however, in another experiment that found increased craving in response to cues (Lundahl & Johanson, 2011). While marijuana-associated pictures consistently increase reports of interest in use, they apparently do not always lead to heightened physiological reactions.

The experience of craving reported by marijuana users is not always consistent with theories of craving development. File-drawer problems and publication bias for significant results (Rosenthal, 1979) also raise questions about the few studies that do suggest that cannabis cues can induce craving in functionally similar ways to other substances of abuse. Nevertheless, limitations in previous cannabis craving studies also might account for modest effect sizes and failures to find effects. Most

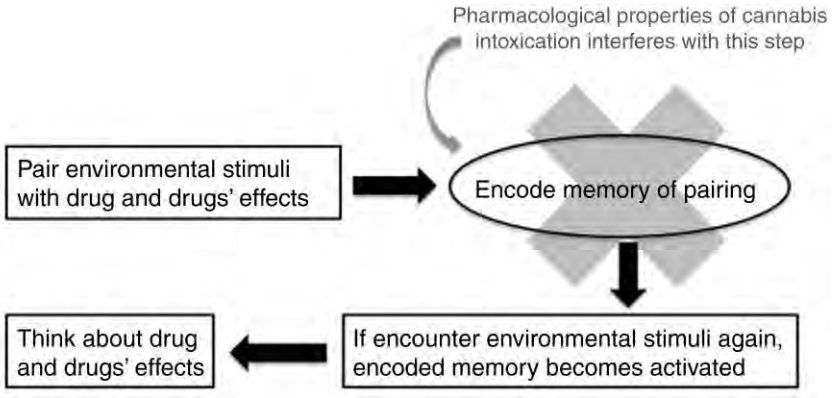


FIGURE 33.4 Craving unlikely to develop if memory encoding is disrupted. Cannabis intoxication impacts associative learning; a necessary step in the development of craving.

FIGURE 33.5 Cue-reactivity experiment using virtual reality. Cue-reactivity experiments have started incorporating the use of virtual reality to try to sidestep research design problems of relying on cues with poor generalizability. *Source: From NASA, and appears in public domain. No permissions are needed to replicate. NASA image use policy: http://www.jpl.nasa.gov/imagepolicy/*

studies use pictures and generic paraphernalia as cues (for additional, see Fig. 33.5). Participants might not use the type of ingestion mechanism presented (eg, glass pipes), and photographs might not generalize well to real world environmental stimuli. Moreover, the very fact that no accepted definition of craving exists in the scientific community suggests that definitions of what craving is likely differ across drug users. Cannabis users' definition of craving might not look the same as that of other substance users.

Findings remain inconsistent as to whether cannabis craving is a real phenomenon, and not just an issue of self-report. If craving really does develop as a response to regular cannabis use, then understanding its etiology will be a necessary task for future cannabis researchers.

MINI-DICTIONARY

Cue-reactivity Measurable reactions to environmental contexts or drug paraphernalia that reliably accompany use of the drug.
Diagnostic and Statistics Manual of Mental Disorders 5 (DSM 5) The most recent classification and diagnostic manual of the American Psychiatric Association (APA).
Instrumental conditioning (also known as Skinnerian conditioning) Process of behavior modification where a subject comes to emit a given response due to manipulation of the antecedents, and consequences of the given behavior.
Item response theory (IRT) analysis (also known as latent trait theory) Examines the response of examinees of a similar theoretical ability or attribute of interest to test whether they respond in similar ways on each item on the test or measure.
Learning theory Related to tenants of behaviorism; where learning is viewed as an observable change in behavior.
Pavlovian conditioning (also known as classical conditioning) Process of behavior modification where a subject comes to emit a given response in the presence of a previously neutral stimulus, because the neutral stimulus was associated with an unconditioned stimulus that elicits the desired response.

References

Adsett, C. A., Schottsteadt, W. W., & Wolf, S. G. (1962). Changes in coronary blood flow and other hemodynamic indicators induced by stressful interviews. *Psychosomatic Medicine*, *24*, 331–336.

American Psychiatric Association. (2014). Diagnostic and statistical manual of mental disorders (5th ed.).

Bernat, E., Patrick, C. J., Benning, S. D., & Tellegan, A. (2006). Effects of picture content and intensity on affective physiological response. *Psychophysiology*, *43*, 93–103.

Berridge, K. C. (2007). The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology*, *191*, 391–431.

Berridge, K. C., & Robinson, T. E. (2003). Parsing reward. *Trends in Neurosciences*, *26*, 507–513.

Blumenthal, D. M., & Gold, M. S. (2010). Neurobiology of food addiction. *Current Opinion in Clinical Nutrition and Metabolic Care*, *13*, 359–365.

Boileau, I., Assaad, J. M., Pihl, R. O., Benkelfat, C., Leyton, M., Diksic, M., et al. (2003). Alcohol promotes dopamine release in the human nucleus accumbens. *Synapse*, *49*, 226–231.

Bossong, M. G., van Berckel, B. N., Boellaard, R., Zuurman, L., Schuit, R. C., Windhorst, A. D., …, & Kahn, R. S. (2009). Δ9-tetrahydrocannabinol induces dopamine release in the human striatum. *Neuropsychopharmacology*, *34*, 759–766.

Breier, A., Su, T. P., Saunders, R., Carson, R. E., Kolachana, B. S., De Bartolomeis, A., et al. (1997). Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. *Proceedings of the National Academy of Sciences of the United States of America*, *94*, 2569–2574.

Brody, A. L., Mandelkern, M. A., Olmstead, R. E., Scheibal, D., Han, E., Schiraga, S., et al. (2006). Gene variants of the brain dopamine pathways and smoking-induced dopamine release in the ventral caudate/nucleus accumbens. *Archives of General Psychiatry*, *63*, 808–816.

Budney, A. J., Vandrey, R. G., Hughes, J. R., Thostenson, J. D., & Bursac, Z. (2008). Comparison of cannabis and tobacoo withdrawal: severity and contribution to relapse. *Journal of Substance Abuse Treatment*, *35*, 362–368.

Carter, B. L., & Tiffany, S. T. (1999). Meta-analysis of cue-reactivity in addiction research. *Addiction*, *94*, 327–340.

Casteel, B., & Valora, J. (2010). *APA announces draft diagnostic criteria for DSM-5.* Available from http://www.dsm5.org/Newsroom/Documents/Diag%20%20Criteria%20General%20FINAL%202.05.pdf

Cepeda-Benito, A., & Tiffany, S. T. (1996). The use of dual-task procedurefor the assessment of cognitive effort associated with cigarette craving. *Psychopharmacology (Berl)*, *127*(2), 155–163.

Chan, K. Y. C., & Lovibond, P. F. (1996). Expectancy bias in trait anxiety. *Journal of Abnormal Psychology*, *105*, 637–647.

Codispoti, M., Surcinelli, P., & Baldaro, B. (2008). Watching emotional movies: affective reactions and gender differences. *International Journal of Psychophysiology*, *69*, 90–95.

Coffey, C., Carlin, J. B., Degenhardt, L., Lynskey, M., Sanci, L., & Patton, G. C. (2002). Cannabis dependence in young adults: an Australian population study. *Addiction*, *97*, 187–194.

Cortese, B. M., Uhde, T. W., LaRowe, S. D., Stein, S. V., Freeman, W. C., McClernon, F. J., …, Hartwell, K. J. (2014). Olfactory cue reactivity in nicotine-dependent adult smokers. *Psychology of Addictive Behaviors*, *29*, 91–96.

Cox, W. M., Fadardi, J. S., & Pothos, E. M. (2006). The addiction-Stroop test: theoretical considerations and procedural recommendations. *Psychological Bulletin*, *132*, 443–476.

Crean, R. D., Crane, N. A., & Mason, B. J. (2011). An evidence based review of acute and long-term effects of cannabis use on executive cognitive functions. *Journal of Addiction Medicine*, *5*, 1–8.

DiFranza, J. R., & Wellman, R. J. (2005). A sensitization—homeostasis model of nicotine craving, withdrawal, and tolerance: integrating the clinical and basic science literature. *Nicotine and Tobacco Research*, *7*, 9–26.

Drobes, D. J., & Thomas, S. E. (1999). Assessing craving for alcohol. *Alcohol Research and Health*, *23*, 179–186.

Drummond, D. C. (2000). What does cue-reactivity have to offer clinical research? *Addiction*, *95*, 129–144.

Dunning, J. P., Parvaz, M. A., Hajcak, G., Maloney, T., Alia-Klein, N., Woicik, P. A., …, Goldstein, R. Z. (2011). Motivated attention to cocaine and emotional cues in abstinent and current cocaine users—an ERP study. *European Journal of Neuroscience*, *33*, 1716–1723.

Duvauchelle, C. L., Ikegami, A., & Castaneda, E. (2000). Conditioned increases in behavioral activity and accumbens dopamine levels produced by intravenous cocaine. *Behavioral Neuroscience*, *114*, 1156–1166.

Ehrman, R. N., Robbins, S. J., Bromwell, M. A., Lankford, M. E., Monterosso, J. R., & O'Brien, C. P. (2002). Comparing attentional bias to smoking cues in current smokers, former smokers, and nonsmokers using a dot-probe task. *Drug and Alcohol Dependence*, *67*, 185–191.

Everitt, B. (1997). Craving cocaine cues: cognitive neuroscience meets drug addiction research. *Trends in Cognitive Science*, *1*, 1.

Feingold, A., & Rounsaville, B. (1995). Construct validity of the dependence syndrome as measured by DSM-IV for different psychoactive substances. *Addiction*, *90*, 1661–1669.

Flannery, B. A., Roberts, A. J., Cooney, N., Swift, R. M., Anton, R. F., & Rohsenow, D. J. (2006). The role of craving in alcohol use, dependence, and treatment. *Alcoholism, Clinical and Experimental Research*, *25*, 299–308.

Franken, I. H., Kroon, L. Y., & Hendriks, V. M. (2000). Influence of individual differences in craving and obsessive cocaine thoughts on attentional processes in cocaine abuse patients. *Addictive Behavior*, *25*, 99–102.

Gillespie, N. A., Neale, M. C., Prescott, C. A., Aggen, S. H., & Kendler, K. S. (2007). Factor and item-response analysis DSM-IV criteria for abuse of and dependence on cannabis, cocaine, hallucinogens, sedatives, stimulants, and opiods. *Addiction*, *102*, 920–930.

Hone-Blanchet, A., Wensign, T., & Fecteau, S. (2014). The use of virtual reality in craving assessment and cue-exposure therapy in substance use disorders. *Frontiers in Human Neuroscience*, *8*, 844.

Hyman, S. E., Malenka, R. C., & Nestler, E. J. (2006). Neural mechanisms of addiction: the role of reward-related learning and memory. *Annual Review of Neuroscience*, *29*, 565–598.

Ilan, A. B., Smith, M. E., & Gevins, A. (2004). Effects of marijuana on neurophysiological signs of working and episodic memory. *Psychopharmacology*, *176*, 214–222.

Jasova, D., Bob, P., & Fedor-Freybergh, P. (2007). Alcohol craving, limbic irritability, and stress. *Medical Science Monitor*, *13*, CR543–CR547.

Koston, T. R., Rounsaville, B. J., Babor, T. F., Spitzer, R. L., & Williams, J. B. (1987). Substance-use disorders in DSM-III-R. Evidence for the dependence syndrome across different psychoactive substances. *The British Journal of Psychiatry*, *151*, 834–843.

Kozlowski, L. T., Mann, R. E., Wilkinson, D. A., & Poulos, C. X. (1989). "Cravings" are ambiguous: ask about urges or desires. *Addictive Behaviors*, *14*, 443–445.

Kreibig, S. D. (2010). Autonomic nervous system activity in emotion: a review. *Biological Psychology*, *84*, 394–421.

Ludwig, A. M., Wikler, A., & Stark, L. H. (1974). The first drink: psychobiological aspects of craving. *Archives of General Psychiatry*, *30*, 539–547.

Lundahl, L. H., & Johanson, C. E. (2011). Cue-induced craving for marijuana in cannabis-dependent adults. *Experimental and clinical psychopharmacology*, *19*, 224.

Lupica, C. R., Riegel, A. C., & Hoffman, A. F. (2004). Marijuana and cannabinoid regulation of brain reward circuits. *British Journal of Pharmacology*, *143*, 227–234.

Marlatt, A. G. (1987). Craving notes. *British Journal of Addiction*, *82*, 42–44.

Martin-Santos, R., Fagundo, A. B., Crippa, J. A., Atakan, Z., Bhattacharyya, S., Allen, P., …, McGuire, P. (2010). Neuroimaging in cannabis use: a systematic review of the literature. *Psychological Medicine*, *40*, 383–398.

McRae, A. L., Hedden, S. L., Malcolm, R. J., Carter, R. E., & Brady, K. T. (2007). Characteristics of cocaine- and marijuana-dependent subjects presenting for medication treatment trials. *Addictive Behavior*, *32*, 1433–1440.

Nestor, L., Roberts, G., Garavan, H., & Hester, R. (2007). Deficits in learning and memory: parahippocampal hyperactivity and frontocortical hypoactivity in cannabis users. *NeuroImage*, *40*, 1328–1339.

Nickerson, L. D., Ravichandran, C., Lundahl, L. H., Rodolico, J., Dunlap, S., …, & Trisak, G. H. (2011). Cue reactivity in cannabis-dependent adolescents. *Psychology of Addictive Behaviors*, *25*, 168–173.

Orford, J. (2001). Addiction as excessive appetite. *Addiction*, *96*, 15–31.

Pickens, R. W., & Johanson, C. E. (1992). Craving: consensus of status and agenda for future research. *Drug and Alcohol Dependence*, *30*, 127–131.

Potenza, M. N. (2008). The neurobiology of pathological gambling and drug addiction: an overview and new findings. *Philosophical Transactions of the Royal Society: Biological Sciences*, *363*, 3181–3189.

Ranganathan, M., & D'Souza, D. C. (2006). The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berl)*, *188*, 425–444.

Ritz, T., Thons, M., Fahrenkrug, S., & Dahme, B. (2005). Airways, respiration, and respiratory sinus arrhythmia during picture viewing. *Psychophysiology*, *42*, 568–578.

Robinson, T. E., & Berridge, K. C. (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Research Review*, *18*, 247–291.

Rosenthal, R. (1979). The file drawer problem and tolerance for null results. *Psychological Bulletin*, *86*, 638–641.

SAMHSA. (2012). Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration, Treatment Episode Data Set (TEDS). Available from http://www-dasis.samhsa.gov/webt/quicklink/US12.htm

Schlaepfer, T. E., Pearlson, G. D., Wong, D. F., Marenco, S., & Dannals, R. F. (1997). PET study of competition between intravenous cocaine and [11C]raclopride at dopamine receptors in human subjects. *American Journal of Psychiatry*, *154*, 1209–1213.

Schlicker, E., & Kathmann, M. (2001). Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends in Pharmacological Science*, *22*, 565–572.

Schultz, W. (2013). Updating dopamine reward signals. *Current Opinion in Neurobiology*, *23*, 229–238.

Singleton, E. G., Trotman, A. J., Zavahir, M., Taylor, R. C., & Heishman, S. J. (2002). Determination of the reliability and validity of the marijuana craving questionnaire using imagery scripts. *Experimental and Clinical Psychopharmacology*, *10*, 47–53.

Skinner, B. F. (1938). *The behavior of organisms: An experimental analysis*. New York, NY: Appleton-Century-Crofts.

Skinner, M. D., & Aubin, H. (2010). Craving's place in addiction theory: contributions of the major models. *Neuroscience and Biobehavioral Reviews*, *34*, 606–623.

Skosnik, P. D., Edwards, C. R., O'Donnell, B. F., Steffen, A., Steinmetz, J. E., & Hetrick, W. P. (2008). Cannabis use disrupts eyeblink conditioning: evidence for cannabinoid modulation of cerebellar-dependent learning. *Neuropsychopharmacology*, *33*, 1432–1440.

Smith, K. S., Berridge, K. C., & Aldridge, J. W. (2011). Disentangling pleasure from incentive salience and learning signals in brain reward circuitry. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 255–264.

Tiffany, S. T., & Wray, J. M. (2012). The clinical significance of drug craving. *Annals of the New York Academy of Sciences*, *1248*, 1–17.

Van Diest, I., Proot, P., Van De Woestijne, K. P., Han, J. N., Devriese, S., Winters, W., & Van Den Bergh, O. (2001). Critical conditions for hyperventilation responses. *Behavior Modifications*, *25*, 621–639.

Verheul, R., van den Brink, W., & Geerlings, P. (1999). A three-pathway psychobiological model of craving for alcohol. *Alcohol and Alcoholism*, *34*, 197–222.

Volkow, N. D., Fowler, J. S., Wang, G. J., Hitzemann, R., Logan, J., Schlyer, D. J., …, Wolf, A. P. (1993). Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. *Synapse*, *14*, 169–177.

Volkow, N. D., Wange, G. J., Fowler, J. S., Logan, J., Gatley, S. J., Wong, C., Hitzemann, R., & Pappas, N. R. (1999). Reinforcing effects of psychostimulants in humans are associated with increases in brain dopamine and occupancy of D-2 receptors. *Journal of Psychopharmacology Experimental Theory*, *291*, 409–415.

West, R. (1987). Use and misuse of craving. *British Journal of Addiction*, *82*, 39–42.

Wise, R. A. (1980). The dopamine synapse and the notion of "pleasure centers" in the brain. *Trends in Neurosciences*, *3*, 91–95.

Wise, R. A. (2004). Dopamine, learning and motivation. *Nature Review of Neuroscience*, *5*, 483–494.

Wolfling, K., Flor, H., & Grusser, S. M. (2008). Psychophysiological responses to drug-associated stimuli in chronic heavy cannabis use. *European Journal of Neuroscience*, *27*, 976–983.

CHAPTER

34

Delta-9-Tetrahydrocannabinol and Catalepsy-Like Immobilization

N. Egashira

Department of Pharmacy, Kyushu University Hospital; Department of Neuropharmacology, Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka, Japan

SUMMARY POINTS

- This chapter focuses on catalepsy-like immobilization induced by THC, the major psychoactive component of marijuana.
- THC induces catalepsy-like immobilization, and this behavior is antagonized by the CB1 receptor antagonist rimonabant.
- Also, the microinjection of THC induces catalepsy-like immobilization when injected into the nucleus accumbens.
- THC-induced catalepsy-like immobilization is prevented by lesions in the nucleus accumbens.
- 5-HT receptor agonists inhibit the THC-induced catalepsy-like immobilization.
- In contrast, 5-HT receptor antagonists enhance the THC-induced catalepsy-like immobilization.
- THC-induced catalepsy-like immobilization is also prevented by NMDA receptor antagonists MK-801 and amantadine.
- THC decreases extracellular 5-HT levels in rat nucleus accumbens, and this effect is antagonized by rimonabant.
- Moreover, MK-801 abolishes this THC-induced decrease in 5-HT release.
- Taken together, these results demonstrate that CB1-mediated, THC-induced catalepsy-like immobilization is mediated by decreased 5-HT neurotransmission in the nucleus accumbens, due to the action of glutamate-containing neurons.

KEY FACTS OF CATALEPSY

- Microinjection of THC induces catalepsy-like immobilization when injected into the nucleus accumbens, central amygdala, lateral hypothalamus, or ventromedial hypothalamus.
- THC-induced catalepsy-like immobilization is prevented by lesion in nucleus accumbens, central amygdala, lateral hypothalamus, or ventromedial hypothalamus.
- Haloperidol-induced catalepsy is abolished by lesion in the caudate putamen, substantia nigra, globus pallidus, central amygdala, or lateral hypothalamus.
- Haloperidol-induced catalepsy is not abolished by lesion in the nucleus accumbens.

KEY FACTS OF RECEPTOR

- THC-induced catalepsy-like immobilization is antagonized by the CB1 receptor antagonist rimonabant, but not by the CB2 receptor antagonist AM630.

KEY FACTS OF SEROTONIN

- The 5-HT precursor 5-HTP, and the 5-HT receptor agonist 5-MeODMT inhibit THC-induced catalepsy-like immobilization.
- The 5-HT_{1A}/5-HT_{7} receptor agonist 8-OH-DPAT inhibits THC-induced catalepsy-like immobilization, and this inhibition is reversed by WAY100635, the selective 5-HT_{1A} receptor antagonist, but not by SB269970, the selective 5-HT_{7} receptor antagonist.
- The 5-HT_{1A} receptor partial agonist buspirone inhibits THC-induced catalepsy-like immobilization.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00038-7
Copyright © 2017 Elsevier Inc. All rights reserved.

- The 5-$HT_{2A/2C}$ receptor agonist DOI inhibits THC-induced catalepsy-like immobilization, and this inhibition is reversed by the 5-HT_{2A} receptor antagonist ketanserin, but not by the 5-HT_{2C} receptor antagonist SB242084.
- The selective 5-HT_{2C} receptor agonist WAY161503 had no effect on THC-induced catalepsy-like immobilization.

KEY FACTS OF GLUTAMATE

- THC-induced catalepsy-like immobilization is prevented by NMDA receptor antagonists, such as MK-801 and amantadine, but not the AMPA receptor antagonist YM-90K.

LIST OF ABBREVIATIONS

AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
CB	Cannabinoid
EPS	Extrapyramidal side effects
GABA	γ-Aminobutyric acid
HPLC-ECD	High-performance liquid chromatography-electrochemical detector
5-HT	5-Hydroxytryptamine, serotonin
5-HTP	5-Hydroxy-L-tryptophan
5-MeODMT	5-Methoxy-*N*,*N*-dimethyltryptamine
NMDA	*N*-methyl-D-aspartic acid
8-OH-DPAT	8-Hydroxy-2-(di-*n*-propylamino) tetralin
SSRIs	Selective 5-HT reuptake inhibitors
THC	Delta-9-tetrahydrocannabinol
WAY100635	*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl) cyclohexane carboxamide dihydrochloride

INTRODUCTION

Delta-9-tetrahydrocannabinol (THC), the principal psychoactive component of marijuana, is known to cause catalepsy in mice and rats (Egashira et al., 2006a; Fujiwara & Egashira, 2004; Tseng & Craft, 2004; Varvel et al., 2005). This effect is attenuated by the cannabinoid (CB)1 receptor antagonist rimonabant (Egashira et al., 2006a; Lichtman & Martin, 1997; Nagai et al., 2006; Sano et al., 2008; Tseng & Craft, 2004; Varvel et al., 2005), indicating that the effect is mediated by CB1 receptors. In contrast, rimonabant is not able to affect catalepsy induced by haloperidol or raclopride (Anderson, Kask, & Chase, 1996; Marchese et al., 2003), indicating that catalepsy induced by dopamine D_2 blockade does not involve stimulation of CB1 receptors. In fact, haloperidol fails to bind to CB1 receptors, and THC and rimonabant do not show appreciable affinity for D_2 receptors (Marchese et al., 2003). Consequently, the mechanism mediating THC-induced catalepsy-like immobilization is different from that mediating D_2 receptor antagonist-induced catalepsy. Moreover, THC-induced catalepsy was inhibited by air blowing and sound stimuli, whereas haloperidol-induced catalepsy was not inhibited by those stimuli (Sano et al., 2008). Additionally, THC did not impair motor coordination in the rota-rod test in mice and rats (Egashira et al., 2006a; Nagai et al., 2006). Thus, THC-induced catalepsy is clearly distinct from that induced by large doses of major tranquilizers, which cause extrapyramidal side effects (EPS), such as akinesia. This cataleptic immobilized state may be thought to represent a decreased motivation toward movement. Therefore, we have called this cataleptic immobilized state catalepsy-like immobilization (Fig. 34.1; Fujiwara & Egashira, 2004).

FIGURE 34.1 Photograph of THC-induced catalepsy-like immobilization. THC-induced catalepsy was inhibited by air blowing and sound stimuli, whereas haloperidol-induced catalepsy was not inhibited by those stimuli. Additionally, THC did not impair motor coordination in the rota-rod test. THC-induced catalepsy is clearly distinct from that induced by large doses of major tranquilizers, which cause extrapyramidal side effects (EPS), such as akinesia. We have called this cataleptic immobilized state catalepsy-like immobilization.

The present article introduces studies related to the THC-induced catalepsy-like immobilization.

INVOLVEMENT OF THE CB1 RECEPTOR IN THC-INDUCED CATALEPSY-LIKE IMMOBILIZATION

Two CB receptors have been cloned: the CB1 receptor in the central nervous system (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990), and the CB2

receptor in immune cells and peripheral tissues (Munro, Thomas, & Abu-Shaar, 1993). THC binds both CB1 and CB2 receptors. We previously reported THC-induced catalepsy-like immobilization in mice and rats, and that this behavior was antagonized by a CB1 receptor antagonist rimonabant (Egashira et al., 2006a; Nagai et al., 2006; Sano et al., 2008). These results are consistent with previous findings (Lichtman & Martin, 1997; Tseng & Craft, 2004; Varvel et al., 2005). However, THC-induced catalepsy-like immobilization was not reversed by the CB2 receptor antagonist AM630 (1 and 10 mg/kg, i.p.) (unpublished data). Moreover, the CB1 receptor agonists HU210 (0.06 mg/kg, i.p.) and WIN55,212-2 (15 mg/kg, i.p.) induced catalepsy-like immobilization in rats (unpublished data). These results suggest that the action of THC is CB1 receptor-mediated. In contrast, cannabidiol did not induce catalepsy-like immobilization (Hayakawa et al., 2008). Cannabidiol is a nonpsychoactive component of cannabis, and is generally known to have a very low affinity for the CB1 and CB2 receptors. Additionally, endocannabinoids anandamide (6, 10, and 20 mg/kg, i.p.; 100, 160, and 240 µg/brain, i.c.v.) and 2-AG (1 and 10 mg/kg, i.p.; 50, 100, and 250 µg/brain, i.c.v.) did not induce catalepsy-like immobilization (unpublished data). These findings suggest that THC induces catalepsy-like immobilization through direct action at CB1 receptors.

INVOLVEMENT OF THE NUCLEUS ACCUMBENS IN THC-INDUCED CATALEPSY-LIKE IMMOBILIZATION

Microinjection of THC into the nucleus accumbens has been reported to induce catalepsy in mice (Onaivi, Chakrabarti, Gwebu, & Chaudhuri, 1995). We reported that the microinjection of THC induced catalepsy-like immobilization when injected into the nucleus accumbens, central amygdala, lateral hypothalamus, or ventromedial hypothalamus that contains CB1 receptors (Table 34.1; Sano et al., 2008). Furthermore, THC-induced catalepsy-like immobilization was prevented by lesion in the nucleus accumbens, central amygdala, lateral hypothalamus, or ventromedial hypothalamus (Sano et al., 2008). However, haloperidol-induced catalepsy was abolished by lesion in the caudate putamen, substantia nigra, globus pallidus, central amygdala, or lateral hypothalamus, whereas it was not abolished by lesion in the nucleus accumbens (Sano et al., 2008). These findings suggest that the brain regions involved in THC-induced catalepsy-like immobilization are different from those involved in haloperidol-induced catalepsy. Also, microinjection of THC into the nucleus accumbens strongly induces catalepsy-like immobilization (Sano et al., 2008), indicating that the nucleus

TABLE 34.1 Characteristic of THC-Induced Catalepsy-Like Immobilization

	Catalepsy	
Brain regions	**THC**	**Haloperidol**
Lesion		
Nucleus accumbens	↓↓↓	–
Caudate putamen	–	↓↓↓
Globus pallidus	–	↓↓↓
Substantia nigra	–	↓↓↓
Lateral hypothalamus	↓	↓↓↓
Ventromedial hypothalamus	↓	
Central amygdala	↓↓↓	↓↓↓
Microinjection		
Nucleus accumbens	↑↑↑	
Caudate putamen	–	
Lateral hypothalamus	↑↑	
Ventromedial hypothalamus	↑↑	
Central amygdala	↑↑	

↑, Potentiation; ↓, decrease; –, no change.
Microinjection of delta-9-tetrahydrocannabinol (THC) induced catalepsy-like immobilization when injected into the nucleus accumbens, central amygdala, lateral hypothalamus, or ventromedial hypothalamus regions. Furthermore, THC-induced catalepsy-like immobilization was prevented by lesions in the nucleus accumbens, central amygdala, lateral hypothalamus, or ventromedial hypothalamus regions. However, haloperidol-induced catalepsy was abolished by lesions in the caudate putamen, substantia nigra, globus pallidus, central amygdala, or lateral hypothalamus, whereas it was not abolished by lesions in the nucleus accumbens.

accumbens plays an important role in THC-induced catalepsy-like immobilization. In fact, this region contains a moderately high density of CB1 receptors (Herkenham et al., 1991; Tsou, Brown, Sanudo-Pena, Mackie, & Walker, 1998). It has been suggested that the nucleus accumbens is a site that directly mediates the rewarding or primary motivational characteristics of natural stimuli, such as food, water, and sex, and it is an interface for the integration of emotional information. Therefore, THC-induced catalepsy-like immobilization may be due to impairment of emotional behavior.

INVOLVEMENT OF THE SEROTONERGIC NEURONAL SYSTEM IN THC-INDUCED CATALEPSY-LIKE IMMOBILIZATION

There is evidence that the serotonergic system is involved in THC-induced behavioral effects. The 5-hydroxytryptamine (5-HT, serotonin) precursor 5-hydroxy-L-tryptophan (5-HTP), clomipramine, citalopram, the 5-HT receptor agonist 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT), the 5-HT_{1A}/5-HT_7 receptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), and the 5-$HT_{2A/2C}$ receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) attenuate THC-induced impairment of spatial memory in the eight-arm radial maze, in rats (Egashira et al., 2002b, 2006b; Inui et al., 2004). THC decreases 5-HT release in the ventral hippocampus, which is an important area of the brain for THC-induced impairment of spatial memory (Egashira, Mishima, Iwasaki, & Fujiwara, 2002; Egashira et al., 2002b). Moreover, citalopram and 8-OH-DPAT reduce the enhancement of immobility by THC in the mouse forced swim test (Egashira et al., 2008). Additionally, THC, CB1 receptor agonists, anandamide, and endocannabinoid modulators inhibit DOI-induced 5-HT_{2A} receptor-mediated behaviors in mice (Darmani, 2001; Egashira et al., 2004, 2011). These findings suggest that there is an interaction between the CB and serotonergic systems.

5-HT has been implicated in the control of motor behavior, including catalepsy. Selective 5-HT reuptake inhibitors (SSRIs), such as paroxetine and sertraline, have been shown to attenuate haloperidol-induced catalepsy in mice (Pires et al., 2005). Moreover, 5-HT_{1A} (Invernizzi, Cervo, & Samanin, 1988; Lucas, Bonhomme, De Deurwaerdere, Le Moal, & Spampinato, 1997; McMillen, Scott, & Davanzo, 1988; Prinssen, Kleven, & Koek, 1999) and 5-HT_{2A} receptor agonists (Elliott, Walsh, Close, Higgins, & Hayes, 1990; Hicks, 1990; Neal-Beliveau, Joyce, & Lucki, 1993; Wadenberg & Ahlenius, 1995) attenuate D_2-receptor-mediated catalepsy. Also, these anticataleptic effects of 5-HT_{1A} and 5-HT_{2A} receptor agonists were antagonized by a selective 5-HT_{1A} receptor antagonist (Bartoszyk, Roos, & Ziegler, 1996) and a 5-HT_2 receptor antagonist (Neal-Beliveau et al., 1993), respectively, indicating that the serotonergic system is involved in the onset of catalepsy. In contrast, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexane carboxamide dihydrochloride (WAY100635), a selective 5-HT_{1A} receptor antagonist, enhances haloperidol-induced catalepsy (Prinssen, Colpaert, & Koek, 2002). Thus, 5-HT_{1A} and 5-HT_{2A} receptors play an important role in the regulation of catalepsy.

Serotonergic drugs, such as 5-HTP and 5-MeODMT inhibited THC-induced catalepsy-like immobilization in rats (Sano et al., 2008). This evidence indicates the involvement of 5-HT receptors in THC-induced catalepsy-like immobilization. The 5-HT_{1A}/5-HT_7 receptor agonist 8-OH-DPAT and 5-HT_{1A} receptor partial agonist buspirone inhibited THC-induced catalepsy-like immobilization, and that this inhibition was reversed by WAY100635 (Egashira et al., 2006a). In contrast, the selective 5-HT_7 receptor antagonist SB269970 did not affect this inhibitory effect of 8-OH-DPAT (Egashira et al., 2006a). These findings therefore indicate that 8-OH-DPAT and buspirone inhibit THC-induced catalepsy-like immobilization through a direct action on 5-HT_{1A} receptors. The 5-$HT_{2A/2C}$ receptor agonist DOI inhibited THC-induced catalepsy-like immobilization (Egashira et al., 2007). By contrast, the selective 5-HT_{2C} receptor agonist WAY161503 had no effect on THC-induced catalepsy-like immobilization (Egashira et al., 2007). Additionally, the 5-HT_{2A} receptor antagonist ketanserin completely reversed the inhibitory effect of DOI on THC-induced catalepsy-like immobilization, whereas the selective 5-HT_{2C} receptor antagonist SB242084 did not affect this inhibitory effect of DOI (Egashira et al., 2007). Thus, these findings indicate that DOI inhibits THC-induced catalepsy-like immobilization through a direct action on 5-HT_{2A} receptors. Furthermore, WAY100635 and ketanserin enhanced the catalepsy-like immobilization induced by THC (Egashira et al., 2006a, 2007). Hence, these findings suggest that 5-HT_{1A} and 5-HT_{2A} receptors are involved in THC-induced catalepsy-like immobilization.

Microinjection of THC into the nucleus accumbens induces catalepsy-like immobilization in rats (Sano et al., 2008). The nucleus accumbens is densely innervated by serotonergic projections from the dorsal raphe nucleus (Van Bockstaele, Biswas, & Pickel, 1993). Also, THC decreased extracellular 5-HT levels in rat nucleus accumbens, and this effect was antagonized by rimonabant (Figs. 34.2 and 34.3; Sano et al., 2008), indicating that the effect is mediated by CB1 receptors. These findings suggest that THC-induced inhibition of 5-HT neurotransmission in the nucleus accumbens is involved in THC-induced catalepsy-like immobilization.

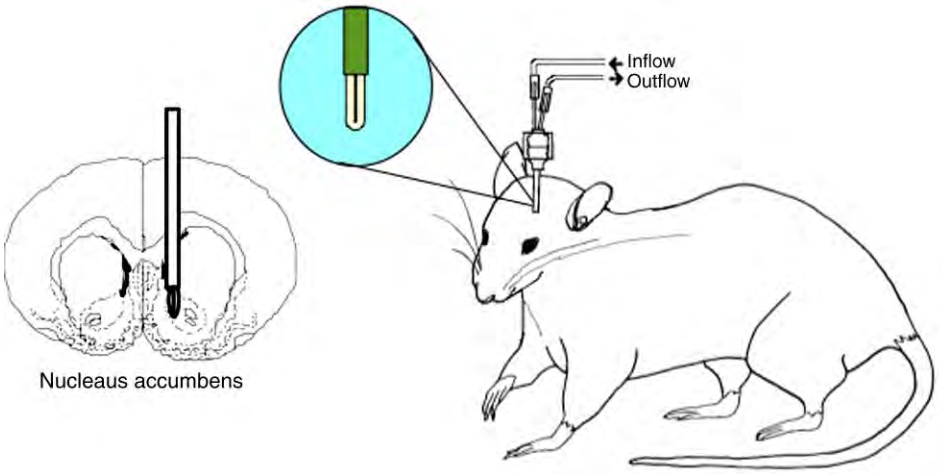


FIGURE 34.2 Diagram of the nucleus accumbens and in vivo microdialysis. Animals are stereotaxically implanted with a guide cannula in the nucleus accumbens, and a microdialysis probe is inserted into the guide cannula, which is perfused with a solution, such as Ringer's solution. The sample is collected, and the sampled concentrations are measured by a high-performance liquid chromatography-electrochemical detector (HPLC-ECD) system.

INVOLVEMENT OF THE GLUTAMATERGIC NEURONAL SYSTEM IN THC-INDUCED CATALEPSY-LIKE IMMOBILIZATION

We previously reported that the glutamatergic neuronal system may be involved in the THC-induced impairment of spatial memory (Fujiwara & Egashira, 2004). We found that THC inhibited the release of glutamate in the dorsal hippocampus, and this inhibition was antagonized by rimonabant in an in vivo microdialysis study, indicating that the release of glutamate is mediated by the CB1 receptor (unpublished data). Moreover, we found that D-cycloserine, a partial agonist at the glycine modulatory site on the *N*-methyl-D-aspartic acid (NMDA) receptor, and aniracetam, a positive modulator of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, improved the THC-induced impairment of spatial memory (unpublished data). These findings suggest that THC impairs spatial memory, at least in part, by inhibiting the release of glutamate through CB1 receptors in the dorsal hippocampus. Thus, the glutamatergic system is also involved in THC-induced behavioral effects.

Administration of NMDA receptor antagonists has been reported to prevent haloperidol-induced catalepsy (Kaur, Ozer, & Starr, 1997; Mehta & Ticku, 1990; Moore, Blackman, Awere, & Leander, 1993; Schmidt & Bubser, 1989; Verma & Kulkarni, 1992; Yanahashi et al., 2004). Moreover, NMDA has been reported to potentiate catalepsy induced by haloperidol (Mehta & Ticku, 1990). These findings indicate that the glutamatergic system is also involved in the induction of catalepsy.

THC-induced catalepsy-like immobilization was also prevented by NMDA receptor antagonists, such as MK-801 and amantadine, but not YM-90K, an AMPA receptor antagonist (Sano et al., 2008). Similarly, MK-801 and selective γ-aminobutyric acid (GABA) reuptake inhibitor tiagabine have been reported to modify THC-induced catalepsy in mice (Kinoshita et al., 1994; Pertwee, Greentree, & Swift, 1988). Furthermore, THC decreased extracellular glutamate levels in rat nucleus accumbens, and this effect was antagonized by rimonabant (Fig. 34.3; Sano et al., 2008), indicating that this effect is also mediated by CB1 receptors. This evidence is consistent with reports of in vitro electrophysiological studies showing that cannabinoids inhibit glutamatergic neurons projecting to the nucleus accumbens (Hoffman & Lupica, 2001; Pistis, Muntoni, Pillolla, & Gessa, 2002; Robbe, Alonso, Duchamp, Bockaert, & Manzoni, 2001). As described earlier, it is suggested that serotonergic and glutamatergic neurons are associated with the induction of catalepsy. It has also been reported that NMDA receptor antagonists MK-801 and phencyclidine increase the release and/or metabolism of 5-HT in the nucleus accumbens (Hernandez, Auerbach, & Hoebel, 1988; Loscher, Annies, & Honack, 1991; Martin, Carlsson, & Hjorth, 1998; Yan, Reith, Jobe, & Dailey, 1997). Moreover, the serotonergic system seems to be significantly involved in the behavioral syndrome induced by NMDA antagonists (Loscher & Honack, 1992; Nabeshima et al., 1984). These studies indicate that serotonergic and glutamatergic neurons interact with each other. Immunocytochemical and electrophysiological studies have demonstrated the presence of CB1 receptors at both GABAergic and glutamatergic synapses in the nucleus accumbens (Manzoni & Bockaert, 2001; Robbe et al., 2001). Also, presynaptic CB1 receptor-mediated inhibition of glutamate and GABA release from neurons in the nucleus accumbens has been demonstrated

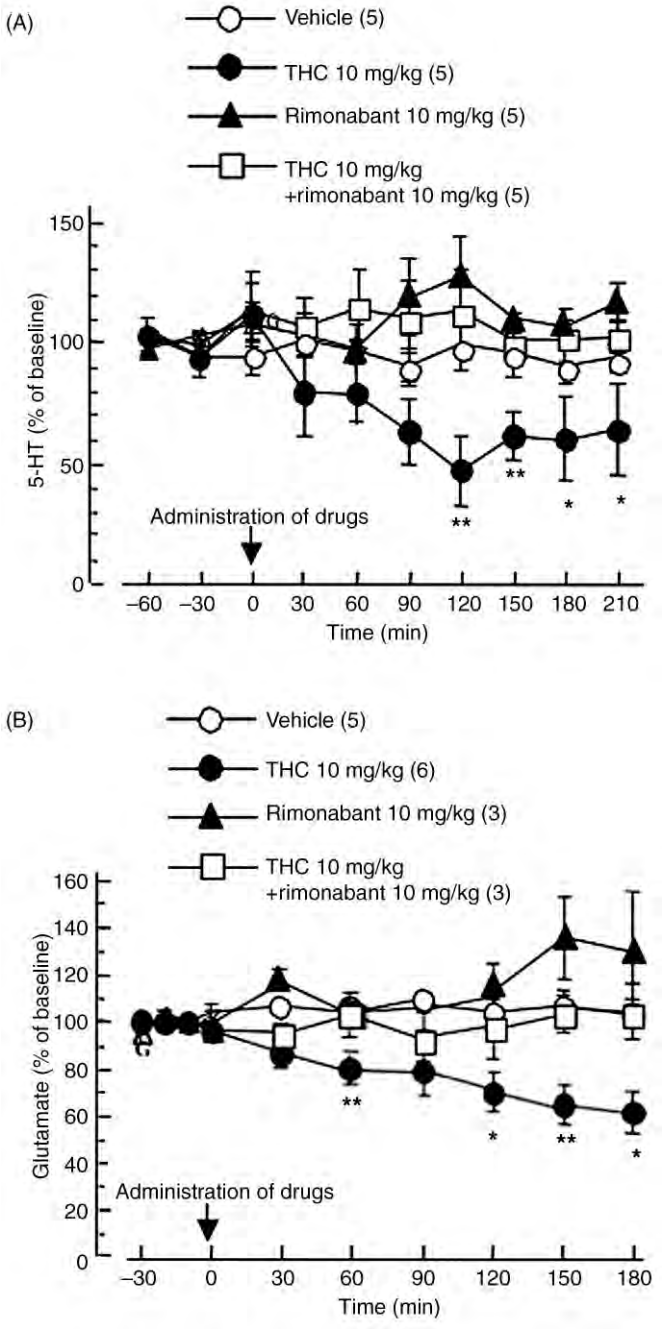


FIGURE 34.3 **Effects of THC on 5-HT and glutamate release in the nucleus accumbens.** THC and rimonabant were administered i.p. 60 min prior to the test. THC significantly decreased (A) 5-HT and (B) glutamate release in the nucleus accumbens. Rimonabant reversed the decrease in 5-HT and glutamate release. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with vehicle. *Source: Reproduced from Sano et al. (2008), with permission.*

(Hoffman & Lupica, 2001; Manzoni & Bockaert, 2001; Robbe et al., 2001). Moreover, we found that MK-801 abolished the THC-induced decrease in 5-HT release in the nucleus accumbens (Fig. 34.4; Sano et al., 2008). Hence, THC inhibits 5-HT release in the nucleus accumbens, at least in part, through glutamatergic mechanisms.

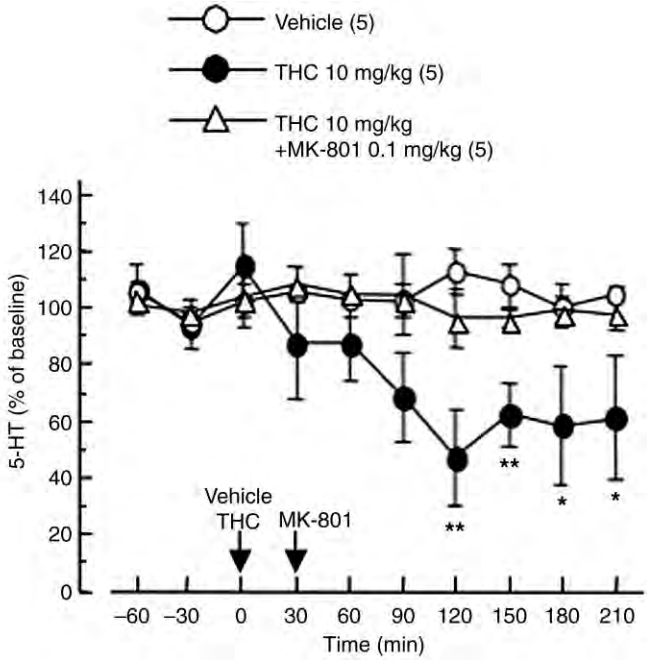


FIGURE 34.4 **Effects of MK-801 on the THC-induced reduction in 5-HT release in the nucleus accumbens.** THC and MK-801 were administered i.p. 60 and 30 min prior to the test, respectively. MK-801 inhibited the reduction in 5-HT release induced by THC. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with vehicle. *Source: Reproduced from Sano et al. (2008), with permission.*

CONCLUSIONS

In conclusion, these studies implicate that THC-induced catalepsy-like immobilization is distinct from that induced by large doses of major tranquilizers, such as haloperidol, and is mediated by decreased 5-HT neurotransmission in the nucleus accumbens, due to the action of glutamate-containing neurons through the CB1 receptors (Fig. 34.5). Moreover, we concluded that 5-HT_{1A} and 5-HT_{2A} receptors are involved in THC-induced catalepsy-like immobilization.

MINI-DICTIONARY

Cannabinoid (CB)1 receptor Two cannabinoid receptors have been cloned, and the CB1 receptor is located in the central nervous system.
Catalepsy-like immobilization THC induces catalepsy. However, THC-induced catalepsy is clearly distinct from that induced by large doses of major tranquilizers. This cataleptic immobilized state is thought to represent a decreased motivation toward movement. Therefore, we have called this cataleptic immobilized state catalepsy-like immobilization.
Delta-9-tetrahydrocannabinol (THC) THC is a major psychoactive component of marijuana. THC induces abnormal behaviors, such as impairment of spatial memory, catalepsy-like immobilization, and aggressive behavior in rats and mice.
Impairment of spatial memory THC impairs performance in the radial maze in rats. We have called this impairment state impairment of spatial memory.

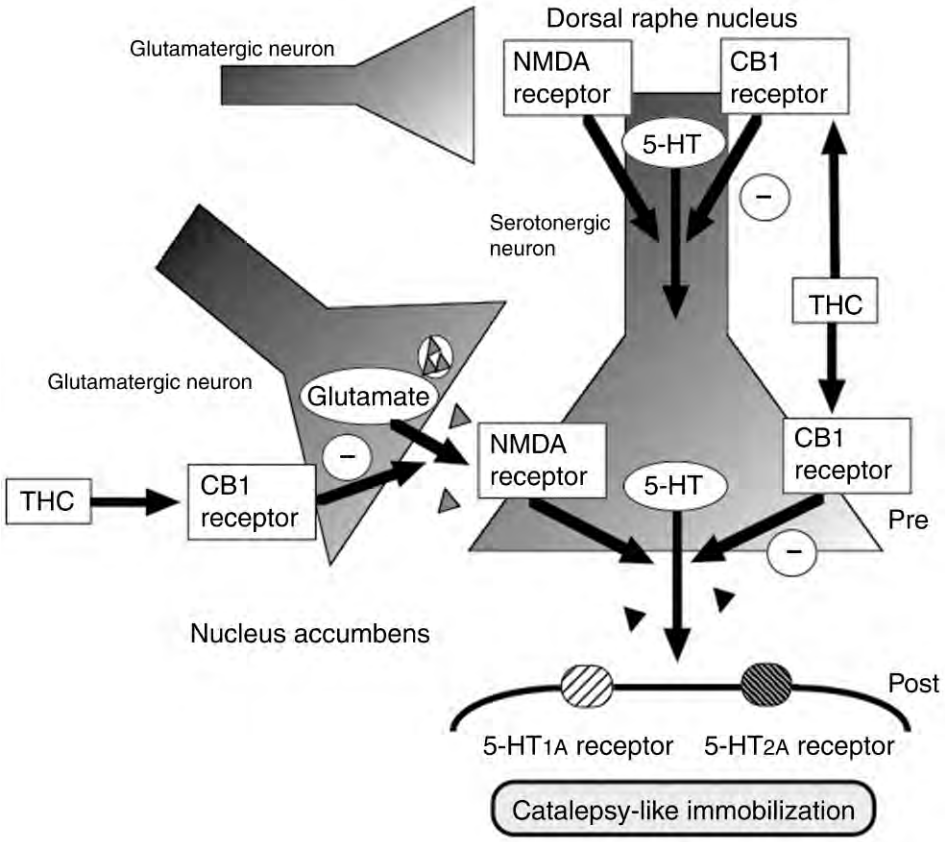


FIGURE 34.5 **A hypothetical overview of the mechanisms underlying THC-induced catalepsy-like immobilization.** THC-induced catalepsy-like immobilization is mediated by decreased 5-HT neurotransmission in the nucleus accumbens, due to the action of glutamate-containing neurons through CB1 receptors. 5-HT_{1A} and 5-HT_{2A} receptors are involved in THC-induced catalepsy-like immobilization.

Microdialysis Microdialysis is a method used for the measurement of the release of brain neurotransmitters. Animals are stereotaxically implanted with a guide cannula, and a microdialysis probe is inserted into the guide cannula, which is perfused with a solution, such as Ringer's solution. The sample is collected, and the sampled concentrations are measured by a high-performance liquid chromatography-electrochemical detector (HPLC-ECD) system.

Microinjection Microinjection is a method for injection into each brain. A stainless steel injection cannula is used to infuse the drug.

References

Anderson, J. J., Kask, A. M., & Chase, T. N. (1996). Effects of cannabinoid receptor stimulation and blockade on catalepsy produced by dopamine receptor antagonists. *European Journal of Pharmacology*, *295*, 163–168.

Bartoszyk, G. D., Roos, C., & Ziegler, H. (1996). 5-HT1A receptors are not involved in clozapine's lack of cataleptogenic potential. *Neuropharmacology*, *35*, 1645–1646.

Darmani, N. A. (2001). Cannabinoids of diverse structure inhibit two DOI-induced 5-HT_{2A} receptor-mediated behaviors in mice. *Pharmacology Biochemistry and Behavior*, *68*, 311–317.

Egashira, N., Koushi, E., Mishima, K., Iwasaki, K., Oishi, R., & Fujiwara, M. (2007). 2,5-Dimethoxy-4-iodoamphetamine (DOI) inhibits Δ^9-tetrahydrocannabinol-induced catalepsy-like immobilization in mice. *Journal of Pharmacological Sciences*, *105*, 361–366.

Egashira, N., Matsuda, T., Koushi, E., Higashihara, F., Mishima, K., Chidori, S., Hasebe, N., Iwasaki, K., Nishimura, R., Oishi, R., & Fujiwar, A. M. (2008). Δ^9-Tetrahydrocannabinol prolongs the immobility time in the mouse forced swim test: involvement of cannabinoid CB_1 receptor and serotonergic system. *European Journal of Pharmacology*, *589*, 117–121.

Egashira, N., Matsuda, T., Koushi, E., Mishima, K., Iwasaki, K., Shoyama, Y., & Fujiwara, M. (2006a). Involvement of 5-hydroxytryptamine$_{1A}$ receptors in Δ^9-tetrahydrocannabinol-induced catalepsy-like immobilization in mice. *European Journal of Pharmacology*, *550*, 117–122.

Egashira, N., Matsumoto, Y., Mishima, K., Iwasaki, K., Fujioka, M., Matsushita, M., Shoyama, Y., Nishimura, R., & Fujiwara, M. (2006b). Low dose citalopram reverses memory impairment and electroconvulsive shock-induced immobilization. *Pharmacology Biochemistry and Behavior*, *83*, 161–167.

Egashira, N., Mishima, K., Iwasaki, K., & Fujiwara, M. (2002a). Intracerebral microinjections of Δ^9-tetrahydrocannabinol: search for the impairment of spatial memory in the eight-arm radial maze in rats. *Brain Research*, *952*, 239–245.

Egashira, N., Mishima, K., Katsurabayashi, S., Yoshitake, T., Matsumoto, Y., Ishida, J., Yamaguchi, M., Iwasaki, K., & Fujiwara, M. (2002b). Involvement of 5-hydroxytryptamine neuronal system in Δ^9-tetrahydrocannabinol-induced impairment of spatial memory. *European Journal of Pharmacology*, *445*, 221–229.

Egashira, N., Mishima, K., Uchida, T., Hasebe, N., Nagai, H., Mizuki, A., Iwasaki, K., Ishii, H., Nishimura, R., Shoyama, Y., & Fujiwara, M. (2004). Anandamide inhibits the DOI-induced head-twitch response in mice. *Psychopharmacology*, *171*, 382–389.

Egashira, N., Shirakawa, A., Okuno, R., Mishima, K., Iwasaki, K., Oishi, R., & Fujiwara, M. (2011). Role of endocannabinoid and glutamatergic systems in DOI-induced head-twitch response in mice. *Pharmacology Biochemistry and Behavior*, *99*, 52–58.

Elliott, P. J., Walsh, D. M., Close, S. P., Higgins, G. A., & Hayes, A. G. (1990). Behavioural effects of serotonin agonists and antagonists in the rat and marmoset. *Neuropharmacology*, *29*, 949–956.

Fujiwara, M., & Egashira, N. (2004). New perspectives in the studies on endocannabinoid and cannabis: abnormal behaviors associate with CB_1 cannabinoid receptor and development of therapeutic application. *Journal of Pharmacological Sciences*, *96*, 362–366.

Hayakawa, K., Mishima, K., Hazekawa, M., Sano, K., Irie, K., Orito, K., Egawa, T., Kitamura, Y., Uchida, N., Nishimura, R., Egashira, N., Iwasaki, K., & Fujiwara, M. (2008). Cannabidiol potentiates pharmacological effects of Δ^9-tetrahydrocannabinol via CB_1 receptor-dependent mechanism. *Brain Research*, *1188*, 157–164.

Herkenham, M., Lynn, A. B., Johnson, M. R., Melvin, L. S., de Costa, B. R., & Rice, K. C. (1991). Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *Journal of Neuroscience*, *11*, 563–583.

Hernandez, L., Auerbach, S., & Hoebel, B. G. (1988). Phencyclidine (PCP) injected in the nucleus accumbens increases extracellular dopamine and serotonin as measured by microdialysis. *Life Sciences*, *42*, 1713–1723.

Hicks, P. B. (1990). The effect of serotonergic agents on haloperidol-induced catalepsy. *Life Sciences*, *47*, 1609–1615.

Hoffman, A. F., & Lupica, C. R. (2001). Direct actions of cannabinoids on synaptic transmission in the nucleus accumbens: a comparison with opioids. *Journal of Neurophysiology*, *85*, 72–83.

Inui, K., Egashira, N., Mishima, K., Yano, A., Matsumoto, Y., Hasebe, N., Abe, K., Hayakawa, K., Ikeda, T., Iwasaki, K., & Fujiwara, M. (2004). The $serotonin_{1A}$ receptor agonist 8-OHDPAT reverses Δ^9-tetrahydrocannabinol-induced impairment of spatial memory and reduction of acetylcholine release in the dorsal hippocampus in rats. *Neurotoxicity Research*, *6*, 153–158.

Invernizzi, R. W., Cervo, L., & Samanin, R. (1988). 8-Hydroxy-2-(di-*n*-propylamino) tetralin, a selective serotonin1A receptor agonist, blocks haloperidol-induced catalepsy by an action on raphe nuclei medianus and dorsalis. *Neuropharmacology*, *27*, 515–518.

Kaur, S., Ozer, H., & Starr, M. (1997). MK 801 reverses haloperidol-induced catalepsy from both striatal and extrastriatal sites in the rat brain. *European Journal of Pharmacology*, *332*, 153–160.

Kinoshita, H., Hasegawa, T., Katsumata, Y., Kameyama, T., Yamamoto, I., & Nabeshima, T. (1994). Effect of dizocilpine (MK-801) on the catalepsy induced by Δ^9-tetrahydrocannabinol in mice. *Journal of Neural Transmission*, *95*, 137–143.

Lichtman, A. H., & Martin, B. R. (1997). The selective cannabinoid antagonist SR 141716A blocks cannabinoid-induced antinociception in rats. *Pharmacology Biochemistry and Behavior*, *57*, 7–12.

Loscher, W., & Honack, D. (1992). The behavioural effects of MK-801 in rats: involvement of dopaminergic, serotonergic and noradrenergic systems. *European Journal of Pharmacology*, *215*, 199–208.

Loscher, W., Annies, R., & Honack, D. (1991). The *N*-methyl-D-aspartate receptor antagonist MK-801 induces increases in dopamine and serotonin metabolism in several brain regions of rats. *Neuroscience Letters*, *128*, 191–194.

Lucas, G., Bonhomme, N., De Deurwaerdere, P., Le Moal, M., & Spampinato, U. (1997). 8-OH-DPAT, a 5-HT1A agonist and ritanserin, a 5-HT2A/C antagonist, reverse haloperidol-induced catalepsy in rats independently of striatal dopamine release. *Psychopharmacology*, *131*, 57–63.

Manzoni, O. J., & Bockaert, J. (2001). Cannabinoids inhibit GABAergic synaptic transmission in mice nucleus accumbens. *European Journal of Pharmacology*, *412*, R3–R5.

Marchese, G., Casti, P., Ruiu, S., Saba, P., Sanna, A., Casu, G., & Pani, L. (2003). Haloperidol, but not clozapine, produces dramatic catalepsy in Δ^9-THC-treated rats: possible clinical implications. *British Journal of Pharmacology*, *140*, 520–526.

Martin, P., Carlsson, M. L., & Hjorth, S. (1998). Systemic PCP treatment elevates brain extracellular 5-HT: a microdialysis study in awake rats. *Neuroreport*, *9*, 2985–2988.

Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., & Bonner, T. I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature*, *346*, 561–564.

McMillen, B. A., Scott, S. M., & Davanzo, E. A. (1988). Reversal of neuroleptic-induced catalepsy by novel aryl-piperazine anxiolytic drugs. *Journal of Pharmacy and Pharmacology*, *40*, 885–887.

Mehta, A. K., & Ticku, M. K. (1990). Role of *N*-methyl-D-aspartate (NMDA) receptors in experimental catalepsy in rats. *Life Sciences*, *46*, 37–42.

Moore, N. A., Blackman, A., Awere, S., & Leander, J. D. (1993). NMDA receptor antagonists inhibit catalepsy induced by either dopamine D1 or D2 receptor antagonists. *European Journal of Pharmacology*, *237*, 1–7.

Munro, S., Thomas, K. L., & Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, *365*, 61–65.

Nabeshima, T., Yamaguchi, K., Hiramatsu, M., Amano, M., Furukawa, H., & Kameyama, T. (1984). Serotonergic involvement in phencyclidine-induced behaviors. *Pharmacology Biochemistry and Behavior*, *21*, 401–408.

Nagai, H., Egashira, N., Sano, K., Ogata, A., Mizuki, A., Mishima, K., Iwasaki, K., Shoyama, Y., Nishimura, R., & Fujiwara, M. (2006). Antipsychotics improve Δ^9-tetrahydrocannabinol-induced impairment of the prepulse inhibition of the startle reflex in mice. *Pharmacology Biochemistry and Behavior*, *84*, 330–336.

Neal-Beliveau, B. S., Joyce, J. N., & Lucki, I. (1993). Serotonergic involvement in haloperidol-induced catalepsy. *Journal of Pharmacology and Experimental Therapeutics*, *265*, 207–217.

Onaivi, E. S., Chakrabarti, A., Gwebu, E. T., & Chaudhuri, G. (1995). Neurobehavioral effects of delta 9-THC and cannabinoid (CB1) receptor gene expression in mice. *Behavioural Brain Research*, *72*, 115–125.

Pertwee, R. G., Greentree, S. G., & Swift, P. A. (1988). Drugs which stimulate or facilitate central GABAergic transmission interact synergistically with Δ^9-tetrahydrocannabinol to produce marked catalepsy in mice. *Neuropharmacology*, *27*, 1265–1270.

Pires, J. G., Bonikovski, V., & Futuro-Neto, H. A. (2005). Acute effects of selective serotonin reuptake inhibitors on neuroleptic-induced catalepsy in mice. *Brazilian Journal of Medical and Biological Research*, *38*, 1867–1872.

Pistis, M., Muntoni, A. L., Pillolla, G., & Gessa, G. L. (2002). Cannabinoids inhibit excitatory inputs to neurons in the shell of the nucleus accumbens: an in vivo electrophysiological study. *European Journal of Neuroscience*, *15*, 1795–1802.

Prinssen, E. P., Colpaert, F. C., & Koek, W. (2002). $5\text{-}HT_{1A}$ receptor activation and anti-cataleptic effects: high-efficacy agonists maximally inhibit haloperidol-induced catalepsy. *European Journal of Pharmacology*, *453*, 217–221.

Prinssen, E. P. M., Kleven, M. S., & Koek, W. (1999). Interactions between neuroleptics and $5\text{-}HT_{1A}$ ligands in preclinical behavioral models for antipsychotic and extrapyramidal effects. *Psychopharmacology*, *144*, 20–29.

Robbe, D., Alonso, G., Duchamp, F., Bockaert, J., & Manzoni, O. J. (2001). Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens. *Journal of Neuroscience*, *21*, 109–116.

Sano, K., Mishima, K., Koushi, E., Orito, K., Egashira, N., Irie, K., Takasaki, K., Katsurabayashi, S., Iwasaki, K., Uchida, N., Egawa, T., Kitamura, Y., Nishimura, R., & Fujiwara, M. (2008). Δ^9-Tetrahydrocannabinol-induced catalepsy-like immobilization is mediated by decreased 5-HT neurotransmission in the nucleus accumbens due to the action of glutamate-containing neurons. *Neuroscience*, *151*, 320–328.

Schmidt, W. J., & Bubser, M. (1989). Anticataleptic effects of the *N*-methyl-D-aspartate antagonist MK-801 in rats. *Pharmacology Biochemistry and Behavior*, *32*, 621–623.

Tseng, A. H., & Craft, R. M. (2004). CB_1 receptor mediation of cannabinoid behavioral effects in male and female rats. *Psychopharmacology*, *172*, 25–30.

Tsou, K., Brown, S., Sanudo-Pena, M. C., Mackie, K., & Walker, J. M. (1998). Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience*, *83*, 393–411.

Van Bockstaele, E. J., Biswas, A., & Pickel, V. M. (1993). Topography of serotonin neurons in the dorsal raphe nucleus that send axon collaterals to the rat prefrontal cortex and nucleus accumbens. *Brain Research*, *624*, 188–198.

Varvel, S. A., Bridgen, D. T., Tao, Q., Thomas, B. F., Martin, B. R., & Lichtman, A. H. (2005). Δ^9-Tetrahydrocannabinol accounts for the antinociceptive, hypothermic, and cataleptic effects of marijuana in mice. *Journal of Pharmacology and Experimental Therapeutics*, *314*, 329–337.

Verma, A., & Kulkarni, S. K. (1992). D1/D2 dopamine and *N*-methyl-D-aspartate (NMDA) receptor participation in experimental catalepsy in rats. *Psychopharmacology*, *109*, 477–483.

Wadenberg, M. L., & Ahlenius, S. (1995). Antagonism by the 5-HT2A/C receptor agonist of raclopride-induced catalepsy in the rat. *European Journal of Pharmacology*, *294*, 247–251.

Yan, Q. S., Reith, M. E., Jobe, P. C., & Dailey, J. W. (1997). Dizocilpine (MK-801) increases not only dopamine but also serotonin and norepinephrine transmissions in the nucleus accumbens as measured by microdialysis in freely moving rats. *Brain Research*, *765*, 149–158.

Yanahashi, S., Hashimoto, K., Hattori, K., Yuasa, S., & Iyo, M. (2004). Role of NMDA receptor subtypes in the induction of catalepsy and increase in Fos protein expression after administration of haloperidol. *Brain Research*, *1011*, 84–93.

CHAPTER

35

The Interactive Nature of Cannabis and Schizophrenia Risk Genes

T. Karl[*,**], *J.C. Arnold*[**,†,‡]

*Neuroscience Research Australia, Randwick, NSW, Australia
**School of Medicine, Western Sydney University, Campbelltown, NSW, Australia
†University of Sydney, Department of Pharmacology, Bosch Institute, Sydney, NSW, Australia
‡Brain and Mind Research Institute, Camperdown, NSW, Australia

SUMMARY POINTS

- Schizophrenia is a multifactorial disease characterized by high heritability, and pronounced sensitivity to environmental factors.
- The neurodevelopmental theory of schizophrenia predicts that genes and environment together affect brain development negatively during critical periods of neuronal development.
- Heavy cannabis abuse, in particular during adolescence, increases the risk of psychosis at least twofold, and enhances the severity of positive symptoms, and the risk of psychotic relapse.
- A recent genetic study revealed an association between an individual's burden of schizophrenia risk alleles and the frequency and quantity of cannabis abuse.
- A functional polymorphism in the catechol-O-methyltransferase (*COMT*) gene confers vulnerability to cannabis-induced psychosis, and magnifies the negative consequences of adolescent cannabis abuse on the development of schizophrenia.
- Extensive mouse model research has provided evidence that the *neuregulin 1* (*NRG1*) gene mediates increased susceptibility to the neurobehavioral effects of cannabis.
- A *COMT* Val158Met polymorphism has recently been associated with the susceptibility to abuse cannabis, and develop cannabis dependence and a single nucleotide polymorphism (SNP) in *NRG1* was associated with the development of cannabis dependence in African Americans.

KEY FACTS OF SCHIZOPHRENIA

- Schizophrenia is a neurodevelopmental disorder where a variety of genetic and environmental risk factors modify brain maturation.
- Schizophrenia has a prevalence of around 1% worldwide
- Patients suffering from the disorder exhibit positive, negative, and cognitive symptoms.
- Current treatment options are limited, as antipsychotic drugs have severe side effects (eg, extrapyramidal symptoms, obesity, and impotence), and do not treat all symptoms.
- Heavy cannabis abuse is a component/cumulative cause for the development of schizophrenia.

KEY FACTS OF CANNABIS

- *Cannabis sativa* has more than 100 different plant constituents called phytocannabinoids.
- The main plant constituent is Δ^9-tetrahydrocannabinol (THC), which has psychotic-like properties, whereas

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00039-9
Copyright © 2017 Elsevier Inc. All rights reserved.

another cannabinoid, cannabidiol (CBD), might actually possess antipsychotic-like potential.
- Cannabis abuse can increase positive symptoms, and the severity of psychotic relapse in patients with schizophrenia.
- Heavy cannabis abuse during adolescence increases the risk to develop psychosis by around twofold.

LIST OF ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
CB_1	Cannabinoid receptor 1
CB_2	Cannabinoid receptor 2
CBD	Cannabidiol
COMT	Catechol-O-methyltransferase
CP	CP 55,940
FLOT1	Flotillin-1
$GABA_A$	γ-Aminobutyric acid receptor (GABA) A
GxE	Gene-environment interaction
$5\text{-}HT_{2A}$	Serotonin 5-HT 2A receptor
LSV	Ventrolateral septum
MAGL	Monoacylglycerol lipase
NAPE-PLD	*N*-Acyl phosphatidylethanolamine phospholipase D
NMDA	*N*-Methyl-D-aspartic acid receptor
NRG1	Neuregulin 1
OF	Open field test
PPI	Prepulse inhibition of the startle response
THC	Δ^9-Tetrahydrocannabinol
WIN	WIN 55,212-2

Cannabis increases the risk of developing schizophrenia in genetically vulnerable individuals. Genetically modified mice offer a means to improve our understanding of the genes that confer vulnerability to the effects of cannabis in humans. Such models have been useful in forward (mouse to human) and reverse (human to mouse) translational research. On one hand, mouse research guides human studies on which genes to focus on; on the other hand, human studies that identify genetic variants can then be modeled in mice in an effort to delineate mechanisms involved in gene–cannabis interactions. This chapter will focus on two genes that are associated with schizophrenia, and appear to confer vulnerability to the effects of cannabis on psychosis—that is, the genes for catechol-O-methyltransferase (*COMT*) and neuregulin 1 (*NRG1*). Importantly, both genes have been investigated in mouse and human studies. *COMT*–cannabis interactions were first identified in human schizophrenia studies that were then reverse translated to mice, in order to appraise basic mechanisms. *NRG1*–cannabis interactions were first identified in mouse studies, as mice hypomorphic for transmembrane domain Nrg1 displayed altered neurobehavioral responses to cannabinoids in schizophrenia-relevant domains. The *Nrg1*-cannabis interaction was then confirmed in human studies. This chapter will focus on mouse preclinical research, which has improved our understanding how *COMT* and *Nrg1* modulate the schizophrenia-relevant effects of cannabinoids.

SCHIZOPHRENIA

Schizophrenia is a complex mental disorder with a prevalence of around 1%, although a number of related diagnoses, such as schizophreniform disorder, schizoaffective disorder, delusional disorder, and drug-induced psychosis could be added to the lifetime prevalence of psychosis. The disease is characterized by positive (eg, hallucinations, delusions), negative (eg, social withdrawal, anhedonia, impairment of sensorimotor gating), and cognitive symptoms. Current treatment options are limited, as typical and atypical antipsychotics are variably effective in ameliorating positive symptoms, and have limited success in treating cognitive symptoms. Furthermore, up to 30% of patients remain treatment resistant, and negative side effects of treatments available (eg, obesity, impotence, extrapyramidal symptoms) result in poor compliance.

GENE-ENVIRONMENT INTERACTIONS IN SCHIZOPHRENIA

Schizophrenia is a multifactorial disease characterized by high heritability (up to 80%), and pronounced sensitivity to environmental factors. The "two-hit hypothesis" of schizophrenia states that genetic and environmental risk factors interactively cause the development of the disorder (Bayer, Falkai, & Maier, 1999; Caspi & Moffitt, 2006). Interactions of genetic and environmental risk factors (GxE) occur when an individual's genetic predispositions are expressed dependent on their environment, or when environmental influences on a trait differ according to the individual's genome (Tsuang, Bar, Stone, & Faraone, 2004). Twin studies have shown that nature and nurture both play a role in the development of schizophrenia, as the concordance rate of monozygotic twins is around 50%. Furthermore, genome-wide association studies have failed to confirm common single nucleotide polymorphisms of so-called "schizophrenia candidate genes" as being accountable for a substantial proportion of the risk to develop the mental disorder (Sanders et al., 2008). Rather, the combined actions of a number of genes with small effect sizes (Owen, Craddock, & O'Donovan, 2005) and a variety of environmental risk factors (van Os, Kenis, & Rutten, 2010; van Os, Rutten, & Poulton, 2008) on brain development appear to cause

schizophrenia. Indeed, there might be subgroups in the population that are more vulnerable to particular environmental risk factors of schizophrenia (eg, cannabis abuse, developmental trauma). Thus, it is important to consider an interplay of genetic and environmental risk factors in order to understand the etiology of the disorder in depth. This is in line with the neurodevelopmental theory of schizophrenia, which predicts that genes and environment together affect brain development negatively during critical periods of neuronal development, thereby causing schizophrenia.

CANNABIS AND SCHIZOPHRENIA

The exact causes of schizophrenia are still unknown. However, cannabis has been suggested as a major environmental risk factor for the disease. Indeed, there is substantial evidence that cannabis use is much higher among schizophrenia patients than in the general population (Green, Young, & Kavanagh, 2005), and cannabis intoxication can trigger an acute transient psychotic episode (Thornicroft, 1990). Furthermore, there is substantial epidemiological evidence showing that heavy cannabis abuse, in particular during adolescence, increases the risk of psychosis by at least twofold, and enhances the severity of positive symptoms, and the risk of psychotic relapse (Arseneault et al., 2002; Henquet, Murray, Linszen, & van Os, 2005). Anecdotal evidence suggests that some schizophrenia patients may self-medicate with cannabis to reduce schizophrenia symptoms, or to alleviate some of the side effects of antipsychotic medication (Compton, Furman, & Kaslow, 2004; Dixon, Haas, Weiden, Sweeney, & Frances, 1990, 1991). However, more recent studies could not confirm a major therapeutic-like effect of cannabis on schizophrenia symptoms (Loberg & Hugdahl, 2009). In conclusion, an ever increasing number of researchers believe that cannabis users with a family history of schizophrenia (ie, genetic vulnerability to the disease) have a higher risk to develop psychotic disorder than the general population, thus classifying heavy cannabis abuse as a component/cumulative cause for schizophrenia (Caspi & Moffitt, 2006).

Recently, Power et al. (2014) investigated whether cannabis is a component cause of schizophrenia because it raises the risk of psychosis directly, or whether the same genes that increase the risk to develop psychosis also increase the likelihood of cannabis abuse (Power et al., 2014). This study was important, as the possibility of reverse causation had not been ruled out, and a few studies suggested that psychosis may be a risk factor for cannabis use (Addington & Duchak, 1997; Spencer, Castle, & Michie, 2002). Furthermore, cannabis use appears to be heritable (Agrawal & Lynskey, 2006; Verweij et al., 2012), and this heritability could result from a shared heritability with schizophrenia. Power's genetic association study revealed an association between an individual's burden of schizophrenia risk alleles and the frequency and quantity of cannabis abuse. Thus, at least part of the association between schizophrenia and cannabis abuse might be due to a shared genetic etiology and, therefore, not simply one of an environmental risk factor, but rather involving gene-environment correlations. It should be noted here that the authors did not rule out the possibility of cannabis being a risk factor for schizophrenia on its own right.

STUDIES EXAMINING GENETIC VULNERABILITY TO CANNABIS-INDUCED PSYCHOSIS

Early rodent work recognized that the rewarding properties of the main psychoactive component of the cannabis plant, Δ^9-tetrahydrocannabinol (THC), or its synthetic analogues, was dependent on genetic background (Arnold, Hunt, & McGregor, 2001; Chen, Paredes, Lowinson, & Gardner, 1991; Lepore, Liu, Savage, Matalon, & Gardner, 1996). Importantly, Lyons and coworkers confirmed in humans that the response to cannabis differed across individuals (Lyons et al., 1997). These studies suggest that the genetic background (or particular genes) influences the actions of cannabinoids. Considering the findings by Power and his colleagues (Power et al., 2014), it appears likely that at least some of these potential genetic candidates are also of relevance to schizophrenia research. Two major gene candidates with relevance to schizophrenia, *COMT* and *NRG1*, will be outlined in the following sections, as both mouse and human research support their role in conferring vulnerability to the propsychotic effects of cannabis.

MAJOR CANDIDATES FOR GENE–CANNABIS INTERACTIONS IN SCHIZOPHRENIA

Catechol-O-Methyltransferase

COMT is an enzyme which inactivates dopamine. A functional polymorphism in this gene conferred vulnerability to cannabis-induced psychosis, and magnified the negative consequences of adolescent cannabis abuse on the development of schizophrenia (Caspi & Moffitt, 2006; Henquet et al., 2006). Subsequently, a genetic mouse model for *COMT* (ie, adolescent homozygous and heterozygous *Comt* mutant mice; for a summary of *Comt* mouse model phenotypes see Table 35.1) was treated chronically with two doses of THC. Adolescent

TABLE 35.1 Overview of Knockout Mouse Model Phenotypes for COMT and NRG1

	Mouse model phenotypes
COMT	Improved spatial working memory Increased aggression Unaltered social behaviors and social recognition memory (reviewed in O'Tuathaigh et al., 2010a; O'Tuathaigh, Kirby, Moran, & Waddington, 2010b)
NRG1	Hyper-locomotive (Karl, Burne, Van den Buuse, & Chesworth, 2011; Stefansson et al., 2002) Moderate prepulse inhibition deficit (Karl et al., 2011; Stefansson et al., 2002) Impaired learning and memory (Chesworth, Downey, Logge, Killcross, & Karl, 2012; Duffy, Cappas, Lai, Boucher, & Karl, 2010; O'Tuathaigh et al., 2007) Deficient social behaviors (O'Tuathaigh et al., 2007) Increased expression of 5-HT_{2A} and NMDA receptors in cortical areas (Dean et al., 2008; Newell, Karl, & Huang, 2013) Altered NMDA receptor expression in the nucleus accumbens and thalamus (Newell et al., 2013) Increased CB_1 expression in the substantia nigra (Newell et al., 2013)

Abbreviations: *CB_1*, cannabinoid receptor 1; *NMDA*, *N*-methyl-D-aspartic acid receptor; *5-HT_{2A}*, serotonin 5-HT 2A receptor; *COMT*, Catechol-O-methyltransferase; *NRG1*, *Neuregulin 1*.

mice were chosen to model the findings from the Caspi study as closely as possible. *Comt* mice were more sensitive to THC-induced working memory impairments and anxiolysis, compared to wild type-like control mice (O'Tuathaigh et al., 2010a). In another study, adolescent *Comt* knockout mice were also more vulnerable to the disruptive effects of long-term treatment with the synthetic cannabinoid receptor 1 (CB_1) agonist WIN 55,212-2 (WIN) on sensorimotor gating (a behavioral hallmark of schizophrenia). On the other hand, knockout mice were less susceptible to the inhibiting effects of WIN on sociability (O'Tuathaigh et al., 2012). These behavioral responses were related to changes in dopamine metabolism in the prefrontal cortex of the mutant mice (O'Tuathaigh et al., 2012; Yavich, Forsberg, Karayiorgou, Gogos, & Mannisto, 2007). Mice also showed a genotype-specific response in components of dopaminergic and γ-aminobutyric acid (GABA)ergic pathways, as well as in the expression of CB_1 receptors. In particular, *Comt*–cannabis treatment interactions were found to influence dopaminergic cell sizes in the ventral tegmental area, expression of hippocampal CB_1 protein, and for parvalbumin-positive GABA interneuron cell size in the prefrontal cortex (Behan et al., 2012). Importantly, the mouse model research also suggested a developmental component of *Comt* genetic vulnerability to the actions of cannabis, as all effects were detected after adolescent but not adult cannabinoid exposure. This might be due to the slow maturation of cannabinoid receptors, which peak in expression around adolescence, and are reduced later in life due to postadolescent pruning events (Belue, Howlett, Westlake, & Hutchings, 1995). Finally, gene-dose effects were observed as the effects described were less prominent in heterozygous than in homozygous *Comt* mice. Interestingly, a recent human study showed that a *COMT* Val158Met polymorphism was associated with the susceptibility to develop cannabis dependence (Baransel Isir et al., 2008).

Neuregulin 1

The protein NRG1 influences key developmental processes, including cardiac development and neuronal migration, myelination, and synaptogenesis in the brain. It is also involved in the expression and activation of $GABA_A$ and *N*-methyl-D-aspartic acid (NMDA) receptors (Harrison & Law, 2006; Mei & Xiong, 2008). Alternative promoter usage and splicing has resulted in more than 30 different NRG1 isoforms, classified into at least six different isoform types (Mei & Xiong, 2008). This has led to the development of an increasing number of germline and conditional genetic mouse models for *NRG1*. The mouse model which has been evaluated in greatest detail and shows face, construct, and predictive validity for schizophrenia research is the heterozygous transmembrane domain *Nrg1* mutant mouse model (TM *Nrg1* HET mice) (Stefansson et al., 2002). The transmembrane domain of *NRG1* has been associated with schizophrenia (Walss-Bass et al., 2006) and TM *Nrg1* HET mice show behavioral characteristics relevant to schizophrenia endophenotypes (Powell & Miyakawa, 2006), including hyperactivity, sensorimotor gating deficits, cognitive and social impairments, some of which are reversible with antipsychotic treatment (Chesworth et al., 2012; Duffy et al., 2010; Karl, Duffy, Scimone, Harvey, & Schofield, 2007; Karl et al., 2011; Stefansson et al., 2002) (for a summary of *Nrg1* mouse model phenotypes see Table 35.1). Furthermore, adult *Nrg1* mutant male mice have increased expression of 5-HT_{2A} and NMDA receptors in cortical areas (Dean et al., 2008; Newell et al., 2013), altered NMDA receptor expression in the nucleus accumbens and thalamus, and increased CB_1 expression in the substantia nigra (Newell et al., 2013). Finally, *Nrg1* mutant mice are more susceptible to environmental factors such as housing and stress (as reviewed in Karl, 2013). Importantly, our team has studied the effects of cannabis constituents in this mouse model extensively.

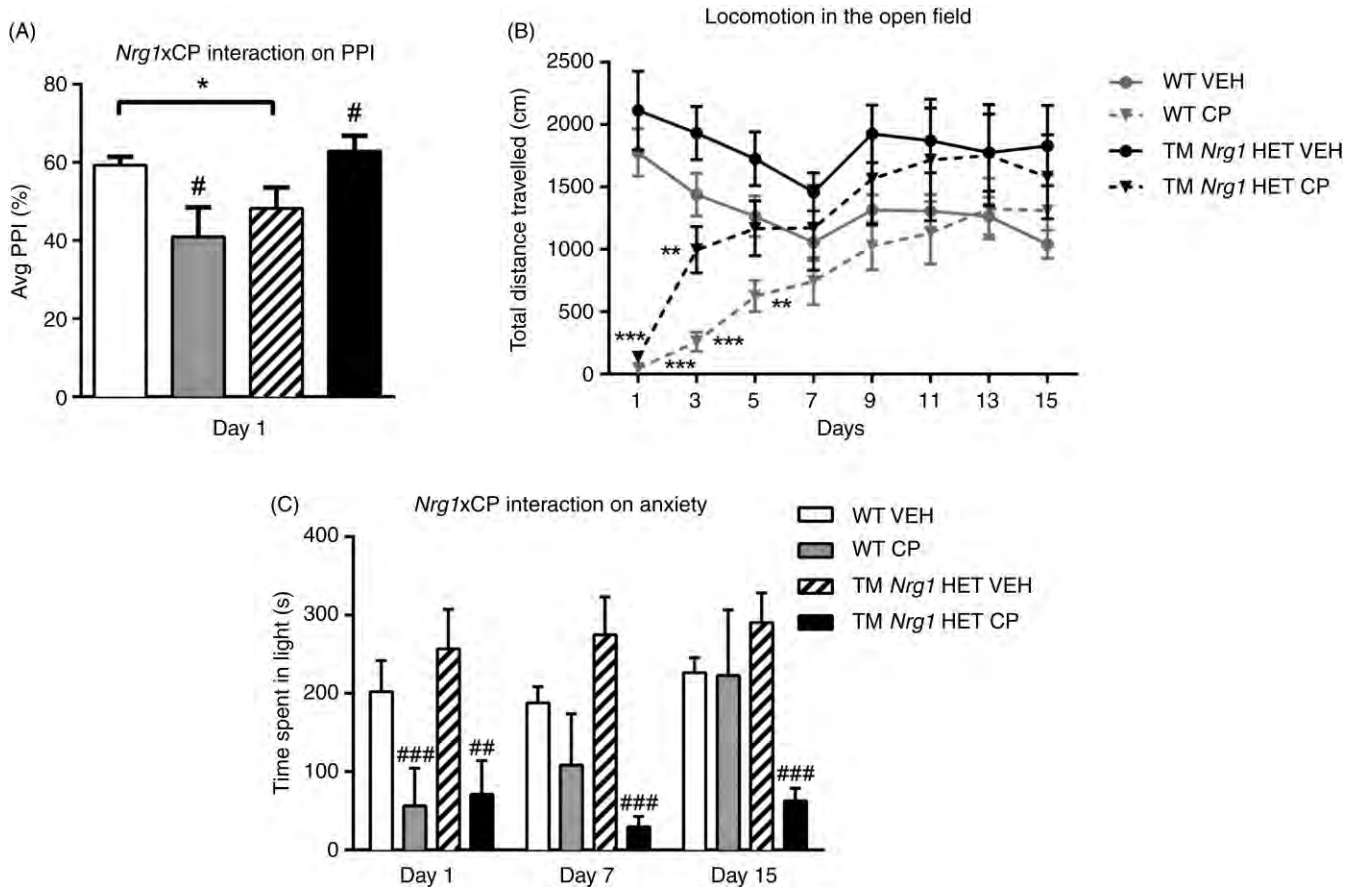


FIGURE 35.1 Mutant Nrg1 interacted with acute and repeated CP 55,940 *(CP)* exposure on (A) sensorimotor gating [as measured by prepulse inhibition *(PPI)*] and (B) behavioral tolerance development in the open field; but not (C) anxiety-relevant light-dark testing. Means ±SEM are shown. Significant effects for "genotype" are indicated by $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ and for "drug treatment" by $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, or $^{\#\#\#}p < 0.001$. Abbreviations: *PPI*, prepulse inhibition; *WT*, control littermates; *TM Nrg1 HET*, transmembrane domain *neuregulin 1* mutant mice; *VEH*, vehicle treatment; *CP*, CP 55,940 treatment. *Source: Modified from Boucher et al. (2011).*

Nrg1 mutant male mice challenged with an acute dose of THC showed an increased susceptibility to the sedative and anxiogenic effects of this cannabinoid, compared to wild type-like control mice (Boucher et al., 2007a). Importantly, acute cannabinoid challenge with THC or the synthetic CB_1 agonist CP 55,940 (CP) ameliorated deficits in sensorimotor gating (Boucher et al., 2007b, 2011), one of the hallmarks of schizophrenia (Geyer, Krebs-Thomson, Braff, & Swerdlow, 2001) (Fig. 35.1A). This beneficial impact of THC on prepulse inhibition of the startle response (PPI) in *Nrg1* mutant mice was unexpected, and could support the idea that initial exposure to cannabis might be beneficial in alleviating schizophrenia endophenotypes, similar to antipsychotic drugs normalizing sensorimotor gating deficits in schizophrenia patients (Geyer et al., 2001). The neural substrates of acute THC–*Nrg1* interaction were examined using c-Fos immunohistochemistry. THC increased c-Fos expression in the ventral part of the lateral septum (LSV) of mutant *Nrg1* mice only (Boucher et al., 2007b). This is significant, as drugs which modulate PPI, including both propsychotic drugs and antipsychotic drugs, elevate c-Fos expression in the lateral septum (Sumner et al., 2004). *Nrg1* HET mice also exhibited a more pronounced enhancement of c-Fos levels in stress-related brain regions (ie, paraventricular nucleus of hypothalamus and central nucleus of amygdala) than control littermates. The finding of increased behavioral sensitivity of *Nrg1* mutant males to THC was sex-specific, as female TM *Nrg1* HET mice exhibited no such sensitivity, and developed resistance to aspects of THC-induced social withdrawal (Long, Chesworth, Arnold, & Karl, 2010). The mechanisms responsible for the sexually dimorphic effects of cannabinoids on *Nrg1* mice require further investigation, as they may offer insight into the observed gender dimorphism in human *NRG1*–cannabis interactions (Han et al., 2012), and gender differences observed in the clinical course of schizophrenia (Fattore et al., 2007; Lacroix-Fralish, Tawfik, Nutile-McMenemy, Harris, & Deleo, 2006; McGregor & Arnold, 2007). In summary, these mouse studies were the first to show convincingly that cannabis might interact with the schizophrenia risk gene *NRG1*, and that genes

and gender may interact in a complex manner to modulate sensitivity to cannabinoids.

To model chronic cannabis abuse in schizophrenia, we also determined the neuroadaptive response of male TM *Nrg1* HET mice to repeated exposure (ie, 15 days) to the synthetic CB_1 agonist CP 55,940 (CP). *Nrg1* mutant mice more rapidly developed tolerance to the locomotor-inhibiting and hypothermic effects of the synthetic cannabinoid than control mice (Boucher et al., 2011) (Fig. 35.1B). On the other hand, *Nrg1* mutant mice failed to develop tolerance to the anxiogenic effects of CP (Fig. 35.1C). All mice developed tolerance to the genotype-specific effects of acute CP on PPI (ie, impairment in WT and facilitation in *Nrg1* HET mice) (Fig. 35.1A). FosB/ΔFosB expression was measured in brain tissue to detect for long-term neuroadaptive changes: CP induced a selective increase in FosB/ΔFosB in the LSV of mutant mice only. Thus, *Nrg1* not only affected the acute effects of cannabinoids, but also the neuroadaptive response to long-term cannabinoid exposure in a brain region implicated in the regulation of stress and anxiety responses. The data also reinforced the notion that the LSV is a critical brain region for *Nrg1*-cannabinoid interactions. The LSV is interconnected to various brain regions, including the hypothalamus, amygdala, hippocampus, and the prefrontal cortex, all implicated in the pathogenesis of schizophrenia, and expressing both Nrg1 and CB_1 receptors (reviewed in Arnold, Boucher, & Karl, 2012). Female TM *Nrg1* HET mice exhibited THC-induced facilitation of sensorimotor gating (measured by PPI), which was not evident in THC-treated control animals, following repeated dosing with THC for 21 days. (Spencer, Chohan, Karl, & Arnold, 2013). However, those effects were only significant for one particular interstimulus interval (ie, 32 ms) of the PPI protocol. Testing female mice again 21 days post THC treatment period revealed no residual effects of THC in either genotype. Thus, the lack of genotype-specific response of female *Nrg1* mice to acute THC discussed earlier (Long et al., 2010a) might be overcome following chronic THC treatment.

As earlier research on COMT–cannabis interactions had identified adolescence as a time of increased vulnerability to the psychotic and detrimental effects of heavy cannabis abuse (Caspi et al., 2005), we carried out an additional experiment where adolescent male TM *Nrg1* HET mice and control littermates where treated chronically with THC. Different to what had been observed in adulthood, *Nrg1* mice were less sensitive to the inhibiting effects of THC on investigative social behavior (Long et al., 2013). *Nrg1* also modulated the effects of adolescent THC on neurotransmitter receptor systems relevant to the pathophysiology of schizophrenia. Vehicle-treated *Nrg1* mutant mice displayed reduced CB_1 levels in the substantia nigra (Fig. 35.2A) and THC treatment increased CB_1 binding in TM *Nrg1* HET mice in this brain region. *Nrg1* also conferred opposing effects of THC on $5\text{-}HT_{2A}$ receptor expression in the insular cortex (ie, increased in *Nrg1* mice, decreased in control mice; Fig. 35.2B), and THC increased NMDA receptor binding in the hippocampus and cingulate cortex in *Nrg1* HET mice but not in WT control mice (Long et al., 2013) (Fig. 35.2C).

To gain insights into the molecular pathways involved in *Nrg1*–cannabis interactions during adolescence proteomic analyses were conducted for the hippocampus (Spencer et al., 2013b). THC treatment increased NMDA receptor binding in the hippocampus of *Nrg1* mice (Long et al., 2013), and our proteomic analysis revealed altered hippocampal expression of proteins involved in intracellular trafficking, and stabilization of NMDA receptors at the synapse in mutant mice, such as G protein signaling modulator 2 (GPSM2) and flotillin 1. This study also revealed that *Nrg1* and cannabinoid systems might interact through modulation of the endocannabinoid system. *Nrg1* mice repeatedly exposed to THC in adolescence showed a selective increase in the anandamide synthesizing enzyme *N*-acyl phosphatidylethanolamine phospholipase D (*NAPE-PLD*), implying a corresponding increase in hippocampal anandamide levels. A recent study reinforces the notion that interplay exists between Nrg1 and endocannabinoid systems, as exposure of cultured hippocampal neurons to recombinant Nrg1 increased the expression of the 2-arachidonoylglycerol (2-AG) degrading enzyme monoacylglycerol lipase (MAGL), lowering levels of 2-AG and disrupting long-term depression, a form of synaptic plasticity (Du, Kwon, & Kim, 2013). Taken together, these studies suggest *Nrg1*–cannabinoid interactions might be subserved through alteration of endocannabinoid levels and disruption of normal synaptic plasticity.

The importance of the *Nrg1* mouse model for research into the neurobehavioral effects of cannabis has recently been confirmed by a genome-wide linkage and single nucleotide polymorphism (SNP) association analysis, which discovered *NRG1* as a major candidate for the development of cannabis dependence in an African American test cohort (Han et al., 2012). Furthermore, *NRG1* may also confer increased sensitivity to the effects of THC on auditory evoked mismatch negativity in humans, as *NRG1* polymorphisms worsened THC-induced information processing dysfunction (Stadelmann et al., 2010).

DO GENES ALSO MODULATE THE EFFECTS OF OTHER PHYTOCANNABINOIDS LIKE CBD?

Some of the contrary reports regarding the role of cannabis in the development of schizophrenia might be due to the phenomenon that the *Cannabis sativa* plant is a mix

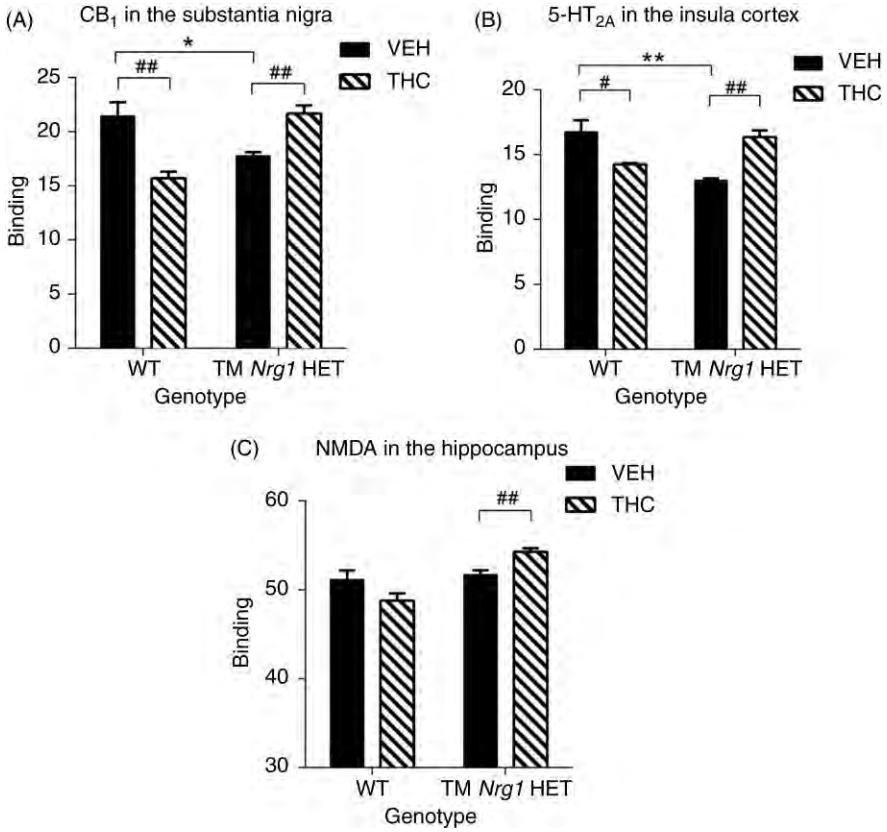


FIGURE 35.2 (A–C) Mutant Nrg1 interacted with repeated THC exposure during adolescence to alter schizophrenia-relevant receptors in the brain. Means ± SEM are shown. Significant effects for "genotype" are indicated by $^{*}p < 0.05$ and $^{**}p < 0.01$ and for "drug treatment" by $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$. Abbreviations: *CB_1*, cannabinoid receptor 1; *NMDA*, *N*-methyl-D-aspartic acid receptor; *5-HT_{2A}*, serotonin 5-HT 2A receptor; *WT*, control littermates; *TM Nrg1 HET*, transmembrane domain *neuregulin 1* mutant mice; *VEH*, vehicle treatment; *THC*, Δ^9-Tetrahydrocannabinol treatment. *Source: Modified from Long et al., (2013).*

of over 100 different cannabinoids, including other cannabinoids that might antagonize the effects of THC, or have antipsychotic properties in their own right, like cannabidiol (CBD). CBD has been shown to block or reverse the effects of THC and other psychotropic drugs, including ketamine and dexamphetamine (Long et al., 2010b; Zuardi, Crippa, Hallak, Moreira, & Guimaraes, 2006). Thus, it is possible that varying levels of THC and CBD in different cannabis strains could influence the consequences of long-term cannabis consumption, and the nature of gene–cannabis interactions. We examined the effects of acute and repeated CBD on adult TM *Nrg1* HET mice, and hypothesized that CBD might offset schizophrenia-relevant behavioral phenotypes in these mice (Long et al., 2012). CBD did not alter the schizophrenia-relevant hyperlocomotive or impaired PPI phenotypes of *Nrg1* mice, although acute high-dose CBD improved PPI in *Nrg1* mutant mice, at a specific prepulse intensity of 86 dB. *Nrg1* mice developed tolerance to this effect, following repeated dosing. Furthermore, chronic high-dose CBD increased investigative social behavior (ie, *anogenital sniffing*) selectively in mutant *Nrg1* mice, but not control littermates. CBD enhanced 5-HT_{2A} receptor binding in the substantia nigra in control mice, but failed to reverse the reduced 5-HT_{2A} receptor binding that was observed in TM *Nrg1* HET mice. Further, CBD selectively increased $GABA_A$ density in mutant mice. Thus, *Nrg1* may not only modulate neurobehavioral actions of THC, but also of CBD in a task- and brain region-specific manner.

In conclusion, both mouse and human translational research has been effective in isolating two genes, *COMT* and *NRG1*, that both moderate the effects of cannabis or particular cannabinoids (summarized in Table 35.2). Mouse research on these vulnerability genes have progressed our understanding of the cellular, molecular, and neurobiological mechanisms of cannabis-induced psychosis. Future large-scale genetic screenings could enable clinicians to identify high risk patient groups (ie, particular SNPs in *COMT* or *NRG1*), who are at heightened risk to develop psychosis when abusing cannabis. In addition, it will be important to carry out novel mouse and human translational research to isolate additional genes that might confer vulnerability to cannabis-induced psychosis.

TABLE 35.2 Overview of Major Research Findings for Gene-Cannabis Interactions in Schizophrenia

	References
COMT	Baransel Isir et al. (2008); Behan et al. (2012); Belue et al. (1995); Caspi and Moffitt (2006); Caspi et al. (2005); Henquet et al. (2006); O'Tuathaigh et al. (2012); O'Tuathaigh et al. (2010a); Yavich et al. (2007)
NRG1	Boucher et al. (2007a, 2007b, 2011); Han et al. (2012); Long et al. (2010a, 2012, 2013); Spencer et al. (2013a, 2013b); Stadelmann et al. (2010)

COMT, Catechol-O-methyltransferase; *NRG1*, *Neuregulin 1*.

MINI-DICTIONARY

Cannabis Taxa of three plants, namely *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis*, containing more than 100 different plant constituents (ie, phytocannabinoids).
Cannabidiol (CBD) CBD can account for up to 40% of the plant's extract (although most cannabis available illegally contains only minimal levels of CBD, (Swift, Wong, Li, Arnold, & McGregor, 2013; Arnold et al., 2012). CBD has been shown to block some of the effects of Δ^9-tetrahydrocannabinol (THC), and is currently evaluated for its therapeutic potential in Alzheimer's disease and schizophrenia.
Catechol-O-methyltransferase (COMT) An enzyme which degrades catecholamines, including dopamine, and has a role in the metabolism of dopamine released into synapses (encoded by the *COMT* gene).
Endophenotype Attempt to separate a complex variety of behavioral disease symptoms into more stable phenotypes with a clear genetic component and mechanistic basis.
Mouse model of schizophrenia A model for genetic or environmental risk factors of schizophrenia, showing one or a limited number of schizophrenia-relevant endophenotypes. These models aim to possess face, construct, and predictive validity for the disease.
Neuregulin 1 (NRG1) A protein that is essential for brain and heart development. It is encoded by the *NRG1* gene in humans, and has been found to be involved in myelination, neuronal migration, and the activity of neurotransmitter receptors.
Prepulse inhibition (ie, model for sensorimotor gating) The inhibition of the startle response to an acoustic or tactile stimulus because of a weaker preceding stimulus. Prepulse inhibition is disrupted in schizophrenia, and deficits can be reversed by antipsychotic treatment.
Schizophrenia A mental disorder characterized by positive (eg, hallucinations and delusions), negative (eg, social withdrawal and anhedonia), and cognitive symptoms (eg, working memory deficits). Genetic and early environmental factors, including psychological and social processes, trigger the onset of the disease.
Schizophrenia candidate gene (also risk gene or susceptibility gene) A gene that has been found in human linkage or association studies to modify an individual's risk to develop schizophrenia. Importantly, a number of recent genome-wide association studies failed to validate any of those candidate genes identified over the last two decades for schizophrenia.
Δ^9-Tetrahydrocannabinol (THC) The main psychoactive component of the cannabis plant. It is a partial agonist for the cannabinoid receptor 1 and 2 (CB_1 and CB_2). THC has a number of behavioral effects, including hypomotility, catalepsy, analgesia, and hypothermia (called the tetrad effect of THC).
Two-hit hypothesis of schizophrenia The two-hit hypothesis of schizophrenia states that a combination of (multiple) genetic and environmental risk factors causes the development of schizophrenia. Interactions of genetic and environmental risk factors (GxE) occur when an individual's genetic predispositions are expressed dependent on their environment, or when environmental influences on a trait differ according to the individual's genome.

ACKNOWLEDGMENTS

TK is supported by the Schizophrenia Research Institute utilizing infrastructure funding from NSW Ministry of Health, a career development fellowship (1045643) of the National Health and Medical Research Council, and a research grant from the Mason Foundation National Medical Program.

References

Addington, J., & Duchak, V. (1997). Reasons for substance use in schizophrenia. *Acta Psychiatrica Scandinavica*, *96*(5), 329–333.
Agrawal, A., & Lynskey, M. T. (2006). The genetic epidemiology of cannabis use, abuse and dependence. *Addiction*, *101*(6), 801–812.
Arnold, J. C., Boucher, A. A., & Karl, T. (2012). The yin and yang of cannabis-induced psychosis: the actions of delta 9-Tetrahydrocannabinol and cannabidiol in rodent models of schizophrenia. *Current Pharmaceutical Design*, *18*(32), 5113–5130.
Arnold, J. C., Hunt, G. E., & McGregor, I. S. (2001). Effects of the cannabinoid receptor agonist CP 55,940 and the cannabinoid receptor antagonist SR 141716 on intracranial self-stimulation in Lewis rats. *Life Sciences*, *70*(1), 97–108.
Arseneault, L., Cannon, M., Poulton, R., Murray, R., Caspi, A., & Moffitt, T. E. (2002). Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. *British Medical Journal*, *325*(7374), 1212–1213.
Baransel Isir, A. B., Oguzkan, S., Nacak, M., Gorucu, S., Dulger, H. E., & Arslan, A. (2008). The catechol-O-methyl transferase Val-158Met polymorphism and susceptibility to cannabis dependence. *American Journal of Forensic Medicine and Pathology*, *29*(4), 320–322.
Bayer, T. A., Falkai, P., & Maier, W. (1999). Genetic and non-genetic vulnerability factors in schizophrenia: the basis of the "two hit hypothesis". *Journal of Psychiatric Research*, *33*(6), 543–548.
Behan, A. T., Hryniewiecka, M., O'Tuathaigh, C. M., Kinsella, A., Cannon, M., Karayiorgou, M., …, Cotter, D. R. (2012). Chronic adolescent exposure to delta-9-tetrahydrocannabinol in COMT mutant mice: impact on indices of dopaminergic, endocannabinoid and GABAergic pathways. *Neuropsychopharmacology*, *37*(7), 1773–1783.
Belue, R. C., Howlett, A. C., Westlake, T. M., & Hutchings, D. E. (1995). The ontogeny of cannabinoid receptors in the brain of postnatal and aging rats. *Neurotoxicology and Teratology*, *17*(1), 25–30.
Boucher, A. A., Arnold, J. C., Duffy, L., Schofield, P. R., Micheau, J., & Karl, T. (2007a). Heterozygous neuregulin 1 mice are more sensitive to the behavioural effects of Delta9-tetrahydrocannabinol. *Psychopharmacology*, *192*(3), 325–336.
Boucher, A. A., Hunt, G. E., Karl, T., Micheau, J., McGregor, I. S., & Arnold, J. C. (2007b). Heterozygous neuregulin 1 mice display greater baseline and Delta(9)-tetrahydrocannabinol-induced c-Fos expression. *Neuroscience*, *149*(4), 861–870.
Boucher, A. A., Hunt, G. E., Micheau, J., Huang, X., McGregor, I. S., Karl, T., & Arnold, J. C. (2011). The schizophrenia susceptibility gene neuregulin 1 modulates tolerance to the effects of cannabinoids. *International Journal of Neuropsychopharmacology*, *14*(5), 631–643.

Caspi, A., & Moffitt, T. E. (2006). Gene-environment interactions in psychiatry: joining forces with neuroscience. *Nature Reviews Neuroscience*, *7*(7), 583–590.

Caspi, A., Moffitt, T. E., Cannon, M., McClay, J., Murray, R., Harrington, H., …, Craig, I. W. (2005). Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biological Psychiatry*, *57*(10), 1117–1127.

Chen, J., Paredes, W., Lowinson, J. H., & Gardner, E. L. (1991). Strain-specific facilitation of dopamine efflux by Δ9-tetrahydrocannabinol in the nucleus accumbens of rat: an in vivo microdialysis study. *Neuroscience Letters*, *129*(1), 136–140.

Chesworth, R., Downey, L., Logge, W., Killcross, S., & Karl, T. (2012). Cognition in female transmembrane domain neuregulin 1 mutant mice. *Behavioural Brain Research*, *226*(1), 218–223.

Compton, M. T., Furman, A. C., & Kaslow, N. J. (2004). Lower negative symptom scores among cannabis-dependent patients with schizophrenia-spectrum disorders: preliminary evidence from an African American first-episode sample. *Schizophrenia Research*, *71*(1), 61–64.

Dean, B., Karl, T., Pavey, G., Boer, S., Duffy, L., & Scarr, E. (2008). Increased levels of serotonin 2A receptors and serotonin transporter in the CNS of neuregulin 1 hypomorphic/mutant mice. *Schizophrenia Research*, *99*(1–3), 341–349.

Dixon, L., Haas, G., Weiden, P., Sweeney, J., & Frances, A. (1990). Acute effects of drug abuse in schizophrenic patients: clinical observations and patients' self-reports. *Schizophrenia Bulletin*, *16*(1), 69–79.

Dixon, L., Haas, G., Weiden, P. J., Sweeney, J., & Frances, A. J. (1991). Drug abuse in schizophrenic patients: clinical correlates and reasons for use. *American Journal of Psychiatry*, *148*(2), 224–230.

Du, H., Kwon, I. K., & Kim, J. (2013). Neuregulin-1 impairs the long-term depression of hippocampal inhibitory synapses by facilitating the degradation of endocannabinoid 2-AG. *Journal of Neuroscience*, *33*(38), 15022–15031.

Duffy, L., Cappas, E., Lai, D., Boucher, A. A., & Karl, T. (2010). Cognition in transmembrane domain neuregulin 1 mutant mice. *Neuroscience*, *170*(3), 800–807.

Fattore, L., Spano, M. S., Altea, S., Angius, F., Fadda, P., & Fratta, W. (2007). Cannabinoid self-administration in rats: sex differences and the influence of ovarian function. *British Journal of Pharmacology*, *152*(5), 795–804.

Geyer, M. A., Krebs-Thomson, K., Braff, D. L., & Swerdlow, N. R. (2001). Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology*, *156*(2–3), 117–154.

Green, B., Young, R., & Kavanagh, D. (2005). Cannabis use and misuse prevalence among people with psychosis. *British Journal of Psychiatry*, *187*, 306–313.

Han, S., Yang, B. Z., Kranzler, H. R., Oslin, D., Anton, R., Farrer, L. A., & Gelernter, J. (2012). Linkage analysis followed by association show NRG1 associated with cannabis dependence in African Americans. *Biological Psychiatry*, *72*(8), 637–644.

Harrison, P. J., & Law, A. J. (2006). Neuregulin 1 and schizophrenia: genetics, gene expression, and neurobiology. *Biological Psychiatry*, *60*, 132–140.

Henquet, C., Murray, R., Linszen, D., & van Os, J. (2005). The environment and schizophrenia: the role of cannabis use. *Schizophrenia Bulletin*, *31*(3), 608–612.

Henquet, C., Rosa, A., Krabbendam, L., Papiol, S., Fananas, L., Drukker, M., …, van Os, J. (2006). An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. *Neuropsychopharmacology*, *31*(12), 2748–2757.

Karl, T. (2013). Neuregulin 1: a prime candidate for research into gene-environment interactions in schizophrenia? Insights from genetic rodent models. *Frontiers in Behavioural Neuroscience*, *7*, 106.

Karl, T., Burne, T. H., Van den Buuse, M., & Chesworth, R. (2011). Do transmembrane domain neuregulin 1 mutant mice exhibit a reliable sensorimotor gating deficit? *Behavioural Brain Research*, *223*(2), 336–341.

Karl, T., Duffy, L., Scimone, A., Harvey, R. P., & Schofield, P. R. (2007). Altered motor activity, exploration and anxiety in heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia. *Genes Brain and Behavior*, *6*(7), 677–687.

Lacroix-Fralish, M. L., Tawfik, V. L., Nutile-McMenemy, N., Harris, B. T., & Deleo, J. A. (2006). Differential regulation of neuregulin 1 expression by progesterone in astrocytes and neurons. *Neuron Glia Biology*, *2*(4), 2227–2234.

Lepore, M., Liu, X., Savage, V., Matalon, D., & Gardner, E. L. (1996). Genetic differences in Δ9-tetrahydrocannabinol-induced facilitation of brain stimulation reward as measured by a rate-frequency curve-shift electrical brain stimulation paradigm in three different rat strains. *Life Sciences*, *58*(25), PL365–PL372.

Loberg, E. M., & Hugdahl, K. (2009). Cannabis use and cognition in schizophrenia. *Frontiers in Human Neuroscience*, *3*, 53.

Long, L. E., Chesworth, R., Arnold, J. C., & Karl, T. (2010a). A follow-up study: acute behavioural effects of Delta(9)-THC in female heterozygous neuregulin 1 transmembrane domain mutant mice. *Psychopharmacology*, *211*(3), 277–289.

Long, L. E., Chesworth, R., Huang, X. F., McGregor, I. S., Arnold, J. C., & Karl, T. (2010b). A behavioural comparison of acute and chronic Delta9-tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice. *International Journal of Neuropsychopharmacology*, *13*(7), 861–876.

Long, L. E., Chesworth, R., Huang, X. F., McGregor, I. S., Arnold, J. C., & Karl, T. (2013). Transmembrane domain Nrg1 mutant mice show altered susceptibility to the neurobehavioural actions of repeated THC exposure in adolescence. *International Journal of Neuropsychopharmacology*, *16*(1), 163–175.

Long, L. E., Chesworth, R., Huang, X. F., Wong, A., Spiro, A., McGregor, I. S., …, Karl, T. (2012). Distinct neurobehavioural effects of cannabidiol in transmembrane domain neuregulin 1 mutant mice. *PLoS One*, *7*(4), e34129.

Lyons, M. J., Toomey, R., Meyer, J. M., Green, A. I., Eisen, S. A., Goldberg, J., …, Tsuang, M. T. (1997). How do genes influence marijuana use? The role of subjective effects. *Addiction*, *92*(4), 409–417.

McGregor, I. S., & Arnold, J. C. (2007). Cannabis reward: biased towards the fairer sex? *British Journal of Pharmacology*, *152*(5), 562–564.

Mei, L., & Xiong, W. C. (2008). Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. *Nature Reviews Neuroscience*, *9*(6), 437–452.

Newell, K. A., Karl, T., & Huang, X. F. (2013). A neuregulin 1 transmembrane domain mutation causes imbalanced glutamatergic and dopaminergic receptor expression in mice. *Neuroscience*, *248*, 670–680.

O'Tuathaigh, C. M., Babovic, D., O'Sullivan, G. J., Clifford, J. J., Tighe, O., Croke, D. T., …, Waddington, J. L. (2007). Phenotypic characterization of spatial cognition and social behavior in mice with 'knockout' of the schizophrenia risk gene neuregulin 1. *Neuroscience*, *147*(1), 18–27.

O'Tuathaigh, C. M., Clarke, G., Walsh, J., Desbonnet, L., Petit, E., O'Leary, C., …, Waddington, J. L. (2012). Genetic vs. pharmacological inactivation of COMT influences cannabinoid-induced expression of schizophrenia-related phenotypes. *International Journal of Neuropsychopharmacology*, *15*(9), 1331–1342.

O'Tuathaigh, C. M., Hryniewiecka, M., Behan, A., Tighe, O., Coughlan, C., Desbonnet, L., …, Waddington, J. L. (2010). Chronic adolescent exposure to Delta-9-tetrahydrocannabinol in COMT mutant mice: impact on psychosis-related and other phenotypes. *Neuropsychopharmacology*, *35*(11), 2262–2273.

O'Tuathaigh, C. M., Kirby, B. P., Moran, P. M., & Waddington, J. L. (2010b). Mutant mouse models: genotype-phenotype relationships to negative symptoms in schizophrenia. *Schizophrenia Bulletin*, *36*(2), 271–288.

Owen, M. J., Craddock, N., & O'Donovan, M. C. (2005). Schizophrenia: genes at last? *Trends in Genetics*, *21*(9), 518–525.

Powell, C. M., & Miyakawa, T. (2006). Schizophrenia-relevant behavioural testing in rodent models: a uniquely human disorder? *Biological Psychiatry*, *59*, 1198–1207.

Power, R. A., Verweij, K. J., Zuhair, M., Montgomery, G. W., Henders, A. K., Heath, A. C., …, Martin, N. G. (2014). Genetic predisposition to schizophrenia associated with increased use of cannabis. *Molecular Psychiatry*, *19*(11), 1201–1204

Sanders, A. R., Duan, J., Levinson, D. F., Shi, J., He, D., Hou, C., … Gejman, P. V. (2008). No significant association of 14 candidate genes with schizophrenia in a large European ancestry sample: implications for psychiatric genetics. *American Journal of Psychiatry*, *165*(4), 497–506.

Spencer, C., Castle, D., & Michie, P. T. (2002). Motivations that maintain substance use among individuals with psychotic disorders. *Schizophrenia Bulletin*, *28*(2), 233–247.

Spencer, J. R., Chohan, T. W., Karl, T., & Arnold, J. C. (2013a). Female neuregulin 1 heterozygous mice require repeated exposure to Delta(9)-tetrahydrocannabinol to alter sensorimotor gating function. *Pharmacopsychiatry*, *46*(7), 286–291.

Spencer, J. R., Darbyshire, K. M., Boucher, A. A., Kashem, M. A., Long, L. E., McGregor, I. S., …, Arnold, J. C. (2013). Novel molecular changes induced by Nrg1 hypomorphism and Nrg1-cannabinoid interaction in adolescence: a hippocampal proteomic study in mice. *Frontiers in Cellular Neuroscience*, *7*, 15.

Stadelmann, A. M., Roser, P., Arning, L., Gallinat, J., Epplen, J. T., & Juckel, G. (2010). Acute effects of delta9-tetrahydrocannabinol on the auditory evoked mismatch negativity are modulated by the NRG1 gene. *Pharmacopsychiatry*, *43*(5), 194–195.

Stefansson, H., Sigurdsson, E., Steinthorsdottir, V., Bjornsdottir, S., Sigmundsson, T., Ghosh, S., …, Stefansson, K. (2002). Neuregulin 1 and susceptibility to schizophrenia. *American Journal of Human Genetics*, *71*(4), 877–892.

Sumner, B. E., Cruise, L. A., Slattery, D. A., Hill, D. R., Shahid, M., & Henry, B. (2004). Testing the validity of c-fos expression profiling to aid the therapeutic classification of psychoactive drugs. *Psychopharmacology*, *171*(3), 306–321.

Swift, W., Wong, A., Li, K. M., Arnold, J. C., & McGregor, I. S. (2013). Analysis of cannabis seizures in NSW, Australia: cannabis potency and cannabinoid profile. *PLoS One*, *8*(7), e70052.

Thornicroft, G. (1990). Cannabis and psychosis. Is there epidemiological evidence for an association? *British Journal of Psychiatry*, *157*, 25–33.

Tsuang, M. T., Bar, J. L., Stone, W. S., & Faraone, S. V. (2004). Gene-environment interactions in mental disorders. *World Psychiatry*, *3*(2), 73–83.

van Os, J., Kenis, G., & Rutten, B. P. (2010). The environment and schizophrenia. *Nature*, *468*(7321), 203–212.

van Os, J., Rutten, B. P., & Poulton, R. (2008). Gene-environment interactions in schizophrenia: review of epidemiological findings and future directions. *Schizophrenia Bulletin*, *34*(6), 1066–1082.

Verweij, K. J., Zietsch, B. P., Liu, J. Z., Medland, S. E., Lynskey, M. T., Madden, P. A., …, Martin, N. G. (2012). No association of candidate genes with cannabis use in a large sample of Australian twin families. *Addiction Biology*, *17*(3), 687–690.

Walss-Bass, C., Liu, W., Lew, D. F., Villegas, R., Montero, P., Dassori, A., …, Raventos, H. (2006). A novel missense mutation in the transmembrane domain of neuregulin 1 is associated with schizophrenia. *Biological Psychiatry*, *60*(6), 548–553.

Yavich, L., Forsberg, M. M., Karayiorgou, M., Gogos, J. A., & Mannisto, P. T. (2007). Site-specific role of catechol-O-methyltransferase in dopamine overflow within prefrontal cortex and dorsal striatum. *Journal of Neuroscience*, *27*(38), 10196–10209.

Zuardi, A. W., Crippa, J. A., Hallak, J. E., Moreira, F. A., & Guimaraes, F. S. (2006). Cannabidiol, a Cannabis sativa constituent, as an antipsychotic drug. *Brazilian Journal of Medical and Biological Research*, *39*(4), 421–429.

CHAPTER

36

Neuroimaging Findings in Adolescent Cannabis Use and Early Phase Psychosis

C.E. Crocker[*,**], *J. Cookey*[*], *P.G. Tibbo*[*]

*Department of Psychiatry, Dalhousie University, Halifax, NS, Canada
**Division of Neurology, Department of Medicine, Dalhousie University, Halifax, NS, Canada

SUMMARY POINTS

- A theory of the development of schizophrenia is the two-hit hypothesis. The first hit may be subtle abnormal brain development that leaves the brain susceptible to a second hit in damage from cannabis use.
- There is damage to oligodendrocytes by cannabis use, demonstrated by reduced integrity of white matter tracts.
- Cannabis use makes the white matter damage in FEP patients (observed as decreased FA values) more pronounced.
- NAA is reduced with cannabis use, suggesting reduced neuronal integrity.
- Glutamate is also reduced with adolescent cannabis use, particularly in female users. This may be indicative of reduced metabolism, and potentially alters the remodeling of the brain in adolescence.
- Unfortunately, we know of no studies examining adolescent cannabis users, FEP patients, and cannabis-using FEP patients to observe directly what the interplay between drug and disease might be on neurometabolism in these groups.

KEY FACTS IN DIFFUSION TENSOR IMAGING

- Diffusion tensor imaging was developed in 1990.
- The complex equations that allow a numerical quantitation of this measurement were developed in 1994.
- Diffusion tensor imaging measures the movement of water inside a tissue, in three dimensions, and generates a value that represents an ellipse pointed in a particular direction.
- Freely moving water is said to be anisotropic; fluid inside axons is said to be constrained and isotropic.
- The direction of water movement can be evaluated in individual blocks of brain images (called voxels) to determine the neuronal pathways.

LIST OF ABBREVIATIONS

Cho	Cytosolic choline
DISC1	Disrupted in schizophrenia 1
DTI	Diffusion tensor imaging
FA	Fractional anisotropy
FEP	First-episode psychosis
GABA	Gamma-aminobutyric acid
Gln	Glutamine
Glu	Glutamate

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00041-7
Copyright © 2017 Elsevier Inc. All rights reserved.

^{1}H-MRS	Magnetic resonance spectroscopy
ILF	Inferior longitudinal fasciculus
MD	Mean diffusivity
mI	Myo-inositol
NRG	Neuregulin
NAA	*N*-acetyl-aspartate
NAA-G	*N*-Acetylaspartylglutamic acid
SLF	Superior longitudinal fasciculus
tCr	Total creatine
TE	Echo time
THC	Delta-9-Tetrahydrocannabinol
TR	Relaxation time

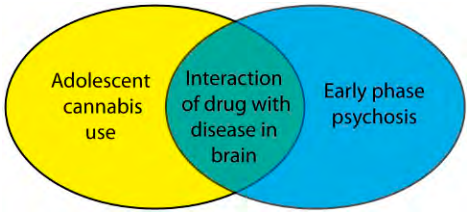


FIGURE 36.1 Venn diagram of chapter aim. Our focus is on two areas of study: cannabis use and first episode psychosis, and how these two areas overlap. This chapter examines our current knowledge in these areas with particular focus on the fields of MRS and DTI.

The human brain undergoes a critical phase of development during adolescence (Dahl, 2004; Spear, 2000). Neuronal maturation and rearrangement takes place with processes, such as myelination, synaptic pruning, and synaptic plasticity occurring. As a result of these adolescent processes, cortical structures (eg, prefrontal cortex) become fully integrated, allowing for better executive functioning, and other higher order functions (Arain et al., 2013). Interestingly, the brain develops in a "posterior to anterior" manner, with the prefrontal cortex the last to mature; this delay is particularly easy to observe when white matter is examined. Adolescents thus have less mature, and physically less, white matter in their frontal lobes than adults (Giedd et al., 1999).

Importantly, from a developmental perspective, as well, white matter tracts can be altered by environmental influences, for example, in humans who have been raised in institutions under conditions of neglect (Hanson et al., 2013). Preclinical studies with mice have shown that oligodendrocyte maturation and myelination are both dependent upon social experience in a critical period after weaning, and that the neuregulin (*NRG1*)/ErbB3 pathway is required for these processes (Makinodan, Rosen, Ito, & Corfas, 2012), in addition to the *disrupted-in-schizophrenia-1* (*DISC1*) gene (Hattori et al., 2014). Both *DISC1* and the *NRG1* signaling pathway have also been implicated as candidate genes for the underlying cause of schizophrenia (Roussos & Haroutunian, 2014). As oligodendrocytes play a key role in ensuring normal brain maturation takes place, it is thus not unreasonable to consider that until myelinogenesis is complete, the brain may be vulnerable to deleterious changes, as a result of environmental insults, which may include cannabis use.

The endocannabinoid system is itself important for cortical development, neural migration, connectivity, and synaptogenesis; interestingly, with cannabinoid receptors being located on human oligodendrocytes (Molina-Holgado et al., 2002). The majority of the work examining what role endocannabinoid receptors may play in oligodendrocyte development, and thus brain development, has occurred in vitro or in animal models. The effects of cannabis exposure on juvenile animals have demonstrated significant changes in the morphology, as well as functioning, of the maturing frontal cortex (Zamberletti et al., 2014). Additionally, the density of cannabinoid receptors in these regions diminishes with progress through adolescence, as well as their distribution, suggesting refinement of the system (Glass, Dragunow, & Faull, 1997).

Schizophrenia affects 1% of the population, has an average age of onset in late adolescence or early adulthood, and often causes significant chronic cognitive and psychosocial impairment. The neurodevelopmental hypothesis for schizophrenia suggests, in part, the prefrontal cortex of the brain does not develop properly; however, this is not apparent until an individual reaches late adolescence, when this region of the brain begins to be more fully integrated into the circuitry of the whole brain (Murray & Lewis, 1987). A variant of this hypothesis is the "two-hit hypothesis" that posits that while the neurodevelopment is disrupted, malfunction does not reach a critical level unless a second environmental insult occurs, further damaging the brain circuitry (Bayer, Falkai, & Maier, 1999). One of the postulated second "hits" is cannabis use (Fig 36.1).

There is clear evidence that use of cannabis increases an individual's risk of developing psychosis. Early work by Andreasson, Allebeck, Engstrom, & Rydberg (1987) conducted a 15-year follow-up study of 50,465 Swedish male conscripts, and found that those who had tried cannabis by age 18 were 2.4 times more likely to be diagnosed with schizophrenia than those who had not. This is a modest increase in risk, as other work has suggested that the risk of developing a psychosis roughly doubles from around 7 in 1000 in nonusers to 14 in 1000, for regular cannabis users (Saha, Chant, Welham, & McGrath, 2005). The effect of cannabis use is more profound for persons with an affected first degree relative (van Os et al., 2002). There is very little disagreement that marijuana use can contribute to the development of psychosis, the link potentially being THC effects on the developing brain (eg, on white matter) at a critical time point.

Human in vivo neuroimaging techniques will play an important role in shedding further light in this area, aiding us in moving the preclinical animal work to adolescent humans. We have chosen to review neuroimaging

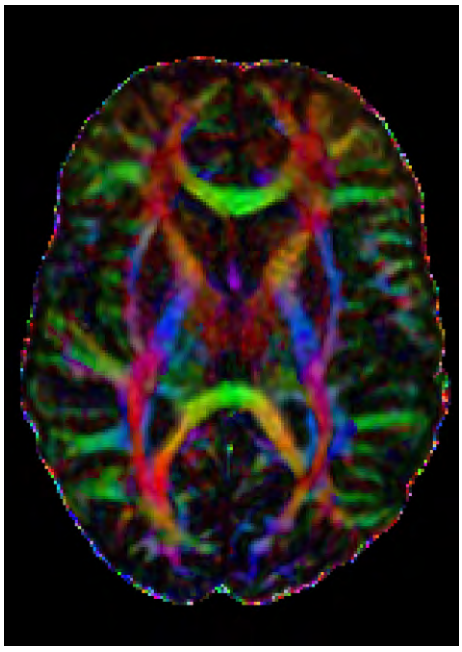


FIGURE 36.2 Example of FA map from diffusion tensor imaging. MRI image showing combined FA map with directional information. The colors indicate the direction of the diffusion. The map is RGB-based, so *red* indicates a left–right direction; *green*, antero-posterior; and *blue*, superior–inferior. *Source: This image was generated in FSL (Analysis Group, FMRIB, Oxford, UK) from a healthy control subject enrolled in one of our studies. The individual gave informed consent for use of their images (unpublished).*

studies in early adolescent cannabis use and early phase psychosis, with a focus on two techniques: diffusion tensor imaging (DTI), and magnetic resonance spectroscopy (MRS) to report the current state of work on this area (Fig. 36.2).

WHAT DOES DIFFUSION TENSOR IMAGING TELL US?

DTI is a magnetic resonance imaging modality that can measure macroscopic axonal organization in the human brain noninvasively, and is important for investigating brain white matter. The technique relies upon measuring the rate of tissue water diffusion in multiple directions at once, in order to estimate the overall direction of the diffusion (anisotropy) in each measured unit (voxel). As the diffusion of water inside the white matter tracts is limited in the fiber bundles, the overall direction of the fiber tracts can be discerned by comparing the dominant direction of diffusion in each adjoining voxel. DTI is usually reported as measures of fractional anisotropy (FA); 0 where there is free diffusion, to 1 where diffusion is highly restricted (anisotropic). FA is highly sensitive to microstructural changes, with higher FA considered representative of thicker myelinated fibers. Further analysis can be done to map the tracts of white matter using diffusion information. This tractography information, when compared between subject groups, gives information on the integrity of the pathways linking various brain regions (Mori & Zhang, 2006).

One particularly interesting DTI tractography study investigated 78 healthy adolescent controls, and compared their results to a metaanalysis of other DTI analyses done in healthy children. Their conclusion was that, bilaterally, the superior longitudinal fasciculus (SLF) was the most important tract in terms of adolescent development. Proper myelination of this tract was an absolute requirement for development of a normal healthy brain (Peters et al., 2012), as the SLF connects parts of the frontal lobe with the parietal, occipital, and temporal lobes. This is particularly interesting, as impaired structure/function of the SLF has also been implicated in schizophrenia, with the impaired working memory often found in schizophrenia being hypothesized to be due, in part, to SLF dysfunction (Peters et al., 2012).

DTI has been employed to explore the hypothesis that, in schizophrenia, abnormal communication between different regions of the brain may be due to disconnected or abnormal axonal tracts, and is well suited to examine cannabis effects on white matter structures, in early phase psychosis. Early studies in schizophrenia focused on medicated patients and, in hindsight, we know that antipsychotic medication can alter oligodendrocytes, resulting in reduced white matter volume and cell density (Konopaske et al., 2008). Thus, this chapter includes only DTI studies reporting on unmedicated early phase psychosis patients. We will explore the literature in early phase psychosis first (illness effects), followed by cannabis in adolescence (drug effects), then overlapping studies (illness and drug effects).

DTI Studies in Early Phase Psychosis

The body of literature examining unmedicated early psychosis patients is not large, and we excluded cohorts with heavy alcohol or illicit drug use, and studies with a sample size of less than 20 in the clinical group. Excluding multidrug and alcohol use removes this as a potential confounding factor, while excluding studies that used a small patient cohort avoids reporting noise discoveries (Ioannidis, 2005a, 2005b). There were 18 studies reported that meet these criteria. In the majority of studies (12 out of 18), the patients have decreased FA relative to healthy controls (Cheung et al., 2008; Gasparotti et al., 2009; Guo et al., 2012; Hao et al., 2006; Kumra et al., 2005; Kyriakopoulos, Vyas, Barker, Chitnis, & Frangou, 2008; Luck et al., 2011; Perez-Iglesias et al., 2010; Szeszko et al., 2008; Tang et al., 2010; Wang et al., 2011; White, Schmidt, & Karatekin, 2009). A recent metaanalysis that did not use our sample size criteria also confirmed the consistent finding of decreased FA in FEP patients, and identified the main tracts involved

as the cingulum bundle, the left inferior longitudinal fasciculus, the left inferior fronto-occipital fasciculus and, interestingly, the intrahemispheric connections of the corpus callosum (Yao et al., 2013). This change in water anisotropy points to changes in cell packing, or less robust myelination, both of which could be the result of decreased number or function of oligodendrocytes. One other interesting paper by Guo et al. (2012) is worth mentioning. This study showed FA reductions in several tracts, but only in the right hemisphere. This work only recruited drug naïve paranoid schizophrenic patients, and raises an interesting point about the consistency of results in psychosis research when broad or narrow classification systems are used (Guo et al., 2012). Similarly, the results of DTI studies may also be impacted by the genetic profile of the participants; both *neuregulin 1* and schizophrenia risk gene *ZNF804A* have both been shown to alter white matter microstructure in healthy individuals (Ikuta et al., 2014; Nickl-Jockschat et al., 2014). Thus, for future DTI research, it will be important to examine rigorously white matter structure in healthy controls and in unmedicated early phase psychosis, with clarity around methodology, samples sizes, diagnosis, and genetic influences, in addition to environmental influences.

DTI Studies in Adolescent Cannabis Use

Examination of DTI measures with cannabis use in adolescence also shows measurable brain changes. There is a small group of three studies to consider that strictly examined cannabis use without polydrug use in adolescents. These studies examined cannabis use in adolescent age subjects, or chronic cannabis use that began in adolescence. The studies have found decreases in FA between adolescent cannabis users and controls, regardless of region (Table 36.1) (Epstein et al., 2014; Gruber, Dahlgren, Sagar, Gonenc, & Lukas, 2014; Zalesky et al., 2012). This finding is not surprising, as the overriding hypothesis posits a reduction in connectivity with cannabis use that is greater, if use is begun in adolescence.

To further examine the possibility of reduced connectivity, tractography and DTI measures of axial and radial connectivity are additional measures that can be employed. Radial diffusivity is the measure of diffusion perpendicular to a fiber, whereas axial diffusivity is diffusion parallel to the fiber tract. Zalesky et al. (2012) examined radial and axial diffusivity in 59 chronic cannabis users, and found that both these measures positively correlated with the age at which regular cannabis use commenced in both fimbria and commissural fiber tracks. All the users commenced use in adolescence or early adulthood. Further work on cannabis using adolescents has been reported that examined alcohol use, as well. A group of 31 adolescents with cannabis use disorder and 20 healthy controls were also found to have decreased FA in prefrontal and parietal cortices. The 31 users were part of a larger group that also had alcohol users, yet statistical analysis showed that the decreased FA was associated with cannabis-related symptoms only, not alcohol (Clark, Chung, Thatcher, Pajtek, & Long, 2012). Neuroanatomical analysis methods showed white matter disorganization in the SLF (Clark et al., 2012). There is one further study of interest to note, although this study violates our 20 subject inclusion criteria. Kim et al. (2011) used deterministic tractography in concert with graph theory analysis to examine carefully the integration of 44 different brain regions, in 12 cannabis users (average age 19 years), and 13 similarly aged healthy controls. The application of this method allowed consideration of brain networks, rather than single paths, and could potentially be a method that would yield a better understanding of changes in global connectivity. This study demonstrated that cannabis-using participants had altered patterns of local network organization in the cingulate region, as well as having reduced global network efficiency (Kim et al., 2011).

TABLE 36.1 DTI studies in Early Psychosis and Cannabis

First author (year)	Group (*n*)	Age (years): mean (SD)	Substance use	Results
1. FIRST-EPISODE PSYCHOSIS PATIENTS				
Chan et al. (2010)	FEP (39) HC (64)	27 (7) 32 (10)	Exclusion of abuse in past 3 months	FEP = HC in FA values
Cheung et al. (2008)	FEP (25) HC (26)	29 (9) 28 (9)	Exclusion of abuse	FEP < HC in FA, in association, callosal, projection and brainstem fiber tract families
Collinson et al. (2014)	FEP (65) SCZ (52) HC (75)	27 (7) 32 (10)	Exclusion of abuse in past 3 months	FEP = HC in FA
Friedman et al. (2008)	FEP (40) HC (39)	26 (6) 25 (6)	Exclusion based on urine test	FEP = HC in FA, in callosal and association fibers

TABLE 36.1 DTI studies in Early Psychosis and Cannabis (*cont.*)

First author (year)	Group (*n*)	Age (years): mean (SD)	Substance use	Results
Gasparotti et al. (2009)	FEP (21) HC (21)	29 (9) 27 (7)	Exclusion of abuse and dependence	FEP < HC in FA, in callosal fiber tract family
Guo et al. (2012)	FEP (20) HC (26)	24 (5) 24 (4)	Exclusion of abuse	FEP < HC in FA, in association, limbic and projection fiber tract families
Hao et al. (2006)	FEP (21) HC (21)	24 (6) 25 (5)	Exclusion of dependence	FEP < HC in FA, in association, callosal, projection, limbic and brainstem tract families
Kumra et al. (2005)	FEP (26) HC (34)	15 (2) 15 (3)	Exclusion of abuse and dependence	FEP < HC in FA, in callosal and limbic fiber tract families
Kyriakopoulos et al. (2009)	FEP (34) HC (34)	20 (3) 20 (3)	Exclusion of substance use disorder	FEP < HC in FA, in association, callosal, and brainstem fiber tract families
Luck et al. (2011)	FEP (44) HC (30)	23 (0) 25 (1)	Exclusion not clearly stated	FEP < HC in FA, in association fiber tracts
Perez et al. (2010)	FEP (62) HC (54)	31 (10) 30 (8)	Exclusion of dependence	FEP < HC in FA, in association, callosal, projection and brainstem
Price et al. (2005)	FEP (20) HC (29)	25 (?) 28 (?)	Exclusion of current use	FEP = HC in FA, in callosal fibers
Szeszko et al. (2008)	FEP (33) HC (30)	25 (4) 26 (5)	Exclusion not clearly stated	FEP < HC in FA, in association fiber tract family
Tang et al. (2010)	FEP (38) HC (38)	16 (1) 17(1)	Exclusion not clearly stated	FEP < HC in FA, in limbic fibers
Wang et al. (2011)	FEP (68) HC (100)	24 (8) 26 (8)	Exclusion of abuse	FEP < controls in FA in association and callosal fiber tract families
White et al. (2009)	FEP (29) HC (40)	14 (3) 15 (3)	Exclusion of dependence and of abuse in past month	FEP < controls in FA, in association, callosal, projection, limbic and brainstem tract families
White et al. (2011)	FEP (31) HC (43)	25 (7) 25 (7)	Exclusion not clearly stated	FEP = controls in FA values
2. CANNABIS USE IN ADOLESCENTS OR BEGUN IN ADOLESCENCE				
Clark et al. (2012)	CAN+ (31) HC (20)	17 (1) 16 (1)	Group of 31 a subset of other substance use disorders	CAN+ < HC in FA in SLF. Significant gender effect
Kim et al. (2011)	CAN+ (12) HC (13)	19 (1) 22 (4)	No other illicit drugs previous 3 months	Decreased regional connectivity in CAN+
Gruber et al. (2014)	CAN+ (25) HC (18)	23 (6) 23 (4)	Exclusion of other illicit drugs	CAN+ < HC in FA. Subanalysis with earlier start (14-year-olds vs 17-year-olds) showed greater FA decrease
Zalesky et al. (2012)	CAN+ (59) HC (33)	33 (11) 32 (12)	Exclusion of other illicit drugs	CAN+ < HC in FA, in callosal and limbic fiber tract families. Use begun in adolescence
3. ADOLESCENT CANNABIS USE IN FEP				
Epstein et al. (2014)	FEP (53) UHR (19) CAN+ (28) HC (53)	17 (2) 16 (3) 18 (2) 17 (3)	Exclusion of other illicit drugs	Corticospinal and left ILF and left inferior fronto-occipital fas were all sig differed between FEP and control. Left ILF sig diff from HC for CAN+
James et al. (2011)	FEP (32) −16 CAN+ −16 CAN− HC (28)	16 (1) 16 (1)	Exclusion of other illicit drugs	FEP < controls in FA, in association, brainstem, callosal and projection fibers. CAN+ FEP < CAN− FEP in FA, in brainstem, projection and association fiber tracts

The minimal threshold for significance in this table is $p < .05$ (uncorrected for comparisons. FEP, first-episode psychosis; HC, healthy control; UHR, high risk for psychosis; CAN+, cannabis using; CAN−, do not use cannabis; FA, fractional anisotropy.

Overlapping DTI Findings

There are only two DTI studies reporting on cannabis use in early phase psychosis. The first considered 32 adolescents with FEP and 28 healthy adolescents. The FEP group was further divided into 16 cannabis users and 16 nonusers. The FEP group overall had significantly reduced FA values, compared to the healthy controls; patients with the cannabis use had the lowest FA values in a subgroup analysis. Changes in the SLF and ILF were seen, as well as the corona radiate, brain stem, and internal capsule (James et al., 2011). The second study compared FEP subjects with people at high risk of developing schizophrenia, adolescents with cannabis use disorder, and healthy controls. Patients had lower FA in four areas, including bilateral corticospinal tracts, left ILF, and left interior fronto-occipital fasciculus. The patients had significantly lower FA values, compared to cannabis users, in the corticospinal tracts and the left ILF. Cannabis-using subjects had FA values that were significantly lower than controls in the left ILF (Epstein et al., 2014). Interestingly, there was overlap in the ILF region, again, between the FEP and the cannabis users.

WHAT DOES MAGNETIC RESONANCE SPECTROSCOPY TELL US?

Proton magnetic resonance spectroscopy (^{1}H-MRS) uses proton information to examine tissue for the presence and concentration of various metabolites (Fig. 36.3). ^{1}H-MRS uses a spectrophotometer inside the MRI

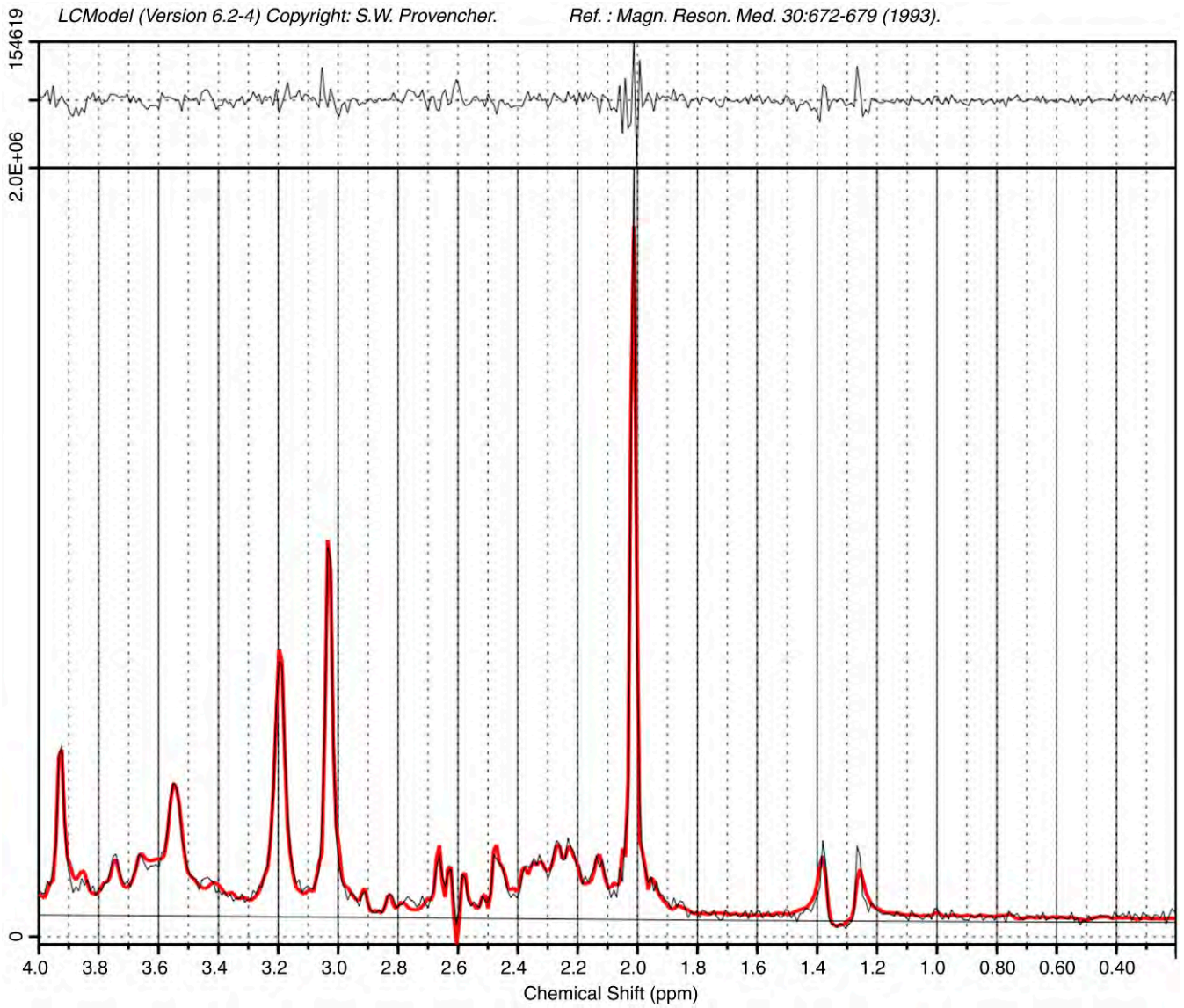


FIGURE 36.3 Example of a ^{1}H-MRS spectroscopy trace. ^{1}H-MRS trace generated at a short echo time (TE35) on a spectroscopy phantom at 1.5 T. *Source: The output is from a LCmodel analysis (Stephen Provencher Incorporated) (unpublished).*

machine to make measurements in vivo noninvasively. This allows quantification of cerebral metabolites, including *N*-acetyl-aspartate (NAA), myo-inositol (mI), total creatine (tCr), cytosolic choline (Cho), gamma-aminobutyric acid (GABA), glutamine (Gln) and glutamate (Glu). NAA is generally considered to be a marker of neuronal health. The tCr signal includes creatine and phosphocreatine, and is a product of the energy metabolism in the brain. MI is a marker of astroglial function, and also plays a role in neuronal energy production. The choline measure is a pooled measurement of free choline, glycerolphosphocholine, and phosphocholine, so Cho is considered a marker of membrane turnover. Gln and Glu can be difficult to differentiate, as Gln is a metabolic product of Glu and, consequently, are sometimes collectively referred to as Glx. Glu is the predominant excitatory neurotransmitter in the brain, and found in greatest concentrations in gray matter (Rothman, Behar, Hyder, & Shulman, 2003).

MRS Studies in Early Psychosis

There are a number of studies examining ^{1}H-MRS in FEP patients. However, again after limiting to papers that had more than 20 subjects, used minimal antipsychotic treatment, and allowed no concurrent recreational drug use, nine studies remained. Seven of the nine papers showed no differences between healthy control subjects and FEP patients, regardless of region examined (Table 36.2) (Bustillo et al., 2008; Crocker et al., 2014; Stanley et al., 1996; Tibbo et al., 2013; Tunc-Skarka et al., 2009; Uhl et al., 2011; Wood et al., 2003). The remaining two papers were from the same group, with one study reporting significant increases in Gln levels, between healthy controls and never treated FEP, in the anterior cingulate and thalamus (Theberge et al., 2002), with another study examining patients at the start of treatment and 30 months later (Theberge et al., 2007). The second study only had 16 subjects in each group, but longitudinal methodology added rigor to the design. The naïve FEP group had elevated Gln; however, after 30 months of treatment, Gln was reduced in the thalamus, but still elevated in the anterior cingulate (Theberge et al., 2007).

The absolute metabolite levels of each cohort may not tell the whole story, though. Work by our group suggests that, within the FEP group, bioenergetics may be affected. In one study, we noted a significant decline in tCr levels with age, not present in the healthy control group (Tibbo et al., 2013). In the other study from our group, we noted the absence of a normal relationship between NAA and Glu in the anterior cingulate of FEP patients (Crocker et al., 2014). Both of these studies suggest that alterations in brain metabolism are present at a young age, and that, by strictly examining group differences between healthy controls and FEP patients, we may be missing brain abnormalities, as measured by MRS within FEP cohorts. Another methodological consideration is that, while the focus of spectroscopy has been in gray matter, with increasing field strength, white matter spectroscopy may be a useful modality to examine changes in oligodendrocyte metabolism.

MRS Studies in Cannabis Use

There are very few studies examining the metabolic effects of chronic cannabis use in adolescents and, thus, the authors had to relax our inclusion criteria of subjects per group. In two studies, adolescent chronic cannabis users demonstrated significantly lower Glu, NAA, tCr and mI, as well as GABA, in the anterior cingulate cortex, compared to healthy age-matched nonusers (Prescot,

TABLE 36.2 ^{1}H-MRS Studies of First Episode Psychosis and Cannabis Use

Authors (year)	Group (*n*)	Design	Medicated (ratio)	^{1}H-MRS region	Findings
1. ^{1}H-MRS IN FEP					
Bustillo et al. (2008)	FEP (32) HC (21)	Between-group TE = 40 ms PRESS ^{1}H-MRS 1.5T	Antipsychotic-naive (15/32) Minimally treated (17/32)	Left Caudate Frontal Occipital right cerebellum	• FEP = HC in baseline levels of NAA, Cr and Cho in each individual region • FEP < HC in baseline levels of Global NAA (pooling NAA values from all four regions of interest)
Crocker et al. (2014)	FEP (29) HC (49)	Between-group TE = 240 ms STEAM ^{1}H-MRS at 3T	Unmedicated at time of scan, 6/29 antipsychotic naïve	Medial prefrontal cortex, including anterior cingulate cortex	• FEP = HC for tCr, Cho, NAA and Glu. • NAA correlated to Glu for HC but not FEP.
Stanley et al. (1996)	FEP (21) HC (24)	Between-group STEAM ^{1}H-MRS at 1.5T	11 naïve and 10 acute medicated	Left dorsolateral prefrontal cortex	• FEP = HC in NAA, Glu, GLN, tCr and Cho

(Continued)

TABLE 36.2 ^{1}H-MRS Studies of First Episode Psychosis and Cannabis Use (*cont.*)

Authors (year)	Group (*n*)	Design	Medicated (ratio)	^{1}H-MRS region	Findings
Theberge et al. (2002)	FEP (21) HC (21)	Between-group TE = 20 ms STEAM ^{1}H-MRS at 4T	Never-treated (21/21)	Left Anterior cingulate Thalamus	• FEP > HC in levels of Gln in each region • FEP = HC in levels of NAA, Tau, Cho, Cr and mI in each region
Theberge et al. (2007)	FEP (16) HC (16)	Between-group and two scans 30 months apart TE = 20 ms STEAM ^{1}H-MRS at 4T	Naïve at 0 but not at 30 months	Thalamus and anterior cingulate cortex	• Significant increases in Glutamine in ACC and thalamus in FEP • Glutamine reduced in thalamus at 30 months
Tibbo et al. (2013)	FEP (33) HC (41)	Between-group TE = 240 ms STEAM ^{1}H-MRS at 3T	Naïve	mPFC	• tCr levels decreased in patients with age but not healthy controls
Uhl et al. (2011)	UHR (30) FEP (24) HC (31)	Between-group TE = 140 ms TR = 1500 ms PRESS ^{1}H-MRS at 1.5T	Some (4/30 in UHR and 4/24 in FEP)	ACC PFC	• No differences between groups • Used ratios to Cr
Wood et al. (2003)	UHR (30) FEP (56) HC (21)	Between-group TE = 135 ms PRESS ^{1}H-MRS at 1.5T	23/56 less than 1 month 24/56 more than 1 month	Left DLPFC	• FEP = HC • UHR elevated NAA/Cr and Cho/Cr
Wood et al. (2009)	FEP (34) HC (19)	Between-group TE = 30 ms PRESS ^{1}H-MRS at 3T	Antipsychotic-naive (15/34) Early treated (19/34)	Bilateral medial temporal	• FEP = HC in levels of NAA, Cr, Cho, mI and Glx • Treated group had significantly elevated tCr and mI when limited to schizophrenia spectrum
2. STUDIES IN CANNABIS USE					
Bitter et al. (2014)	CAN+ (13) HC (15)	Between-group TE = 23 ms PRESS ^{1}H-MRS at 3T		Left ventral lateral prefrontal cortex	• HC = CAN+ in levels of NAA
Muetzel et al. (2013)	CAN+ (27) HC (26)	Between-group TE = 70.8 ms STEAM ^{1}H-MRS at 3T		Right basal ganglia	• HC = CAN+ for Glx, mI, Cho, NAA. • Two gender effects, higher mI and lower Glx in females
Prescot et al. (2011)	CAN+ (17) HC (17)	Between-group TE = 30 ms PRESS ^{1}H-MRS at 3T		Anterior cingulate cortex	• HC > CAN+ for mI, Glu, NAA and tCr
Prescot et al. (2013)	CAN+ (13) HC (16)	Between-group TE = 30 ms PRESS ^{1}H-MRS at 3T		Anterior cingulate cortex	• HC > CAN+ for Glu and GABA
Silveri et al. (2011)	CAN+ (13) HC (10)	Between-group TE = 30-330 ms PRESS ^{1}H-MRS at 3T		Anterior cingulate cortex, basal ganglia, thalamus, temporal, parietal, and occipital lobe	• HC > CAN+ for global mI • HC = CAN+ for NAA, Cr, Cho, Glu

The minimal threshold for significance in this table is $p < .05$ (uncorrected for comparisons of multiple neurochemicals), two-tailed. FEP, first episode psychosis; HC, healthy controls; UHR, ultrahigh risk; SCZ, established schizophrenia; NAA, *N*-acetyl-aspartate and *N*-acetyl-aspartyl-glutamate; Cho, choline-containing compounds (trimethylamines), the two main constituents of the Cho signal are phosphorylcholine (PC) and glycerophosphorylcholine (GPC); Glu: glutamate; Gln, glutamine; GABA, gamma-amino butyric acid; Glx, GABA, glutamate and glutamine quantified together; Cr, creatine and phosphocreatine; mI, myoInositol; Tau, taurine.

Locatelli, Renshaw, & Yurgelun-Todd, 2011; Prescot, Renshaw, & Yurgelun-Todd, 2013). Covarying for gender increased the significance of the Glu levels in cannabis-using subjects (Prescot et al., 2011). A more recent study looking at combined Glu plus Gln in the dorsal striatum did not see an overall group effect but, again, when adjusted for gender, there was a significant decrease in Glu + Gln for female users. Similarly for mI, when covaried for gender, mI was increased in female users (Muetzel et al., 2013). These results could have been the result of differing voxel locations; however, they raise interesting thoughts on gender effects that need to be more fully explored. A recent study comparing cannabis-using adolescents to bipolar cannabis-using adolescents and healthy controls found no differences in NAA between cannabis users and healthy controls (Bitter et al., 2014). There is one further ^{1}H-MRS study in adult chronic users of cannabis, with use begun in adolescence, which is useful to report. Thirteen cannabis-using males and 13 male controls were examined by ^{1}H-MRS with a voxel in the dorsolateral prefrontal cortex, a region known to have abnormalities in schizophrenia. There was again a significant decrease in NAA/Cr ratio in users, compared to nonusers (Hermann et al., 2007). If chronic cannabis use in FEP compounds effects on the DLPFC remains to be examined.

The lowering of Glu is a particularly troubling finding in the adolescent brain. There are a number of maturational changes that take place due to the glutamatergic system during adolescence, including subtype switching and synaptic pruning (Egerton, Fusar-Poli, & Stone, 2012). There is also evidence from preclinical studies that application of glutamatergic antagonists results in neuronal apoptosis and schizophrenia-like symptoms (Egerton et al., 2012). It can thus be inferred that decreasing Glu through the intake of cannabis could adversely affect cortical structure. Glu is also the precursor for GABA and NAA-G, both of which would be adversely affected by the decreased Glu levels. Altering both the major excitatory (Glu) and inhibitory (GABA) neurotransmitters in the brain is likely to alter synaptic plasticity. It is not clear how long such changes will last. However, a PET imaging study has shown that there is anterior cingulate cortex hypoactivity after 25 days of cannabis abstinence (Eldreth, Matochik, Cadet, & Bolla, 2004).

There are no reports, to our knowledge, of MRS studies that examine both early psychosis and cannabis use combined. We are currently conducting a multimodal study combining DTI and ^{1}H-MRS in cannabis-using FEP patients (Bernier et al., 2013). The complete absence of direct MRS measurement of brain metabolism in FEP patients who have used/are using cannabis needs to be addressed, as this is especially important, given the strong evidence of a link between cannabis use and onset of psychosis.

CONCLUSIONS

DTI can tell us that there are changes occurring in the structure of the white matter tracts with FEP and cannabis use, and that these changes are different in location and extent from illness effects and cannabis effects alone (Fig. 36.4). These changes are reflected in decreased FA values, indicating greater anisotropy of tissue water and, thus, less restricted diffusion. DTI tractography suggests that the ILF and the SLF are both candidates for consideration of where the white matter tract dysfunction (connectedness) may occur. DTI's limitation is that it cannot tell us anything specific about the nature of the changes we are measuring, for example, if oligodendrocytes may be dying, or if myelination is simply less robust. DTI can confirm that white matter structures can be affected; however, further research into the process of how this occurs needs to be developed. Even with the limitations of DTI, valuable information can be obtained on outcomes of illness combined with cannabis effects on white matter structures. A focus on refined methodology with larger sample sizes will help inform us better, and drive research in the future.

MRS tells us that there are changes in NAA levels with cannabis use, in various brain regions; however, little work has been completed on combined illness and cannabis effects. A limitation of MRS is that we are unsure, at this stage, of what cell types are contributing to this change, and if the decreased levels of NAA may reflect changes in the bioenergetics of the neuronal cells, or changes in cell

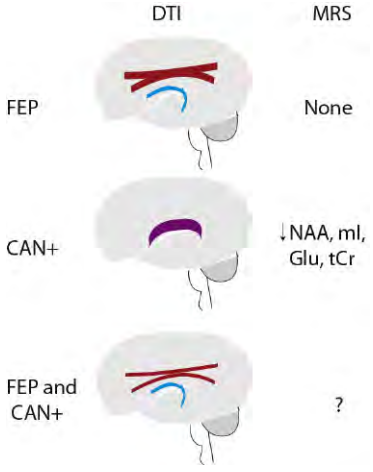


FIGURE 36.4 Summary of the presented findings. For DTI: the best evidence suggests ILF and SLF are affected in FEP, whereas fimbria and commisures are affected in cannabis, and when combined in FEP patients that use cannabis, the ILF and SLF are more dramatically affected. For MRS: there are no global group differences in FEP patients, but in cannabis users there are decreases in NAA, mI, Glu, and tCr. There is currently no information on MRS in Cannabis using FEP (unpublished).

numbers. As with DTI, continued work is needed to improve our understanding of the effects of cannabis in the developing brain at risk for schizophrenia.

What we hope to have illustrated in this chapter is the need for better research into the mechanisms of the action of cannabis on the living human brain. There is a paucity of research using neuroimaging techniques to look into cannabis use. Given what we now know from epidemiological studies about the link between psychosis and cannabis use, and increasing legal access to cannabis, there is an urgent need for well-designed, suitably powered studies into neuroimaging and cannabis use.

MINI-DICTIONARY

Adolescence Phase of human development generally from 12 to 17 years of age, but sometimes up to 24 years. A second surge of neurodevelopment takes place at this time.
Diffusion Tensor Imaging A magnetic resonance imaging technique that allows the measurement of water movement (diffusion) within a tissue of interest, in vivo.
First episode psychosis First psychotic episode, but also the first 5 years of schizophrenia, a time when early intervention can substantially improve overall disease outcome.
Fractional anisotropy (FA) A measure of the diffusion of water inside a specified area of the brain.
Glutamate Major excitatory neurotransmitter in the human brain involved in neuronal energy metabolism.
Longitudinal fasciculi The inferior (ILF) and superior longitudinal fasculi (SLF) are white matter tracts. The SLF connects the frontal lobe to the occipital lobe. The ILF connects the occipital lobe to the temporal lobe.
Magnetic Resonance Spectroscopy A technique used in conjunction with magnetic resonance imaging that allows measurement of biochemical compounds within a tissue, noninvasively.
NAA Amino acid present in the brain at high concentrations. Thought to play a role in neuronal energy metabolism.
Oligodendrocyte A type of neuroglia with myelination as their major function.
Tractography A three-dimensional representation of the white matter connections within the brain, usually between two specified regions.

References

Andreasson, S., Allebeck, P., Engstrom, A., & Rydberg, U. (1987). Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. *Lancet*, *2*(8574), 1483–1486.

Arain, M., Haque, M., Johal, L., Mathur, P., Nel, W., Rais, A., …, Sharma, S. (2013). Maturation of the adolescent brain. *Neuropsychiatric Disease and Treatment*, *9*, 449–461.

Bayer, T. A., Falkai, P., & Maier, W. (1999). Genetic and non-genetic vulnerability factors in schizophrenia: the basis of the "two hit hypothesis". *Journal of Psychiatric Research*, *33*(6), 543–548.

Bernier, D., Cookey, J., McAllindon, D., Bartha, R., Hanstock, C. C., Newman, A. J., …, Tibbo, P. G. (2013). Multimodal neuroimaging of frontal white matter microstructure in early phase schizophrenia: the impact of early adolescent cannabis use. *BMC Psychiatry*, *13*, 264.

Bitter, S. M., Weber, W. A., Chu, W. J., Adler, C. M., Eliassen, J. C., Strakowski, S. M., & Delbello, M. P. (2014). *N*-acetyl aspartate levels in adolescents with bipolar and/or cannabis use disorders. *Journal of Dual Diagnosis*, *10*(1), 39–43.

Bustillo, J. R., Rowland, L. M., Jung, R., Brooks, W. M., Qualls, C., Hammond, R., …, Lauriello, J. (2008). Proton magnetic resonance spectroscopy during initial treatment with antipsychotic medication in schizophrenia. *Neuropsychopharmacology*, *33*(10), 2456–2466.

Chan, W. Y., Yang, G. L., Chia, M. Y., Lau, I. Y., Sitoh, Y. Y., Nowinski, W. L., & Sim, K. (2010). White matter abnormalities in first-episode schizophrenia: a combined structural MRI and DTI study. *Schizophrenia Research*, *119*, 52–60.

Cheung, V., Cheung, C., McAlonan, G. M., Deng, Y., Wong, J. G., Yip, L., …, Chua, S. E. (2008). A diffusion tensor imaging study of structural dysconnectivity in never-medicated, first-episode schizophrenia. *Psychological Medicine*, *38*(6), 877–885.

Clark, D. B., Chung, T., Thatcher, D. L., Pajtek, S., & Long, E. C. (2012). Psychological dysregulation, white matter disorganization and substance use disorders in adolescence. *Addiction*, *107*(1), 206–214.

Collinson, S. L., Gan, S. C., Woon, P. S., Kuswanto, C., Sum, M. Y., Yang, G. L., Lui, J. M., Sitoh, Y. Y., Nowinski, W. L., & Sim, K. (2014). Corpus callosum morphology in first-episode and chronic schizophrenia: combined magnetic resonance and diffusion tensor imaging study of Chinese Singaporean patients. *British Journal of Psychiatry*, *204*, 55–60.

Crocker, C. E., Bernier, D. C., Hanstock, C. C., Lakusta, B., Purdon, S. E., Seres, P., & Tibbo, P. G. (2014). Prefrontal glutamate levels differentiate early phase schizophrenia and methamphetamine addiction: a H MRS study at 3Tesla. *Schizophrenia Research*, *157*, 231–237.

Dahl, R. E. (2004). Adolescent brain development: a period of vulnerabilities and opportunities. Keynote address. *Annals of New York Academy of Sciences*, *1021*, 1–22.

Egerton, A., Fusar-Poli, P., & Stone, J. M. (2012). Glutamate and psychosis risk. *Current Pharmaceutical Design*, *18*(4), 466–478.

Eldreth, D. A., Matochik, J. A., Cadet, J. L., & Bolla, K. I. (2004). Abnormal brain activity in prefrontal brain regions in abstinent marijuana users. *Neuroimage*, *23*(3), 914–920.

Epstein, K. A., Cullen, K. R., Mueller, B. A., Robinson, P., Lee, S., & Kumra, S. (2014). White matter abnormalities and cognitive impairment in early-onset schizophrenia-spectrum disorders. *Journal of the American Academy of Child and Adolescent Psychiatry*, *53*(3), 362–372e 361–e362.

Friedman, J. I., Tang, C., Carpenter, D., Buchsbaum, M., Schmeidler, J., Flanagan, L., Golembo, S., Kanellopoulou, I., Ng, J., Hof, P. R., Harvey, P. D., Tsopelas, N. D., Stewart, D., & Davis, K. L. (2008). Diffusion tensor imaging findings in first-episode and chronic schizophrenia patients. *American Journal of Psychiatry*, *165*, 1024–1032.

Gasparotti, R., Valsecchi, P., Carletti, F., Galluzzo, A., Liserre, R., Cesana, B., & Sacchetti, E. (2009). Reduced fractional anisotropy of corpus callosum in first-contact, antipsychotic drug-naive patients with schizophrenia. *Schizophrenia Research*, *108*(1–3), 41–48.

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., …, Rapoport, J. L. (1999). Brain development during childhood and adolescence: a longitudinal MRI study. *Nature Neuroscience*, *2*(10), 861–863.

Glass, M., Dragunow, M., & Faull, R. L. (1997). Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience*, *77*(2), 299–318.

Gruber, S. A., Dahlgren, M. K., Sagar, K. A., Gonenc, A., & Lukas, S. E. (2014). Worth the wait: effects of age of onset of marijuana use on white matter and impulsivity. *Psychopharmacology*, *231*(8), 1455–1465.

Guo, W., Liu, F., Liu, Z., Gao, K., Xiao, C., Chen, H., & Zhao, J. (2012). Right lateralized white matter abnormalities in first-episode, drug-naive paranoid schizophrenia. *Neuroscience Letters*, *531*(1), 5–9.

Hanson, J. L., Adluru, N., Chung, M. K., Alexander, A. L., Davidson, R. J., & Pollak, S. D. (2013). Early neglect is associated with alterations

in white matter integrity and cognitive functioning. *Child Development*, *84*(5), 1566–1578.

Hao, Y., Liu, Z., Jiang, T., Gong, G., Liu, H., Tan, L., …, Zhang, Z. (2006). White matter integrity of the whole brain is disrupted in first-episode schizophrenia. *Neuroreport*, *17*(1), 23–26.

Hattori, T., Shimizu, S., Koyama, Y., Emoto, H., Matsumoto, Y., Kumamoto, N., …, Ito, A. (2014). DISC1 (disrupted-in-schizophrenia-1) regulates differentiation of oligodendrocytes. *PLoS One*, *9*(2), e88506.

Hermann, D., Sartorius, A., Welzel, H., Walter, S., Skopp, G., Ende, G., & Mann, K. (2007). Dorsolateral prefrontal cortex *N*-acetylaspartate/total creatine (NAA/tCr) loss in male recreational cannabis users. *Biological Psychiatry*, *61*(11), 1281–1289.

Ikuta, T., Peters, B. D., Guha, S., John, M., Karlsgodt, K. H., Lencz, T., …, Malhotra, A. K. (2014). A schizophrenia risk gene, ZNF804A, is associated with brain white matter microstructure. *Schizophrenia Research*, *155*(1–3), 15–20.

Ioannidis, J. P. (2005a). Microarrays and molecular research: noise discovery? *Lancet*, *365*(9458), 454–455.

Ioannidis, J. P. (2005b). Why most published research findings are false. *PLoS Medicine*, *2*(8), e124.

James, A., Hough, M., James, S., Winmill, L., Burge, L., Nijhawan, S., ..., Zarei, M. (2011). Greater white and grey matter changes associated with early cannabis use in adolescent-onset schizophrenia (AOS). *Schizophrenia Research*, *128*(1–3), 91–97.

Kim, D. J., Skosnik, P. D., Cheng, H., Pruce, B. J., Brumbaugh, M. S., Vollmer, J. M., …, Newman, S. D. (2011). Structural network topology revealed by white matter tractography in cannabis users: a graph theoretical analysis. *Brain Connectivity*, *1*(6), 473–483.

Konopaske, G. T., Dorph-Petersen, K. A., Sweet, R. A., Pierri, J. N., Zhang, W., Sampson, A. R., & Lewis, D. A. (2008). Effect of chronic antipsychotic exposure on astrocyte and oligodendrocyte numbers in macaque monkeys. *Biological Psychiatry*, *63*(8), 759–765.

Kumra, S., Ashtari, M., Cervellione, K. L., Henderson, I., Kester, H., Roofeh, D., …, Szeszko, P. R. (2005). White matter abnormalities in early-onset schizophrenia: a voxel-based diffusion tensor imaging study. *Journal of the American Academy of Child and Adolescent Psychiatry*, *44*(9), 934–941.

Kyriakopoulos, M., Vyas, N. S., Barker, G. J., Chitnis, X. A., & Frangou, S. (2008). A diffusion tensor imaging study of white matter in early-onset schizophrenia. *Biological Psychiatry*, *63*(5), 519–523.

Kyriakopoulos, M., Perez-Iglesias, R., Woolley, J. B., Kanaan, R. A., Vyas, N. S., Barker, G. J., Frangou, S., & McGuire, P. K. (2009). Effect of age at onset of schizophrenia on white matter abnormalities. *British Journal of Psychiatry*, *195*, 346–353.

Luck, D., Buchy, L., Czechowska, Y., Bodnar, M., Pike, G. B., Campbell, J. S., …, Lepage, M. (2011). Fronto-temporal disconnectivity and clinical short-term outcome in first episode psychosis: a DTI-tractography study. *Journal of Psychiatric Research*, *45*(3), 369–377.

Makinodan, M., Rosen, K. M., Ito, S., & Corfas, G. (2012). A critical period for social experience-dependent oligodendrocyte maturation and myelination. *Science*, *337*(6100), 1357–1360.

Molina-Holgado, E., Vela, J. M., Arevalo-Martin, A., Almazan, G., Molina-Holgado, F., Borrell, J., & Guaza, C. (2002). Cannabinoids promote oligodendrocyte progenitor survival: involvement of cannabinoid receptors and phosphatidylinositol-3 kinase/Akt signaling. *Journal of Neuroscience*, *22*, 9742–9753.

Mori, S., & Zhang, J. (2006). Principles of diffusion tensor imaging and its applications to basic neuroscience research. *Neuron*, *51*(5), 527–539.

Muetzel, R. L., Marjanska, M., Collins, P. F., Becker, M. P., Valabregue, R., Auerbach, E. J., …, Luciana, M. (2013). In vivo H magnetic resonance spectroscopy in young-adult daily marijuana users. *Neuroimage Clinical*, *2*, 581–589.

Murray, R. M., & Lewis, S. W. (1987). Is schizophrenia a neurodevelopmental disorder? *British Medical Journal*, *295*(6600), 681–682.

Nickl-Jockschat, T., Stocker, T., Krug, A., Markov, V., Huang, R., Schneider, F., …, Kircher, T. (2014). A Neuregulin-1 schizophrenia susceptibility variant causes perihippocampal fiber tract anomalies in healthy young subjects. *Brain Behaviour*, *4*(2), 215–226.

Perez-Iglesias, R., Tordesillas-Gutierrez, D., Barker, G. J., McGuire, P. K., Roiz-Santianez, R., Mata, I., de Lucas, E. M., Quintana, F., Vazquez-Barquero, J. L., …, Crespo-Facorro, B. (2010). White matter defects in first episode psychosis patients: a voxelwise analysis of diffusion tensor imaging. *NeuroImage*, *49*, 199–204.

Peters, B. D., Szeszko, P. R., Radua, J., Ikuta, T., Gruner, P., DeRosse, P., …, Malhotra, A. K. (2012). White matter development in adolescence: diffusion tensor imaging and meta-analytic results. *Schizophrenia Bulletin*, *38*(6), 1308–1317.

Prescot, A. P., Locatelli, A. E., Renshaw, P. F., & Yurgelun-Todd, D. A. (2011). Neurochemical alterations in adolescent chronic marijuana smokers: a proton MRS study. *Neuroimage*, *57*(1), 69–75.

Prescot, A. P., Renshaw, P. F., & Yurgelun-Todd, D. A. (2013). Gamma-amino butyric acid and glutamate abnormalities in adolescent chronic marijuana smokers. *Drug and Alcohol Dependence*, *129*(3), 232–239.

Price, G., Bagary, M. S., Cercignani, M., Altmann, D. R., & Ron, M. A. (2005). The corpus callosum in first episode schizophrenia: a diffusion tensor imaging study. *Journal of Neurology, Neurosurgery, & Psychiatry*, *76*, 585–587.

Rothman, D. L., Behar, K. L., Hyder, F., & Shulman, R. G. (2003). In vivo NMR studies of the glutamate neurotransmitter flux and neuroenergetics: implications for brain function. *Annual Review of Physiology*, *65*, 401–427.

Roussos, P., & Haroutunian, V. (2014). Schizophrenia: susceptibility genes and oligodendroglial and myelin related abnormalities. *Frontiers in Cellular Neuroscience*, *8*, 5.

Saha, S., Chant, D., Welham, J., & McGrath, J. (2005). A systematic review of the prevalence of schizophrenia. *PLoS Medicine*, *2*(5), e141.

Silveri, M. M., Jensen, J. E., Rosso, I. M., Sneider, J. T., & Yurgelun-Todd, D. A. (2011). Preliminary evidence for white matter metabolite differences in marijuana-dependent young men using 2D J-resolved magnetic resonance spectroscopic imaging at 4 Tesla. *Psychiatry Research*, *191*, 201–211.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neuroscience and Biobehavioral Reviews*, *24*(4), 417–463.

Stanley, J. A., Williamson, P. C., Drost, D. J., Rylett, R. J., Carr, T. J., Malla, A., & Thompson, R. T. (1996). An in vivo proton magnetic resonance spectroscopy study of schizophrenia patients. *Schizophrenia Bull*, *22*(4), 597–609.

Szeszko, P. R., Robinson, D. G., Ashtari, M., Vogel, J., Betensky, J., Sevy, S., …, Bilder, R. M. (2008). Clinical and neuropsychological correlates of white matter abnormalities in recent onset schizophrenia. *Neuropsychopharmacology*, *33*(5), 976–984.

Tang, J., Liao, Y., Zhou, B., Tan, C., Liu, T., Hao, W., …, Chen, X. (2010). Abnormal anterior cingulum integrity in first episode, early-onset schizophrenia: a diffusion tensor imaging study. *Brain Research*, *1343*, 199–205.

Theberge, J., Bartha, R., Drost, D. J., Menon, R. S., Malla, A., Takhar, J., …, Williamson, P. C. (2002). Glutamate and glutamine measured with 4.0 T proton MRS in never-treated patients with schizophrenia and healthy volunteers. *American Journal of Psychiatry*, *159*(11), 1944–1946.

Theberge, J., Williamson, K. E., Aoyama, N., Drost, D. J., Manchanda, R., Malla, A. K., …, Williamson, P. C. (2007). Longitudinal grey-matter and glutamatergic losses in first-episode schizophrenia. *British Journal of Psychiatry*, *191*, 325–334.

Tibbo, P. G., Bernier, D., Hanstock, C. C., Seres, P., Lakusta, B., & Purdon, S. E. (2013). 3-T proton magnetic spectroscopy in unmedicated first episode psychosis: a focus on creatine. *Magnetic Resonance in Medicine*, *69*(3), 613–620.

Tunc-Skarka, N., Weber-Fahr, W., Hoerst, M., Meyer-Lindenberg, A., Zink, M., & Ende, G. (2009). MR spectroscopic evaluation of N-acetylaspartate's T2 relaxation time and concentration corroborates white matter abnormalities in schizophrenia. *Neuroimage*, *48*(3), 525–531.

Uhl, I., Mavrogiorgou, P., Norra, C., Forstreuter, F., Scheel, M., Witthaus, H., …, Juckel, G. (2011). 1 H-MR spectroscopy in ultra-high risk and first episode stages of schizophrenia. *Journal of Psychiatric Research*, *45*(9), 1135–1139.

van Os, J., Bak, M., Hanssen, M., Bijl, R. V., de Graaf, R., & Verdoux, H. (2002). Cannabis use and psychosis: a longitudinal population-based study. *American Journal of Epidemiology*, *156*(4), 319–327.

Wang, Q., Deng, W., Huang, C., Li, M., Ma, X., Wang, Y., …, Li, T. (2011). Abnormalities in connectivity of white-matter tracts in patients with familial and non-familial schizophrenia. *Psychological Medicine*, *41*(8), 1691–1700.

White, T., Schmidt, M., & Karatekin, C. (2009). White matter 'potholes' in early-onset schizophrenia: a new approach to evaluate white matter microstructure using diffusion tensor imaging. *Psychiatry Research*, *174*(2), 110–115.

White, T., Magnotta, V. A., Bockholt, H. J., Williams, S., Wallace, S., Ehrlich, S., Mueller, B. A., Ho, B. C., Jung, R. E., Clark, V. P., Lauriello, J., Bustillo, J. R., Schulz, S. C., Gollub, R. L., Andreasen, N. C., Calhoun, V. D., & Lim, K. O. (2011). Global white matter abnormalities in schizophrenia: a multisite diffusion tensor imaging study. *Schizophrenia Bulletin*, *37*, 222–232.

Wood, S. J., Berger, G., Velakoulis, D., Phillips, L. J., McGorry, P. D., Yung, A. R., …, Pantelis, C. (2003). Proton magnetic resonance spectroscopy in first episode psychosis and ultra high-risk individuals. *Schizophrenia Bulletin*, *29*(4), 831–843.

Wood, S. J., Berger, G. E., Wellard, R. M., Proffitt, T. M., McConchie, M., Berk, M., McGorry, P. D., & Pantelis, C. (2009). Medial temporal lobe glutathione concentration in first episode psychosis: a 1H-MRS investigation. *Neurobiology of Disease*, *33*, 354–357.

Yao, L., Lui, S., Liao, Y., Du, M. Y., Hu, N., Thomas, J. A., & Gong, Q. Y. (2013). White matter deficits in first episode schizophrenia: an activation likelihood estimation meta-analysis. *Progress in Neuropsychopharmacology*, *45*, 100–106.

Zalesky, A., Solowij, N., Yucel, M., Lubman, D. I., Takagi, M., Harding, I. H., …, Seal, M. (2012). Effect of long-term cannabis use on axonal fibre connectivity. *Brain*, *135*(Pt 7), 2245–2255.

Zamberletti, E., Beggiato, S., Steardo, L., Jr., Prini, P., Antonelli, T., Ferraro, L., …, Parolaro, D. (2014). Alterations of prefrontal cortex GABAergic transmission in the complex psychotic-like phenotype induced by adolescent delta-9-tetrahydrocannabinol exposure in rats. *Neurobiology of Disease*, *63*, 35–47.

CHAPTER

37

Cannabis Smoking in Adult Schizophrenia: A Cognitive and Functional Magnetic Resonance Imaging Perspective

K. Paquin[*,**], *T. Lecomte*[*,**], *S. Potvin*[*,†]

*Research Centre of the University of Montreal Institute for Mental Health, Montreal, QC, Canada
**Department of Psychology, University of Montreal, Montreal, QC, Canada
†Department of Psychiatry, Faculty of Medicine, University of Montreal, Montreal, QC, Canada

SUMMARY POINTS

- Cognitive deficits are a hallmark of schizophrenia present in close to 80% of individuals.
- Cognitive deficits are present at an early stage of the disorder.
- In the general population, cannabis smoking is associated with a two- to threefold increase in psychosis outcomes.
- Cannabis smoking can trigger first episodes of psychosis in vulnerable individuals.
- Cannabis use in healthy individuals causes small-to-moderate deficits in working memory, executive functions, attention, and episodic memory.
- In schizophrenia, cannabis smoking seems to be paradoxically associated with better cognition.
- fMRI studies using cognitive and emotional stimuli have shown that schizophrenia patients using cannabis have increased prefrontal activity, relative to nonusing schizophrenia patients.
- Factors that may explain these paradoxical findings include the neuroprotective properties of cannabis, social factors related to the lifestyle of cannabis smoking, and uncontrolled premorbid differences.
- Limitations: most studies did not assess all the cognitive domains known to be impaired in schizophrenia, did not confirm self-reports of cannabis smoking with urine drug analyses, did not take into account the potential confounding influence of socio-demographic variables, and did not use longitudinal designs.

KEY FACTS OF SCHIZOPHRENIA

- Schizophrenia is a severe psychiatric disorder characterized by positive and negative symptoms, as well as disorganized thinking/behavior.
- Schizophrenia is associated with high rates of hospitalizations, homelessness, and unemployment.
- The lifetime prevalence of substance use disorder is close to 50% in schizophrenia.
- The life expectancy of schizophrenia patients is 20% shorter than that of the general population.
- 7–8% of schizophrenia patients will end their life by committing suicide.

KEY FACTS OF THE ENDOCANNABINOID SYSTEM

- The endogenous cannabinoid system is composed of (at least) two ligands (anandamide and 2-arachidonylglycerol).

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00042-9
Copyright © 2017 Elsevier Inc. All rights reserved.

- Endocannabinoids bind to (at least) two cannabinoid receptors (CB_1 and CB_2).
- CB_1 receptors are mostly found in the prefrontal cortex, basal ganglia, hippocampus, anterior cingulate cortex, and cerebellum.
- These brain regions are known to be dysfunctional in schizophrenia.
- Delta-9-Tetrahydrocannabinol (Δ^9-THC), the main psychoactive ingredient in cannabis, binds to CB_1 receptors.

LIST OF ABBREVIATIONS

Δ^9-THC	Delta-9-tetrahydrocannabinol
CUD	Cannabis use disorder
dlPFC	Dorso-lateral prefrontal cortex
DD	Schizophrenia group with cannabis abuse
fMRI	Functional magnetic resonance imaging
MATRICS	Measurement and Treatment Research to Improve Cognition in Schizophrenia
mPFC	Medial prefrontal cortex
PCC	Posterior cingulate cortex
Sz + Can	Schizophrenia patients smoking cannabis
Sz	Schizophrenia patients not smoking cannabis
SUD	Substance use disorders

COGNITION IN SCHIZOPHRENIA

The lifetime prevalence of schizophrenia is lower than 1% worldwide. Schizophrenia is a severe psychiatric disorder, characterized by positive (delusions and hallucinations) and negative (flat affect, anhedonia) symptoms, as well as disorganized thinking/behavior. A source of considerable social and economic burden, schizophrenia has been ranked by the World Health Organization as one of the top 10 causes of disability in developed countries (Tandon, Keshavan, & Nasrallah, 2008a). Over the years, cognitive dysfunctions have emerged as being of critical importance in schizophrenia. Given that cannabis smoking is highly prevalent in schizophrenia, and that it has deleterious effects on cognition in nonpsychosis individuals, the potential cognitive effects of cannabis on cognition in schizophrenia have recently attracted growing interest.

It is widely known that individuals with schizophrenia have to cope with a variety of cognitive deficits (Goldberg, David, & Gold, 2011), with close to 80% of them struggling with either socio- or neurocognitive deficits or both. Factor analyses have revealed that six neurocognitive domains are significantly impaired in schizophrenia, namely speed of processing, attention/vigilance, working memory, verbal and visual learning, as well as reasoning, and problem solving. The socio-cognitive domains known to be impaired in schizophrenia include deficits in social knowledge, emotion recognition, Theory of Mind, attribution style, as well as emotion regulation. When compared to the general population, the cognitive deficits of schizophrenia range from 1 to 1.5 standard deviations below the normal scores. Mild deficits are also present, even in the prodromal phase of the disorder, indicating that they could partly be independent of duration of illness and medication intake. Furthermore, nonaffected family members of individuals with schizophrenia tend to show similar though attenuated cognitive deficits, suggesting that the cognitive impairments of schizophrenia are partially heritable. Finally, research has shown that cognitive impairments in schizophrenia are relatively stable over time, even when psychotic symptoms are in remission. Cognitive dysfunctions are, therefore core features of schizophrenia, known to severely impede upon recovery and rehabilitation. Indeed, numerous studies have shown that cognition (especially social cognition) is a better predictor of social and occupational functioning, and overall recovery, than the negative or positive symptoms of schizophrenia. As a result, interest in investigating cognition in schizophrenia has rapidly grown over the past years, in the hopes of pinpointing where the deficits specifically lie, in order to identify better treatments. As such, the National Institute of Mental Health created a standardized battery of tests to assess cognition in clinical settings: the MATRICS Consensus Cognitive Battery, which measures seven domains of cognition (six neuro-cognitive, one socio-cognitive; Table 37.1) (Nuechterlein et al., 2008).

On neurobiological grounds, considerable efforts have been made in order to identify the neurobiological bases of the cognitive impairments of schizophrenia, considered as putative endophenotypes of the disorder. Thus far, functional imaging studies have mostly paid attention to deficits in working memory, executive functions, emotion processing, Theory of Mind, and episodic memory in schizophrenia. This vast functional imaging literature has shown that schizophrenia is a complex psychiatric disorder characterized by impaired fronto-parietal, fronto-striatal, fronto-limbic, and frontal-temporal networks (Delvecchio, Sugranyes, & Frangou, 2013; Minzenberg, Laird, Thelen, Carter, & Glahn, 2009; Ragland et al., 2009; Sugranyes, Kyriakopoulos, Corrigall, Taylor, & Frangou, 2011; Van Snellenberg, Torres, & Thornton, 2006) (Table 37.2).

CANNABIS AND PSYCHOSIS OUTCOMES

The administration of delta-9-tetrahydrocannabinol (Δ^9-THC, the main psychoactive agent of cannabis) to healthy subjects has been shown to consistently produce

TABLE 37.1 MATRICS Cognitive Domains and Associated Tests

Cognitive domain	MATRICS test beta-battery	Most frequently used tests from 20 studies reported in the table
Speed of processing	Category fluency, animal naming	Groove pegboard
	Trail making test (TMT)	CogState
	Wechsler Adult Intelligence Scale, 3rd ed. (WAIS-III), digit symbol-coding subtest	Rapid visual information processing from Cambridge neuropsychological test automated battery
	Brief assessment of cognition in schizophrenia (BACS), symbol coding subtest	Forced-choice span of apprehension test
Attention/vigilance	3–7 Continuous performance test, shortened version	Digit span test (forward version), from the WAIS-III
	Continuous performance test—identical pairs version	Spatial span forward items of the Wechsler Memory Scale-III (WMS-III)
Working memory	BACS, digit sequencing subtest	Bergen N-back paradigm
	WAIS-III, letter-number sequencing subtest	Working memory–mental arithmetic test
	Letter-number span test	CPT identical pairs test
	Wechsler Memory Scale, 3rd ed., spatial span subtest	
	Spatial delayed response task	
Verbal learning/memory	Neuropsychological assessment battery, daily living memory subtest	Rey auditory verbal learning test
	Hopkins verbal learning test—revised, immediate recall	California verbal learning task
		Controlled oral word association test
Visual learning/memory	Neuropsychological assessment battery, shape learning subtest	Spatial-delayed response test (SDR)
	Brief visuospatial memory test—revised	Pattern recognition memory from the Cambridge neuropsychological test automated battery
Reasoning and problem solving	WAIS-III, block design subtest	Wisconsin card sorting test
	BACS, Tower of London subtest	Delis Kaplan executive functioning system
	Neuropsychological assessment battery, mazes subtest	Stroop color word test
Social cognition	Mayer-Salovey-Caruso emotional intelligence test, perceiving emotions branch	Hinting task for theory of mind
	Mayer-Salovey-Caruso emotional intelligence test, managing emotions branch	Degraded facial affect recognition task for emotional recognition

This table includes all the cognitive domains of the MATRICS battery and the associated tests used to measure them.

positive-like and negative-like symptoms, as well as disorganized thinking (D'Souza et al., 2004)—therefore reproducing the complex phenomenology of schizophrenia. Similarly, in some individuals, it has also been observed that cannabis can induce a transient psychotic disorder mimicking the positive symptoms of schizophrenia. The psychomimetic effects of cannabis has led some authors to propose that cannabis may be a risk factor for psychosis, an idea that was first substantiated by the classic study from Andreasson, Engström, Allebeck, and Rydberg (1987) among 45,570 conscripts from the Swedish army. Since then, better controlled studies have been performed, which have provided substantial evidence that cannabis smoking is associated with a two- to three-fold increase in psychotic symptoms or schizophrenia-spectrum diagnoses (Moore et al., 2007). The magnitude of the relationship between cannabis smoking and psychosis outcomes seems to be dose-dependent, and to be influenced by age of cannabis smoking initiation, traumatic experiences, premorbid psychosis vulnerability, and genetic factors (Gage, Zammit, & Hickman, 2013).

In individuals diagnosed with schizophrenia, the risk of compulsive cannabis smoking is increased by 3–6, compared to the general population. A recent metaanalysis

TABLE 37.2 Neurofunctional Alterations in Schizophrenia

Cognitive function	Example(s) of task	Hypoactivations	Hyperactivations	References
Working memory	N-Back	*High working memory load* • dlPFC • Parietal cortex	*Low working memory load* • dlPFC • Parietal cortex	Van Snellenberg et al. (2006)
Executive functions	Stroop; Go-No-Go	• dl and vlPFC • ACC • Striatum	—	Minzenberg et al. (2009)
Emotion processing	Angry/fearful faces (implicit or explicit processing)	• Amygdala • Parahippocampal gyrus • Fusiform gyrus • mPFC • ACC • Insula	—	Delvecchio et al. (2013)
Episodic memory (*encoding*)	Verbal/visual learning/memory	• dl and vlPFC	• Parahippocampal gyrus • ACC • Medial temporal cortex	Ragland et al. (2009)
Episodic memory (*retrieval*)	Verbal/visual learning/memory	• dl and vlPFC • ACC • Medial temporal cortex	• Parahippocampal gyrus	Ragland et al. (2009)
Theory of mind	Judgment of mental states from pictures or eyes	• mPFC • Medial temporal cortex • Thalamus	—	Sugranyes et al. (2011)

This table shows the brain regions known to function abnormally in schizophrenia patients performing various cognitive and emotional tasks.
Abbreviations: *dlPFC*, dorsolateral prefrontal cortex; *vlPFC*, ventrolateral prefrontal cortex; *ACC*, anterior cingulated cortex; *mPFC*, medial prefrontal cortex.

of 35 studies reported that the current rate of cannabis use disorder is 16%, and the lifetime rate is 27.1% in this population (Koskinen, Löhönen, Koponen, Isohanni, & Miettunen, 2009). Cannabis smoking negatively interferes with the course and treatment of schizophrenia, as it is associated with higher rates of hospitalizations, more severe positive symptoms, noncompliance, and more suicide attempts (Bourque et al., 2013). Finally, based on 80 studies, it has been shown that cannabis smoking is associated with an earlier age of psychosis onset (~2 years), after controlling for the influence of sex, smoking chronicity, and other psycho-active substances (Myles, Newall, Nielssen, & Large, 2012). Though no firm inference can be made on causality, based on cross-sectional studies, these results raise the possibility that, in vulnerable people, cannabis smoking is a powerful trigger of psychosis.

THE COGNITIVE EFFECTS OF CANNABIS IN NONPSYCHOSIS INDIVIDUALS

In nonpsychiatric smokers, intoxication with cannabis or Δ^9-THC has been consistently shown to impair working memory, executive functions, attention, and episodic memory (Crean, Crane, & Mason, 2011). The residual cognitive effects of cannabis use have also been studied. While the short-term abstinence from cannabis is associated with small-to-moderate deficits in episodic memory, executive functions, and attention, the magnitude of the long-term residual cognitive effects of cannabis smoking remains unclear (Crean et al., 2011). Study results seem influenced by the quantities of cannabis smoked by participants, as well as the length of abstinence. While some studies have shown that it usually takes about 28 days of abstinence from cannabis smoking to recover one's cognitive abilities, others have reported that certain cognitive deficits may last longer. The magnitude of the cognitive effects of cannabis may also be influenced by earlier age of smoking initiation, as shown in a 25-year cohort study involving 1037 subjects (Meier et al., 2012).

Neuroimaging studies performed on individuals without psychiatric disorders have shown that acute Δ^9-THC intoxication and chronic cannabis smoking both produce neuro-functional alterations echoing the neurophysiologic disturbances seen in schizophrenia (Bossong, Jansma, Bhattacharyya, & Ramsey, 2014). Indeed, cannabinoids have been shown to disrupt working memory-related activity in dorso-lateral prefrontal and parietal cortices, to impair amygdala reactivity during

the viewing of fearful or angry faces, to alter the functioning of frontal and striatal regions during response inhibition tasks, and, finally, to disrupt prefrontal and medial temporal regions during episodic memory tasks. Regardless of heterogeneity of results, cannabinoids seem to reproduce (at least, partially) the fronto-parietal, fronto-striatal, fronto-limbic, and frontal-temporal alterations observed in schizophrenia (Table 37.2).

COGNITIVE PERFORMANCE IN CANNABIS-USING PATIENTS WITH SCHIZOPHRENIA

Considering the (mild) cognitive impairments associated with cannabis smoking, the logical expectation would be that schizophrenia patients who smoke cannabis (Sz + Can) would have more prominent cognitive deficits, compared to schizophrenia patients who do not smoke cannabis (Sz). More than 20 studies have been performed on the topic, and surprisingly, most of them have shown the opposite, namely that Sz + Can patients have *fewer* cognitive deficits than Sz patients (Table 37.3). It must be mentioned, however, that results have been inconsistent across studies, with a minority of studies showing that Sz + Can patients have *more* cognitive deficits than Sz patients, while others failed to show between-group differences (Table 37.3). Theoretically, this heterogeneity of results may reflect the heterogeneity of the recruited samples (schizophrenia, schizo-affective disorder and/or psychotic disorders), as well as the heterogeneous definitions of cannabis smoking status (use, heavy use, abuse, dependence; lifetime, current) (Table 37.3). However, the paradoxical cognitive performance of Sz + Can patients has been observed in various studies, regardless of diagnosis type, and of cannabis use frequency (Table 37.3).

In view of the diversity of results, systematic quantitative reviews have been performed (Table 37.4). Two of them focused solely on cannabis (Rabin, Zakzanis, George, 2011; Yücel et al., 2010), and both showed that cannabis smoking is associated with better cognitive functioning (eg, attention, planning, speed of processing, visual memory, and visuo-spatial abilities) in schizophrenia. Both reviews revealed between-group differences in the small-to-moderate range.

Interestingly, two metaanalyses examined the effects of other psychoactive substances on cognition in schizophrenia, which showed that the better cognitive performance of substance-using schizophrenia patients is mostly related to cannabis. In a metaanalysis of 23 studies, Potvin, Joyal, Pelletier, and Stip (2008) showed that substance-using patients had faster speed of processing, regardless of the substance used. Secondary analyses revealed that alcohol was associated with increased deficits in working memory; cocaine, with contradictory findings; and cannabis, with better global cognition. A subsequent metaanalysis from Donoghue and Doody (2012) showed that cannabis smoking is associated with better cognition in schizophrenia, and that cocaine use/abuse is associated with small-to-moderate impairments in verbal memory. Overall, the evidence suggests that alcohol and cocaine compromise cognition in schizophrenia, whereas cannabis is associated with better cognition. Noteworthy, a metaregression analysis performed by Potvin et al. (2008) showed that there is a negative relationship between age and cognition in substance-using schizophrenia patients. That is, older substance-using schizophrenia patients (>30 years) have worse cognitive functioning than schizophrenia-only patients, whereas younger substance-using schizophrenia patients have better cognition than schizophrenia-only patients. In these studies, older schizophrenia patients tended to prefer alcohol, whereas younger ones preferred cannabis. These results suggest that age × substance type interactions may explain heterogeneity in findings across studies.

SCHIZOPHRENIA, CANNABIS, AND COGNITION: THE FUNCTIONAL IMAGING STUDIES

Despite the wide interest in the psychosis-cannabis link, only six functional imaging studies have been performed in Sz + Can patients (Table 37.5). In the first two functional magnetic resonance imaging (fMRI) studies performed in schizophrenia patients with SUD, negative emotional stimuli were shown to patients while in the scanner (Mancini-Marië et al., 2006; Potvin, Mancini-Marië, Fahim, Mensour, & Stip, 2007). In both studies, schizophrenia patients with SUD had stronger emotional reactions than Sz patients, which translated into increased activations in the medial prefrontal cortex—which plays a critical role in self-relevancy processes. Unfortunately, both studies involved schizophrenia patients who abused both cannabis *and* alcohol. Moreover, the study lacked a control group, making it difficult to determine the exact meaning of the increased activations of the medial prefrontal cortex found in the substance-abusing group. Subsequently, Løberg et al. (2012) performed an fMRI study examining the neural correlates of attention in schizophrenia patients with and without lifetime cannabis smoking. The study showed that Sz + Can patients had increased activations in the posterior cingulate gyrus, the inferior parietal gyrus, and the precentral gyrus, compared to Sz patients. Again, the lack of a control group made it difficult to determine if Sz patients had hypoactivations, or if Sz + Can patients actually had hyperactivations during the task. Recently, our group performed

TABLE 37.3 Studies Showing Intact, Better, or Worse Cognition in Schizophrenia Patients Who Smoke Sannabis

Authors	N (cannabis only)	Mean age	Drugs assessed	Use profile	Illness profile	MATRICS domains	Main results	Confounding factors
Bahorik et al. (2013)	974 (128)	39.21 (drug users)	Cannabis, cocaine, and methamphetamine	Positive radioimmunoassay of hair for drug use	Sz diagnosis according to DSM-IV criteria	Reasoning/problem solving; speed of processing; verbal learning/memory; vigilance	No between group differences (cannabis use versus no drug use)	High male-to-female ratio in Sz + Can group
Coulston et al. (2007)	52 (18)	26.4 (Sz)	Cannabis	DSM-IV criteria for lifetime cannabis abuse/dependence. "High" frequency: at least weekly or more frequent basis "Medium" frequency: between two to four times per month	Diagnosis of Sz or schizoaffective disorder according to DSM-IV criteria	Attention; speed of processing; reasoning/problem solving; verbal learning/memory; visual learning/memory	Better attention, reasoning and problem solving and speed of processing in DD patients	
DeRosse et al. (2010)	455 (175)	37.3	Cannabis	Cannabis use disorder (abuse/dependence)	Sz or schizoaffective disorder	Verbal learning/memory; speed of processing	Better speed of processing/verbal fluency and verbal memory in DD patients	High male-to-female ratio in Sz + Can group
Ferraro et al. (2013)	119 (86)	29.6	Cannabis	Lifetime cannabis use (used at least once)	Affective psychosis and nonaffective psychosis diagnosis according to ICD-10 criteria for psychosis	Other: IQ	Higher current and premorbid IQ in psychotic patients who had smoked cannabis at least once	High male-to-female ratio in Sz + Can group
Jockers-Scherübl et al. (2007)	39 (19)	27.7	Cannabis	Cannabis abuse defined as having consumed an average of $\geq$ 0.5 g/day of cannabis for a minimum of 2 years before the onset of the disorder. Substance use disorders, other than cannabis, were excluded	Sz diagnosis according to DSM-IV criteria	Verbal learning/memory; attention; visual learning/memory; speed of processing; reasoning/problem solving; other: language abilities	Better language abilities and speed of processing in DD patients	High male-to-female ratio in Sz + Can group Younger in Sz + Can group
Leeson et al. (2012)	99 (65)	23.4	Cannabis	High-frequency cannabis users (daily or almost daily use), and low-frequency cannabis users (1–2 days/week to less than monthly). Substance misuse, other than cannabis, was excluded	First psychotic episode	Verbal learning/memory; working memory; reasoning/problem solving	Better on all measure for DD patients except on working memory	Younger in Sz + Can group

Lev-Ran et al. (2012)	28 (12)	18–45 n/a	Cannabis	Cannabis dependence diagnosis according to DSM-IV criteria. Substance use, other than cannabis, was excluded	Sz diagnosis according to DSM-IV criteria	Attention; visual learning/memory; working memory; reasoning/ problem solving	Worse sustained attention and speed of processing (shorter initial thinking time) in DD patients	
Løberg and Hugdahl (2009)	29 (13)	n/a	Cannabis	Current substance use and former substance use	Acute psychosis and Sz	Attention; reasoning/ problem solving; speed of processing; verbal learning/memory; working memory	Better attention, reasoning, and problem solving, speed of processing, verbal memory, and working memory in DD patients	
Mata et al. (2007)	132 (61)	23.4	Cannabis	Cannabis abuse: at least weekly use of cannabis during the previous year. Drug dependence was excluded	First episode of a schizophrenia-spectrum psychosis	Verbal learning/ memory; speed of processing; working, memory; reasoning/ problem solving; other: decision-making and IQ	Worse decision-making in DD patients	High male-to-female ratio in Sz + Can group Younger in Sz + Can group
Meijer et al. (2012)	956 (632)	27.3	Cannabis	Lifetime cannabis use and current use were included	Nonaffective psychosis	Verbal learning/ memory; attention/ vigilance; speed of processing; working memory; reasoning/ problem solving; social cognition	Poorer performance on verbal learning, processing speed and working memory in patients with current cannabis use. Better performance on acquired knowledge, facial affect recognition and social cognition in patients with lifetime cannabis use	High male-to-female ratio in Sz + Can group Younger in Sz + Can group
Rabin et al. (2011)	58 (18)	31.6	Cannabis	Current or former cannabis dependence diagnosis according to DSM-IV criteria	Sz or schizoaffective disorder diagnosis according to DSM-IV criteria	Attention; speed of processing; visual learning/ memory; verbal learning/memory; working memory; reasoning/ problem solving	Better psychomotor speed in patients with lifetime cannabis dependence	Younger in Sz + Can group
Ringen et al. (2010)	140 (23)	33.3	Cannabis	Cannabis abuse/dependency diagnosis according to DSM-IV criteria. Substance use other than cannabis in the previous 6 months was excluded	Sz, schizophreni-form disorder, or schizo-affective disorder diagnosis according to DSM-IV criteria	Speed of processing; attention; working memory; verbal learning/memory; reasoning/ problem solving	Worse reasoning and problem solving, verbal memory, and working memory in DD patients	

(*Continued*)

TABLE 37.3 Studies Showing Intact, Better, or Worse Cognition in Schizophrenia Patients Who Smoke Sannabis (*cont.*)

Authors	N (cannabis only)	Mean age	Drugs assessed	Use profile	Illness profile	MATRICS domains	Main results	Confounding factors
Ringen et al. (2013)	364 (57)	31.2	Cannabis	Lifetime DSM-IV diagnosis of cannabis abuse/addiction. Cannabis-positive urine group (n = 21) was compared to cannabis-negative urine group (n = 343)	Sz, schizophreniform disorder, and schizoaffective disorder diagnosis according to DSM-IV criteria	Speed of processing; attention; working memory; verbal learning/memory; reasoning/problem solving; other: IQ	No between-group differences after adjusting for premorbid functioning	
Rodríguez-Sánchez et al. (2010)	104 (47)	23.6	Cannabis	Cannabis use: at least weekly use of cannabis during the previous year. Drug dependence was excluded	First episode of nonaffective psychosis	Verbal learning/memory; visual learning/memory; speed of processing; working memory; attention; reasoning/problem solving	Better speed of processing in DD patients	High male-to-female ratio in Sz + Can group Younger in Sz + Can group
Sánchez-Torres et al. (2013)	42 (n/a)	37.02	Cannabis, alcohol, stimulants, other drugs (eg, opioids) and tobacco use	Lifetime cannabis use, excluding patients with a diagnosis of cannabis dependence	Sz, schizoaffective disorder, psychotic mood disorder and brief psychotic disorder	Speed of processing; attention/vigilance; working memory; social cognition; reasoning/problem solving	Lifetime cannabis consumption was not associated with negative cognitive outcomes Lifetime cannabis consumption was associated to a negative effect on performance in a social cognition task	
Schnell et al. (2009)	69 (35)	27.1	Cannabis	Cannabis abuse/dependence diagnosis according to DSM-IV criteria. No additional substance use disorders, except for nicotine, were included	Sz or schizoaffective disorder diagnosis according to DSM-IV criteria	Verbal learning/memory; working memory; visual learning/memory; attention; speed of processing; reasoning/problem solving; other: IQ	Better speed of processing, verbal memory and working memory in DD patients.	
Scholes and Martin-Iverson (2010)	71 (22)	35.8	Cannabis	Current use of cannabis	Sz or schizo-affective disorder diagnosis	Attention; working memory; reasoning/problem solving	No between-group differences	

Sevy et al. (2007)	27 (14)	29.0	Cannabis	Current DSM-IV diagnosis for cannabis abuse/dependence, and having cannabis as the main drug of choice	Sz or schizoaffective disorder diagnosis according to DSM-IV criteria. Substance induced psychotic disorder was excluded	Visual learning/ memory; working memory; attention; verbal learning/ memory; speed of processing	Better working memory in DD patients	
Wobrock et al. (2013)	498 (115)	23.9 (drug users)	Cannabis, cocaine, amphetamine or other stimulants, heroin, XTC, LSD or other hallucinogens	Current or former substance use disorder (abuse/dependence) diagnosis according to DSM-IV criteria	First episode of Sz, schizophreniform disorder, and schizoaffective disorder diagnosis according to DSM-IV criteria	Verbal learning/ memory; speed of processing; attention; visual learning/memory; other: complex visual scanning	Better complex visual scanning and psychomotor speed and reasoning/ problem solving (cognitive flexibility) with longer duration of cannabis use	High male-to-female ratio in Sz + Can group Younger in Sz + Can group
Yücel et al. (2010)	85 (59)	20.7	Cannabis	Regular cannabis use (defined as >2 years of use and >2 g/week)	First-episode psychosis	Speed of processing; attention; working memory; verbal learning/memory; reasoning/ problem solving	Better reasoning/ problem solving and working memory in DD patients	

This table summarizes the studies reporting that patients with both schizophrenia and cannabis smoking have intact, better or worse cognition. The relevant cognitive domains are cited.

References

Bahorik, A. L., Newhill, C. E., & Eack, S. M. (2013). Neurocognitive functioning of individuals with schizophrenia: Using and not using drugs. *Schizophrenia Bulletin*, 1–12.

Coulston, C. M., Perdices, M., & Tennant, C. C. (2007). The neuropsychological correlates of cannabis use in schizophrenia: Lifetime abuse/dependence, frequency of use, and recency of use. *Schizophrenia Research, 96*, 169–184.

DeRosse, P., Kaplan, A., Burdick, K. E., Lencz, T., & Malhotra, A. K. (2010). Cannabis use disorders in schizophrenia: Effects on cognition and symptoms. *Schizophrenia Research, 120*, 95–100.

Jockers-Scherübl, M. C., Wolf, T., Radzei, N., Schlattmann, P., Rentzsch, J., Castro, A. G.-C. d., & Kühl, K.-P. (2007). Cannabis induces different cognitive changes in schizophrenic patients and in healthy controls. *Neuro-Psychopharmacology & Biological Psychiatry, 31*, 1054–1063.

Leeson, V. C., Harrison, I., Ron, M. A., Barnes, T. R. E., & Joyce, E. M. (2012). The effect of cannabis use and cognitive reserve on age at onset and psychosis outcomes in first-episode schizophrenia. *Schizophrenia Bulletin, 38* (4), 873–880.

Lev-Ran, S., Segev, A., Braw, Y., & Levkovitz, Y. (2012). Neurocognitive functions of heavy cannabis using schizophrenia patients. *European Psychiatry, 27*, 365–368.

Løberg, E.-M., & Hugdahl, K. (2009). Cannabis use and cognition in schizophrenia *Frontiers in Human Neuroscience, 3*, 1–8.

Meijer, J. H., Dekker, N., Koeter, M. W., Quee, P. J., Beveren, N. J. M. v., & Meijer, C. J. (2012). Cannabis and cognitive performance in psychosis: a cross-sectional study in patients with non-affective psychotic illness and their unaffected siblings. *Psychological Medicine, 42*, 705–716.

Rabin, R. A., Zakzanis, K. K., Daskalakis, Z. J., & George, T. P. (2013). Effects of cannabis use status on cognitive function, in males with schizophrenia. *Psychiatry Research, 206*, 158–165.

Ringen, P. A., Melle, I., Berg, A. O., Agartz, I., Spigset, O., Simonsen, C., Sundet, K., & Andreassen, O. A. (2013). Cannabis use and premorbid functioning as predictors of poorer neurocognition in schizophrenia spectrum disorder. *Schizophrenia Research, 143*, 84–89.

Ringen, P. A., Vaskinn, A., Sundet, K., Engh, J. A., Jónsdóttir, H., Simonsen, C., Friis, S., Opjordsmoen, S., Melle, I., & Andreassen, O. A. (2010). Opposite relationships between cannabis use and neurocognitive functioning in bipolar disorder and schizophrenia. *Psychological Medicine, 40*, 1337–1347.

Rodríguez-Sánchez, J. M., Ayesa-Arriola, R., Mata, I., Moreno-Calle, T., Perez-Iglesias, R., González-Blanch, C., Periañez, J. A., Vazquez-Barquero, J. L., & Crespo-Facorro, B. (2010). Cannabis use and cognitive functioning in first-episode schizophrenia patients. *Schizophrenia Research, 124*, 142–151.

Sánchez-Torres, A. M., Basterra, V., Rosa, A., Fañanás, L., Zarzuela, A., Ibáñez, B., Peralta, V., & Cuesta, M. J. (2013). Lifetime cannabis use and cognition in patients with schizophrenia spectrum disorders and their unaffected siblings. *European Archives of Psychiatry and Clinical Neurosciences, 263*, 643–653.

Scholes, K. E., & Martin-Iverson, M. T. (2010). Cannabis use and neuropsychological performance in healthy individuals and patients with schizophrenia. *Psychological Medicine, 40*, 1635–1646.

Sevy, S., Burdick, K. E., Visweswaraiah, H., Abdelmessih, S., Lukin, M., Yechiam, E., & Bechara, A. (2007). Iowa Gambling Task in schizophrenia: A review and new data in patients with schizophrenia and co-occurring cannabis use disorders. *Schizophrenia Research, 92*, 74–84.

Wobrock, T., Falkai, P., Schneider-Axmann, T., Hasan, A., Galderisi, S., Davidson, M., Kahn, R. S., Derks, E. M., Boter, H., Rybakowski, J. K., Libiger, J., Dollfus, S., López-Ibor, J. J., Peuskens, J., Hranov, L. G., Gaebel, W., & Fleischhacker, W. W. (2013). Comorbid substance abuse in first-episode schizophrenia: Effects on cognition and psychopathology in the EUFEST study. *Schizophrenia Research, 147*, 132–139.

TABLE 37.4 Systematic Quantitative Reviews on the Cognitive Effects of Psychoactive Substances in Schizophrenia Patients

Authors	Number of studies	Substances	MATRICS?	Main results
Donoghue and Doody (2012)	21	11 on polysubstance, seven studies on cocaine, three on cannabis	No	For polysubstance: • Substance users performed better on verbal learning and memory, executive function, and attention and psychomotor speed, and worse on spatial working memory task than nonusers For cocaine: • Cocaine users performed better on attention and psychomotor speed, and worse on verbal learning and memory than nonusers For cannabis: • Cannabis users performed better on executive function, attention and psychomotor speed, and verbal learning and memory than nonusers
Potvin et al. (2008)	28	Mixed psychoactive substances	Yes	Individuals with comorbid substance use had faster speed of processing Secondary analyses revealed significant associations with age and substance type
Rabin et al. (2011)	8	Cannabis	No	Patients smoking cannabis were better in all domains assessed: general cognitive ability and intelligence, sustained and divided attention, executive abilities, working memory, learning, retrieval and recognition, receptive and expressive language abilities, and visuo-spatial and constructional abilities
Yücel et al. (2010)	10	Cannabis	Yes	Patients smoking cannabis had better processing speed, visual memory, working memory, executive functioning, and sustained attention

This table summarizes the systematic reviews that investigated the effects of psychoactive-substances on the cognitive domains known to be impaired in schizophrenia.

two fMRI experiments in 14 Sz + Can patients, 14 Sz patients, and 21 healthy volunteers. Patients with schizoaffective or schizophreniform disorders were excluded, as well as those receiving antidepressants, and those who abused other psychoactive substances than cannabis. In a first experiment, participants performed an emotional memory task. At the cognitive level, Sz + Can patients had a better mnesic performance than Sz patients, but a poorer performance than healthy controls. Neurally, both patient groups had hypoactivations, compared to controls—again, with Sz + Can patients showing more activation than Sz patients, for both negative and positive stimuli (Figs. 37.1 and 37.2). However, Sz + Can patients had increased activations in the dorso-lateral prefrontal cortex, compared to Sz patients (Bourque et al., 2013). In a second experiment, participants performed a mental rotation task that measures visuo-spatial abilities. Although both patient groups performed worse than controls on the mental rotation task, Sz patients had decreased activations in the superior parietal gyrus, whereas the functioning of this region was normal in Sz + Can patients (Potvin et al., 2013). Taken together, these preliminary fMRI results *tentatively* suggest that the brain functioning of Sz + Can patients is less impaired than that of Sz patients.

EXPLANATORY MODELS

To explain these seemingly paradoxical results, three main models have been proposed: the neuro-protection hypothesis, the social hypothesis, and the hypothesis of a lower vulnerability to psychosis in Sz + Can patients. Whereas the first hypothesis postulates that the better cognition of Sz + Can patients is caused by cannabis smoking, the latter two hypotheses propose, on the contrary, that the better cognitive performance of some Sz patients is primary, not secondary, to cannabis smoking.

The Neuro-Protection Hypothesis

The first model trying to explain these seemingly paradoxical findings is a neurobiological one. In preclinical studies, it has been shown that cannabinoids have anti-inflammatory, antioxidant, and neuroprotective effects, and that the endocannabinoid system (Pertwee, 2005), which mediates the effects of cannabis in the brain, is critically involved in various neurodevelopmental processes (neuronal specification, axonal elongation, and synaptogenesis) (Sarne & Mechoulam, 2005). Thus, it

TABLE 37.5 Neurofunctional Alterations in Cannabis-Using Schizophrenia Patients

Authors	Population	Task	Main results	Confounds/limitations
Bourque et al. (2013)	21 healthy controls; 14 Sz patients; 14 Sz patients with CUD	Emotion recognition (positive and negative images)	• Better emotion recognition in cannabis-abusing Sz patients • Increased dlPFC activations in the cannabis-abusing Sz patients, compared to nonusing Sz patients	Lack of a group of nonpsychosis cannabis abusers
Løberg et al. (2009)	26 Sz patients; 13 cannabis users; 13 nonusers	Auditory dichotic listening task	• Increased activations of the PCC, the inferior parietal gyrus and the precentral gyrus in the cannabis-using group	Lack of a control group of healthy volunteers; lack of a group of nonpsychosis cannabis users
Mancini-Marië et al. (2006)	11 Sz patients; 12 Sz patients with SUD (cannabis and alcohol)	Aversive emotional pictures	• Higher emotional ratings in substance abusing Sz patients • Increased activations in the mPFC in Sz patients with SUD	Sz patients were also abusing from alcohol; lack of a control group of healthy volunteers; lack of a group of nonpsychosis cannabis users
Potvin et al. (2013)	21 healthy controls; 14 Sz patients; 14 Sz patients with CUD	Mental rotation task	• Decreased activations of the superior parietal gyrus in Sc patients without CUD, compared to controls	Lack of a group of nonpsychosis cannabis abusers
Potvin et al. (2007)	11 Sz patients; 12 Sz patients with SUD (cannabis and alcohol)	Social emotions (sad movie clips)	• Higher emotional ratings in substance abusing Sz patients; • Increased activations in the mPFC	Sz patients were also abusing from alcohol; lack of a control group of healthy volunteers; lack of a group of nonpsychosis cannabis users

This table summarizes the results of the functional brain imaging studies performed in schizophrenia patients using cannabis
Abbreviations: *Sz*, schizophrenia; *CUD*, cannabis use disorder; *dlPFC*, dorsolateral prefrontal cortex; *PCC*, posterior cingulate cortex; *SUD*, substance-use disorder; *mPFC*, medial prefrontal cortex.

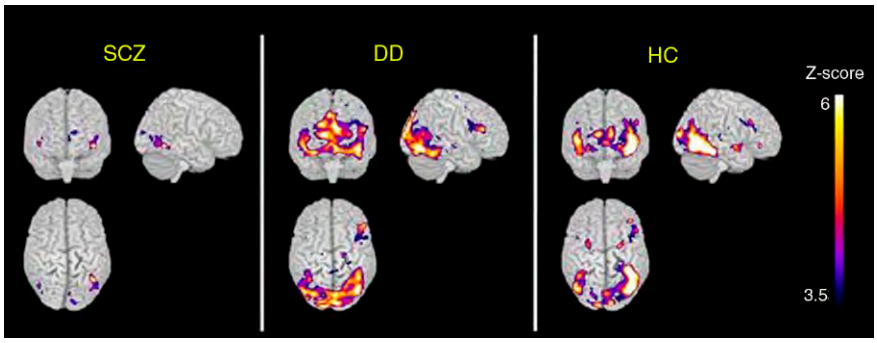


FIGURE 37.1 **Brain activation corresponding to recognition of negatively valenced images.** *SCZ*, schizophrenia group without cannabis abuse; *DD*, schizophrenia group with cannabis abuse; *HC*, healthy controls. *Source: Adapted from Bourque et al. (2013) with permission from the publishers.*

can be hypothesized that cannabis may improve cognition in schizophrenia due to its neuroprotective properties. Although intriguing, this hypothesis seems unlikely, for three main reasons. First of all, cannabinoids produce cognitive impairments, not cognitive enhancement effects, in individuals who do not suffer from psychiatric disorders. Moreover, functional imaging studies have shown that cannabinoids produce brain alterations similar to those seen in schizophrenia patients performing executive or memory tasks. Finally, D'Souza et al. (2004, 2005) administered Δ^9-THC to healthy participants and to schizophrenia patients, and found that Δ^9-THC impaired verbal memory, attention, verbal fluency, and working memory in healthy participants, and that

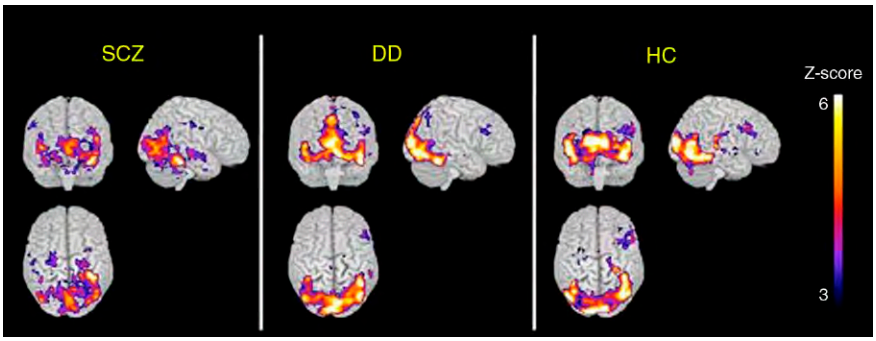


FIGURE 37.2 **Brain activation corresponding to recognition of positively valenced images.** *SCZ*, schizophrenia group without cannabis abuse; *DD*, schizophrenia group with cannabis abuse; *HC*, healthy controls. *Source: Adapted from Bourque et al. (2013) with permission from the publishers.*

it impaired verbal memory and attention in schizophrenia patients. More importantly, the trial showed that the amnesic effects of Δ^9-THC were more pronounced in schizophrenia patients than in controls. These results suggest that acute Δ^9-THC intoxication has more deleterious cognitive effects in schizophrenia than it does in nonpsychiatric individuals. Such results are inconsistent with the notion that cannabinoids may improve cognitive performance in schizophrenia.

The Social Hypothesis

An alternative hypothesis proposes that the better cognitive performance of Sz + Can patients has to do with the set of skills required to sustain the lifestyle of substance use. In order to get substances, the user needs to be able to manage money, to make contacts with dealers, and to strike deals—which means that minimally, intact relational, emotional, and cognitive abilities are required. According to this alterative hypothesis, first proposed by Mueser et al. (2000), the better cognitive performance of Sz + Can patients would not be the result from the pharmacological effects of cannabis, but would rather reflect a difference *primary* to cannabis use/abuse. One of the main implications of this hypothesis is that cognitive results should vary, based on the difficulty to access the substance. According to this hypothesis, Sz + Can patients should perform cognitively better than Sz patients in countries where cannabis is illegal or less common, but not in countries where cannabis smoking has been prevalent for many years. However, two main criticisms can be raised against this hypothesis. First, cannabis is now a substance easily accessible in many Western countries, regardless of its legal status (Schnell, Koethe, Daumann, & Gouzoulis-Mayfrank, 2009). Second, although cocaine is a psychoactive substance harder to access than cannabis, it is cannabis smoking that is associated with better cognition in schizophrenia, not cocaine use.

The Lower Vulnerability Hypothesis

A final hypothesis proposes that the relatively better cognition of Sz + Can patients reflects a lower vulnerability to psychosis (Schnell et al., 2009). According to this hypothesis, the paradoxical performance of Sz + Can patients has to be interpreted in light of the studies showing that cannabis smoking is a risk factor for psychosis outcomes (Moore et al., 2007). As explained by Schnell et al. (2009), one of the plausible implications of the literature showing that cannabis increases the risk for psychosis is that Sz + Can patients might have not developed schizophrenia had they not regularly smoked cannabis. From a developmental perspective, the vulnerability to psychosis, during the prodromal phase, may be lower in Sz + Can patients than it is in Sz patients, who go on to develop schizophrenia in the absence of the effects of cannabis. If so, we would expect Sz + Can patients to have milder deficits on core features of schizophrenia, such as cognitive impairments, especially during the early phase of the disorder.

As in the case of the social hypothesis, the lower vulnerability hypothesis assumes that the paradoxical cognitive performance of Sz + Can patients reflects a difference primary, not secondary, to cannabis use/abuse in schizophrenia. Obviously, only longitudinal studies performed in adolescents, before the onset of the disorder, could resolve this critical issue. Meanwhile, Ferraro et al. (2013) have shown that Sz + Can patients have higher premorbid IQ than Sz patients, a fact that raises the possibility that the better cognitive performance of Sz + Can patients is explained by this premorbid difference.

STUDY LIMITATIONS

Although the current state of the literature suggests that Sz + Can patients have better cognitive functioning than Sz patients, it is important to keep in mind that group differences are not of a large magnitude.

Additionally, the literature is plagued with limitations that will need to be addressed before concluding that it is a proven fact that cannabis smoking is associated with better cognition in schizophrenia. Thus far, most studies in the field have measured a limited number of cognitive functions that do not cover the whole range of cognitive dysfunctions associated with schizophrenia. Importantly, only a few studies have measured sociocognitive variables in Sz + Can patients, although social cognition deficits significantly impede upon recovery and rehabilitation in schizophrenia. Similarly, only a few studies have measured inferior executive functions in Sz + Can patients, although deficits in emotional decision-making, risk-taking, response inhibition, and delay discounting are core features of SUD (Potvin, Stavro, Rizkallah, & Pelletier, 2014; Stavro, Pelletier, & Potvin, 2013). A notable exception is the study from Mata et al. (2008) that measured emotional decision-making, and found that cannabis smoking in schizophrenia was associated with poorer performance.

Another limitation of the literature is that the grouping of Sz patients into cannabis smokers and nonsmokers has relied, most of the time, on self-report, and that smoking status has not been confirmed by urine drug analyses. Moreover, the quantities of cannabis smoked by patients have not been measured in most studies, and insufficient attention has been paid to the potential influence of other psychoactive substances. Deficits in attention, episodic memory, executive functions,, and speed of processing have been consistently observed in nonpsychiatric individuals with alcohol use and cocaine use disorders (Potvin et al., 2014; Stavro et al., 2013). In addition, alcohol use disorders have been shown to exacerbate the cognitive deficits of schizophrenia, while cocaine intake has been associated with mixed findings. As a result, the unreliable measurement of those substances, in either Sz + Can or Sz patients, may significantly confound results.

Socio-demographic variables may also have influenced study outcomes. For instance, in close to 50% of studies, the Sz + Can group had an elevated male-to-female ratio, compared to the Sz group (see Table 37.3). Theoretically, this between-group difference may explain some of the discrepancies in results, since there are subtle effects of sex-differences on cognition in schizophrenia (Mendrek & Stip, 2011). Similarly, in close to 50% of studies, Sz + Can patients were younger than Sz patients (see Table 37.3). Theoretically, it may be hypothesized that Sz + Can patients outperform Sz patients simply because they are younger. Although very plausible, both explanations do not necessarily rule out the presence of a real difference between Sz patients with and without cannabis smoking. Indeed, a better performance of Sz + Can patients, compared to Sz patients, has been found in studies in which the sex ratio did not differ between groups, as well as in studies in which Sz + Can patients were not younger than Sz patients (see Table 37.3).

Arguably, one of the main limitations of the literature on the cognitive effects of cannabis smoking in schizophrenia is that all studies have employed cross-sectional design thus far. While attempts at controlling for premorbid functioning have improved the methodological quality of studies, these attempts are not sufficient to paint a clear picture of the effect of cannabis on cognition in schizophrenia. Ideally, longitudinal studies initiated in adolescence until early adulthood in individuals with and without psychotic vulnerability, with and without cannabis smoking, would be required. However, given the low prevalence of schizophrenia, such studies would require very large sample sizes that could possibly compromise feasibility. In the meantime, it would be highly needed to perform longitudinal studies in first-episode schizophrenia patients, with and without cannabis smoking, and to measure their cognitive performance both when they are smoking cannabis, and after they stop. If the better cognitive performance of Sz + Can patients is not secondary to cannabis use, then, Sz + Can patients should have better cognition then Sz patients not only when they are actively smoking, but also after they cease.

CONCLUSIONS

Cognitive dysfunctions are core features of schizophrenia, significantly impeding upon social and occupational functioning in these patients. A significant risk factor for psychosis, cannabis smoking is highly prevalent in schizophrenia. In nonpsychosis individuals, cannabis smoking has been shown to impair cognition, and to alter the functioning of brain networks known to be impaired in schizophrenia. Paradoxically however, most studies performed in Sz + Can patients have shown that cannabis smoking is associated with better, not worse, cognition in schizophrenia. Due to the cross-sectional nature of studies, it remains to be determined if the better cognitive functioning of Sz + Can patients is primary or secondary to cannabis smoking. In the future, large-scale longitudinal studies will need to be performed in psychosis-prone and schizophrenia patients who smoke cannabis.

MINI-DICTIONARY

Comorbid A disorder that co-occurs with another one is said to be comorbid.
Endo-phenotype Traits that are purported as having a genetic component.
Mnesic Pertaining to memory.
Negative symptoms Deficient emotional and behavioral responses that are normally present in individuals from the general population. Example: amotivation.

Neurocognition Includes speed of processing, attention/vigilance, working memory, verbal and visual learning, as well as reasoning and problem solving.
Neuroprotection Refers to the preservation of functions and/or structures of the central nervous system.
Positive symptoms Ideas and perceptions that are not usually present in individuals from the general population, such as delusions and hallucinations (eg, hearing voices).
Prodromal Early symptoms preceding the onset of the disease.
Self-relevancy Something that has strong implications for an individual.
Socio-cognition Refers to how people process, store, and apply social information. Includes social knowledge, emotion recognition, Theory of Mind, attribution style, as well as emotion regulation.

References

Andreasson, S., Engström, A., Allebeck, P., & Rydberg, U. (1987). Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. *The Lancet*, *330*(8574), 1483–1486.

Bossong, M. G., Jansma, J. M., Bhattacharyya, S., & Ramsey, N. F. (2014). Role of the endocannabinoid system in brain functions relevant for schizophrenia: an overview of human challenge studies with cannabis or Δ9-tetrahydrocannabinol (THC). *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *52*, 53–69.

Bourque, J., Mendrek, A., Durand, M., Lakis, N., Lipp, O., Stip, E., Lalonde, P., Grignon, S., & Potvin, S. (2013). Cannabis abuse is associated with better emotional memory in schizophrenia: a functional magnetic resonance imaging study. *Psychiatry Research: Neuroimaging*, *214*(1), 24–32.

Crean, R. D., Crane, N. A., & Mason, B. J. (2011). An evidence based review of acute and long-term effects of cannabis use on executive cognitive functions. *Journal of Addiction Medicine*, *5*(1), 1–15.

D'Souza, D. C., Perry, E., MacDougall, L., Ammerman, Y., Cooper, T., Wu, Y. T., Braley, G., Gueorguieva, R., & Krystal, J. H. (2004). The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology*, *29*(8), 1558–1572.

D'Souza, D. C., Abi-Saab, W. M., Madonick, S., Forselius-Bielen, K., Doersch, A., Braley, G., Gueorguieva, R., Cooper, T. B., & Krystal, J. H. (2005). Dela-9-Tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biological Psychiatry*, *57*, 594–608.

Delvecchio, G., Sugranyes, G., & Frangou, S. (2013). Evidence of diagnostic specificity in the neural correlates of facial affect processing in bipolar disorder and schizophrenia: a meta-analysis of functional imaging studies. *Psychological Medicine*, *43*(03), 553–569.

Donoghue, K., & Doody, G. A. (2012). Effect of illegal substance use on cognitive function in individuals with a psychotic disorder, a review and meta-analysis. *Neuropsychology*, *26*(6), 785–801.

Ferraro, L., Russo, M., O'Connor, J., Wiffen, B. D., Falcone, M. A., Sideli, L., Gardner-Sood, P., Stilo, S., Trotta, A., Dazzan, P., Mondelli, V., Taylor, H., Friedman, B., Sallis, H., La Cascia, C., La Barbera, D., David, A. S., Reichenberg, A., Murray, R. M., & Di Forti, M. (2013). Cannabis users have higher premorbid IQ than other patients with first onset psychosis. *Schizophrenia Research*, *150*, 129–135.

Gage, S. H., Zammit, S., & Hickman, M. (2013). Stronger evidence is needed before accepting that cannabis plays an important role in the aetiology of schizophrenia in the population. *F1000 Medicine Reports*, *5*.

Goldberg, T. E., David, A., & Gold, J. M. (2011). Neurocognitive impairments in schizophrenia: their character and role in symptom formation. In D. R. Weinberger, & P. J. Harrison (Eds.), *Schizophrenia* (3rd ed., pp. 142–162). Oxford, UK: Wiley-Blackwell.

Koskinen, J., Löhönen, J., Koponen, H., Isohanni, M., & Miettunen, J. (2009). Rate of cannabis use disorders in clinical samples of patients with schizophrenia: a meta-analysis. *Schizophrenia Bulletin*, *36*(6), 1115–1130.

Løberg, E. M., Nygard, M., Berle, J. O., Johnsen, E., Kroken, R. A., Jorgensen, H. A., & Hugdahl, K. (2012). An fMRI study of neuronal activation in schizophrenia patients with and without previous cannabis use. *Frontiers in Psychiatry*, *3*(94), 1–11.

Mancini-Marië, A., Potvin, S., Fahim, C., Mensour, B., Beauregard, M., Roy, J. Y., & Stip, E. (2006). Neural correlates of the affect regulation model in schizophrenia patients with a substance use history: a functional magnetic resonance imaging study. *Journal of Clinical Psychiatry*, *67*(3), 342–350.

Mata, I., Rodríguez-Sánchez, J. M., Pelayo-Terán, J. M., Pérez-Iglesias, R., González-Blanch, C., Ramírez-Bonilla, M., Martínez-García, O., Vázquez-Barquero, J. L., & Crespo-Facorro, B. (2008). Cannabis abuse is associated with decision-making impairment among first-episode patients with schizophrenia-spectrum psychosis. *Psychological Medicine*, *38*, 1257–1266.

Meier, M. H., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R. S., McDonald, K., Ward, A., Poulton, R., & Moffitt, T. E. (2012). Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(40), E2657–E2664.

Mendrek, A., & Stip, E. (2011). Sexual dimorphism in schizophrenia: is there a need for gender-based protocols? *Expert Review of Neurotherapeutics*, *11*(7), 951–959.

Minzenberg, M. J., Laird, A. R., Thelen, S., Carter, C. S., & Glahn, D. C. (2009). Meta-analysis of 41 functional neuroimaging studies of executive function in schizophrenia. *Archives of General Psychiatry*, *66*(8), 811–822.

Moore, T. H. M., Zammit, S., Lingford-Hughes, A., Barnes, T. R. E., Jones, P. B., Burke, M., & Lewis, G. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet*, *370*(9584), 319–328.

Mueser, K. T., Yarnold, P. R., Rosenberg, S. D., Swett, C., Miles, K. M., & Hill, D. (2000). Substance use disorder in hospitalized severely mentally ill psychiatric patients: prevalence, correlates, and subgroups. *Schizophrenia Bulletin*, *26*(1), 179–192.

Myles, N., Newall, H., Nielssen, O., & Large, M. (2012). The association between cannabis use and earlier age at onset of schizophrenia and other psychoses: meta-analysis of possible confounding factors. *Current Pharmaceutical Design*, *18*(32), 5055–5069.

Nuechterlein, K. H., Green, M. F., Kern, R. S., Baade, L. E., Barch, D. M., Cohen, J. D., Essock, S., Fenton, W. S., Frese, F. J., Gold, J. M., Goldberg, T., Heaton, R. K., Keefe, R. S. E., Kraemer, H., Mesholam-Gately, R., Seidman, L. J., Stover, E., Weinberger, D. R., Young, A. S., Zalcman, S., & Marder, S. R. (2008). The MATRICS consensus cognitive battery, part 1: test selection, reliability, and validity. *American Journal of Psychiatry*, *165*(2), 203–213.

Pertwee, R. G. (2005). Pharmacological actions of cannabinoids. In R. G. Pertwee (Ed.), *Cannabinoids* (pp. 1–51). Berlin and Heidelberg, Germany: Springer, In the Handbook of Experimental Pharmacology series.

Potvin, S., Mancini-Marië, A., Fahim, C., Mensour, B., & Stip, E. (2007). A functional magnetic resonance imaging study of social emotion in schizophrenia patients with and without comorbid substance use disorder. *Social Neuroscience*, *2*(2), 1–11.

Potvin, S., Joyal, C. C., Pelletier, J., & Stip, E. (2008). Contradictory cognitive capacities in substance-abusing schizophrenia patients: a meta-analysis. *Schizophrenia Research*, *100*, 242–251.

Potvin, S., Bourque, J., Durand, M., Lipp, O., Lalonde, P., Stip, E., Grignon, S., & Mendrek, A. (2013). The neural correlates of mental rotation abilities in cannabis-abusing patients with schizophrenia: a functional magnetic resonance imaging study. *Schizophrenia Research and Treatment*, *1*(10), Article ID 543842.

Potvin, S., Stavro, K., Rizkallah, E., & Pelletier, J. (2014). Cocaine and cognition: a systematic quantitative review. *Journal of Addiction Medicine*, *8*(5), 368–376.

Rabin, R. A., Zakzanis, K. K., & George, T. P. (2011). The effects of cannabis use on neurocognition in schizophrenia: a meta-analysis. *Schizophrenia Research*, *128*(1–3), 111–116.

Ragland, J. D., Laird, A. R., Ranganath, C., Blumenfeld, R. S., Gonzales, S. M., & Glahn, D. C. (2009). Prefrontal activation deficits during episodic memory in schizophrenia. *American Journal of Psychiatry*, *166*(8), 863–874.

Sarne, Y., & Mechoulam, R. (2005). Cannabinoids: between neuroprotection and neurotoxicity. *Current Drug Targets-CNS and Neurological Disorders*, *4*(6), 677–684.

Schnell, T., Koethe, D., Daumann, J., & Gouzoulis-Mayfrank, E. (2009). The role of cannabis in cognitive functioning of patients with schizophrenia. *Psychopharmacology*, *205*, 45–52.

Stavro, K., Pelletier, J., & Potvin, S. (2013). Alcoholism has widespread and sustained effects on cognition: a meta-analysis. *Addiction Biology*, *18*(2), 203–213.

Sugranyes, G., Kyriakopoulos, M., Corrigall, R., Taylor, E., & Frangou, S. (2011). Autism spectrum disorders and schizophrenia: meta-analysis of the neural correlates of social cognition. *PLoS One*, *6*(10), e25322.

Tandon, R., Keshavan, M. S., & Nasrallah, H. A. (2008a). Schizophrenia, "just the facts." What we know in 2008. Overview. *Schizophrenia Research*, *100*(1–3), 4–19.

Van Snellenberg, J. X., Torres, I. J., & Thornton, A. E. (2006). Functional neuroimaging of working memory in schizophrenia: task performance as a moderating variable. *Neuropsychology*, *20*(5), 497–510.

Yücel, M., Bora, E., Lubman, D. I., Solowij, N., Brewer, W. J., Cotton, S. M., Conus, P., Takagi, M. J., Fornito, A., Wood, S. J., McGorry, P. D., & Pantelis, C. (2010). The impact of cannabis use on cognitive functioning in patients with schizophrenia: a meta-analysis of existing findings and new data in a first-episode sample. *Schizophrenia Bulletin*, *38*(2), 316–330.

CHAPTER

38

The Long-Lasting Effects of Cannabis Use on Movement and Brain Regions that Control Movement

G. Todd, J.M. White

School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA, Australia

SUMMARY POINTS

- This chapter focuses on the long-lasting effects of cannabis use on movement and movement-related brain regions.
- Very few studies have investigated movement in individuals with a history of cannabis use.
- History of cannabis use is associated with abnormal performance of rhythmical movement, but tremor is unaffected.
- Subtle changes in the integration of sensory and motor information also appear to be evident in abstinent cannabis users.
- Not all types of movement have been investigated in individuals with a history of cannabis use.
- Long-lasting abnormalities in movement-related brain regions in former cannabis users suggest that other movement abnormalities are likely to be present.
- Further research is needed to fully understand the long-lasting consequences of cannabis use on movement, and the clinical implications of movement dysfunction.

KEY FACTS OF MOVEMENT

- The control of movement involves much of the central and peripheral nervous system.
- Several steps are involved.
- The conscious "will" to move is followed by formation of a movement plan.
- The movement plan involves coordinated activation of muscles involved in the task, and those involved in stabilizing the limbs and maintaining posture and balance.
- The movement plan is stored and then implemented at the appropriate time.
- Ongoing adjustment of movement in response to internal and external factors (via afferent feedback) takes place during each phase of the movement.
- We are generally unaware of these steps, and they occur over many milliseconds.
- Damage or disease to an area of the nervous system involved in movement usually results in movement dysfunction.

LIST OF ABBREVIATIONS

Δ^9-THC	Δ-9-Tetrahydrocannabinol

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00044-2
Copyright © 2017 Elsevier Inc. All rights reserved.

INTRODUCTION

In recent years, there has been considerable advancement in the understanding of the effect of cannabis use on cognition, mental health, and a range of behaviors. However, very little is known about a behavior that plays a key role in activities of daily living: movement. Many of the movements that we perform each day happen without the need to engage a large amount of conscious thought. For example, once we decide to make ourselves a cup of coffee, we perform the required sequence of movements without consciously assessing our grip on the spoon or the trajectory of the cup to our mouth. This would seem to imply that movement may be a simple brain function, but the planning, initiation, and ongoing adjustment of movement are complex, and involve much of the central and peripheral nervous system. Changes in any one of the regions involved in movement can lead to movement dysfunction.

In this chapter, the long-lasting effects of cannabis use on movement and movement-related brain regions will be discussed. The discussion on movement will focus on knowledge gained from studies involving (1) objective assessment of movement in humans, and (2) participants with a history of cannabis use, but no history of illicit stimulant use. Use of illicit stimulant drugs, such as methamphetamine, ecstasy, and cocaine, is associated with acute and long-lasting changes in monoamine neurotransmission in movement-related brain regions (Benowitz, 1993; Green, Mechan, Elliott, O'Shea, & Colado, 2003; Yamamoto, Moszczynska, & Gudelsky, 2010). The existing literature on the long-lasting effects of other drugs is less clear. The literature on animal movement will not be discussed in detail because it is not readily transferable to humans, due to large differences in movement between species, particularly in gait (bipedal versus quadrupedal) and hand dexterity.

Before considering the long-lasting effects of cannabis use on movement, it is important to have an understanding of the acute effects of cannabis on movement in healthy adults.

ACUTE EFFECTS OF CANNABIS ON MOVEMENT

Not all types of movement are affected by acute cannabis use. However, use of the hand appears to be particularly vulnerable to cannabis intoxication. The ability to track a moving target on a computer screen with movements of the hand (ie, visuomotor tracking) is impaired for up to 7 h after cannabis use (eg, Barnett, Licko, & Thompson, 1985; Kiplinger, Manno, Rodda, & Forney, 1971; Manno, Kiplinger, Scholz, & Forney, 1971; Weil, Zinberg, & Nelsen, 1968; cf Zuurman et al., 2008).

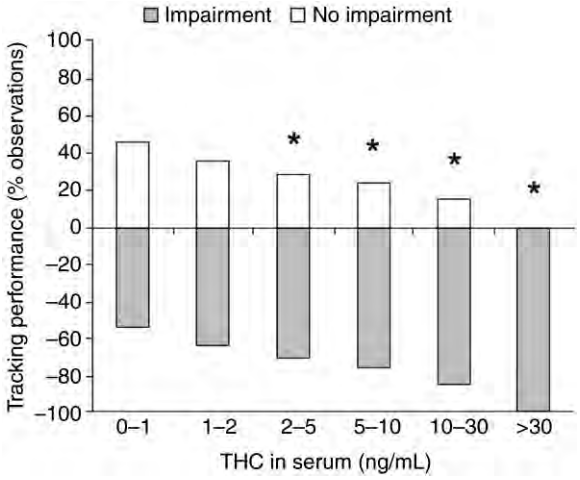


FIGURE 38.1 Acute changes in performance of a visuomotor tracking task after cannabis administration. Performance at varying levels of plasma Δ^9-THC are shown. Data are distribution of observations showing performance "impairment" (negative change score from placebo) and "no impairment" (change score equal to or greater than zero).*, $P < 0.05$. *Reprinted from Ramaekers et al. (2006) with permission from Elsevier.*

Fig. 38.1 shows that significant impairment in visuomotor tracking is evident at low levels (eg, 2–5 ng/mL) of plasma Δ-9-Tetrahydrocannabinol (Δ^9-THC), and the magnitude of impairment increases with increasing level of plasma Δ^9-THC (Ramaekers et al., 2006). Acute decreases in the frequency of maximal finger tapping (plasma Δ^9-THC level: 1–2 ng/mL) (Roser et al., 2009) and accuracy of hand movement (Milstein, MacCannell, Karr, & Clark, 1975), and a dose-related increase in reaction time and motor response time have also been observed after cannabis administration (Borg, Gershon, & Alpert, 1975).

Cannabis use is also associated with acute disturbances in balance (Evans et al., 1973; Kiplinger et al., 1971). Balance is commonly assessed by measurement of body sway during quiet standing with the eyes closed. Zuurman et al. (2008) measured balance up to 90 min after incremental doses of cannabis that resulted in plasma Δ^9-THC levels of approximately 10, 30, 45, and 65 ng/mL, respectively. Fig. 38.2 shows that body sway increases when plasma Δ^9-THC levels exceed 30 ng/mL (Zuurman et al., 2008), suggesting that cannabis use disturbs balance in a concentration-dependent manner.

Acute changes in the control of eye movement (Fant, Heishman, Bunker, & Pickworth, 1998; Ploner et al., 2002; Zuurman et al., 2008), speech production (prolonged pause and syllable length) (Zeidenberg et al., 1973), and level of physical activity (Babor, Mendelson, & Kuehnle, 1976) have also been reported after cannabis use (plasma Δ^9-THC: >3 ng/mL and cannabis cigarette, respectively). It is important to note that the acute effects of cannabis on movement are influenced by the dose and population investigated (naïve or experienced cannabis

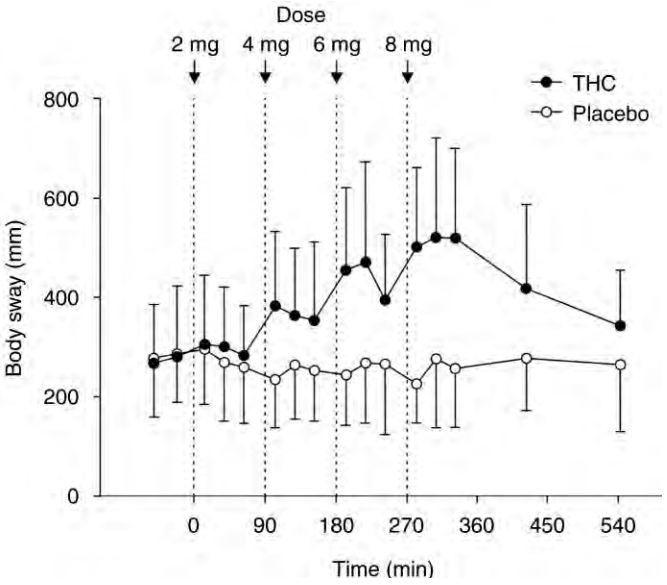


FIGURE 38.2 The acute effect of inhaled THC extract on balance (body sway). Group data are mean + SD. Four incremental doses (2, 4, 6, 8 mg THC) were administered at 0, 90, 180, and 270 min, resulting in plasma Δ^9-THC levels of approximately 10, 30, 45, and 65 ng/mL, respectively. *Adapted from Zuurman et al. (2008) with permission from Elsevier.*

users) (for review see Moskowitz, 1985). Objective investigation of gait immediately after cannabis use has not been conducted, and grip strength (Steadward & Singh, 1975), skin sensation (Milstein, Maccannell, Karr, & Clark, 1974), maximum number of finger or foot taps performed in 10 s (Milstein et al., 1975), and subjective assessment of tremor appear to be unchanged up to 24 h after cannabis use (Kurzthaler et al., 1999).

The underlying mechanism responsible for the change in movement is difficult to differentiate from the acute effect of cannabis on attention (eg, Hunault et al., 2009). Attention is known to affect performance on visuomotor tracking tasks, independent of cannabis use (Kam et al., 2012). However, several pieces of evidence suggest that cannabis has a direct effect on movement. First, the density of CB_1 receptors is high in brain regions that are involved in movement, particularly in the basal ganglia, cerebral cortex, and cerebellum (Howlett et al., 2002), and CB_1 receptors are present in the dorsal and ventral horn of the spinal cord in primates (Ong & Mackie, 1999). Second, activation of presynaptic CB_1 receptors in the central and peripheral nervous system can inhibit neurotransmitter release. For example, reduced GABAergic transmission is evident in several rat brain structures after administration of Δ^9-THC (Maneuf, Nash, Crossman, & Brotchie, 1996), including brain regions involved in movement (Chan, Chan, & Yung, 1998). Reduced $GABA_A$-mediated intracortical inhibition is evident in human motor cortex when plasma Δ^9-THC levels exceed 2 ng/mL in heavy cannabis users (Fitzgerald, Williams, & Daskalakis, 2009). Third, cannabis use is associated with acute changes in baseline regional cerebral blood flow in the frontal, insular, and anterior cingulate cortex (Mathew et al., 2002), and in the motor cortex and cerebellum during an auditory reaction time task (O'Leary et al., 2002).

CHANGES IN MOVEMENT FOLLOWING LONG-TERM CANNABIS USE

Repeated cannabis use over a long period of time is also associated with long-lasting changes in the neurophysiology of movement-related brain regions. Studies involving transcranial magnetic brain stimulation suggest that history of cannabis use is associated with changes in the excitability of circuitry in the motor cortex and corticomotoneuronal pathway in conscious humans. Fig. 38.3 shows that $GABA_A$-mediated short-interval intracortical inhibition in motor cortex is reduced for up to 7 days after cannabis use, in individuals with a history of moderate to heavy use (1–4 times per week) (Fitzgerald et al., 2009), but not in individuals with a longer duration of abstinence (average 0.7 ± 1.6 years) (Flavel, White, & Todd, 2012b). Conversely, there does not appear to be

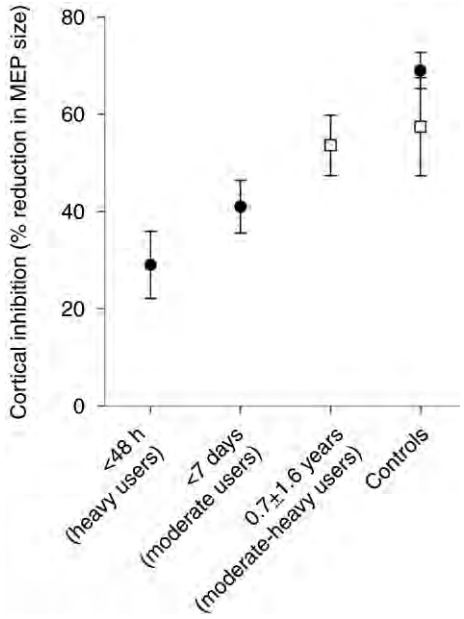


FIGURE 38.3 Short-interval intracortical inhibition in the motor cortex of cannabis users and nondrug using control subjects. Short-interval intracortical inhibition was measured in the hand region of the motor cortex with paired-pulse transcranial magnetic brain stimulation (2 ms interstimulus interval). The intensity of the conditioning pulse was set at approximately 70% of resting motor threshold, and the intensity of the test pulse was set to evoke a motor-evoked potential of approximately 1 mV in amplitude. Group data are mean ± SEM and are derived from Fitzgerald et al. (2009; *circles*) and Flavel et al. (2012b; *squares*). The *x*-axis labels indicate lifetime use and duration of abstinence from cannabis use.

an acute or long-lasting change in resting corticomotoneuronal excitability and $GABA_B$-mediated intracortical inhibition within motor cortex (ie, cortical silent period). However, there is a trend for increased corticomotoneuronal excitability during movement in individuals who have been abstinent from cannabis for 0.7 ± 1.6 years (Flavel et al., 2012b). Prolongation of the latency of the response evoked by transcranial magnetic stimulation in abstinent cannabis users also suggests altered conduction of descending cortical volleys (Flavel et al., 2012b).

Neuroimaging suggests that cannabis use is associated with long-lasting changes in brain activation, metabolism, and regional cerebral blood flow. Studies involving positron emission tomography and magnetic resonance spectroscopy suggest that chronic cannabis users exhibit decreased glutamate in the basal ganglia (Chang, Cloak, Yakupov, & Ernst, 2006), and changes in regional blood flow, metabolism, and activation in movement-related brain regions, particularly the cerebellum (for review see Batalla et al., 2013).

It is unclear if the long-lasting changes in neurophysiology of movement-related brain regions are accompanied by changes in brain morphology. Morphometric magnetic resonance imaging studies show no change in gray and white matter volume (Block et al., 2000; Tzilos et al., 2005), but studies involving voxel-based morphometry suggest that gray matter density is decreased in the right parahippocampal gyrus, and increased in the precentral gyrus and thalamus of abstinent (20 days) heavy cannabis users (Matochik, Eldreth, Cadet, & Bolla, 2005). The morphology of the substantia nigra, viewed with transcranial ultrasound, is unaltered in abstinent cannabis users (Todd et al., 2013). However, decreased white matter density has been observed in chronic cannabis users (Matochik et al., 2005). The morphological findings have not been uniform across studies, and factors such as current age, age of first cannabis use, history of cannabis use, and duration of abstinence from cannabis may have contributed to variability in the findings.

There is evidence that long-lasting changes in the neurophysiology of movement-related brain regions in abstinent cannabis users have functional consequences for certain types of movement. Movement speed and rhythmicity is abnormal in abstinent cannabis users during maximal finger tapping, a task that is used widely in clinical and experimental settings to assess motor changes associated with a range of neurological disorders. Individuals that had abstained from heavy cannabis use for 28 days exhibited reduced performance on a 10 s finger tapping task. Heavy cannabis users (78–117 joints per week; $n = 7$) performed 45–47 taps in 10 s, less than individuals with a history of light (2–14 joints per week; 49–51 taps, $n = 7$) to moderate (18–70 joints per week; 49–50 taps, $n = 8$) cannabis use. However, the effect only reached statistical significance on the left side, possibly due to the small sample size ($n = 7$–8 per group) (Bolla, Brown, Eldreth, Tate, & Cadet, 2002). The finding needs to be interpreted with care because the study lacked a nondrug using control group, and history of other illicit drug use was not reported.

Our group explored long-lasting changes in movement speed and rhythmicity, further, by investigating finger tapping in abstinent cannabis users with no history of illicit stimulant or opioid use, and nondrug using controls. Fig. 38.4 shows that abstinent cannabis users exhibit a slow onset frequency of tapping, and increased variability of tapping over a 5 s period, but the overall number of taps performed did not differ between groups (Flavel, White, & Todd, 2013). The abnormality appeared to be long-lasting (it was observed in individuals that had been abstinent from cannabis for 0.6 ± 1.6 years), and was observed in individuals with normal neuropsychological performance (Flavel et al., 2013). Slow initial speed of finger movement is likely to contribute to the prolonged reaction time observed on simple tasks in abstinent heavy cannabis users (Bolla et al., 2002).

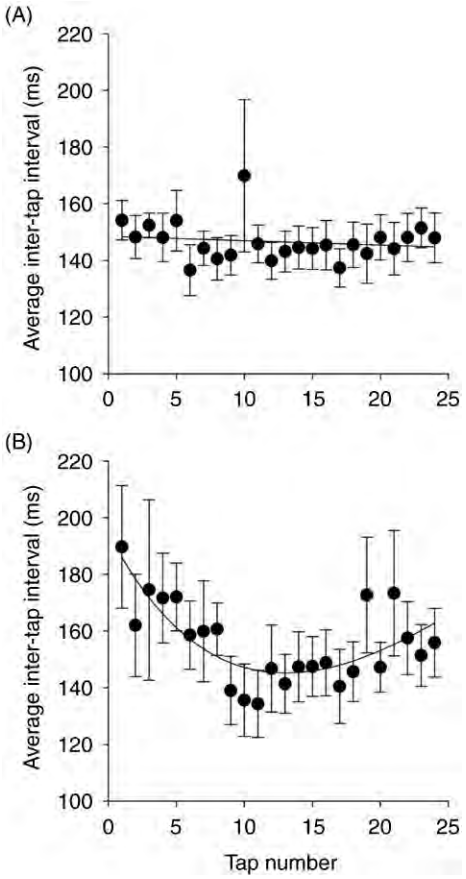


FIGURE 38.4 **Relationship between performance (intertap interval) and time (tap number) during a maximal finger tapping task (duration 5 s).** (A) Nondrug control group with linear regression. (B) Abstinent cannabis users with exponential regression. Group data are mean + SEM. *Reprinted from Flavel et al. (2013) with permission from John Wiley and Sons.*

Cannabis use may also be associated with long-lasting changes in sensorimotor integration, and hand dexterity. A study on a small group of abstinent (28 days) cannabis users found a correlation between lifetime cannabis use and performance on the grooved pegboard test. The grooved pegboard test involves placing 25 key-shaped pegs into corresponding holes, as fast as possible. Performance declined with increasing lifetime cannabis use (joints per week) but the effect only reached statistical significance on the nondominant hand (Bolla et al., 2002). The effect is unlikely to be related to peripheral changes in skin sensation, because acute cannabis use does not affect tactile acuity (two-point discrimination) or sensitivity to touch (Milstein et al., 1974). Another study involving subjects with a longer period of abstinence from cannabis use (52 ± 17 months) reported unaltered performance on the grooved pegboard test (Chang et al., 2006).

Cannabis use does not have a long-lasting effect on all types of movement. The amplitude and frequency of tremor during relaxation and movement of the hand appears to be normal in abstinent individuals with a history of cannabis use (Flavel, Koch, White, & Todd, 2012a). The level of daily physical activity also does not differ between adults with a history of moderate to heavy cannabis use and nondrug using controls (Babor et al., 1976). Further research is needed to fully understand the long-lasting consequences of cannabis use on movement, and the clinical implications of movement dysfunction.

CONCLUSIONS

Investigation of the long-lasting effect of cannabis use on movement is challenging. The studies undertaken so far have been cross sectional in design and, thus, interpretation of the data has been limited to an association between history of cannabis use and movement dysfunction. Studies with a longitudinal design are needed to eliminate the possibility that movement dysfunction predated onset of cannabis use. It is also important to study movement in cannabis users that have *no* history of illicit stimulant use. Illicit stimulant drugs, such as methamphetamine, ecstasy, and cocaine, are associated with acute and long-lasting changes in monoamine neurotransmission (for review, see Boghdadi & Henning, 1997; Green et al., 2003; Yamamoto et al., 2010). Methamphetamine in particular is associated with damage to dopaminergic neurons, and the importance of dopaminergic neurons in movement is evidenced by the symptoms associated with Parkinson's disease, a disease that is characterized by loss of dopaminergic neurons in the substantia nigra (Fearnley & Lees, 1991). Even minimal use of illicit stimulant drugs has the potential to cause long-lasting changes in movement-related brain regions, if the dose is sufficiently high. Thus, the results of studies performed on subjects that were "not dependent" on other illicit drugs, or lack information on use of other illicit drugs, need to be interpreted with care. Other factors that need to be considered include possible tolerance to the impairing effects of cannabis in daily cannabis users (for review see Gonzalez, Cebeira, & Fernandez-Ruiz, 2005), age of first cannabis use, and higher rates of symptoms of depression (eg, Lev-Ran et al., 2014; Roebke, Vadhan, Brooks, & Levin, 2014; Todd et al., 2013), and lifetime use of alcohol and tobacco (eg, Fletcher et al., 1996; Todd et al., 2013) in cannabis users, compared to controls.

The long-lasting effects of cannabis use on movement and movement-related brain regions also need to be considered in the proposed therapeutic use of cannabis in patients with certain neurological disorders. Although the effects of cannabis use on movement tend to be negative in otherwise healthy subjects, studies suggest that cannabis use may reduce the severity of movement symptoms in some patient populations. In patients with multiple sclerosis, cannabis extract appears to be beneficial for reducing the severity of muscle spasticity and stiffness (eg, Zajicek, Hobart, Slade, Barnes, & Mattison, 2012), but not tremor, although disturbed balance is a noted movement related side-effect (for review see Koppel et al., 2014; Syed, McKeage, & Scott, 2014). A small number of studies have investigated the therapeutic efficacy of cannabis extract in patients with Huntington's disease (eg, Consroe et al., 1991; Curtis, Mitchell, Patel, Ives, & Rickards, 2009), Tourette syndrome (Muller-Vahl et al., 2002), dystonia (Consroe, Sandyk, & Snider, 1986), and levodopa-induced dyskinesia in Parkinson's disease (Sieradzan et al., 2001), but the sample sizes were too small to draw meaningful conclusions.

MINI-DICTIONARY

Body sway A parameter used to investigate balance. Movement of the body in the posterior–anterior and medial–lateral direction is measured, usually during quiet standing.
Grooved pegboard A test used to assess sensorimotor integration and hand dexterity. It involves recording the time taken to place 25 key-shaped pegs into corresponding holes.
Maximal tapping A test used to assess movement speed and rhythmicity. It involves tapping, usually the index finger, on a keyboard, or strain gauge as fast as possible for 5–10 s.
Transcranial magnetic brain stimulation A noninvasive and painless technique used to investigate excitability of the motor cortex and corticomotoneuronal pathway, in conscious humans.
Tremor An involuntary, rapid, and rhythmic oscillation of a body part.
Visuomotor tracking The ability to track a moving target with movement of a limb (most commonly the arm).

References

Babor, T. F., Mendelson, J. H., & Kuehnle, J. (1976). Marihuana and human physical activity. *Psychopharmacology*, *50*, 11–19.

Barnett, G., Licko, V., & Thompson, T. (1985). Behavioral pharmacokinetics of marijuana. *Psychopharmacology*, *85*, 51–56.

Batalla, A., Bhattacharyya, S., Yucel, M., Fusar-Poli, P., Crippa, J. A., Nogue, S., Torrens, M., Pujol, J., Farre, M., & Martin-Santos, R. (2013). Structural and functional imaging studies in chronic cannabis users: a systematic review of adolescent and adult findings. *PLoS One*, *8*, e55821.

Benowitz, N. L. (1993). Clinical pharmacology and toxicology of cocaine. *Pharmacology and Toxicology*, *72*, 3–12.

Block, R. I., O'Leary, D. S., Ehrhardt, J. C., Augustinack, J. C., Ghoneim, M. M., Arndt, S., & Hall, J. A. (2000). Effects of frequent marijuana use on brain tissue volume and composition. *Neuroreport*, *11*, 491–496.

Boghdadi, M. S., & Henning, R. J. (1997). Cocaine: pathophysiology and clinical toxicology. *Heart and Lung*, *26*, 466–483.

Bolla, K. I., Brown, K., Eldreth, D., Tate, K., & Cadet, J. L. (2002). Dose-related neurocognitive effects of marijuana use. *Neurology*, *59*, 1337–1343.

Borg, J., Gershon, S., & Alpert, M. (1975). Dose effects of smoked marihuana on human cognitive and motor functions. *Psychopharmacologia*, *42*, 211–218.

Chan, P. K., Chan, S. C., & Yung, W. H. (1998). Presynaptic inhibition of GABAergic inputs to rat substantia nigra pars reticulata neurones by a cannabinoid agonist. *Neuroreport*, *9*, 671–675.

Chang, L., Cloak, C., Yakupov, R., & Ernst, T. (2006). Combined and independent effects of chronic marijuana use and HIV on brain metabolites. *Journal of Neuroimmune Pharmacology*, *1*, 65–76.

Consroe, P., Sandyk, R., & Snider, S. R. (1986). Open label evaluation of cannabidiol in dystonic movement disorders. *International Journal of Neuroscience*, *30*, 277–282.

Consroe, P., Laguna, J., Allender, J., Snider, S., Stern, L., Sandyk, R., Kennedy, K., & Schram, K. (1991). Controlled clinical trial of cannabidiol in Huntington's disease. *Pharmacology, Biochemistry and Behavior*, *40*, 701–708.

Curtis, A., Mitchell, I., Patel, S., Ives, N., & Rickards, H. (2009). A pilot study using nabilone for symptomatic treatment in Huntington's disease. *Movement Disorders*, *24*, 2254–2259.

Evans, M. A., Martz, R., Brown, D. J., Rodda, B. E., Kiplinger, G. F., Lemberger, L., & Forney, R. B. (1973). Impairment of performance with low doses of marihuana. *Clinical Pharmacology and Therapeutics*, *14*, 936–940.

Fant, R. V., Heishman, S. J., Bunker, E. B., & Pickworth, W. B. (1998). Acute and residual effects of marijuana in humans. *Pharmacology, Biochemistry and Behavior*, *60*, 777–784.

Fearnley, J. M., & Lees, A. J. (1991). Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain*, *114*, 2283–2301.

Fitzgerald, P. B., Williams, S., & Daskalakis, Z. J. (2009). A transcranial magnetic stimulation study of the effects of cannabis use on motor cortical inhibition and excitability. *Neuropsychopharmacology*, *34*, 2368–2375.

Flavel, S. C., Koch, J. D., White, J. M., & Todd, G. (2012a). Illicit stimulant use in humans is associated with a long-term increase in tremor. *PLoS One*, *7*, e52025.

Flavel, S. C., White, J. M., & Todd, G. (2012b). Motor cortex and corticospinal excitability in humans with a history of illicit stimulant use. *Journal of Applied Physiology*, *113*, 1486–1494.

Flavel, S. C., White, J. M., & Todd, G. (2013). Abnormal maximal finger tapping in abstinent cannabis users. *Human Psychopharmacology*, *28*, 612–614.

Fletcher, J. M., Page, J. B., Francis, D. J., Copeland, K., Naus, M. J., Davis, C. M., Morris, R., Krauskopf, D., & Satz, P. (1996). Cognitive correlates of long-term cannabis use in Costa Rican men. *Archives of General Psychiatry*, *53*, 1051–1057.

Gonzalez, S., Cebeira, M., & Fernandez-Ruiz, J. (2005). Cannabinoid tolerance and dependence: a review of studies in laboratory animals. *Pharmacology, Biochemistry and Behavior*, *81*, 300–318.

Green, A. R., Mechan, A. O., Elliott, J. M., O'Shea, E., & Colado, M. I. (2003). The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). *Pharmacological Reviews*, *55*, 463–508.

Howlett, A. C., Barth, F., Bonner, T. I., Cabral, G., Casellas, P., Devane, W. A., Felder, C. C., Herkenham, M., Mackie, K., Martin, B. R., Mechoulam, R., & Pertwee, R. G. (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacological Reviews*, *54*, 161–202.

Hunault, C. C., Mensinga, T. T., Bocker, K. B., Schipper, C. M., Kruidenier, M., Leenders, M. E., de Vries, I., & Meulenbelt, J. (2009). Cognitive and psychomotor effects in males after smoking a combination of tobacco and cannabis containing up to 69 mg delta-9-tetrahydrocannabinol (THC). *Psychopharmacology*, *204*, 85–94.

Kam, J. W., Dao, E., Blinn, P., Krigolson, O. E., Boyd, L. A., & Handy, T. C. (2012). Mind wandering and motor control: off-task thinking disrupts the online adjustment of behavior. *Frontiers in Human Neuroscience*, *6*, 329.

Kiplinger, G. F., Manno, J. E., Rodda, B. E., & Forney, R. B. (1971). Dose-response analysis of the effects of tetrahydrocannabinol in man. *Clinical Pharmacology and Therapeutics*, *12*, 650–657.

Koppel, B. S., Brust, J. C., Fife, T., Bronstein, J., Youssof, S., Gronseth, G., & Gloss, D. (2014). Systematic review: efficacy and safety of medical marijuana in selected neurologic disorders: report of the Guideline Development Subcommittee of the American Academy of Neurology. *Neurology*, *82*, 1556–1563.

Kurzthaler, I., Hummer, M., Miller, C., Sperner-Unterweger, B., Gunther, V., Wechdorn, H., Battista, H. J., & Fleischhacker, W. W. (1999). Effect of cannabis use on cognitive functions and driving ability. *Journal of Clinical Psychiatry*, *60*, 395–399.

Lev-Ran, S., Roerecke, M., Le Foll, B., George, T. P., McKenzie, K., & Rehm, J. (2014). The association between cannabis use and depression: a systematic review and meta-analysis of longitudinal studies. *Psychological Medicine*, *44*, 797–810.

Maneuf, Y. P., Nash, J. E., Crossman, A. R., & Brotchie, J. M. (1996). Activation of the cannabinoid receptor by delta 9-tetrahydrocannabinol reduces gamma-aminobutyric acid uptake in the globus pallidus. *European Journal of Pharmacology*, *308*, 161–164.

Manno, J. E., Kiplinger, G. F., Scholz, N., & Forney, R. B. (1971). The influence of alcohol and marihuana on motor and mental performance. *Clinical Pharmacology and Therapeutics*, *12*, 202–211.

Mathew, R. J., Wilson, W. H., Turkington, T. G., Hawk, T. C., Coleman, R. E., DeGrado, T. R., & Provenzale, J. (2002). Time course of tetrahydrocannabinol-induced changes in regional cerebral blood flow measured with positron emission tomography. *Psychiatry Research*, *116*, 173–185.

Matochik, J. A., Eldreth, D. A., Cadet, J. L., & Bolla, K. I. (2005). Altered brain tissue composition in heavy marijuana users. *Drug and Alcohol Dependence*, *77*, 23–30.

Milstein, S. L., Maccannell, K., Karr, G. W., & Clark, S. (1974). Marijuana produced changes in cutaneous sensitivity and affect: users and non-users. *Pharmacology, Biochemistry and Behavior*, *2*, 367–374.

Milstein, S. L., MacCannell, K., Karr, G., & Clark, S. (1975). Marijuana-produced impairments in coordination. Experienced and nonexperienced subjects. *Journal of Nervous and Mental Disease*, *161*, 26–31.

Moskowitz, H. (1985). Marihuana and driving. *Accident, Analysis and Prevention*, *17*, 323–345.

Muller-Vahl, K. R., Schneider, U., Koblenz, A., Jobges, M., Kolbe, H., Daldrup, T., & Emrich, H. M. (2002). Treatment of Tourette's syndrome with Delta 9-tetrahydrocannabinol (THC): a randomized crossover trial. *Pharmacopsychiatry*, *35*, 57–61.

O'Leary, D. S., Block, R. I., Koeppel, J. A., Flaum, M., Schultz, S. K., Andreasen, N. C., Ponto, L. B., Watkins, G. L., Hurtig, R. R., & Hichwa,

R. D. (2002). Effects of smoking marijuana on brain perfusion and cognition. *Neuropsychopharmacology*, *26*, 802–816.

Ong, W. Y., & Mackie, K. (1999). A light and electron microscopic study of the CB1 cannabinoid receptor in the primate spinal cord. *Journal of Neurocytology*, *28*, 39–45.

Ploner, C. J., Tschirch, A., Ostendorf, F., Dick, S., Gaymard, B. M., Rivaud-Pechoux, S., Sporkert, F., Pragst, F., & Stadelmann, A. M. (2002). Oculomotor effects of delta-9-tetrahydrocannabinol in humans: implications for the functional neuroanatomy of the brain cannabinoid system. *Cerebral Cortex*, *12*, 1016–1023.

Ramaekers, J. G., Moeller, M. R., van Ruitenbeek, P., Theunissen, E. L., Schneider, E., & Kauert, G. (2006). Cognition and motor control as a function of Delta9-THC concentration in serum and oral fluid: limits of impairment. *Drug and Alcohol Dependence*, *85*, 114–122.

Roebke, P. V., Vadhan, N. P., Brooks, D. J., & Levin, F. R. (2014). Verbal learning in marijuana users seeking treatment: a comparison between depressed and non-depressed samples. *American Journal of Drug and Alcohol Abuse*, *40*(4), 274–279.

Roser, P., Gallinat, J., Weinberg, G., Juckel, G., Gorynia, I., & Stadelmann, A. M. (2009). Psychomotor performance in relation to acute oral administration of Delta9-tetrahydrocannabinol and standardized cannabis extract in healthy human subjects. *European Archives of Psychiatry and Clinical Neuroscience*, *259*, 284–292.

Sieradzan, K. A., Fox, S. H., Hill, M., Dick, J. P., Crossman, A. R., & Brotchie, J. M. (2001). Cannabinoids reduce levodopa-induced dyskinesia in Parkinson's disease: a pilot study. *Neurology*, *57*, 2108–2111.

Steadward, R. D., & Singh, M. (1975). The effects of smoking marihuana on physical performance. *Medicine and Science in Sports*, *7*, 309–311.

Syed, Y. Y., McKeage, K., & Scott, L. J. (2014). Delta-9-tetrahydrocannabinol/cannabidiol (sativex((R))): a review of its use in patients with moderate to severe spasticity due to multiple sclerosis. *Drugs*, *74*, 563–578.

Todd, G., Noyes, C., Flavel, S. C., Della Vedova, C. B., Spyropoulos, P., Chatterton, B., Berg, D., & White, J. M. (2013). Illicit stimulant use is associated with abnormal substantia nigra morphology in humans. *PLoS One*, *8*, e56438.

Tzilos, G. K., Cintron, C. B., Wood, J. B., Simpson, N. S., Young, A. D., Pope, H. G., Jr., & Yurgelun-Todd, D. A. (2005). Lack of hippocampal volume change in long-term heavy cannabis users. *American Journal on Addictions*, *14*, 64–72.

Weil, A. T., Zinberg, N. E., & Nelsen, J. M. (1968). Clinical and psychological effects of marihuana in man. *Science*, *162*, 1234–1242.

Yamamoto, B. K., Moszczynska, A., & Gudelsky, G. A. (2010). Amphetamine toxicities: classical and emerging mechanisms. *Annals of the New York Academy of Sciences*, *1187*, 101–121.

Zajicek, J. P., Hobart, J. C., Slade, A., Barnes, D., & Mattison, P. G. (2012). Multiple sclerosis and extract of cannabis: results of the MUSEC trial. *Journal of Neurology, Neurosurgery and Psychiatry*, *83*, 1125–1132.

Zeidenberg, P., Clark, W. C., Jaffe, J., Anderson, S. W., Chin, S., & Malitz, S. (1973). Effect of oral administration of delta9 tetrahydrocannabinol on memory, speech, and perception of thermal stimulation: results with four normal human volunteer subjects. Preliminary report. *Comprehensive Psychiatry*, *14*, 549–556.

Zuurman, L., Roy, C., Schoemaker, R. C., Hazekamp, A., den Hartigh, J., Bender, J. C., Verpoorte, R., Pinquier, J. L., Cohen, A. F., & van Gerven, J. M. (2008). Effect of intrapulmonary tetrahydrocannabinol administration in humans. *Journal of Psychopharmacology*, *22*, 707–716.

CHAPTER

39

Assessment of Cannabis Acute Effects on Driving Skills: Laboratory, Simulator, and On-Road Studies

P. Bondallaz[*], *H. Chtioui*[**], *B. Favrat*[*,†], *E. Fornari*[‡], *C. Giroud*[§], *P. Maeder*[¶]

[*]Traffic Medicine and Psychology Unit, University Center of Legal Medicine, University Hospital of Geneva, Geneva, Switzerland

[**]Department of Clinical Pharmacology and Toxicology, University Hospital of Lausanne, Lausanne, Switzerland

[†]Department of Ambulatory Care and Community Medicine, University Hospital of Lausanne, Lausanne, Switzerland

[‡]CIBM, University Hospital of Lausanne, Lausanne, Switzerland

[§]Forensic Toxicology and Chemistry Unit, University Center of Legal Medicine, University Hospital of Lausanne, Lausanne, Switzerland

[¶]Department of Radiology, University Hospital of Lausanne, Lausanne, Switzerland

SUMMARY POINTS

- Traffic legislation-related knowledge shows growing concerns about acute effects of cannabis on psychomotor performance involved in safe driving.
- Laboratory studies assessing the acute effects of cannabis on cognitive functions involved in normal driving tasks found that working memory, motor inhibition, and divided-attention or tracking tasks were mostly impaired.
- In simulator and on-road experiments, cannabis-induced effects mostly altered mean headway and road tracking by increasing lane position variability.
- The correlation between blood levels and psychoactive effects of THC is challenged by a nonlinear time relationship, making the detection of DUIC difficult.

KEY FACTS IN TRAFFIC MEDICINE

- Cannabinoids were present in 5.3–35.7% of the blood samples collected from European impaired drivers suspected of drug use, and were the most frequently encountered illicit drugs on the roads.
- Many laboratory, simulator, and on-road experimental studies have shown an alteration in several cognitive functions.
- Brain imagery has shown changes in activations of brain areas and networks potentially involved in drivers' impairment after cannabis smoking.
- According to the duration of the majority of psychomotor impairments, it is recommended that patients using marijuana as a self-treatment abstain from driving for 8 h after a subjective "high."
- A driver's whole blood THCCOOH concentration higher than 40 µg/L is proposed as the indication of heavy use, requiring medical assessment of the fitness to drive.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00045-4
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

11-OH-THC	11-Hydroxy-THC
BAC	Blood alcohol concentration
BART	Balloon analog risk task
CTT	Critical tracking task
DAT	Divided attention task
DSST	Digit-symbol substitution test
DUIC	Driving under the influence of cannabis
DUID	Driving under the influence of drug
EDT	Experiential discounting task
fMRI	Functional magnetic resonance imaging
RT	Reaction time
SDLP	Standard deviation of lateral position
SST	Stop signal task
THC	Δ^9-Tetrahydrocannabinol
THCCOOH	11-Nor-9-carboxy-THC
WCST	Wisconsin card sorting test

INTRODUCTION

The prevalence of drivers under the influence of cannabis in the entire driving population or in the population of drivers involved in traffic accidents is poorly estimated. One main reason is the inability to collect reliable and comprehensive statistical information from abstinent, sober, or stoned drivers. Collection of data about these drivers depends on political decisions, police strategies, and also on the drivers' and the public's consent. Methodological choices and differences in statistical processing may also influence the results. In Europe, the project "Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID)," cofunded by the European Commission, estimated that the proportions of positive cannabis drivers involved in accidents vary from 4% to 14%, while 1% to 7% drive undetected under the influence of cannabis (DUIC cases), and without being involved in traffic accidents. In Switzerland, a nationwide study, conducted on 4794 blood samples gathered from suspected impaired drivers, showed that cannabinoids were present in 48% of the total number of cases and, thus, were the most frequently encountered drugs (Senna et al., 2010). A systematic review of observational studies with metaanalysis performed by Asbridge, Hayden, and Cartwright (2012), including nine studies (49,411 participants), concluded that the risk of a motor vehicle collision under the effects of acute cannabis consumption was almost twice the risk observed for sober drivers. According to a case-control study conducted by Blows et al. (2005), car crash injuries appear strongly associated with frequent use of marijuana.

DRIVING ABILITY AFTER ACUTE CANNABIS EXPOSURE

Many European and American studies show that the involvement of cannabis users is frequent in car accidents, and raises the question of the influence of cannabis effects on driving ability. Experimental studies have assessed the acute effects of cannabis on neurocognitive functions required in normal driving tasks. Cannabis-induced impairment of various aspects of a driver's performance, such as driving coordination, reaction time (RT), decision-making, critical tracking, and attention, has been evaluated (Table 39.1). Working memory and reasoning were also tested.

To reach these goals, researchers used laboratory and simulator testing, as well as on-road studies, in real situations or on closed courses and, more recently, brain imaging. Findings made in past experimental studies were often equivocal or focused on very specific psychomotor and cognitive functions (Moskowitz, 1985; Rafaelsen et al., 1973; Smiley, 1998; Ward & Dye, 1999). Subsequent studies (Kurzthaler et al., 1999; Robbe, 1998; Sexton et al., 2000) have shown that acute cannabis inhalation impairs psychomotor skills in a dose-related manner (Liguori, Gatto, & Robinson, 1998; Ramaekers et al., 2006). The tests that show the biggest effects of cannabis are those involving reaction time measurement (Lenné et al., 2010; Liguori et al., 1998; MacDonald, Schleifer, Richards, & de Wit, 2003; Rafaelsen et al., 1973; Ramaekers et al., 2006; Ronen et al., 2008), a divided attention task (DAT), a critical tracking task (CTT) (Chait & Perry, 1994; Lenné et al., 2010), or an urgent task (Moskowitz, 1985). In contrast to drunk

TABLE 39.1 Recommendations on the Levels of Behavior Predicting Crash Risks According to the International Council on Alcohol, Drugs, and Traffic Safety

Automative behaviors	Tracking and steering	Road tracking Critical tracking Compensatory tasks
	Sustained attention	Mackworth Clock Test
	Motor performance and maneuvers	Reaction time Car following
Control behaviors	Divided attention	Dual attention task
	Perception	Time-to-collision task
	Risk-taking and impulsivity	Stop signal task Iowa gambling task
Executive planning	Attention and information processing	Choice reaction time Selective attention task Focused attention task
	Cognition and judgment	Tower of London task

Adapted from Walsh, Verstraete, Huestis, and Morland (2008).

drivers, cannabis-intoxicated drivers seem to be aware of their impairment, and try to compensate by driving more cautiously, or by forgoing driving (Anderson, Rizzo, Block, Pearlson, & O'Leary, 2010; Lenné et al., 2010; Lamers & Ramaekers, 2001; Ménétrey et al., 2005; Robbe & O'Hanlon, 1999; Ronen et al., 2008). In on-road and driving simulator studies, cannabis-induced effects on psychomotor function alter braking latency, headway, speed, and mostly road tracking, by increasing lane position variability, and steering wheel variability (Lenné et al., 2010; Ménétrey et al., 2005; Robbe, 1998; Ronen et al., 2008).

Results of relevant experimental studies of cannabis-induced acute effects on neurocognitive functions that may affect the performance of driving are detailed later in the chapter and summarized in Tables 39.2A,B, 39.3, and 39.4. Subjects were considered occasional smokers when cannabis use occurred less than once a week.

Laboratory Studies

Working Memory

Several studies demonstrated that cannabis consumption impairs working memory (as reviewed by Crean, Crane, & Mason, 2011). Kurzthaler et al. (1999) tested the verbal memory of subjects having consumed a 290 µg/kg dose of cannabis, and observed a moderate defect of learning immediately after consumption, reflecting a difficulty in quickly processing new information after cannabis use. Impairment in working memory was also found in frequent and occasional smokers, after 3.9% Δ^9-tetrahydrocannabinol (THC) smoking, or after intravenous (IV) administration of 2.5 mg dronabinol (synthetic THC) (Hart, van Gorp, Haney, Foltin, & Fischman, 2001; Morrison et al., 2009).

Time Perception

Different studies resulted in conflicting findings about cannabis effects on time perception: on one hand, Chait and Perry (1994) found an underestimation of time intervals after smoking two 3.6% THC cigarettes, while MacDonald et al. (2003) only observed an increased estimation of the duration of short time intervals. These latter results were not replicated with chronic daily cannabis smokers using THC doses of 13 and 17 mg (Weinstein et al., 2008b). One study involving IV administration showed that an acute THC dose, ranging from 0.015 to 0.05 mg/kg, induced a time overestimation in infrequent cannabis users, while no differences was found in chronic smokers (Sewell et al., 2013).

Motor Inhibition and Impulsivity

Among the few studies that have assessed cannabis-induced acute effects on impulsivity and inhibition of inappropriate behavior, two found an increased measure of impulsivity in a group of recreational users (MacDonald et al., 2003; Ramaekers et al., 2006). Moreover, it has been shown that a 13 or 17 mg THC inhalation resulted in a dose-related significant impairing effect in a Wisconsin card sorting test (WCST) (Weinstein et al., 2008b). These results were replicated using a stop signal task (SST) used to investigate neurocognitive performance during acute THC intoxication (500 µg/kg) in occasional and heavy users (Ramaekers, Kauert, Theunissen, Toennes, & Moeller, 2009). Interestingly, Metrik et al. (2012) found that, for some regular marijuana users, the assumption of having smoked THC induced a compensation for expected intoxication effects, and resulted in less impulsive and risky decisions, while direct pharmacological effects are not compensated.

Decision Making

Some discrepancies among studies appear concerning the effects of cannabis use on decision making or planning, as well as on reaction time (RT). Ramaekers et al. (2006) found that, compared to the placebo group, recreational smokers receiving 500 µg/kg THC (≈35 mg) were significantly less likely to make correct decisions in the Tower of London test. Additionally, those in the THC-treated groups had an increased response latency. Subsequently, it has been shown that frequent smokers were not significantly affected. However, RT was not significantly increased either in occasional or frequent smokers (Ramaekers et al., 2009, 2011). Several investigators studied the acute effects of cannabis on risk-taking tasks. High doses (3.6% THC) resulted in significantly increased risk-taking behavior, compared to lower doses, in one study (Lane, Cherek, Tcheremissine, Lieving, & Pietras, 2005). MacDonald et al. (2003) and Ramaekers et al. (2006) found no significant differences in performance between groups. In another study, 36 chronic, daily cannabis users were tested after smoking placebo, low (1.8%), or high (3.9%) THC cigarettes (Vadhan et al., 2007), and their performance was not altered. However, more time was necessary to perform the task in THC groups. Conversely, Weinstein et al. (2008b) reported that neither 13 mg nor 17 mg THC significantly affected speed in a gambling task, although regular users of marijuana chose least-likely outcomes after the higher dose.

Attention

Both divided and sustained attention are required for safe driving, and are known to be mediated by the frontal lobes. The CTT is a simple test of psychomotor functioning that measures eye-hand coordination and delays in visual motor response, shown to be particularly sensitive to the impairing acute effects of cannabis for a wide range of doses (Moskowitz, 1985). However, Ward and Dye (1999) reported that, while both divided and sustained attention were affected, it was not in a dose-related manner. Then, Sexton et al. (2000) confirmed a significant performance decrease in a DAT after a high dose of

TABLE 39.2A Acute Effects of Cannabis on Neurocognitive Function in Laboratory Studies

References	*n* (M/F)	Cannabis history	THC doses	Recall tasks	Digit span tasks	Time perception	WCST	Stroop test	Stop signal task	Go-No-Go task	BART	EDT	Tower of London	Gambling task
Sewell et al. (2013)	44	O; F	0.015–0.05 mg/kg (IV)			X[a]								
Metrik et al. (2012)	136	F	2.80%					X	X		NS	NS		
Theunissen et al. (2012)	24	O; F	500 µg/kg 13% (≈250–270 mg)						X[b]					
Ramaekers et al. (2011)	15/6	F	400 µg/kg (≈ 28 mg)						NS				NS	
Ramaekers et al. (2009)	12	O; F	500 µg/kg (≈ 35 mg)						X				NS	
Weinstein et al. (2008b)	14	F	13; 17 mg			NS	X							X
Weinstein et al. (2008a)	12	F	17 mg			NS	X							NS
Vadhan et al. (2007)	36	F	1.8%; 3.9%											X
Ramaekers et al. (2006)	14/6	ns	250; 500 µg/kg (≈ 17.5; 35 mg}						X				X	NS
Lane et al. (2005)	5/5	O; F	1.77%; 3.58%											X
MacDonald et al. (2003)	18/19	O	7.5; 15 mg (oral)	NS	X	X			X	NS				NS
Hart et al. (2001)	10/8	F	1.8%; 3.9%	X	NS									
Chait and Perry (1994)	14	ns	3.60%	NS	NS	X								

O, Occasional; F, frequent; NS, not specified; ns, not significant; X, impaired

[a] *In occasional smokers.*

[b] *Event-related potentials.*

TABLE 39.2B Acute Effects of Cannabis on Neurocognitive Function in Laboratory Studies

References	*n* (M/F)	Cannabis history	THC doses	DSST	Tracking tasks	Virtual maze	Divided attention task	Reaction time	Visual Processing	Trail making test	Benton
Battistella et al. (2013)	31	O	42 mg		X[a]						
Schwope, Bosker, Ramaekers, Gorelick, and Huestis (2012)	9/1	F	6.80%		NS		NS				
Theunissen et al. (2012)	24	O; F	500 µg/kg 13% (≈ 250–270 mg)				X[b,c]				
Ramaekers et al. (2011)	15/6	F	400 µg/kg (≈ 28 mg)		NS		X				
Ramaekers et al. (2009)	12	O; F	500 µg/kg (≈ 35 mg)		X[b]		X[b]				
Weinstein et al. (2008b)	14	F	13; 17 mg			X					
Weinstein et al. (2008a)	12	F	17 mg			X[d]					
Ramaekers et al. (2006)	14/6	ns	250; 500 µg/kg (≈ 17.5; 35 mg)		X						
MacDonald et al. (2003)	18/19	O	7.5; 15 mg (oral)	NS							
Hart et al. (2001)	10/8	F	1.8%; 3.9%	NS			X[e]	X	NS		
Sexton et al. (2000)	15	NS	1.7%; 2.67%				X	NS			
Haney, Ward, Comer, Foltin, and Fischman (1999)	12	F	1.8%; 3.1%	X			X[e]				
Kurzthaler et al. (1999)	34/26	Non-users	290 µg/kg							NS	NS
Chait and Perry (1994)	14	ns	3.60%	X			X				

O, Occasional; F, frequent; NS, not specified; ns, not significant; X, impaired.

[a] *fMRI.*

[b] *In occasional users.*

[c] *Event-related potentials.*

[d] *PET.*

[e] *Improved.*

TABLE 39.3 Acute Effects of Cannabis on Neurocognitive Function in Simulator Studies

References	*n* (M/F)	Cannabis history	THC doses	Reaction time	Brake latency	Mean speed	SD speed	Mean headway	SD headway	Road tracking	SDLP	Divided attention task	Collisions	Visual search	Rapid judgment
Anderson et al. (2010)	50/35	ns	22.9 mg	NS		X	NS				NS	X			
Lenné et al. (2010)	47	ns	19; 38 mg	X		X	X	X	X		X	X			
Ronen et al. (2010)	7/5	O	13 mg			X	NS				NS	X	X		
Ronen et al. (2008)	10/4	O	13; 17 mg	X		X	X				X		X		
Ménétrey et al. (2005)	8	O	16.5; 45.7 mg (oral); 20 mg, dronabinol							X				X	
Papafotiou, Carter, and Stough (2005)	26/14	ns	14; 52 mg							X					
Sexton et al. (2000)	15	ns	1.7%; 2.67%	X		X					X				
Liguori, Gatto, and Robinson (1998)	7/3	F	1.77%; 3.95%	NS	X										NS
Rafaelsen et al. (1973)	8	O	8: 12; 16 mg (oral)	X		NS									

O, Occasional; F, frequent; NS, not specified: ns, not significant; X, impaired.

TABLE 39.4 Effects of Cannabis on Neurocognitive Function in On-Road Studies

References	*n* (M/F)	Cannabis history	THC doses	RT	Mean speed	SD speed	Mean headway	SD headway	Road tracking	Time out of lane	Mean lateral position	SDLP	Divided attention task	Maneuvers	Emergency	Visual search
Lamers and Ramaekers (2001)	8/8	O	100 µg/kg													NS
Ramaekers, Robbe, and O'Hanlon (2000)	9/9	ns	100; 200 µg/kg	NS			X			NS		X				
Robbe (1998; s 1)	12/12	ns	100; 200; 300 µg/kg		NS	NS					NS	X				
Robbe (1998; s 2)	8/8	ns	100; 200; 300 µg/kg	NS			X					X				
Robbe (1998; s 3)	8/8	ns	100 µg/kg						NS					NS		
Robbe (1998; s 4)	9/9	ns	100; 200 µg/kg	NS				X				X				
Smiley (1986)	63	ns	7; 14 mg		NS		X						NS		NS	

O, Occasional; NS, not specified; ns, not significant; X, impaired.

THC (2.67%), both in the CTT and in the proportion of correct answers concerning the additional task. These results were replicated in occasional smokers (with 250 or 500 µg/kg or with 2.5% THC), but regular smokers were only affected in the DAT (Morrison et al., 2009; Ramaekers et al., 2006, 2009, 2011), suggesting that an increased mental load may impair the information processing and, thus, the performance on a central task. Weinstein et al. (2008b) found a significant impairment during a virtual maze task, in a regular smokers group, after a 17 mg THC cigarette inhalation, and this effect was not detected with a 13 mg THC dose. Then, Schwope et al. (2012) showed that no significant differences were observed for CTT or DAT performance in a cohort of 10 heavy, chronic cannabis smokers after smoking a 6.8% THC cannabis cigarette. Interestingly, other studies found that after acute THC intoxication, daily cannabis users significantly improved their performance in sustained attention tasks and DAT tests (Haney et al., 1999; Hart et al., 2001), raising the question of the existence of a tolerance, or of a functional adaptation to long-term cannabis exposure. An alleviation of withdrawal symptoms or the feeling of expected and rewarding effects of cannabis can be also put forward to explain these positive effects.

Brain Imaging

In a study by Theunissen et al. (2012), using neuropsychological tests such as DAT and SST on occasional or heavy cannabis smokers after a 500 µg/kg THC, event-related potentials recordings confirm that heavy cannabis smokers develop tolerance to some of the impairing behavioral effects of cannabis that cannot be attributed to behavioral compensation alone.

Weinstein et al. (2008a) have shown that regular marijuana smokers undergoing PET scan under the effects of 17 mg THC were impaired in a virtual maze task, and that during task performance their brain metabolism was increased in the middle and medial frontal cortices and anterior cingulate, in areas related to motor coordination and attention, while it was reduced in the occipital lobes in areas associated with visual integration of motion. These observations suggest an immediate effect of marijuana on cognitive–motor skills and brain mechanisms that modulate coordinated movement and driving. Battistella et al. (2013), using a brain fMRI paradigm based on a visuomotor tracking task, found that, even at low THC blood concentrations, psychomotor skills were impaired. This effect was correlated with an alteration of the activity of the brain networks involved in cognition. Brain areas that were concerned were the anterior insula, the dorsomedial thalamus, and the striatum, as well as regions of the right superior parietal cortex, and the dorsolateral prefrontal cortex. These brain areas involve the Control Executive network known to operate once the most relevant stimuli (ie, the saliencies) are identified. Moreover, it increases activity in the rostral anterior cingulate cortex, and ventromedial prefrontal cortices, suggesting an increase in self-oriented mental activity leading to insufficient attention to task performance. In addition, these effects were not significantly correlated with the blood levels of THC, but with subjective felt side-effects.

Simulator Studies

Driving simulator studies have been useful for better describing the effects of cannabis on specific driving performances, such as variability of lateral position, decision making in case of emergency, and speed control. Since the 1980s, several former experiments cited by Smiley (1998) have shown that the behavior of subjects under the influence of cannabis is altered when they have to face an unexpected situation, indicating decreased risk taking (speed decrease, mean headway increase), and confirming an impairment during DATs. Then, Liguori et al. (1998) proposed a rapid judgment task consisting in asking the subject to maintain a speed and to choose the correct lane in a crossing, or to avoid hitting a fence that suddenly appears. The scores did not differ across those conditions after a 1.77 or 3.95% THC dose, although brake latency was marginally increased after the higher dose, similarly to prior results in subjects with breath alcohol concentration near 0.5 g/L. According to Sexton et al. (2000), who observed an increased variability in the lateral position, in participants under the influence of a 2.67% (≈ 20 mg) THC dose (not significant with a lower dose: 1.70% ≈ 10 mg), standard deviation of lateral position (SDLP) appeared to be one of the most sensitive road-tracking measures associated with THC impairment. However, variability in the cannabis history of subjects, the THC dose ranges and the time lapse between the end of the consumption and the beginning of the task can explain several discrepancies observed in the results. Other studies confirmed an SDLP increase after 13 and 17 mg THC smoked by occasional smokers (Ronen et al., 2008), or after 19 and 38 mg doses (Lenné et al., 2010). Conversely, no significant increases in SDLP were found after a 13 mg dose in occasional smokers (Ronen et al., 2010), or after a 22.9 mg dose in a heterogeneous group of smokers (1–10 times/month) (Anderson et al., 2010). In addition, other authors used various road-tracking outcomes to demonstrate a THC-induced impairment. In occasional smokers, Ménétrey et al. (2005) revealed obvious and statistically significant differences in a road-tracking task, as well as in a visual search and processing testing, after ingestion of 16.5 or 45.7 mg THC decoction, or 20 mg synthetic THC (dronabinol), compared to placebo, especially when the strongest decoction was used. Papafotiou et al. (2005) observed altered performances 80 min after 14 or 52 mg THC was

smoked, while not present at 30 min. A decrease in mean speed was also observed in several studies, after THC doses from ≈ 10 to 38 mg (Anderson et al., 2010; Lenné et al., 2010; Ronen et al., 2008, 2010; Sexton et al., 2000), confirming previous results that suggest subjects' awareness of their impairment leads to more careful driving. In a group of regular smokers (at least weekly but not daily) no significant cannabis-induced effect was noticed (Liguori et al., 1998). Some studies found a dose-related increase in RT (Lenné et al., 2010; Rafaelsen et al., 1973; Ronen et al., 2008), while others produced nonsignificant results (Anderson et al., 2010; Liguori et al., 1998; Liguori, Gatto, & Jarrett, 2002). Experiments using DATs have shown a dose-related increase in mean headway and SD, after smoking 13 and 17 mg, or 19 and 38 mg THC doses (Lenné et al., 2010; Ronen et al., 2008). One study failed to detect any effect after smoking a 22.9 mg THC dose (Anderson et al., 2010).

On-Road Studies

On-road studies strengthened the previous experimental results, excepting those of Smiley (1986), who found no significant effect on RT in situations when anticipation was possible. In the Netherlands, Robbe (1998) conducted a series of studies most relevant to the effect of cannabis on driving skills. Recreational cannabis users were exposed to three different THC doses (100, 200, and 300 µg/kg), according to a pilot study that defined the maximal administered dose necessary to achieve the desired psychological effect (called "high") corresponding to 20.8 mg THC (308 µg/kg). Several experimental designs were then based on similar doses. In a restricted highway study, a significant increase in the lateral position variability, measured as the SDLP, was observed, whether the 100, 200, or 300 µg/kg dose of cannabis was used, resulting in an effect similar to a blood alcohol concentration (BAC) from 0.3 to 0.7 g/L, whereas blood plasma concentrations of THC were not related to the degree of impairment. In a second study including a road-tracking task and a car-following task on a normal highway, the lateral position variability appeared to be the variable most sensitive to the effects of THC. The SDLP increased in a dose-related manner, although the slight effect produced by the lowest dose was not significant. The mean headway was increased, corresponding to distance variability during deceleration maneuvers of 8, 6, and 2 m, respectively, after a 100, 200, or 300 µg/kg THC dose, suggesting an effect of practice. RTs increased, compared to placebo, but not in a significant way. Robbe (1998) concluded that a different type of information processing is required in each test. Road-tracking is basically controlled by an automatic information processing system which is relatively impervious to environmental changes, but highly vulnerable to internal factors such as THC or other drugs. On the other hand, the car-following task depends more on controlled information processing and, thus, compensatory mechanisms can work to restore the situation. Other studies replicated these results. Ramaekers et al. (2000) found that the driving tasks previously described were impaired 30 and 75 min after a 100 or 200 µg/kg THC dose inhalation. Moreover, higher SDLP values were reported in the second drive. In a subsequent experiment, similar THC doses showed the same SDLP impairment 40 and 100 min after smoking (Lamers & Ramaekers, 2001).

DETECTION OF DRIVERS UNDER THE INFLUENCE OF CANNABIS (DUIC)

Two broad approaches can be used to determine whether the driver was driving under the influence of cannabis or not. First, we can evaluate the blood levels of psychoactive cannabinoids. Indeed, several authors tried to associate specific THC blood levels with increased risk of driving accidents. However, this correlation is challenged by the nonlinear time relationship between blood levels and effects, and the complex metabolism of THC, making the detection of DUIC uneasy. Furthermore, large variations exist between individuals, with respect to the subjective and objective effects of cannabis, even at similar concentrations. THC is rapidly metabolized into psychoactive 11-hydroxy-THC (11-OH-THC), and a broad range of other metabolites. 11-OH-THC is then oxidized in the liver into 11-nor-9-carboxy-THC (THC-COOH). This nonpsychoactive metabolite is glucuronidated and excreted into bile and urine. The urinary metabolite THCCOOH can be easily detected by a large panel of immunoassays and chromatographic methods. Its detection demonstrates cannabis exposure, but provides no indication with regard to the level of impairment, and the time of use. However, some minor cannabinoids (THC-glucuronide, cannabidiol, cannabinol) have been advocated as possible markers of recent cannabis use (Desrosiers et al., 2014; Mareck et al., 2009). The second strategy is based on determining the time of exposure, and relating it to the duration of impairment found in most pharmacodynamic studies. If the time-lapse between cannabis use and the relevant event (eg, a car crash) is short (less than 6–8 h), we can assume than the relevant event happened under the influence of cannabis. This is in agreement with a recent review by Neavyn, Blohm, Babu, and Bird (2014), which notes that the majority of psychomotor impairments occur in the first 2 h and disappear within 3–6 h after smoking, and recommends that patients using marijuana as self-treatment for medical purposes abstain from driving for 8 h after a subjective "high." Calculation of the time of exposure relies mainly on the different pharmacokinetic

(PK) models suggested by Huestis, Barnes, and Smith (2005). Their predictive PK models provide an estimation of the time between cannabis smoking and blood sampling, based on THC concentration alone or on the THCCOOH/THC concentration ratio in a single blood or plasma specimen.

Assessment of Cannabis Effects From Blood Levels

Although the blood concentrations of psychoactive THC are not directly correlated with those in the brain, where they exert their adverse behavioral, cognitive, and motor side-effects (Mura, Kintz, Dumestre, Raul, & Hauet, 2005), blood sampling remains the most effective way to confirm or refute recent consumption of cannabis, and to evaluate the extent of its effects. THC blood level rises rapidly, reaches peak concentrations within 10 min, and then drops rapidly, though remaining detectable for about 4–8 h. However, whether single THC blood concentration values are suitable to estimate the level of driver impairment or not needs to be discussed, considering the nonlinear relationship and counterclockwise hysteresis between THC blood levels and cannabis effects: just after smoking, the concentration rapidly reaches high levels, while the effects are still low. During the elimination phase, the effects still persist, while the concentrations have already sharply declined. On the other hand, THCCOOH can be detected in the blood up to several hours or even days after smoking. Bergamaschi et al. (2013) observed that cannabinoids are still detectable in the blood of chronic daily cannabis smokers after a month of sustained abstinence.

Other Biological Matrices for Cannabis Screening (Urine, Oral Fluid, Hair)

THCCOOH can be detected in the urine from 30 min after ingestion to several weeks, and cannot be used to determine when cannabis use occurred. Thus, urine specimens can be "cannabis" positive while driving skills are completely unaffected. Conversely, a urine specimen taken just after smoking can test "negative" while the smoker's performances are impaired. Currently, the evaluation of oral fluid for cannabis detection is an active area of research. THC contamination of the oral cavity during cannabis smoking could result in very high THC levels in saliva. On the other hand, THCCOOH, an indicator of actual exposure to cannabis, is found only in trace levels in this matrix. The European Rosita projects examined several drug testing devices between 2003 and 2005, and established criteria for accurate and reliable tests, but none of the devices met the criteria. A comprehensive review by Lee and Huestis (2014) supported oral fluid as an interesting matrix for clinical and forensic cannabinoid screening, although a better understanding is still needed of the mechanisms underlying cannabinoid disposition in oral fluid, as well as of the relationship between the selection of oral fluid THC concentration cutoffs and impairment. For example, a significant correlation with performance on the Tower of London but not on the CTT was mentioned. Furthermore, a strong correlation with blood concentrations was also noted. Conversely, the integrated project DRUID concluded that there is no reliable correlation between oral fluid and blood THC concentrations (Verstraete et al., 2011).

Hair analysis is not appropriate to detect recent use, but may provide information concerning the extent of chronic use, in the context of abstinence control. However, as discussed by Fabritius et al. (2014), this matrix could be contaminated by passive cannabis smoke exposure, or by a urine-contaminated environment, or hand contact. In this regard, experimental studies failed to establish significant correlations between hair and urine THCCOOH concentration or self-reported frequency of use.

Assessing Heavy Versus Occasional Use

The long-term fitness to drive can be influenced by many parameters, such as the frequency of cannabis use, suggesting the need for a specific criterion to distinguish between regular and occasional cannabis use. Considering that cannabis history is of limited utility with respect to performing relevant driving ability evaluations, objective data are preferable. Fabritius et al. (2014) investigated differences in THCCOOH levels of 797 whole blood specimens collected in these two populations of smokers, and proposed a THCCOOH threshold concentration to help determine to which group they belong. In agreement with previously published experimental data, a concentration higher than 40 µg/L of whole blood was found to strongly suggest heavy use. Thus, the threshold of 40 µg/L could be used as a cutoff to detect drivers who require further investigation of their long-term fitness to drive. Moreover, it is proposed that a THCCOOH level below 3 µg/L strongly suggests occasional use, and that no medical assessment of the fitness to drive is required.

MINI-DICTIONARY

Divided attention task Neuropsychological tests related to the ability of a subject to perform two different tasks simultaneously.
Executive functions Neuropsychological term referring to a group of integrated cognitive processes, including working memory, reasoning, attention and planning, that regulate adaptive behavior.
Fitness to drive Long-term ability to drive safely, considering the driver's physical and mental health.

functional Magnetic Resonance Imaging Noninvasive technique to map brain activity by evaluating changes in blood oxygenation level.

Standard deviation of lateral position Measure of dispersion of the distances from the car position to a virtual central line on the lane.

ACKNOWLEDGMENTS

The financial support for writing this chapter is provided by the Swiss National Scientific Research Foundation (grant SNF 320030_127507/1) and the Faculty of Biology and Medicine of the University of Lausanne (multidisciplinary project FBM). The authors declare no conflict of interest.

References

Anderson, B. M., Rizzo, M., Block, R. I., Pearlson, G. D., & O'Leary, D. S. (2010). Sex differences in the effects of marijuana on simulated driving performance. *Journal of Psychoactive Drugs*, *42*(1), 19–30.

Armentano, P. (2013). Should per se limits be imposed for cannabis? Equating cannabinoid blood concentrations with actual driver impairment: practical limitations and concerns. *Humboldt Journal of Social Relations*, *35*, 41–51.

Asbridge, M., Hayden, J. A., & Cartwright, J. L. (2012). Acute cannabis consumption and motor vehicle collision risk: systematic review of observational studies and meta-analysis. *British Medical Journal*, *344*, e536.

Battistella, G., Fornari, E., Thomas, A., Mall, J. F., Chtioui, H., Appenzeller, M., Annoni, J. -M., Favrat, B., Maeder, P., & Giroud, C. (2013). Weed or wheel! FMRI, behavioural, and toxicological investigations of how cannabis smoking affects skills necessary for driving. *PLoS One*, *8*(1), e52545.

Bergamaschi, M. M., Karschner, E. L., Goodwin, R. S., Scheidweiler, K. B., Hirvonen, J., Queiroz, R. H., & Huestis, M. A. (2013). Impact of prolonged cannabinoid excretion in chronic daily cannabis smokers' blood on per se drugged driving laws. *Clinical Chemistry*, *59*(3), 519–526.

Blows, S., Ivers, R. Q., Connor, J., Ameratunga, S., Woodward, M., & Norton, R. (2005). Marijuana use and car crash injury. *Addiction*, *100*(5), 605–611.

Chait, L. D., & Perry, J. L. (1994). Acute and residual effects of alcohol and marijuana, alone and in combination, on mood and performance. *Psychopharmacology (Berlin)*, *115*(3), 340–349.

Crean, R. D., Crane, N. A., & Mason, B. J. (2011). An evidence based review of acute and long-term effects of cannabis use on executive cognitive functions. *Journal of Addiction Medicine*, *5*(1), 1–8.

Desrosiers, N. A., Himes, S. K., Scheidweiler, K. B., Concheiro-Guisan, M., Gorelick, D. A., & Huestis, M. A. (2014). Phase I and II cannabinoid disposition in blood and plasma of occasional and frequent smokers following controlled smoked cannabis. *Clinical Chemistry*, *60*(4), 631–643.

Fabritius, M., Favrat, B., Chtioui, H., Battistella, G., Annoni, J. M., Appenzeller, M., Dao, K., Fornari, E., Lauer, E., Mall, J. -F., Maeder, P., Mangin, P., Staub, C., & Giroud, C. (2014). THCCOOH concentrations in whole blood: are they useful in discriminating occasional from heavy smokers? *Drug Testing and Analysis*, *6*(1–2), 155–163.

Haney, M., Ward, A. S., Comer, S. D., Foltin, R. W., & Fischman, M. W. (1999). Abstinence symptoms following smoked marijuana in humans. *Psychopharmacology (Berlin)*, *141*, 395–404.

Hart, C. L., van Gorp, W., Haney, M., Foltin, R. W., & Fischman, M. W. (2001). Effects of acute smoked marijuana on complex cognitive performance. *Neuropsychopharmacology*, *25*(5), 757–765.

Huestis, M. A., Barnes, A., & Smith, M. L. (2005). Estimating the time of last cannabis use from plasma delta9-tetrahydrocannabinol and 11-nor-9-carboxy-delta9-tetrahydrocannabinol concentrations. *Clinical Chemistry*, *51*(12), 2289–2295.

Kurzthaler, I., Hummer, M., Miller, C., Sperner-Unterweger, B., Gunther, V., Wechdorn, H., Battista, H. -J., & Fleischhacker, W. W. (1999). Effect of cannabis use on cognitive functions and driving ability. *Journal of Clinical Psychiatry*, *60*(6), 395–399.

Lamers, C. T., & Ramaekers, J. G. (2001). Visual search and urban driving under the influence of marijuana and alcohol. *Human Psychopharmacology*, *16*(5), 393–401.

Lane, S. D., Cherek, D. R., Tcheremissine, O. V., Lieving, L. M., & Pietras, C. J. (2005). Acute marijuana effects on human risk taking. *Neuropsychopharmacology*, *30*(4), 800–809.

Lee, D., & Huestis, M. A. (2014). Current knowledge on cannabinoids in oral fluid. *Drug Testing and Analysis*, *6*(1–2), 88–111.

Lenné, M. G., Dietze, P. M., Triggs, T. J., Walmsley, S., Murphy, B., & Redman, J. R. (2010). The effects of cannabis and alcohol on simulated arterial driving: influences of driving experience and task demand. *Accident Analysis and Prevention*, *42*(3), 859–866.

Liguori, A., Gatto, C. P., & Jarrett, D. B. (2002). Separate and combined effects of marijuana and alcohol on mood, equilibrium and simulated driving. *Psychopharmacology*, *163*, 399–405.

Liguori, A., Gatto, C. P., & Robinson, J. H. (1998). Effects of marijuana on equilibrium, psychomotor performance, and simulated driving. *Behavioral and Pharmacology*, *9*(7), 599–609.

MacDonald, J., Schleifer, L., Richards, J. B., & de Wit, H. (2003). Effects of THC on behavioral measures of impulsivity in humans. *Neuropsychopharmacology*, *28*(7), 1356–1365.

Mareck, U., Haenelt, N., Geyer, H., Guddat, S., Kamber, M., Brenneisen, R., Thevis, M., & Schänzer, W. (2009). Temporal indication of cannabis use by means of THC glucuronide determination. *Drug Testing and Analysis*, *1*(11–12), 505–510.

Ménétrey, A., Augsburger, M., Favrat, B., Pin, M. A., Rothuizen, L. E., Appenzeller, M., Buclin, T., Mangin, P., & Giroud, C. (2005). Assessment of driving capability through the use of clinical and psychomotor tests in relation to blood cannabinoids levels following oral administration of 20 mg dronabinol or of a cannabis decoction made with 20 or 60 mg Delta9-THC. *Journal of Analytical Toxicology*, *29*(5), 327–338.

Metrik, J., Kahler, C. W., Reynolds, B., McGeary, J. E., Monti, P. M., Haney, M., de Wit, H., & Rohsenow, D. J. (2012). Balanced placebo design with marijuana: pharmacological and expectancy effects on impulsivity and risk taking. *Psychopharmacology (Berlin)*, *223*(4), 489–499.

Morrison, P. D., Zois, V., McKeown, D. A., Lee, T. D., Holt, D. W., Powell, J. F., Kapur, S., & Murray, R. M. (2009). The acute effects of synthetic intravenous Delta9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. *Psychological Medicine*, *39*(10), 1607–1616.

Moskowitz, H. (1985). Marihuana and driving. *Accident Analysis and Prevention*, *17*(4), 323–345.

Mura, P., Kintz, P., Dumestre, V., Raul, S., & Hauet, T. (2005). THC can be detected in brain while absent in blood. *Journal of Analytical Toxicology*, *29*(8), 842–843.

Neavyn, M. J., Blohm, E., Babu, K. M., & Bird, S. B. (2014). Medical marijuana and driving: a review. *Journal of Medical Toxicology*, *10*, 269–279.

Papafotiou, K., Carter, J. D., & Stough, C. (2005). The relationship between performance on the standardised field sobriety tests, driving performance and the level of Delta9-tetrahydrocannabinol (THC) in blood. *Forensic Science International*, *155*(2–3), 172–178.

Rafaelsen, O. J., Bech, P., Christiansen, J., Christrup, H., Nyboe, J., & Rafaelsen, L. (1973). Cannabis and alcohol: effects on stimulated car driving. *Science*, *179*(4076), 920–923.

Ramaekers, J. G., Kauert, G., Theunissen, E. L., Toennes, S. W., & Moeller, M. R. (2009). Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. *Journal of Psychopharmacology*, *23*(3), 266–277.

Ramaekers, J. G., Kauert, G., van Ruitenbeek, P., Theunissen, E. L., Schneider, E., & Moeller, M. R. (2006). High-potency marijuana impairs executive function and inhibitory motor control. *Neuropsychopharmacology*, *31*(10), 2296–2303.

Ramaekers, J. G., Robbe, H. W., & O'Hanlon, J. F. (2000). Marijuana, alcohol and actual driving performance. *Human Psychopharmacology*, *15*(7), 551–558.

Ramaekers, J. G., Theunissen, E. L., de Brouwer, M., Toennes, S. W., Moeller, M. R., & Kauert, G. (2011). Tolerance and cross-tolerance to neurocognitive effects of THC and alcohol in heavy cannabis users. *Psychopharmacology (Berlin)*, *214*(2), 391–401.

Robbe, H. W. J. (1998). Marijuana's impairing effects on driving are moderate when taken alone but severe when combined with alcohol. *Human Psychopharmacology, Clinical and Experimental*, *13*, S70–S78.

Robbe, H. W. J., & O'Hanlon, J. F. (1999). *Marijuana, alcohol and actual driving performance*. Washington, DC: National Highway Traffic Safety Administration, pp. 1-43, Report DOT HS 808 939.

Ronen, A., Chassidim, H. S., Gershon, P., Parmet, Y., Rabinovich, A., Bar-Hamburger, R., Cassuto, Y., & Shinar, D. (2010). The effect of alcohol, THC and their combination on perceived effects, willingness to drive and performance of driving and non-driving tasks. *Accident Analysis and Prevention*, *42*(6), 1855–1865.

Ronen, A., Gershon, P., Drobiner, H., Rabinovich, A., Bar-Hamburger, R., Mechoulam, R., Cassuto, Y., & Shinar, D. (2008). Effects of THC on driving performance, physiological state and subjective feelings relative to alcohol. *Accident Analysis and Prevention*, *40*(3), 926–934.

Schwope, D. M., Bosker, W. M., Ramaekers, J. G., Gorelick, D. A., & Huestis, M. A. (2012). Psychomotor performance, subjective and physiological effects and whole blood Delta(9)-tetrahydrocannabinol concentrations in heavy, chronic cannabis smokers following acute smoked cannabis. *Journal of Analytical Toxicology*, *36*(6), 405–412.

Senna, M. C., Augsburger, M., Aebi, B., Briellmann, T. A., Donze, N., Dubugnon, J. L., Iten, P. X., Staub, C., Sturm, W., & Sutter, K. (2010). First nationwide study on driving under the influence of drugs in Switzerland. *Forensic Science International*, *198*(1–3), 11–16.

Sewell, R. A., Schnakenberg, A., Elander, J., Radhakrishnan, R., Williams, A., Skosnik, P. D., Pittman, B., Ranganathan, M., & D'Souza, D. C. (2013). Acute effects of THC on time perception in frequent and infrequent cannabis users. *Psychopharmacology (Berlin)*, *226*(2), 401–413.

Sexton, B. F., Tunbridge, R. J., Brook-Carter, N., Jackson, P. G., Wright, K., Stark, M. M., & Englehart, K. (2000). *The influence of cannabis on driving*. Wokingham, UK: TRL, TRL Report 477.

Smiley, A. (1986). Marijuana: on-road and driving simulator studies. *Alcohol, Drugs and Driving Abstracts and Reviews*, *2*, 121–134.

Smiley, A. (1998). Marijuana: on-road and driving simulator studies. In H. Kalant, W. Corrigala, W. Hall, & R. G. Smart (Eds.), *The health effects of cannabis* (pp. 173–191). Toronto: Centre for Addiction and Mental Health.

Theunissen, E. L., Kauert, G. F., Toennes, S. W., Moeller, M. R., Sambeth, A., Blanchard, M. M., & Ramaekers, J. G. (2012). Neurophysiological functioning of occasional and heavy cannabis users during THC intoxication. *Psychopharmacology (Berlin)*, *220*(2), 341–350.

Vadhan, N. P., Hart, C. L., van Gorp, W. G., Gunderson, E. W., Haney, M., & Foltin, R. W. (2007). Acute effects of smoked marijuana on decision making, as assessed by a modified gambling task, in experienced marijuana users. *Journal of Clinical and Experimental Neuropsychology*, *29*, 357–364.

Verstraete, A., Knoche, A., Jantos, R., Skopp, G., Gjerde, H., Vindenes, V., Morland, J., Langel, K., & Lillsunde, P. (2011). *Per se limits—Methods of defining cut-off values for zero tolerance*. Bergisch Gladbach, Germany: Driving under the Influence of Drugs, Alcohol and Medicines (DRUID), Deliverable D1.4.2..

Walsh, J. M., Verstraete, A. G., Huestis, M. A., & Morland, J. (2008). Guidelines for research on drugged driving. *Addiction*, *103*(8), 1258–1268.

Ward, N. J., & Dye, L. (1999). *Cannabis and driving. A review of the literature and commentary*. London, UK: Department of the Environment, Transport and the Regions, London road safety research report no. 12, 94 p.

Weinstein, A., Brickner, O., Lerman, H., Greemland, M., Bloch, M., Lester, H., Chisin, R., Mechoulam, R., Bar-Hamburger, R., Freedman, N., & Even-Sapir, E. (2008a). Brain imaging study of the acute effects of Delta9-tetrahydrocannabinol (THC) on attention and motor coordination in regular users of marijuana. *Psychopharmacology (Berlin)*, *196*(1), 119–131.

Weinstein, A., Brickner, O., Lerman, H., Greemland, M., Bloch, M., Lester, H., Chisin, R., Sarne, Y., Mechoulam, R., Bar-Hamburger, R., Freedman, N., & Even-Sapir, E. (2008b). A study investigating the acute dose-response effects of 13 mg and 17 mg Delta 9- tetrahydrocannabinol on cognitive-motor skills, subjective and autonomic measures in regular users of marijuana. *Journal of Psychopharmacology*, *22*(4), 441–451.

CHAPTER

40

Chronic Cannabis Use and Axonal Fiber Connectivity

N. Solowij[*], *A. Zalesky*[**], *V. Lorenzetti*[**,†], *M. Yücel*[**,†]

*School of Psychology, University of Wollongong, Wollongong, NSW, Australia
**Melbourne Neuropsychiatry Centre, The University of Melbourne and Melbourne Health, Melbourne, VIC, Australia
†Brain & Mental Health Laboratory, Monash Institute of Cognitive and Clinical Neurosciences, School of Psychological Sciences, Monash University, Clayton, VIC, Australia

SUMMARY POINTS

- Evidence that long-term cannabis use may be hazardous to white matter in the developing brain has been accumulating.
- Early onset cannabis use in particular is thought to decrease white matter structural integrity during the critical neurodevelopment occurring in adolescence.
- We report specific localized axonal connectivity disturbances in adult long-term heavy cannabis users, rather than any widespread dysconnectivity throughout the brain, with 84–88% reductions in streamlines in the fimbria of the hippocampus, and commissural fibers extending to the precuneus. We found associations between the white matter integrity within these fiber bundles and the age of onset of cannabis use. Our findings require replication.
- The endocannabinoid system is critically involved in axonal growth in the developing brain; mechanisms underlying axonal morphological alterations following exposure to cannabis in utero have been identified.
- Mechanisms that may be specifically perturbed by cannabis use impacting the neurodevelopment and brain maturational processes that occur during adolescence require further research.
- Dysfunctional connectivity may underlie a wide range of cognitive disturbances and psychological symptoms, including vulnerability to psychosis, depression, and anxiety disorders, all of which are significant public health concerns.

KEY FACTS OF CONNECTIVITY DISTURBANCES

- The brain is an amazingly complex network. Mounting evidence suggests that disorders of the brain and mind are associated with disturbances to the axonal fibers interconnecting different cortical and subcortical regions.
- Advances in brain imaging have enabled comprehensive mapping of the human brain's axonal connectivity architecture, giving rise to the human connectome, while novel statistical techniques have been developed to pinpoint specific circuit-level, topological, and connectome-wide disturbances.
- The human connectome provides a powerful framework to formulate hypotheses about how the brain responds, either adaptively or maladaptively, to pathological insult. For example, the brain is particularly vulnerable to disturbances at highly connected hub regions concentrated in cortical association areas.
- Connectivity disturbances are disease specific, and may provide novel targets for targeted interventions and drug therapies.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00046-6
Copyright © 2017 Elsevier Inc. All rights reserved.

KEY FACTS OF CANNABIS USE

- Cannabis use is highly prevalent throughout the world, with adolescence being the prime time for experimentation and progression to regular use.
- Long-term or heavy cannabis use is associated with cognitive dysfunction, functional and structural brain alterations, and elevated psychological symptoms.
- The hippocampus is a key brain region thought to be impacted by long-term and heavy cannabis use, and verbal learning and memory are frequently impaired in cannabis users.
- There is rapidly growing evidence to suggest that early onset cannabis use during adolescence may be detrimental to the developing brain, and lead to a range of adverse consequences.
- There is a paucity of studies aimed at assessing the extent to which the adverse cognitive, brain, and psychological effects of cannabis may recover following prolonged abstinence.

LIST OF ABBREVIATIONS

AD	Axial diffusivity
CB1R	Cannabinoid receptor type 1
DW-MRI	Diffusion-weighted magnetic resonance imaging
eCB	Endogenous cannabinoid or endocannabinoid
FA	Fractional anisotropy
MD	Mean diffusivity
NAA	*N*-acetylaspartate
RD	Radial diffusivity

INTRODUCTION

Chronic exposure to cannabis has been associated with cognitive impairment, elevated psychological symptoms (eg, anxious, depressive and psychotic), and alterations of brain function and structure (Solowij, Yücel, Lorenzetti, & Lubman, 2009). Neurofunctional alterations are increasingly being demonstrated across diverse cognitive tasks (Batalla et al., 2013), while the most consistent morphological alterations include reduced hippocampal volume, and changes in the gray matter density of medial temporal brain regions (Batalla et al., 2013; Lorenzetti, Solowij, Fornito, Lubman, & Yücel, 2014; Yücel et al., 2008). While examination of the activity and structure of individual brain regions informs understanding of the adverse neurobiological effects of cannabis, this corpus of research does not provide information about the detrimental effect of cannabis on the human connectome: the brain's complex network of interconnecting structures and nexus of communication (Sporns, Tononi, & Kötter, 2005). Advances in brain imaging technology have enabled comprehensive mapping of the human connectome in vivo, and statistical comparison of its structure between different groups.

Interrogating the connectome may ultimately lead to a better understanding of the mechanisms by which exposure to cannabis leads to gross morphological changes in the brain, and the functional significance and behavioral consequences of such alterations. Connectivity disturbances have risen to prominence as the neural substrates of complex phenotypes that characterize a range of other clinical and psychiatric disorders (eg, schizophrenia; Fornito, Zalesky, Pantelis, & Bullmore, 2012), with increasing interest in the field of addictions (Filbey, 2013; Gulley & Juraska, 2013). A further reason to focus on brain connectivity is because its establishment forms the major part of the normal maturational processes in brain development during adolescence. It is precisely during this period of brain maturation that the vast majority of cannabis users are first exposed to the drug, and progress to regular use. During adolescence, substantial restructuring of neural connections occurs, involving apoptosis, dendritic pruning, and ongoing myelination. Gradual reduction of gray matter and increasing white matter reflect the strengthening of important and elimination of redundant connections and synapses to promote efficiency of neural conductivity (Dennis et al., 2013; Lebel & Beaulieu, 2011), with concomitant changes in associated neurotransmitter receptor density, gene expression, and signaling. Notably, the endocannabinoid (eCB) system, which mediates the effect of cannabinoids on the brain, is thought to be critically involved in shaping neurodevelopment (Harkany, Keimpema, Barabas, & Mulder, 2008; Schneider, 2008).

Cannabinoid type 1 receptors (CB1R) are located on white matter structures that comprise the physical foundations of connectivity in the brain: terminal boutons, axons, myelin sheaths, and associated oligodendrocytes and astrocytes (Molina-Holgado et al., 2002). Their presence is associated with guiding white matter development during brain maturation (Malone, Hill, & Rubino, 2010), and their concentrations are high in white matter tracts during development, progressively diminishing during adulthood (Glass, Dragunow, & Faull, 1998; Romero et al., 1997). As such, developing white matter may be at greater risk of damage during this neurodevelopmental period due to its higher concentration of cannabinoid receptors, relative to the mature brain, and downregulation of the eCB system with repeated exposure to cannabis. Indirect support for this claim comes from many studies finding an association between an earlier age of onset of cannabis use and a range of greater adverse outcomes, including cognitive

impairment (Solowij & Pesa, 2012). Further to this, an association between cannabis use and the development of schizophrenia (Malone et al., 2010) speaks to the potential impact of cannabis on the brain developmental processes thought to go awry in this disorder during adolescence and young adulthood, the peak period for the development of the disorder.

MOUNTING EVIDENCE FOR DYSCONNECTIVITY IN CANNABIS USERS

White matter abnormalities such as altered volume, density, and hyperintensities have been observed in cannabis users using standard magnetic resonance imaging (Batalla et al., 2013; Solowij et al., 2011). More advanced imaging methods such as diffusion weighted MRI (DW-MRI, of which diffusion tensor imaging (DTI) is one particular model fitted to DW-MRI data) provide a means to map the trajectories followed by white matter axonal tracts, and assess the integrity of white matter microstructure with fractional anisotropy (FA), radial (RD), axial (AD), and mean diffusivity (MD) indices (see Mini-dictionary for definitions).

White matter microstructural alterations in adult and adolescent cannabis users—most often reduced FA—have been reported in the corpus callosum, the largest fiber bundle interconnecting the two hemispheres (Arnone et al., 2008; Bava et al., 2009; Gruber, Silveri, Dahlgren, & Yurgelun-Todd, 2011), frontotemporal regions including the arcuate or superior longitudinal fasciculus (Ashtari et al., 2009; Bava et al., 2009; Bava, Jacobus, Mahmood, Yang, & Tapert, 2010; Clark, Chung, Thatcher, Pajtek, & Long, 2012; Jacobus et al., 2009; Yücel et al., 2010), and in white matter surrounding the hippocampus (Yücel et al., 2010). Bava et al. (2009, 2010) also reported increased MD in the corpus callosum, in adolescent cannabis users. Associations between reduced frontal FA or increased MD and a younger age of onset of cannabis use have been reported (Gruber et al., 2011, 2014). Some studies included extensive other substance use in addition to cannabis, especially alcohol use, and reported widespread white matter alterations (Bava et al., 2009, 2010; Clark et al., 2012; Jacobus et al., 2009). The findings from these studies are summarized in Table 40.1. Abnormalities in FA have also been observed in cannabis-using patients with early psychosis, in these same brain regions (Dekker et al., 2010; James et al., 2011; Peters et al., 2009). While most often FA was found to be reduced, some studies reported increased FA which might reflect increased oligodendrocyte transcription factor and expression of myelin basic protein due to CB1R stimulation (Arevalo-Martin et al., 2007), or premature myelination (DeBellis et al., 2008). Taken together, these studies suggest cannabis use may be particularly detrimental to the white matter structures of the developing brain, both in healthy volunteers and patients with recent onset schizophrenia.

The functional significance of the observed white matter abnormalities in cannabis users remains underinvestigated. Bava et al. (2010) showed that lower FA in temporal regions in heavy cannabis and alcohol-using adolescents was associated with poorer cognitive performance, on tasks of attention, working memory, and processing speed, but higher FA in the anterior internal capsule was associated with poor verbal memory performance. Traditional structure–function relationships may be altered by exposure to cannabis in the context of brain maturation. A further means of interrogating structure–function relationships in cannabis users is to probe functional connectivity in the brain, assessed by examining the temporal correlation of neural signals across brain regions, using indices such as independent component analysis applied to functional magnetic resonance imaging data during the performance of a cognitive task or at rest. There is a growing literature reporting mostly increased functional connectivity in cannabis users (for a review see Batalla et al., 2013), particularly in association with early onset use. These methods can be informative regarding the communication nexus of the connectome; increased functional connectivity may reflect decreased white matter integrity, requiring greater signal between network elements to compensate for white matter loss (Filbey, 2013).

Few DW-MRI studies of cannabis users have moved beyond a focus on measures of microstructural integrity (mostly FA). One study assessed structural brain connectivity measures derived from DW-MRI and white matter tractography, by means of a graph theoretic analysis of both global and local brain network properties in adult cannabis users. Kim et al. (2012) found reduced global network efficiency, and increased localized clustering, particularly altered in the cingulate region, reflecting less efficiently integrated networks, and altered regional connectivity. Higher resolution imaging coupled with advances in DW-MRI methods have enabled tractography, through more reliable measurement of directional diffusion information, to reconstruct the neural fiber tracts throughout the brain.

AN INVESTIGATION OF AXONAL FIBER CONNECTIVITY IN LONG-TERM HEAVY CANNABIS USERS

In a study aimed at improving upon previous research in this area, DW-MRI and tractography were used to interrogate axonal fiber pathways in long-term heavy cannabis users (see Zalesky et al., 2012 for details). We recruited from the general community a sample of cannabis users substantially larger than that of previous studies, carefully screened for neurological conditions,

TABLE 40.1 White Matter Integrity Perturbations in Cannabis Users

Authors	Nature of sample	Extent of cannabis use	Regions where alterations were observed	Findings
Arnone et al. (2008)	Young adult males, *n* = 11, 19–21 y, no other illicit substance use	Daily use ≥ 2 y, mean 9 y	Corpus callosum	↑MD in prefrontal subregion of corpus callosum, trend association duration of use
Ashtari et al. (2009)	Adolescent and young adult males in treatment for CUD, *n* = 14, 18–21 y, no other illicit substance use, some past alcohol abuse	Mean 5.3 y, mean 5.8 j/day, abstinent mean 6.7 m	Arcuate fasciculus, internal capsule/thalamic radiation, corpus callosum, prefrontal cortex	↓FA, ↓AD, ↑RD in most regions, ↓FA in the right arcuate, ↓AD in motor fibers
Bava et al. (2009, 2010, 2013)	Adolescent, *n* = 36, 16–19 y, with 18 m follow-up of *n* = 41, 16–21 y, significant alcohol use, some extensive other substance use in follow-up sample	Lifetime occasions of use mean 552, 453 at baseline in follow-up sample with 652 uses during the follow-up period	Superior longitudinal fasciculus, thalamic radiations, corpus callosum, temporal regions and other widespread alterations	↓FA in 10 clusters, ↑MD in other clusters; some associations with NP/↓FA in 3 clusters and ↑MD, ↑RD, ↑AD in multiple clusters at follow-up, related more to alcohol than cannabis use during that period
Clark et al. (2012)	Adolescents in treatment for substance use disorders (most with CUD), *n* = 35, 14–19 y, other substance use and psychological disorders	Onset age substance use disorder 11–18 y, mean 14.7 y, use in month prior to testing mean 6.6 days (SD 11.1)	Prefrontal, temporal, and parietal regions	↓FA, correlated with CUD symptoms and psychological dysregulation
Gruber et al. (2011, 2014)	Adult, predominantly male, *n* = 15/*n* = 25, mean 25 y (SD 8.4)/mean 23 y (SD 5.9), no other illicit substance use	Mean 10 y, 25 j/week ≥2500 occasions of use	Left frontal, right genu/left and right genu of corpus callosum	↓FA, ↑MD, correlate with age of onset and impulsivity
Jacobus et al. (2009); Jacobus, Squeglia, Bava, and Tapert (2013)	Adolescent, *n* = 14 binge drinkers with cannabis use, 16–19 y/*n* = 21 for 36 m follow-up, 19–22 y; no other illicit substance use	180–1800 occasions of use, mean 550, abstinent 23–61 days	Superior longitudinal fasciculus; corona radiate/widespread white matter alterations at 36 m follow-up	↓FA, inversely correlated with lifetime use/↓FA in 14/15 regions at follow-up but no difference between binge drinkers with or without cannabis use
Yücel et al. (2010)	Adolescent/young adult, *n* = 11	≥1 y, 0.8 g/day (SD 0.5)	Superior longitudinal fasciculus, white matter surrounding hippocampus	↓FA
Zalesky et al. (2012)	Adult, *n* = 59, mean 33.4 y (SD 10.9); no other illicit substance use	Mean 15.6 y (SD 9.5), near daily, ≈26,000 lifetime j	Right fimbria to splenium of corpus callosum, commissural fiber from splenium to right precuneus	↓Streamline count,↓FA; RD and MD correlated with age of onset

AD, Axial diffusivity; CUD, cannabis use disorder; FA, fractional anisotropy; j, joints; m, months; MD, mean diffusivity; NP, neuropsychological performance; RD, radial diffusivity; y, years.

psychiatric diagnoses, and other substance use confounds, including heavy alcohol use. Fifty nine cannabis users with 15.6 years of near daily cannabis use, consuming 147 joints per month on average, were compared with 33 age-, IQ-, education-, and gender-matched non-user controls. The mean age at which regular cannabis use commenced was 16.7 years (SD 3.3).

The DW-MRI data were processed using a validated fiber-tracking algorithm to create high-resolution white matter connectivity maps indicating the extent of axonal connectivity between thousands of distinct voxel pairs. Specifically, millions of streamlines (3D curves following the trajectory of a particular fiber bundle) were generated to reconstruct the brain's entire network of axonal fibers. The reconstructed trajectories followed the direction of maximum water diffusion, aligned with the path of underlying axonal fiber bundles (Fig. 40.1). A connectivity matrix was mapped for each participant, to provide a

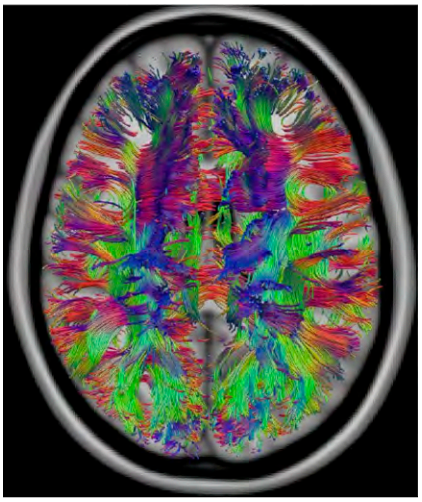


FIGURE 40.1 **Whole-brain tractography results registered to standard space, shown here for a representative nonuser control.** *Source: Adapted with permission from Zalesky, A., Solowij, N., Yücel, M., Lubman, D. I., Takagi, M., Harding, I. H., Lorenzetti, V., Wang, R., Searle, K., Pantelis, C., Seal, M. (2012). Effect of long-term cannabis use on axonal fibre connectivity.* Brain, 135, 2245–2255.

measure of the extent to which each voxel pair from spatially distinct brain regions was anatomically (axonally) connected. Using a data-driven whole brain analysis (as opposed to predefined regions of interest as in previous studies), a novel network-based statistic (Zalesky, Fornito, & Bullmore, 2010) was applied to control the family wise error rate in comparing the 169,863 voxel pairs that were interconnected by at least one streamline and, hence, identify between-group connectivity differences. Two indices of white matter microstructure, axonal and radial diffusivity (AD and RD), were averaged over all voxels intersected by at least one streamline, yielding a network-wide measure of AD and RD for each significant network. AD and RD were regressed against the age at which regular cannabis use commenced, cumulative lifetime dose of exposure to cannabis, and duration of regular use, with biological age included as a nuisance regressor to control for maturation effects.

Whole brain volume did not differ between groups (p = 0.9), and we found no evidence of brain-wide connectivity disturbances. Approximately 1.19 ± 0.1 million streamlines were found in cannabis users which did not differ in number from nonuser controls (p = 0.9), and there was no evidence of any brain-wide shortening of axonal fibers. However, two specific localized networks were found to be interconnected with significantly fewer streamlines in users, compared to nonusers, indicating tract-specific disturbances in axonal integrity. The first network (Fig. 40.2) implicated the right fimbria (crus of the fornix), a prominent band of fibers running alongside the medial periphery of the hippocampus and extending to the hippocampal commissure, as well as

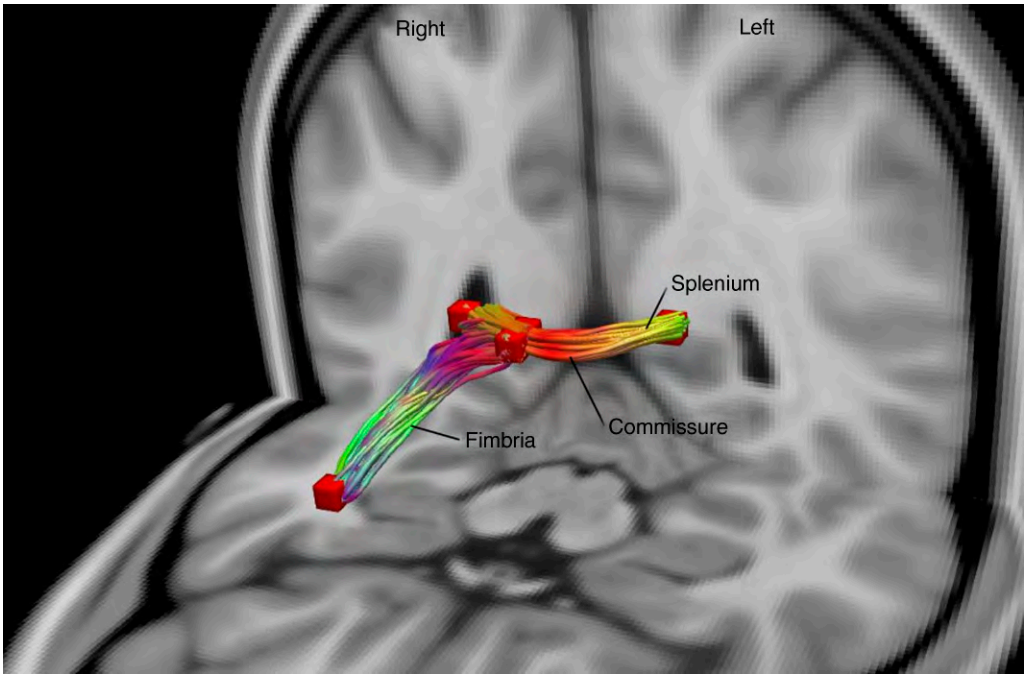


FIGURE 40.2 **The right fimbria of the hippocampus, hippocampal commissure, and splenium, comprising 84% fewer streamlines in cannabis users compared to nonusers.** Voxels interconnected by fewer streamlines are colored *red*, and the corresponding streamlines via which they are interconnected are colored such that: left–right is *red*, superior–inferior is *blue*, and anterior–posterior is *green*. *Source: Adapted with permission from Zalesky, A., Solowij, N., Yücel, M., Lubman, D. I., Takagi, M., Harding, I. H., Lorenzetti, V., Wang, R., Searle, K., Pantelis, C., Seal, M. (2012). Effect of long-term cannabis use on axonal fibre connectivity.* Brain, 135, 2245–2255.

the splenium of the corpus callosum bilaterally. This network comprised 7.0 ± 1.6 streamlines in users, and 44.0 ± 6.4 in nonusers, representing an 84% reduction ($p < 0.05$, corrected). The second network (Fig. 40.3) exclusively implicated a commissural fiber bundle beginning at the splenium of the corpus callosum, and extending to the right precuneus, which comprised 9.0 ± 1.7 streamlines in users, and 78.0 ± 15.1 in nonusers—an 88% reduction ($p < 0.05$, corrected). No significant gender effects were found, other than a trend level greater reduction in male users in the commissural fiber. FA was significantly lower in the corpus callosum in the cannabis users ($p = 0.01$) and FA, RD, and AD indices in the fimbria correlated with those in the corpus callosum ($p < .001$).

Group membership (user/nonuser) was the only significant regressor for streamline counts in both networks (fimbria: $p < 0.001$; commissural: $p < 0.001$), with alcohol, tobacco, trait anxiety, and depressive symptoms nonsignificant in the models. RD and AD were positively correlated with the age at which regular cannabis use commenced in both the fimbria (RD: $p = 0.02$, AD: $p = 0.06$) and commissural fiber (RD and AD: $p < 0.001$), remaining significant after controlling for age and duration of regular cannabis use. No associations with duration of regular cannabis use and cumulative lifetime dose were found for RD or AD, indicating a specific effect of age of onset of use during adolescence, and not simple brain maturation or dose-dependent effects.

Our findings are particularly compelling, given that our whole-brain investigation of white matter exclusively localized some of the very few fiber pathways that are particularly abundant with cannabinoid receptors during brain development (Romero et al., 1997; Molina-Holgado et al., 2002), and all of the cannabis users in our study commenced regular use during adolescence. The significant associations observed with age of onset of regular cannabis use provide evidence for white matter reacting differently to cannabis exposure commencing during adolescence, compared with adulthood, most likely due to the high concentration of cannabinoid receptors in the corpus callosum and fornix during adolescence.

Although users had greater trait anxiety and depressive symptoms, and lower Global Assessment of Functioning scores, these were not found to be significantly associated with measures of axonal integrity or connectivity, and nor were measures of impaired verbal learning and memory, or scores from a range of other cognitive tests. Future research should aim to replicate these findings, and determine the functional significance of such dramatic reductions in connectivity in cannabis users. Further, it is important to determine the extent to which these white matter abnormalities may recover following a substantial period of abstinence.

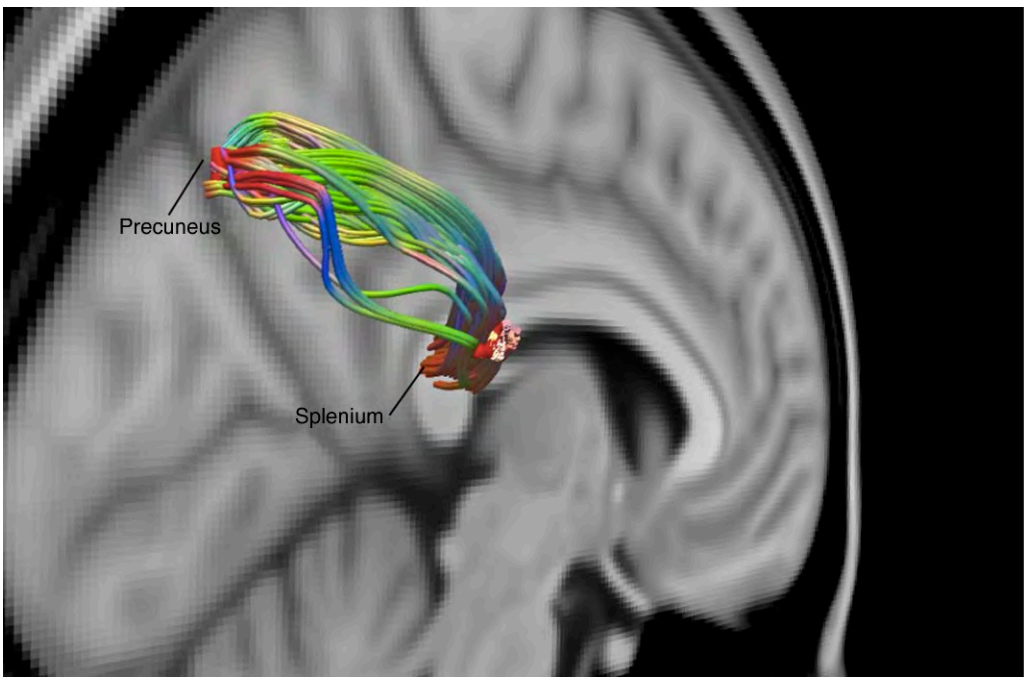


FIGURE 40.3 Streamlines interconnecting the right precuneus with the splenium, comprising an 88% reduction in cannabis users compared to nonusers. Voxels interconnected by fewer streamlines are colored *red*, and the corresponding streamlines via which they are interconnected are colored such that: left–right is *red*, superior–inferior is *blue*, and anterior–posterior is *green*. *Source: Adapted with permission from Zalesky, A., Solowij, N., Yücel, M., Lubman, D. I., Takagi, M., Harding, I. H., Lorenzetti, V., Wang, R., Searle, K., Pantelis, C., Seal, M. (2012). Effect of long-term cannabis use on axonal fibre connectivity.* Brain, 135, *2245–2255.*

FURTHER EVIDENCE

Additional studies continue to extend on prior findings. Bava et al. (2013) and Jacobus et al. (2013) recently reported on the longitudinal follow-up of their adolescent sample with substantial cannabis and alcohol use. Bava et al. (2013) report that greater alcohol consumption over an 18-month period predicted higher diffusivity in the superior longitudinal fasciculus and posterior corona radiate (thalamic fiber tracts), but there were no cannabis-related effects on diffusivity or FA. By the 3-year follow-up, Jacobus et al. (2013) report that 15 white matter clusters within projection, association, and interhemispheric fiber tracts differed between adolescent controls, alcohol, and heavy alcohol and cannabis users. In 14 of those clusters, the heavy alcohol and cannabis group showed significantly lower FA than controls, but no difference from those with heavy alcohol use alone and, in the remaining cluster (right uncinate fasciculus), the cannabis-exposed group showed higher FA than the group exposed to alcohol alone. Significant declines in white matter integrity from baseline to 3-year follow-up were not associated with poorer cognitive performance. Gruber et al. (2014) report reduced FA in a number of brain regions of cannabis users, particularly in early onset users, and an association between these changes and greater impulsivity. Yücel et al. (2010) report reduced FA in the corpus callosum of cannabis and inhalant users. White matter disturbances may be implicated from several further functional connectivity studies that have been reported (Filbey & Yezhuvath, 2013; Harding et al., 2012; Houck, Bryan, & Feldstein Ewing, 2013; Orr et al., 2013; Pujol et al., 2014). Direct investigations of the relatedness of functional and structural dysconnectivity are required in cannabis users, and are now possible by means of novel multimodal network imaging and analysis methods (Nixon et al., 2014; Preti et al., 2014; Sporns, 2014).

MECHANISMS OF AXONAL DISTURBANCE

CB1R are critically involved in the axonal growth process, and cannabinoid exposure during brain development is thought to disrupt neuronal specification, structural organization and, hence, synaptic wiring of neuronal networks (Berghuis et al., 2007; Cristino & Di Marzo, 2014). Pharmacological manipulation of CB1R had previously been shown to introduce corticofugal axon fasciculation errors, in particular enlargement of axons with reduced myelination (Mulder et al., 2008). Prenatal exposure to cannabinoids is now known to alter CB1R-mediated specification, and distance migration of neurons, axonal growth, and synapse formation in fetuses (Cristino & Di Marzo, 2014). In a series of elegant studies in mice and in human fetal postmortem brain tissue, Tortoriello et al. (2014) identify the molecular signaling cascade mechanisms directly involved in this disruption, thought to permanently impair wiring during corticogenesis. Superior Cervical Ganglion 10 (SCG10)/stathmin-2 was identified as a substrate of altered neuronal connectivity and axonal morphology, particularly in the hippocampus. This is a microtubule-binding protein in axons that contributes to the maintenance of cytoskeletal instability required for axonal growth and synaptic plasticity at Schaffer collaterals. Tortoriello et al. (2014) showed that repeated cannabinoid exposure in pregnant dams disrupted eCB signaling in offspring, particularly CB1R temporal dynamics and molecular cascades, to induce cytoskeletal modification in neuronal specification, disrupting the wiring of fetal cortical circuitry, particularly involving hippocampus, and ultimately altering axon morphology. The axonal specificity of CB1R-dependent SCG10 degradation was established, specifically implicating this mechanism in the modulation of axonal growth and guidance. The authors suggest that the mode of action of exogenous cannabinoids involves the disrupted development and maintenance of synapses critical for highly ordered executive and cognitive functions, and that altered neuronal integrity and connectivity may result in a range of adverse phenotypes, including cognitive deficits, psychological symptomatology, and risk-taking behaviors.

Gilbert and Soderstrom (2014) showed that repeated cannabinoid exposure in zebra finch during brain development (coinciding with song learning that these researchers have repeatedly demonstrated to be impaired) but not during adulthood, produces persistent changes in cytoskeletal proteins critical to both dendritic (MAP2) and axonal (Nf-200) morphology in CB1R-expressing brain regions, demonstrating that exogenous cannabinoids can alter normal brain development through these mechanisms. These same markers were previously shown to be altered in pubertal rats following chronic cannabinoid treatment (Tagliaferro et al., 2006).

FUTURE DIRECTIONS

Multimodal imaging approaches are particularly useful for furthering the field. Bernier et al. (2013) report a study protocol for examining the impact of early adolescent onset of cannabis use on white matter tissue integrity in young adults with schizophrenia (diagnosed within the past 5 years). They propose to assess indices of diffusion tensor imaging, proton magnetic resonance spectroscopy, transverse relaxation time constants of regional tissue water and of *N*-acetylaspartate (NAA) in a large frontal white matter fiber bundle – the left superior

longitudinal fasciculus, connecting the dorsal prefrontal and caudal-inferior parietal regions of the brain. Abnormalities in this fiber bundle have been reported in cannabis users as described earlier, as well as in people with schizophrenia. The combined approach will provide information on underlying cellular abnormalities of white matter in schizophrenia, and how this is perturbed by exposure to cannabis use during adolescence. Previous research has used magnetic resonance spectroscopy to assess neuronal integrity in cannabis users, finding reduced NAA levels in the dorsolateral prefrontal cortex (Hermann et al., 2007) and anterior cingulate cortex (Prescot, Locatelli, Renshaw, & Yurgelun-Todd, 2011; Prescot, Renshaw, & Yurgelun-Todd, 2013). NAA is the most concentrated molecule in the brain, after glutamate. We found an association between reduced NAA in hippocampus and smaller hippocampal volumes in adult cannabis users (Yücel et al., 2013), likely reflecting neuronal loss or damage, mitochondrial anomalies, or reduced synaptic connections. There is preliminary evidence also for an altered metabolic profile in white matter (Silveri, Jensen, Rosso, Sneider, & Yurgelun-Todd, 2011), and Bernier et al. (2013) propose to specifically interrogate white matter tissue where reduced NAA would indicate insufficient availability of acetate, one of the main building blocks necessary for myelin repair and maintenance. Examining the interplay between NAA, FA, and T2 relaxation time constant outcomes will enable greater attribution of specificity of disturbances to myelin sheaths, axons themselves, or involvement of oligodendrocytes. Other means by which the specificity of axons or myelin could be interrogated from MRI measures include the magnetization transfer ratio (MTR) contrast (eg, Chen et al., 2008) or *g*-ratio—the ratio of inner axon to total outer, including myelin, and circumference (eg, Chomiak & Hu, 2009).

Replication of our findings of 84–88% reduction in the streamline count of two specific localized fiber bundles in long-term very heavy cannabis users are required. Given these dramatic reductions, further research needs to identify their functional significance. Application of advanced DW-MRI methods will overcome inherent technical problems, particularly regarding crossing fibers that could potentially lead to spurious measures of reduced FA (Baker, Yücel, Fornito, Allen, & Lubman, 2013). Additional research might aim to address the extent to which abnormal white matter microstructure might precede the onset of substance use, and increase the risk for the development of substance use disorders. Prospective and longitudinal studies would be informative for understanding vulnerability to, and biological underpinnings of addiction, exposure consequences differentiated from those of other substance use, and potential recovery of white matter microstructure following abstinence (Baker et al., 2013). The mechanisms described earlier for specific eCB involvement in axonal structural development, and exogenous cannabinoid perturbation of these processes during gestation, provide solid evidence for persistent consequences of cannabinoid exposure at neurodevelopmentally critical times—whether similar mechanisms and their perturbation by cannabis use during adolescent neural maturation may underlie structural connectivity disturbances in cannabis users is an important question for future research.

MINI-DICTIONARY

AD Axial diffusivity refers to the magnitude of diffusion parallel to fiber tracts. Lower AD might reflect axonal injury, reduced axonal caliber, or less coherent orientation of axons. There is evidence that AD is not influenced by myelin.

DW-MRI Diffusion weighted magnetic resonance imaging is a neuroimaging technique that enables mapping of the diffusion of water molecules in brain tissue, which is highly directional in proximity to white matter tracts. The hydrophobic phospholipid bilayer of the myelin sheath restricts the flow of water perpendicular to axonal processes, resulting in anisotropic diffusion (see FA later). Analysis of DW-MRI measures involves tractography using tract-based spatial statistics of whole brain white matter, a whole brain voxelwise approach, or interrogation of hypothesis-driven regions of interest (ROI).

FA Fractional anisotropy refers to the fraction of diffusion that is directionally dependent (anisotropic). Lower FA might reflect damage to the myelin sheath surrounding axons, enlarged axonal diameter, reduced axonal packing density, or increased membrane permeability, but FA is not myelin specific.

MD Mean diffusivity is the overall directionally averaged magnitude of diffusion, and its increase reflects reduced white matter integrity due to either axonal or myelin degradation.

RD Radial diffusivity refers to the magnitude of diffusion perpendicular to fiber tracts. RD may be relatively more sensitive to myelin, but higher RD might reflect myelin loss, or loss of axons and/or reduced axonal packing density.

ACKNOWLEDGMENTS

This work was supported by the National Health and Medical Research Council of Australia (NHMRC Project Grant 459111 to N.S.; Career Development Fellowship GNT1047648 to A.Z.; Senior Research Fellowship Award 1021973 to M.Y.); and the Australian Research Council (ARC Future Fellowship FT110100752 to N.S.; DP0986320 to A.Z.).

References

Arevalo-Martin, A., Garcia-Ovejero, D., Rubio-Araiz, A., Gomez, O., Molina-Holgado, F., & Molina-Holgado, E. (2007). Cannabinoids modulate Olig2 and polysialylated neural cell adhesion molecule expression in the subventricular zone of post-natal rats through cannabinoid receptor1 and cannabinoid receptor2. *European Journal of Neuroscience*, *26*, 1548–1559.

Arnone, D., Barrick, T. R., Chengappa, S., Mackay, C. E., Clark, C. A., & Abou-Saleh, M. T. (2008). Corpus callosum damage in heavy marijuana use: preliminary evidence from diffusion tensor tractography and tract-based spatial statistics. *Neuroimage*, *41*, 1067–1074.

Ashtari, M., Cervellione, K., Cottone, J., Ardekani, B. A., Sevy, S., & Kumra, S. (2009). Diffusion abnormalities in adolescents and young adults with a history of heavy cannabis use. *Journal of Psychiatry Research*, *43*, 189–204.

Baker, S. T. E., Yücel, M., Fornito, A., Allen, N. B., & Lubman, D. I. (2013). A systematic review of diffusion weighted MRI studies of white matter microstructure in adolescent substance users. *Neuroscience and Biobehavioral Reviews*, *37*, 1713–1723.

Batalla, A., Bhattacharyya, S., Yücel, M., Fusar-Poli, P., Crippa, J. A., Nogue, S., Torrens, M., Pujol, J., Farre, M., & Martin-Santos, R. (2013). Structural and functional imaging studies in chronic cannabis users: a systematic review of adolescent and adult findings. *PLoS One*, *8*, e55821.

Bava, S., Frank, L. R., McQueeny, T., Schweinsburg, B. C., Schweinsburg, A. D., & Tapert, S. F. (2009). Altered white matter microstructure in adolescent substance users. *Psychiatry Research*, *173*, 228–237.

Bava, S., Jacobus, J., Mahmood, O., Yang, T. T., & Tapert, S. F. (2010). Neurocognitive correlates of white matter quality in adolescent substance users. *Brain and Cognition*, *72*, 347–354.

Bava, S., Jacobus, J., Thayer, R. E., & Tapert, S. F. (2013). Longitudinal changes in white matter integrity among adolescent substance users. *Alcoholism: Clinical and Experimental Research*, *37*(1), 181–189.

Berghuis, P., Rajnicek, A. M., Morozov, Y. M., Ross, R. A., Mulder, J., Urban, G. M., Monory, K., Marsicano, G., Matteoli, M., Canty, A., Irving, A. J., Katona, I., Yanagawa, Y., Rakic, P., Lutz, B., Mackie, K., & Harkany, T. (2007). Hardwiring the brain: endocannabioids shape neuronal connectivity. *Science*, *316*, 1212–1216.

Bernier, D., Cookey, J., McAllindon, D., Bartha, R., Hanstock, C. C., Newman, A. J., Stewart, S. H., & Tibbo, P. G. (2013). Multimodal neuroimaging of frontal white matter microstructure in early phase schizophrenia: the impact of early adolescent cannabis use. *BMC Psychiatry*, *13*, 264.

Chen, J. T., Collins, D. L., Atkins, H. L., Freedman, M. S., & Arnold, D. L. The Canadian MS/BMT Study Group. (2008). Magnetization transfer ratio evolution with demyelination and remyelination in multiple sclerosis lesions. *Annals of Neurology*, *63*, 254–262.

Chomiak, T., & Hu, B. (2009). What is the optimal value of the g-ratio for myelinated fibers in the rat CNS? A theoretical approach. *PLoS One*, *4*, e7754.

Clark, D. B., Chung, T., Thatcher, D. L., Pajtek, S., & Long, E. C. (2012). Psychological dysregulation, white matter disorganization and substance use disorders in adolescence. *Addiction*, *107*, 206–214.

Cristino, L., & Di Marzo, V. (2014). Fetal cannabinoid receptors and the "dis-joint-ed" brain. *The EMBO Journal*, *33*, 665–667.

De Bellis, M. D., Van Voorhees, E., Hooper, S. R., Gibler, N., Nelson, L., Hege, S. G., et al. (2008). Diffusion tensor measures of the corpus callosum in adolescents with adolescent onset alcohol use disorders. *Alcoholism: Clinical and Experimental Research*, *32*, 395–404.

Dekker, N., Schmitz, N., Peters, B. D., van Amelsvoort, T. A., Linszen, D. H., & de Haan, L. (2010). Cannabis use and callosal white matter structure and integrity in recent-onset schizophrenia. *Psychiatry Research*, *181*, 51–56.

Dennis, E. L., Jahanshad, N., McMahon, K. L., de Zubicaray, G. I., Martin, N. G., Hickie, I. B., Toga, A. W., Wright, M. J., & Thompson, P. M. (2013). Development of brain structural connectivity between ages 12 and 30: a 4-Tesla diffusion imaging study in 439 adolescents and adults. *Neuroimage*, *64*, 671–684.

Filbey, F. (2013). An introduction to "The addiction connectome: brain connectivity in drug and alcohol addiction". *American Journal of Drug and Alcohol Abuse*, *39*, 341–342.

Filbey, F., & Yezhuvath, U. (2013). Functional connectivity in inhibitory control networks and severity of cannabis use disorder. *American Journal of Drug and Alcohol Abuse*, *39*, 382–391.

Fornito, A., Zalesky, A., Pantelis, C., & Bullmore, E. (2012). Schizophrenia, neuroimaging and connectomics. *Neuroimage*, *62*, 2296–2314.

Gilbert, M. T., & Soderstrom, K. (2014). Developmental but not adult cannabinoid treatments persistently alter axonal and dendritic morphology within brain regions important for zebra finch vocal learning. *Brain Research*, *1558*, 57–73.

Glass, M., Dragunow, M., & Faull, R. L. (1998). Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience*, *77*, 299–318.

Gruber, S. A., Silveri, M. M., Dahlgren, M. K., & Yurgelun-Todd, D. (2011). Why so impulsive? White matter alterations are associated with impulsivity in chronic marijuana smokers. *Experimental and Clinical Psychopharmacology*, *19*, 231–242.

Gruber, S. A., Dahlgren, M. K., Sagar, K. A., Gonenc, A., & Lukas, S. E. (2014). Worth the wait: effects of age of onset of marijuana use on white matter and impulsivity. *Psychopharmacology (Berlin)*, *231*(8), 1455–1465.

Gulley, J. M., & Juraska, J. M. (2013). The effects of abused drugs on adolescent development of corticolimbic circuitry and behavior. *Neuroscience*, *249*, 3–20.

Harding, I. H., Solowij, N., Harrison, B. J., Tajagi, M., Lorenzetti, V., Lubman, D. I., Seal, M. L., Pantelis, C., & Yucel, M. (2012). Functional connectivity in brain networks underlying cognitive control in chronic cannabis users. *Neuropsychopharmacology*, *37*, 1923–1933.

Harkany, T., Keimpema, E., Barabas, K., & Mulder, J. (2008). Endocannabinoid functions controlling neuronal specification during brain development. *Molecular and Cellular Endocrinology*, *286*, S84–S90.

Hermann, D., Sartorius, H., Welzel, H., Walter, S., Skopp, G., Ende, G., & Mann, K. (2007). Dorsolateral prefrontal cortex *N*-acetylaspartate/total creatine (NAA/tCr) loss in male recreational cannabis users. *Biological Psychiatry*, *61*, 1281–1289.

Houck, J. M., Bryan, A. D., & Feldstein Ewing, S. W. (2013). Functional connectivity and cannabis use in high-risk adolescents. *American Journal of Drug and Alcohol Abuse*, *39*, 414–423.

Jacobus, J., McQueeny, T., Bava, S., Schweinsburg, B. C., Frank, L. R., Yang, T. T., & Tapert, S. F. (2009). White matter integrity in adolescents with histories of marijuana use and binge drinking. *Neurotoxicology and Teratology*, *31*, 349–355.

Jacobus, J., Squeglia, L. M., Bava, S., & Tapert, S. F. (2013). White matter characterization of adolescent binge drinking with and without co-occurring marijuana use: A 3-year investigation. *Psychiatry Research: Neuroimaging*, *214*, 374–381.

James, A., Hough, M., James, S., Winmill, L., Burge, L., Nijhawan, S., Matthews, P. M., & Zarei, M. (2011). Greater white and grey matter changes associated with early cannabis use in adolescent-onset schizophrenia (AOS). *Schizophrenia Research*, *128*, 91–97.

Kim, D.-J., Skosnik, P. D., Cheng, H., Pruce, B. J., Brumbaugh, M. S., Vollmer, J. M., Hetrick, W. P., O'Donnell, B. F., Sporns, O., Puce, A., & Newman, S. D. (2012). Structural network topology revealed by white matter tractography in cannabis users: a graph theoretical analysis. *Brain Connectivity*, *1*, 473–483.

Lebel, C., & Beaulieu, C. (2011). Longitudinal development of human brain wiring continues from childhood into adulthood. *Journal of Neuroscience*, *31*, 10937–10947.

Lorenzetti, V., Solowij, N., Fornito, A., Lubman, D. I., & Yücel, M. (2014). The association between regular cannabis exposure and alterations of human brain morphology: an updated review of the literature. *Current Pharmaceutical Design*, *20*, 2138–2167.

Malone, D. T., Hill, M. N., & Rubino, T. (2010). Adolescent cannabis use and psychosis: epidemiology and neurodevelopmental models. *British Journal of Pharmacology*, *160*, 511–522.

Molina-Holgado, E., Vela, J. M., Arevalo-Martin, A., Almazan, G., Molina-Holgado, F., Borrell, J., & Guaza, C. (2002). Cannabinoids promote oligodendrocyte progenitor survival: involvement of cannabinoid receptors and phosphatidylinositol-3 kinase/Akt signaling. *Journal of Neuroscience*, *22*, 9742–9753.

Mulder, J., Aguado, T., Keimpema, E., Barabas, K., Ballester Rosado, C. J., Nguyen, L., Monory, K., Marsicano, G., Di Marzo, V., Hurd, Y. L., Guillemot, F., Mackie, K., Lutz, B., Guzman, M., Lu, H. C., Galve-Roperh, I., & Harkany, T. (2008). Endocannabinoid signaling controls pyramidal cell specification and long-range axon patterning. *Proceedings of the National Academy of Sciences USA*, *105*, 8760–8765.

Nixon, N. L., Liddle, P. F., Nixon, E., Worwood, G., Liotti, M., & Palaniyappan, L. (2014). Biological vulnerability to depression: linked structural and functional brain network findings. *British Journal of Psychiatry*, *204*, 283–289.

Orr, C., Morioka, R., Behan, B., Datwani, S., Doucet, M., Ivanovic, J., Kelly, C., Weierstall, K., Watts, R., Smyth, B., & Garavan, H. (2013). Altered resting state connectivity in adolescent cannabis users. *American Journal of Drug and Alcohol Abuse*, *39*, 372–381.

Peters, B. D., de Haan, L., Vlieger, E. J., Majoie, C. B., den Heeten, G. J., & Linszen, D. H. (2009). Recent-onset schizophrenia and adolescent cannabis use: MRI evidence for structural hyperconnectivity? *Psychopharmacology Bulletin*, *42*, 75–88.

Prescot, A. P., Locatelli, A. E., Renshaw, P. F., & Yurgelun-Todd, D. A. (2011). Neurochemical alterations in adolescent chronic marijuana smokers: a proton MRS study. *Neuroimage*, *57*, 69–75.

Prescot, A. P., Renshaw, P. F., & Yurgelun-Todd, D. A. (2013). γ-Amino butyric acid and glutamate abnormalities in adolescent chronic marijuana smokers. *Drug and Alcohol Dependence*, *129*, 232–239.

Preti, M. G., Makris, N., Papadimitriou, G., Lagana, M. M., Griffanti, L., Clerici, M., Nemni, R., Westin, C. F., Baselli, G., & Baglio, F. (2014). A novel approach of groupwise fMRI-guided tractography allowing to characterize the clinical evolution of Alzheimer's disease. *PLoS One*, *9*, e92026.

Pujol, J., Blanco-Hinojo, L., Batalla, A., López-Solà, M., Harrison, B. J., Soriano-Mas, C., Crippa, J. A., Fagundo, A. B., Deus, J., de la Torre, R., Noguém, S., Farré, M., Torrens, M., & Martín-Santos, R. (2014). Functional connectivity alterations in brain networks relevant to self-awareness in chronic cannabis users. *Journal of Psychiatric Research*, *51*, 68–78.

Romero, J., Garcia-Palomero, E., Berrendero, F., Garcia-Gil, L., Hernandez, M. L., Ramos, J. A., & Fernandez-Ruis, J. J. (1997). Atypical location of cannabinoid receptors in white matter areas during rat brain development. *Synapse*, *26*, 317–323.

Schneider, M. (2008). Puberty as a highly vulnerable developmental period for the consequences of cannabis exposure. *Addiction Biology*, *13*, 253–263.

Silveri, M. M., Jensen, J. E., Rosso, I. M., Sneider, J. T., & Yurgelun-Todd, D. A. (2011). Preliminary evidence for white matter metabolite differences in marijuana-dependent young men using 2D J-resolved magnetic resonance spectroscopic imaging at 4 Tesla. *Psychiatry Research Neuroimaging*, *191*, 201–211.

Solowij, N., & Pesa, N. (2012). Cannabis and cognition: short and long-term effects. In D. Castle, R. Murray, & D. C. D'Souza (Eds.), *Marijuana and madness* (2nd ed., pp. 91–102). Cambridge, UK: Cambridge University Press.

Solowij, N., Yücel, M., Lorenzetti, V., & Lubman, D. I. (2009). Structural brain alterations in cannabis users: association with cognitive deficits and psychiatric symptoms. In M. S. Ritsner (Ed.), *The handbook of neuropsychiatric biomarkers, endophenotypes, and genes. Vol. II: Neuroanatomical and neuroimaging endophenotypes and biomarkers* (pp. 215–225). Dordrecht, the Netherlands: Springer.

Solowij, N., Yücel, M., Respondek, C., Whittle, S., Lindsay, E., Pantelis, C., & Lubman, D. I. (2011). Cerebellar white matter changes in cannabis users with and without schizophrenia. *Psychological Medicine*, *41*, 2349–2359.

Sporns, O. (2014). Contributions and challenges for network models in cognitive neuroscience. *Nature Neuroscience*, *17*, 652–660.

Sporns, O., Tononi, G., & Kötter, R. (2005). The human connectome: a structural description of the human brain. *PLoS Computational Biology*, *1*, e42.

Tagliaferro, P., Ramosa, A. J., Onaivi, E. S., Evrarda, S. G., Lujilde, J., & Brusco, A. (2006). Neuronal cytoskeleton and synaptic densities are altered after a chronic treatment with the cannabinoid receptor agonist WIN 55,212-2. *Brain Research*, *1085*, 163–176.

Tortoriello, G., Morris, C. V., Alpar, A., Fuzik, J., Shirran, S. L., Calvigioni, D., Keimema, E., Botting, C. H., Reinecke, K., Herdegen, T., Courtney, M., Hurd, Y. L., & Harkany, T. (2014). Miswiring the brain: Δ^9-tetrahydrocannabinol disrupts cortical development by inducing an SCG10/stathmin-2 degradation pathway. *EMBO Journal*, *33*, 668–685.

Yücel, M., Lorenzetti, V., Takagi, M., Harding, I. H., Pantelis, C., Fornito, A., Seal, M., Lubman, D. I., & Solowij, N. (2013). Reduced concentration of N-acetyl-aspartate associated with volume reduction of the hippocampus in heavy cannabis users. *Biological Psychiatry*, *73*, 311S.

Yücel, M., Solowij, N., Respondek, C., Whittle, S., Fornito, A., Pantelis, C., & Lubman, D. I. (2008). Regional brain abnormalities associated with long-term heavy cannabis use. *Archives of General Psychiatry*, *65*, 694–701.

Yücel, M., Zalesky, A., Takagi, M., Bora, E., Fornito, A., Ditchfield, M., et al. (2010). White-matter abnormalities in adolescents with long-term inhalant and cannabis use: a diffusion magnetic resonance imaging study. *Journal of Psychiatry and Neuroscience*, *35*, 409–412.

Zalesky, A., Fornito, A., & Bullmore, E. T. (2010). Network-based statistic: identifying differences in brain networks. *Neuroimage*, *53*, 1197–1207.

Zalesky, A., Solowij, N., Yücel, M., Lubman, D. I., Takagi, M., Harding, I. H., Lorenzetti, V., Wang, R., Searle, K., Pantelis, C., & Seal, M. (2012). Effect of long-term cannabis use on axonal fibre connectivity. *Brain*, *135*, 2245–2255.

CHAPTER

41

Microglial Activation and Cannabis Exposure

L. Cutando, R. Maldonado, A. Ozaita

Department of Experimental and Health Sciences, Laboratory of Neuropharmacology, School of Health and Life Sciences, Pompeu Fabra University, Barcelona, Spain

SUMMARY POINTS

- Microglia is a surveillance cell population sensitive to changes in the brain parenchyma composition, and to cytokines and other cell mediators.
- Microglial cells may change their phenotype from a resting/surveillance state to an activated state, characterized by morphological but also altered genes expression that results in neuroinflammation.
- Components of the endocannabinoid system, such as cannabinoid receptors, are differentially expressed in the diverse stages on microglia reactivity.
- Cannabis components may produce an antineuroinflammatory response through the interaction with cannabinoid receptors in activated microglia.
- Chronic Δ^9-tetrahydrocannabinol (THC) administration affects cerebellar microglia due to the downregulation of cannabinoid receptor type I (CB1 receptors) in parallel fiber terminals.
- After THC withdrawal, cerebellar microglia reactivity increases due to the deregulation of CB1 receptors, affecting cerebellar function. Under these conditions, inhibitors of microglial reactivity normalize cerebellar function even when CB1 receptors remain downregulated.

KEY FACTS OF CYTOKINES

- Term applied to small protein and polypeptide mediators synthesized and released by immune cells, such as microglia, during inflammation.
- Cytokines include interleukins, chemokines, interferons, colony-stimulating factors, growth factors, and tumor necrosis factors.
- They affect cell function through autocrine and paracrine mechanisms, by the activation of specific, high-affinity receptors usually coupled to kinase activity or to G-proteins.
- They have a role in the induction of the immune response, but also as pro- and antiinflammatory agents.
- Interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) are the primary proinflammatory cytokines.

LIST OF ABBREVIATIONS

Abn-CBD	Abnormal-cannabidiol receptors
2-AG	2-Arachidonoylglycerol
AEA	*N*-arachidonoylethanolamine or anandamide
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ATP	Adenosine-5′-triphosphate
CB1 receptor	Type I cannabinoid receptor
CB2 receptor	Type II cannabinoid receptor
CBD	Cannabidiol

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00047-8
Copyright © 2017 Elsevier Inc. All rights reserved.

CNS	Central nervous system
DAMP	Danger-associated molecular pattern molecules
eCB	Endocannabinoids
ECS	Endogenous cannabinoid system
GPR 18	G protein-coupled receptor 18
GPR 55	G protein-coupled receptor 55
IFN-gamma	Interferon gamma
IL	Interleukin
LPS	Lipopolysaccharide
NAGly	*N*-arachidonoyl glycine
NF-κβ	Nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	*N*-methyl-D-aspartic acid or *N*-methyl-D-aspartate receptor
NO	Nitric oxide
PAMP	Pathogen-associated molecular pattern molecules
PEA	Palmitoylethanolamide
PPAR	Peroxisome proliferator-activated receptor
SIRPα	Signal regulatory protein alpha receptor
TGF-beta	Transforming growth factor beta
THC	Δ^9-Tetrahydrocannabinol
TLR	Toll-like receptors
TNF-α	Tumor necrosis alpha
TREM2	Triggering receptor expressed on myeloid cells 2
TRPV	Transient receptor potential cation channel subfamily vanilloid
UTP	Uridine-5′-triphosphate

OVERVIEW OF MICROGLIA

Microglial cells are the immune effector cells in the central nervous system (CNS), where they comprise approximately 10% of the total cell brain population (Lawson, Perry, Dri, & Gordon, 1990; Mittelbronn, Dietz, Schluesener, & Meyermann, 2001). They are considered the macrophages of the brain. Microglial cells are differently distributed along the brain parenchyma, where their density varies depending on the brain region, going from 5% in corpus callosum to 12% in substantia nigra (Lawson et al., 1990), and are more abundant in gray matter compared to white matter (Kofler & Wiley, 2011). Microglial cells have a hematopoietic origin and, in mammals, they derive from the invasion of peripheral mesodermal primitive macrophages arising from the yolk sac (Alliot, Godin, & Pessac, 1999). During early embryogenesis, they migrate into the CNS and, under the influence of different molecules and transcription microglial precursors, differentiate and proliferate from an amoeboid to a more mature ramified phenotype. By the time of birth, an important amount of microglial cells are present in the brain, forming a dense network along the parenchyma contributing to brain homeostasis (Saijo & Glass, 2011). Microglial cells have been implicated in cellular and synaptic maintenance, myelin homeostasis, and responses to brain damage. These cells act as local sentinels of the CNS, playing also a key role in the innate immune response of the brain. Once microglial cells are activated by the so-called "On" signals (Fig. 41.1), they express different surface markers and release diverse substances such as cytokines, reactive oxygen species, and nitric oxide (NO), which may exacerbate the surrounding neuronal damage, and complicate the synaptic homeostasis (Kofler & Wiley, 2011; Benarroch, 2013). Upon infection or brain injury, activated microglial cells efficiently phagocyte pathogens and damage dying cells. It is well known that excessive microglial cell activation may produce pathological situations contributing to the pathogenesis of neurodegenerative diseases (Hanisch & Kettenmann, 2007). However, under certain conditions, microglial cells have an alleviating role, promoting neuroprotective and neurotrophic functions (Saijo & Glass, 2011). In addition, microglial cells have an important role removing synapses from injured neurons, a process called *synaptic stripping* (Kettenmann, Kirchhoff, & Verkhratsky, 2013). Thus, microglial cells may play a key role exerting proinflammatory, cytotoxic, immunoregulatory, and repairing functions. Because of this wide variety of processes and functions, this cell type has been the focus of much research in recent years. The accumulated information shows that microglia plays a crucial role in the control of neuronal activity, becoming an extremely important player in brain function.

MICROGLIAL ACTIVATION PROCESS

Microglial cells may be found in the brain in two different states, depending on their morphology and the expression of activation markers: "resting state" and "activated state". Under basal conditions (absence of brain injury or alteration), microglial cells present a "resting" phenotype characterized by small soma and highly ramified and elongated branches (Cuadros & Navascués, 1998). In these quiescent conditions, microglial cells form an organized net of cells along the brain parenchyma, where each microglial cell scavengers its own immediate area without overlapping their processes with those of the neighboring microglial cell. In the ramified form, microglial cells actively move their processes without translocation of the soma. Indeed, in vivo two-photon laser-scanning microscopy experiments revealed that microglial cells present highly dynamic processes in intact healthy brains, and can elongate or retract more than 1–3 μm/min, voiding the idea that microglia from healthy brain is a resting component (Nimmerjahn,

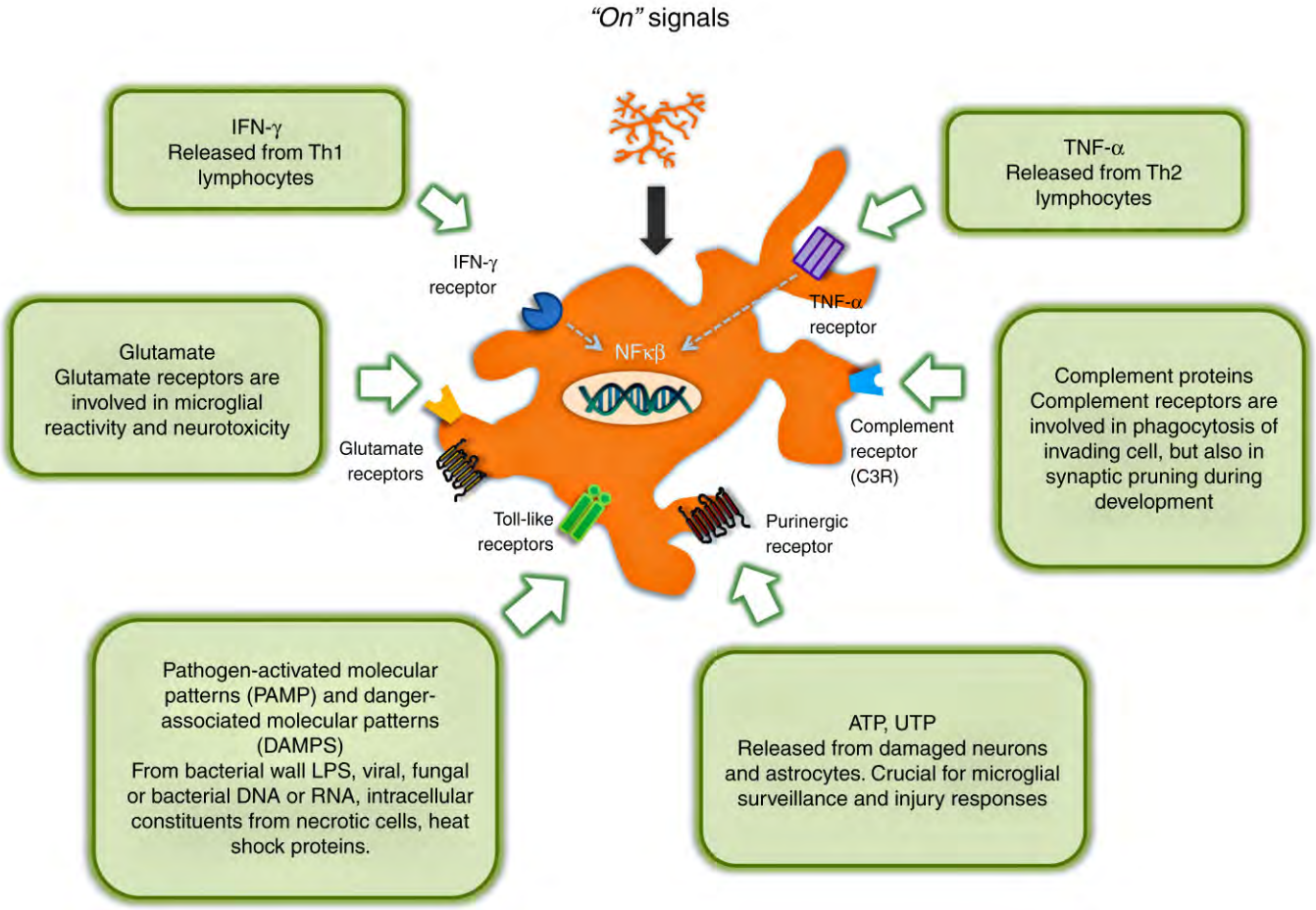


FIGURE 41.1 **Schematic drawing of "On" signals that trigger the microglial activation.** Resting/surveillance microglia turns activated in response to the presence in the brain parenchyma of IFN-gamma, glutamate, PAMPs or DAMPs, ATP or UTP, complement proteins or TNF-α.

Kirchhoff, & Helmchen, 2005). In this regard, microglial cells act as "active sensors" of their environment, in order to survey their own parcel of parenchyma. Microglial motility control depends on extracellular adenosine-5′-triphosphate (ATP) concentration level, thus a reduction in ATP concentration by ATP-hydrolyzing enzyme apyrase decreases microglial motility, whereas artificial ATP gradients enhance their motility (Davalos et al., 2005). Purinergic P2Y12 metabotropic receptors seem to be involved in these responses (Haynes et al., 2006). Therefore, microglial resting state is an active surveillance state of the surrounding extracellular milieu.

After brain injury or upon recognition of a pathogen, microglial cells become activated, retracting their processes and enhancing the perimeter of their soma, and move following chemotactic gradients to the site of lesion, or to contact the aggressor pathogen. Activated microglial cells are really heterogeneous and plastic cells, and they could present different phenotypes, depending on the type of stimulus, or the environmental factors that produced their activation. Thus, activated microglial cells executing a number of adaptive responses to a given challenge are also known as "effector cells," and they may present really different phenotypes, such as amoeboid, rod, or multinucleated morphologies (Hanisch & Kettenmann, 2007).

Microglial activation is tightly controlled, and sensitive to different signals that can turn on or inhibit this process in order to orchestrate the wide range of microglial functions. As mentioned above, microglial activation may occur due to "On" signals that appear in the brain, and mediate the microglial activation, or from the reduced expression of "Off" signals (those that maintain microglial cells in resting state) (Biber, Neumann, Inoue, & Boddeke, 2007). Multiple "On" signals have been described to activate microglia through microglial toll-like receptors (TLR), such as infection signals like bacterial wall lipopolysaccharides, bacterial and viral RNA or DNA, viral structural envelopes, intracellular elements released from necrotic cells, heat shock proteins, among others (PAMPs and DAMPs). Other molecules, such as complement proteins, interferon-γ cytokine (IFN) released from Th1 lymphocytes or TNF-α released from Th2 lymphocytes, activate innate immune responses

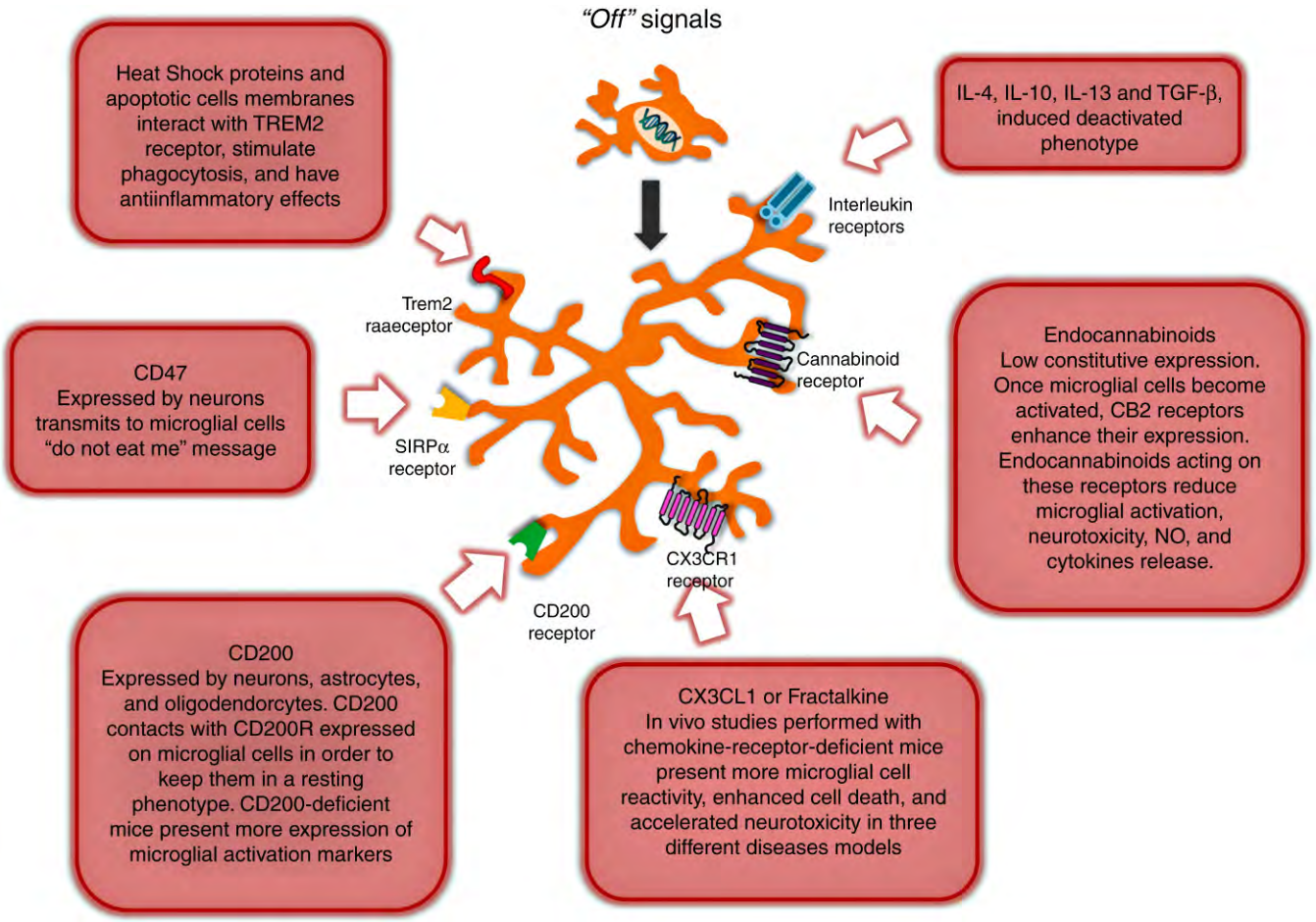


FIGURE 41.2 **Schematic drawing of "Off" signals that reduce microglial activation.** Activated microglia is sensitive to Heat Shock proteins and apoptotic cell membranes, CD47, CD200, CX3CL1, cannabinoid receptor agonists, antiinflammatory cytokines (IL-4, IL-10, IL-13, TGF-beta).

triggering the transcriptional activation of proinflammatory genes through the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ) (Benarroch, 2013; Hanisch & Kettenmann, 2007; Kierdorf & Prinz, 2013; Kettenmann, Hanisch, Noda, & Verkhratsky, 2011). In addition, microglial cells also present purinergic P2X ionotropic receptors (mainly P2X4 and P2X7) and/or P2Y metabotropic receptors (P2Y2, P2Y6, and P2Y12) for UTP and ATP, which are crucial for the role of microglial cells in surveillance and injury responses. ATP released from damaged neurons and astrocytes may induce microglial activation and processes motility (Inoue & Tsuda, 2012; Suzuki et al., 2004, Ohsawa et al., 2007). Furthermore, microglial cells also express AMPA-type glutamate receptors, kainate receptors, and metabotropic glutamate receptors. Activation of AMPA receptors is linked to TNF-α release, and glutamate-induced chemotaxis, while group II metabotropic glutamate receptors trigger neurotoxicity and microglial reactivity (Kettenmann et al., 2011).

Finally, microglial cells also present adenosine, cholinergic, adrenergic, dopamine, and cytokine receptors. The stimulation of these receptors may modulate microglial proliferation, cytokines release, gene expression, and NO production (Kettenmann et al., 2011).

On the other hand, "Off" signals produced by cells surrounding microglia have the purpose to keep microglial cells in a "resting" phenotype imposing a "calming" influence on microglial cells (Fig. 41.2). One of these molecules is CD200, expressed by neurons, astrocytes, and oligodendrocytes that interacts with CD200 receptor (CD200R) expressed by microglial cells. In vivo studies using CD200R-deficient mice demonstrated that CD200R expressed by microglia is important to maintain these cells in a resting phenotype. The lack of this receptor produces an enhancement in CD45 and CD11b expression (markers of microglial activation), together with a less ramified morphology in the microglial cells (Cardona et al., 2006). Other studies clearly demonstrated the crucial role of CX3CL1 chemokine (also known as fractalkine or neurotactin) in the regulation of the microglial cell reactivity, when bound to CX3CR1 receptors expressed in microglial cells. CX3CL1 is highly expressed in CNS parenchyma under healthy conditions.

In vivo studies demonstrated that chemokine-receptor-deficient mice present microglial cells with an overactivated phenotype, enhanced cell death, and accelerated neurotoxicity in different disease models (Cardona et al., 2006; Sheridan & Murphy, 2013). In addition, other inhibitory surface receptors or molecules expressed by microglial cells interact with ligands secreted or expressed by neurons. One of these molecules is the signal regulatory protein α (SIRPα) receptor which interacts with CD47 expressed by neurons to prevent phagocytosis (Biber et al., 2007). Furthermore, another molecule expressed by microglial cells is the triggering receptor expressed on myeloid cells 2 (TREM2), associated with the adapter protein DAP12. This molecule is essential for phagocytosis of membranes from apoptotic cells and microglial clearance function, and its activation has antiinflammatory effects (Takahashi, Prinz, Stagi, Chechneva, & Neumann, 2007). Moreover, it is known that the interactions between IL-4, IL-10, IL-13, and the transforming growth factor-β (TGF-β) with their receptors expressed on microglial cells reduces the microglial activated phenotype (Kettenmann et al., 2011). Finally, microglial cells also express type I (CB1) and type II (CB2) cannabinoid receptors, as well as other cannabinoid-like receptors (Fig. 41.2). The constitutive expression of these receptors is very low in resting microglial cells, and a rapid enhancement of CB2 receptor expression appeared once microglial cells become activated (Stella, 2009, 2010). The stimulation of cannabinoid receptors has been reported to reduce microglial activation and neurotoxicity (Stella, 2009, 2010).

ENDOGENOUS CANNABINOID SYSTEM IN MICROGLIA

The possibility to reduce the microglial proinflammatory responses in some neurodegenerative diseases or after brain injury, maintaining their antiinflammatory properties, has been extensively studied. A number of evidences underline the interest to target the endogenous cannabinoid system (ECS) for these purposes. In this regard, microglial cells express different types of cannabinoid receptors that regulate microglial cell migration and activation, as well as proinflammatory responses. Moreover, microglial cells can produce several neurotransmitters, including endocannabinoids (eCBs) that can directly affect neuronal functions. As explained in the next paragraphs, the impact of cannabis exposure on microglia will depend on the activation state of this cell population, but also on the adaptive changes that cannabis compounds produce on the ECS that may modulate microglial reactivity. In this chapter we will explain the effects of cannabis on microglia, focusing our attention on its main psychoactive component, Δ^9-tetrahydrocannabinol (THC). This interaction has an impact on cerebellar functions, and the mechanisms involved have been recently addressed (Cutando et al., 2013).

Direct Effect of Cannabinoid Agonists on Cannabinoid-Like Receptors

As previously reported, CB1 and CB2 receptors are expressed in microglial cells, although their expression depends on the microglial activation profile. Thus, microglial cells become activated from resting/surveillance phenotype in response to "On" signals. A very low expression of CB1 and CB2 receptors is present in resting microglia, which can only be found in intact healthy brain tissue. Microglial activation rapidly upregulates CB2 receptors "on demand," being highly expressed in activated states (Carlisle, Marciano-Cabral, Staab, Ludwick, & Cabral, 2002; Walter et al., 2003; Stella, 2010). Moreover, it has been demonstrated that microglial primary cultures are intrinsically activated because of the transferring procedure to the culture plate (Becher & Antel, 1996). Indeed, both microglial primary cultures, and microglial cell lines such as BV-2, frequently used in research, express high levels of CB2 receptors (Carlisle et al., 2002; Stella, 2009; Carrier et al., 2004). Furthermore, it has been described that CB2 receptor expression is upregulated in pathological conditions such as Alzheimer disease, Huntington disease, or AIDS-associated dementia (Benito et al., 2003; Palazuelos et al., 2009; Kim, Shin, & Thayer, 2011). Yiangou et al. (2006) also found enhanced levels of CB2 receptor during amyotrophic lateral sclerosis and multiple sclerosis development in spinal cord samples. Therefore, cannabinoids may have therapeutic potential in some of these disorders, acting on the CB2 receptor. In this sense, cannabinoids may improve motor symptoms in a model of Huntington disease targeting CB2 receptors (Palazuelos et al., 2009), and prevent microglial reactivity and the neurotoxicity induced by β-amyloid in models of Alzheimer disease (Ramírez, Blázquez, Gómez del Pulgar, Guzmán, & de Ceballos, 2005). Furthermore, an upregulation of CB2 mRNA expression related with an enhancement in the microglial reactivity process in the spinal cord has been reported in a model of neuropathic pain (but not in a model of chronic inflammatory pain) (Zhang et al., 2003). In summary, experimental evidences show that the stimulation of CB2 receptors by agonists (Fig. 41.3) modulates microglial reactivity, but also chemotaxis, proliferation, phagocytosis, migration, and infiltration into brain areas with neuroinflammation. As a consequence, CB2 receptor agonists have been postulated for the treatment of different neurodegenerative diseases.

On the other hand, it has been described that microglial cells from different species also express CB1 receptors.

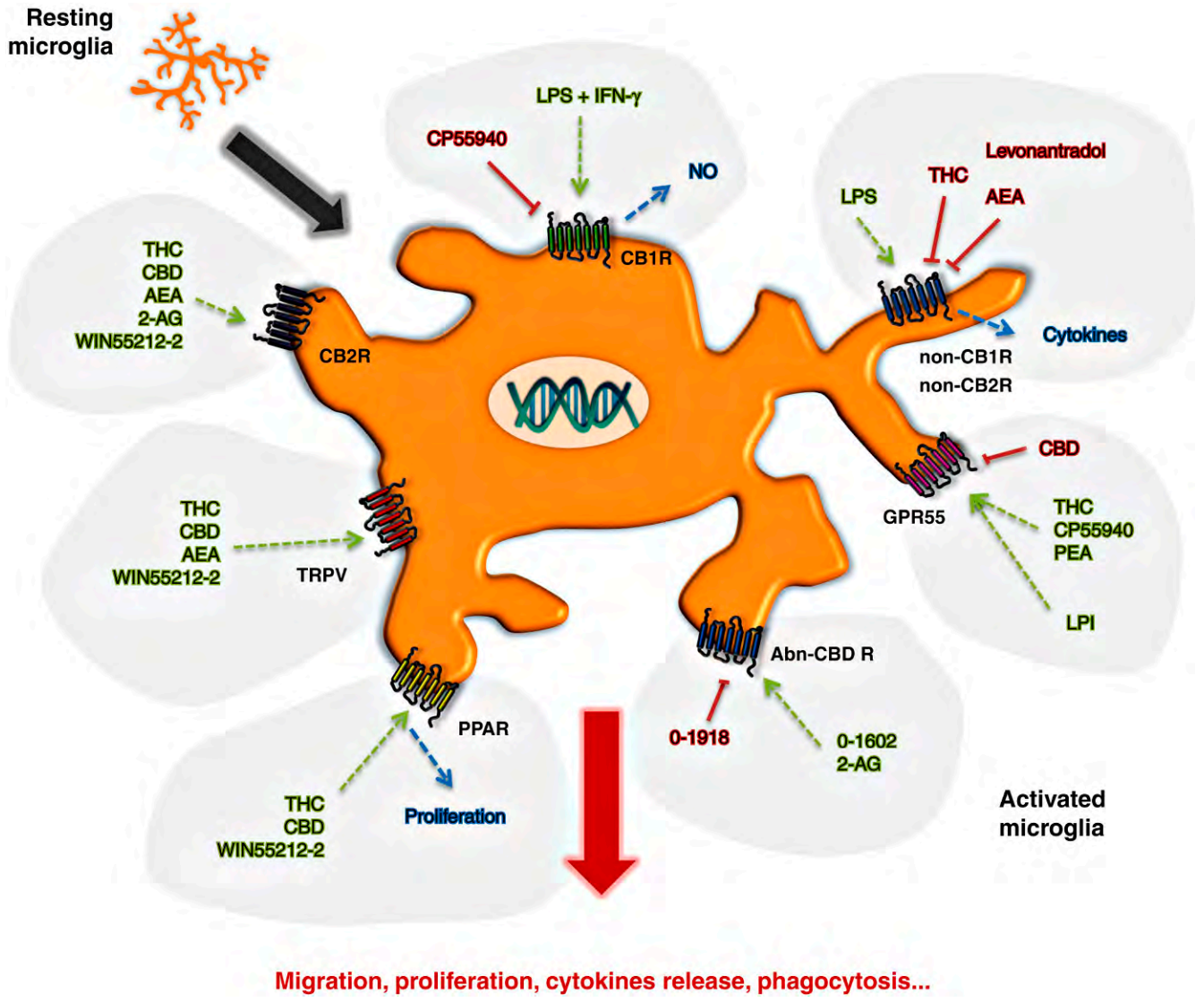


FIGURE 41.3 **Schematic drawing of potential sites in activated microglia sensitive to cannabinoid compounds.** Activated microglia is sensitive to cannabinoid drugs through different receptors described in microglial cells: CB1, CB2, GPR55, abn-CBD, PPAR, TRPV. *Green arrows* denote a decrease in microglial activation, while *red bars* denote an increase in microglial reactivity. *Blue arrows* indicate a cellular response significant for microglia state change.

This cannabinoid receptor has been related with NO production, an inflammatory mediator released from activated microglial cells (Waksman, Olson, Carlisle, & Cabral, 1999). Indeed, NO production induced by lipopolysaccharide (LPS) and interferon-γ was partially inhibited by the cannabinoid agonist CP55940. Moreover, functional studies performed previously also demonstrated that the CB1-selective antagonist SR141716A could prevent the inhibition of NO production mediated by CP55940 (Rinaldi-Carmona et al., 1994). Altogether, these results clearly demonstrate the role of CB1 receptors regulating NO production by activated microglial cells (Fig. 41.3).

In addition, other studies demonstrated that cannabinoids, such as THC, and anandamide, inhibit LPS-inducible cytokine mRNA expression for IL-6, IL-1β, IL-1α, and TNF-α in cortical rat neonatal microglial cells. The same inhibitory outcome was observed with the synthetic cannabinoid analogs of dronabinol, the enantiomers levonantradol and dextronantradol. Neither the CB1-selective antagonist SR141716A, nor the CB2-selective antagonist SR144528 were able to reverse the inhibition of cytokine mRNA expression by levonantradol, suggesting that the blockade of cytokine mRNA expression by cannabinoids is apparently mediated through cannabinoid receptors, unlike CB1 and CB2 receptor. Indeed, other cannabinoid-like receptors different from CB1 and CB2 may be involved in the microglial immune-related response, when high concentrations of cannabinoid agonists are used (Puffenbarger, Boothe, &

Cabral, 2000). Candidates are G protein-coupled receptor 55 (GPR55), abnormal-cannabidiol receptors (abn-CBD), peroxisome proliferator-activated receptor (PPAR), transient receptor potential cation channel (TRP), and WIN 55,212-2-like receptors (Fig. 41.3). All these receptors are expressed in microglial cells, and respond to endogenous or synthetic cannabinoids in different ways in order to modulate microglial activation processes.

GPR55 is expressed in healthy brain and exhibits 13.5 and 14.1% sequence homology to CB1 and CB2 receptors, respectively (Baker, Pryce, Davies, & Hiley, 2006). GPR55 mRNA is expressed and functional in BV-2 cells and in mouse microglia, and its activation mediates similar changes to those determined in CB2 receptor (Pietr et al., 2009). These results suggest that GPR55 and CB2 are involved in neuroinflammation processes, and that their expression depends on the activation state of the microglial cells. A role for GPR55 has been recently reported in neuroprotection using an excitotoxicity model on rat organotypic hippocampal slice cultures (Kallendrusch et al., 2013). Indeed, the GPR55 selective agonist l-α-lysophosphatidylinositol (LPI) prevents dentate gyrus granule cells excitotoxic damaged and reduces the number of activated microglia after NMDA induced lesions, suggesting a clear role of GPR55 receptor in neuroprotection (Fig. 41.3).

Moreover, it has been also demonstrated that the synthetic atypical cannabinoid closely related to cannabidiol O-1602 can activate cannabinoid-like receptors, such the abn-CBD receptor GPR18 and GPR55. It has been shown that the effect of O-1602 was antagonized by the synthetic compound O-1918, also related to cannabidiol (Ashton, 2012). In addition, McHugh, Wager-Miller, Page, and Bradshaw, (2012) determined that GPR18 was the abn-CBD receptor involved in the BV-2 microglial cell migration and chemotaxis. These researchers demonstrated that NAGly (an enzymatically oxygenated metabolite of the endocannabinoid *N*-arachidonoyl ethanolamide, anandamide AEA) initiates directed microglial migration in the CNS through activation of GPR18 (McHugh et al., 2012). Similarly, it was previously shown that 2-arachidonoylglycerol (2-AG) can stimulate BV-2 cell chemotaxis and migration (Franklin & Stella, 2003). In addition, 2-AG acting on abn-CBD receptors expressed on microglial cells reduces microglial reactivity and toxicity on excitotoxically lesioned dentate gyrus granule cells (Kreutz et al., 2009) (Fig. 41.3).

PPAR are also expressed in microglial cells. Different cannabinoids like 2-AG, AEA, palmitoylethanolamide (PEA), WIN 55,212-2, CBD, and THC interact with PPAR to modulate their activity. Some studies demonstrated the role of these receptors in the regulation of microglial activation process, after brain injury. Indeed, PPAR-γ from microglial cells control ischemia-induced microglial activation and neuronal damage/death in the dentate gyrus of the hippocampus, after transient global cerebral ischemia (Lee & Won, 2014). In addition, an inflammatory and neuroprotective action of PPAR-γ agonists through the modulation of CD200, mainly expressed in neurons but also in astrocytes, and CD200R, mainly present in microglia, has been recently demonstrated (Dentesano et al., 2014). CD200R receptors are involved in maintenance of the microglial proinflammatory activated phenotype, and alterations in this mechanism are related to neurodegenerative diseases. Considering the therapeutic value of these targets, more intensive research will be necessary to increase our understanding of the possible consequences of the modulation of PPAR receptors.

Microglial cells are also TRPV receptors. It has been demonstrated that TRPV1 and TRPV4 stimulation from BV-2 and microglial cells may modulate the abnormal microglial activation process in an acute model of inflammation (Raboune et al., 2014), and are induced by LPS (Konno et al., 2012). Cannabinoid agonists including THC, CBD, WIN 55,212-2, AEA, and the antagonist SR141716 have been described to operate on this ion channel at micromolar concentrations, and modify its action. In this sense, CBD enhances microglial phagocytosis via TRPV channel activation, evidencing the pharmacological potential of TRPV channel for treating neuroinflammatory disorders involving changes in microglial function (Hassan et al., 2014).

As mentioned above, endocannabinoids act on microglial cells to modulate their functions and activation. Several studies demonstrated the effect of 2-AG on the regulation of microglial reactivity. 2-AG, acting on CB2 receptors, triggers microglial migration, decreases proinflammatory molecule release, and enhances BDNF release and microglial proliferation (Carrier et al., 2004). These results evidence that, after brain damage, the ECS responds by enhancing local 2-AG production in order to recruit new microglial cells to face and solve the brain damage.

Similar results were found for PEA which interacts with an orphan receptor expressed by microglial cells, and regulates microglial activation and recruitment after focal cerebral ischemia (FCI) damage induction (Franklin & Stella, 2003; Stella, 2010). The neuroinflammation process in the injured brain area after FCI damage enhanced microglial cell motility, as well as the PEA levels. PEA acts through an orphan Gi/o-coupled receptor, and this receptor would be distinct from CB1, CB2, and abn-CBD receptors (Franklin & Stella, 2003). PEA interacts with PPAR-α in the micromolar range, and reduces the number of microglial cells, protecting dentate gyrus granule cells in excitotoxically damaged organotypic hippocampal slice cultures (Koch et al., 2011). These results suggest PPAR-α receptors as a possible target for therapeutic interventions to mitigate symptoms of secondary neuronal damage.

Taken together, these studies evidence the importance of the ECS in microglial physiology. Cannabinoid

agonists acting through microglial cannabinoid-like receptors modulate several processes in microglial cells, such as activation, proliferation, cytokines, and NO release, migration and/or phagocytosis postulating the microglial ECS as a key element in the treatment of neuroinflammation

Microglia as a Downstream Effector of ECS Deregulation: Cerebellar Motor Impairment

Chronic cannabis exposure may affect brain microglial reactivity through an indirect mechanism recently described that results in motor coordination deficits beyond the cannabis consumption (Cutando et al., 2013). The repeated administration of THC in healthy naïve mice enhances microglial reactivity in the cerebellum (Fig. 41.4). This effect was related to the impact that repeated cannabis administration has on CB1 receptor expression. In rodents receiving THC, but also in heavy cannabis smokers, repeated cannabis exposure results in strong downregulation and desensitization of CB1 receptors. In the cerebellum, a brain region crucial for motor learning and fine motor coordination, the downregulation of CB1 receptor by chronic THC was

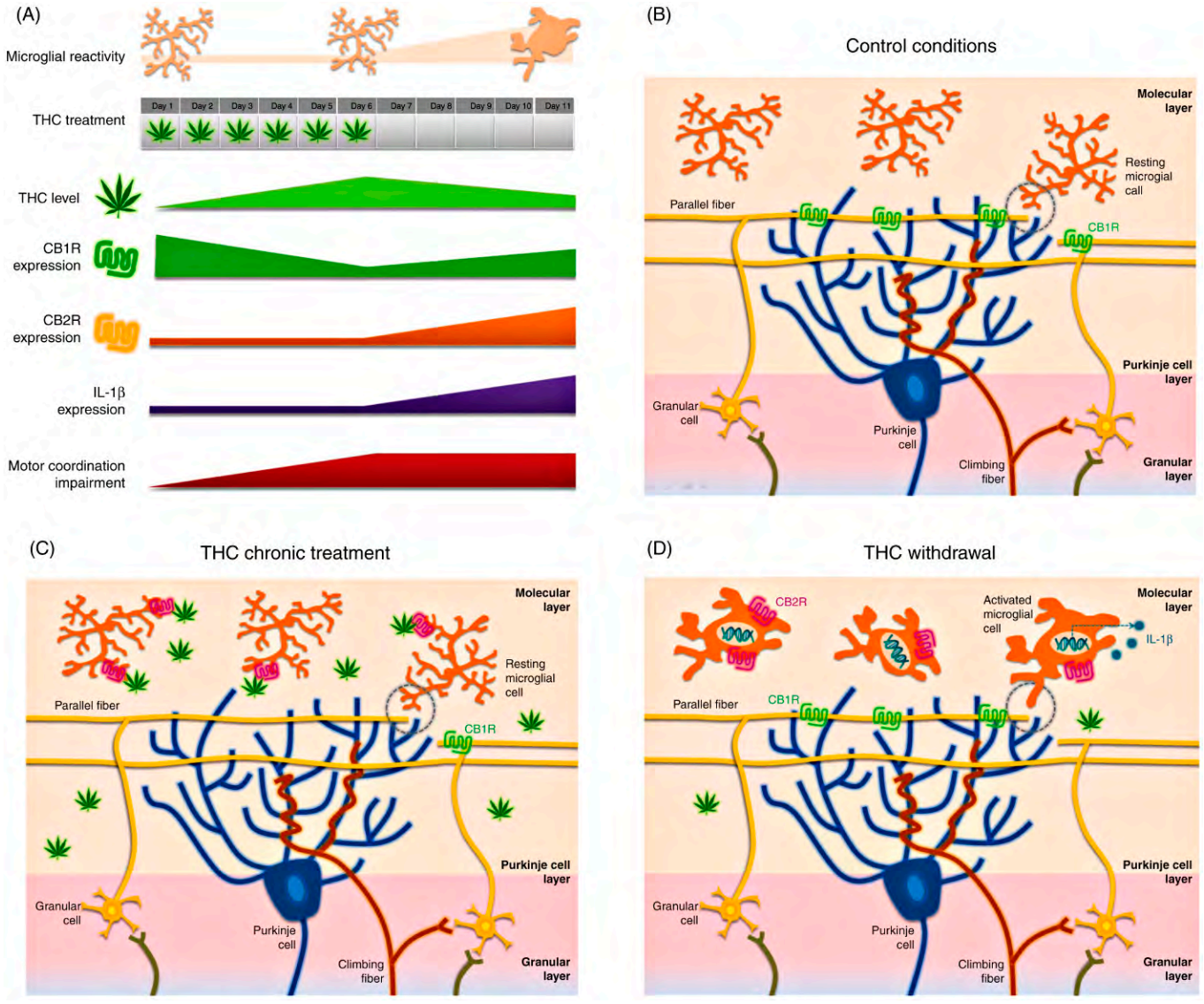


FIGURE 41.4 Effect of THC exposure in the neuronal circuit of the cerebellar cortex. (A) Schematic time-course for repeated THC exposure over the days of cannabinoid administration and subsequent withdrawal, representing the microglial reactivity, the measured levels of CB1 and CB2 receptors, the expression of IL-1β, and the cerebellar functional assessment in behavioral paradigms in mice; (B) neuronal circuit in the cerebellar cortex under control conditions, with the main cell populations (Purkinje cells, granular cells, and resting microglia), and the main fibers contacting Purkinje neurons (climbing fibers and parallel fibers); (C) chronic THC exposure downregulates CB1 receptor expression in the cerebellar parallel fibers, and may have antineuroinflammatory effects on microglial reactivity; (D) after THC withdrawal, microglial cells in the cerebellar cortex become activated, produce IL-1β, and affect cerebellar function.

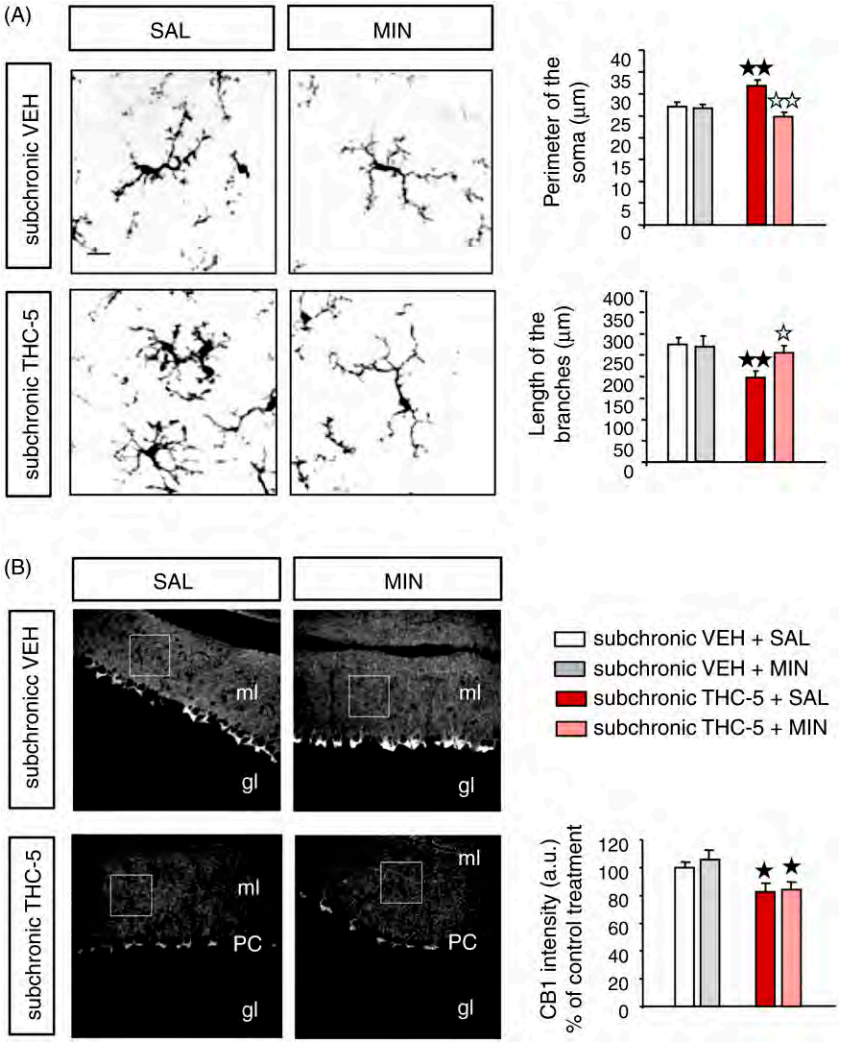


FIGURE 41.5 Experimental data supporting Fig. 41.4, on the activation of cerebellar microglia and CB1 receptor expression after THC withdrawal. (A) Immunofluorescence detection of Iba1 to reveal microglial morphology in the cerebellum of mice after subchronic THC exposure. The administration of minocycline, a tetracycline antibiotic that reduces microglial activation, normalizes microglial cell morphology in this brain area. Note the increase in the perimeter of the soma and the reduced branch length after subchronic THC administration, a microglial phenotype that is reversed by minocycline treatment; (B) immunofluorescence detection of CB1 receptor and signal density quantification in the molecular layer of the cerebellum, after chronic THC administration on mice that received subsequent minocycline. Note that minocycline treatment does not affect CB1 receptor downregulation in this cerebellar layer (modified from Cutando et al., 2013). Data are expressed as mean ± SEM. *$p < 0.05$, **$p < 0.01$ (compared to vehicle-treated group); *$p < 0.05$, **$p < 0.01$ (compared to THC-treated group) *Source: From Cutando et al. (2013), reproduced with permission of the* Journal of Clinical Investigation.

responsible for the appearance of reactive microglial cells at the cerebellar cortex molecular layer, as well as the enhanced excitability at Purkinje cells, the main output cell of the cerebellar cortex. This microglial activation process produced by chronic cannabis exposure is accompanied by changes in CB2 and IL-1β expression, as well as alterations in the microglial morphology toward an activated phenotype (Fig. 41.4). Moreover, constitutive genetic removal of CB1 receptor, or conditional removal of CB1 receptor from the parallel fibers of the cerebellum in mice, also resulted in reactive microglia in the cerebellar molecular layer. This result points to the necessary deregulation of CB1 receptor expression for the appearance of microglial reactivity, accompanied by an overactivation of Purkinje cell excitability. The pharmacological inhibition of microglial reactivity was sufficient to prevent the deficits in cerebellar function produced by repeated cannabis exposure. Indeed, the coat-hanger test for fine motor coordination, and the eye-blink conditioning test for conditioned motor learning (Fig. 41.6) were normalized with the microglial inhibition, even while CB1 regulating expression was below control levels (Fig. 41.5). In addition, the inhibition of microglial reactivity reduced Purkinje cell excitability. This recent study provides new insights into the role of the ECS on the microglial reactivity, and highlights the

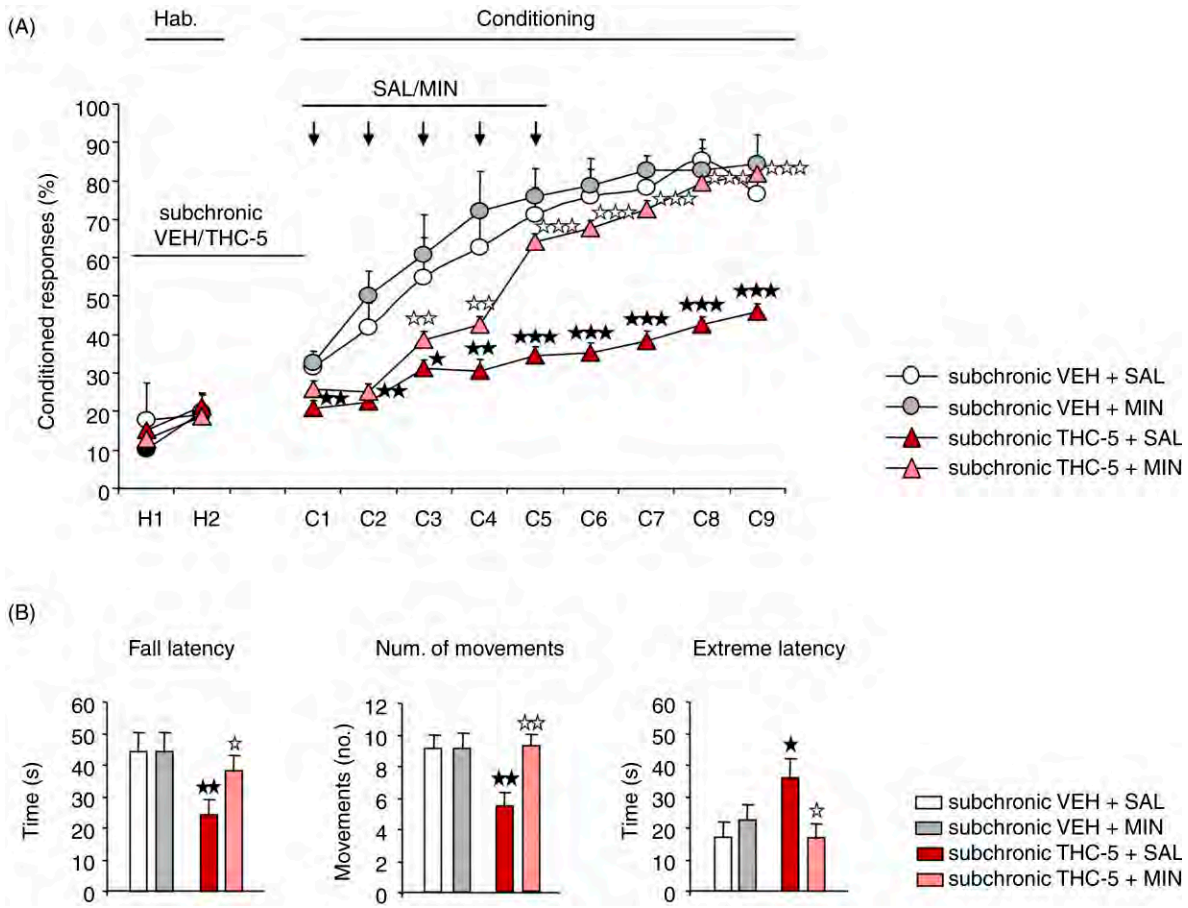


FIGURE 41.6 **Experimental data on the impact of subchronic exposure to THC and its withdrawal on conditioned cerebellar learning and motor coordination.** (A) Conditioned cerebellar learning in the eye-blink conditioning in mice that received THC followed by the administration of minocycline *(arrows)*. The number of conditioned responses in the eye-blink conditioning is a measure of cerebellar function. After two days of habituation to the procedure (H1, H2), mice were trained during nine consecutive days (C1–C9) where the number of conditioned responses to a conditioned stimulus (sound) was measured. THC-treated mice showed a clear deficit *(red triangles)* that was fully prevented by minocycline treatment *(arrows)* during THC withdrawal *(pink triangles)*; (B) motor coordination measured in the coat-hanger test was impaired after THC withdrawal *(red bars)*. Preventing microglial activation with minocycline normalized motor coordination. Data are expressed as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (compared to vehicle-treated group), $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (compared to THC-treated group) *Source: From Cutando et al. (2013), reproduced with permission of the* Journal of Clinical Investigation.

important role of microglial cells in brain function and pathological states.

MINI-DICTIONARY

Chemokines Type of chemoattractant cytokines that regulate the migration of immune cells, and help to coordinate immune and inflammatory reactions.

Coat-hanger test Behavioral test to evaluate fine motor coordination. The experimental animal is located on a coat-hanger above a cushioned surface. Then the animal movements and time on the coat-hanger while it moves to reach the hanging bar are analyzed to reveal cerebellar deficits.

Danger-associated molecular pattern molecules (DAMPs) Intracellular constituents (DNA or intracellular proteins) from necrotic cells that can initiate the noninfectious innate immune response in macrophages.

Eye-blink conditioning Neurophysiological test of cerebellar function where the eyelid movement of the experimental subject is analyzed. The subject must learn the association of a conditioned stimulus (sound) with an unconditioned stimulus (electric stimulus or air puff to the eye). Proper cerebellar function will produce an increasing number of conditioned responses over the days of training sessions. Cannabis smokers show a marked deficit in this cerebellar test, which is mimicked by mice after THC administration.

Interferon gamma Cytokine produced by T lymphocytes as part of the immunological response to viral and nonviral antigens, as well as to other cytokines.

Interleukins Type of cytokines with relevant functions in immune cell modulation. This is the most diverse family of cytokines, with more than 30 types. There are proinflammatory (IL-1β) but also antiinflammatory interleukins (IL-4, IL-10, or IL-13).

Microglial activation Change in microglial state from a surveillance/resting state to a reactive or activated state. The microglial activation process involves changes in gene expression and cell surface marker, such as CD11b, but also changes in cell morphology from a ramified cell with small soma to a cell with amoeboid phenotype, characterized by short or no branches, and an enlarged soma.

Neuroinflammation Refers to a central nervous system status in which reactive microglial cells and astrocytes, but not

peripheral immune cells, are usually implicated in the secretion of proinflammatory factors.

Pathogen-associated molecular pattern molecules (PAMPs) Conserved components that are widely common to pathogens (viruses, bacteria, or fungi) that initiate the innate immune response through Toll (pattern recognition) receptors on macrophages.

Th1 and Th2 lymphocytes Types of T helper lymphocytes derived from a common clone of T cells. The presence of specific interleukins promotes the development of one or the other type of Th cell. Usually, Th1 lymphocytes promote macrophage/cell-mediated response, while Th2 lymphocytes intervene in the antibody-mediated response.

References

Alliot, F., Godin, I., & Pessac, B. (1999). Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain Research Developmental Brain Research*, *117*, 145–152.

Ashton, J. C. (2012). The atypical cannabinoid O-1602: targets, actions, and the central nervous system. *Central Nervous System Agents in Medicinal Chemistry*, *12*, 233–239.

Baker, D., Pryce, G., Davies, W. L., & Hiley, C. R. (2006). In silico patent searching reveals a new cannabinoid receptor. *Trends in Pharmacological Sciences*, *27*, 1–4.

Becher, B., & Antel, J. P. (1996). Comparison of phenotypic and functional properties of immediately ex vivo and cultured human adult microglia. *Glia*, *18*, 1–10.

Benarroch, E. E. (2013). Microglia: multiple roles in surveillance, circuit shaping, and response to injury. *Neurology*, *81*, 1079–1088.

Benito, C., Núñez, E., Tolón, R. M., Carrier, E. J., Rábano, A., Hillard, C. J., & Romero, J. (2003). Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *Journal of Neuroscience*, *23*, 11136–11141.

Biber, K., Neumann, H., Inoue, K., & Boddeke, H. W. (2007). Neuronal 'On' and 'Off' signals control microglia. *Trends in Neuroscience*, *30*, 596–602.

Cardona, A. E., Pioro, E. P., Sasse, M. E., Kostenko, V., Cardona, S. M., Dijkstra, I. M., Huang, D., Kidd, G., Dombrowski, S., Dutta, R., Lee, J. C., Cook, D. N., Jung, S., Lira, S. A., Littman, D. R., & Ransohoff, R. M. (2006). Control of microglial neurotoxicity by the fractalkine receptor. *Nature Neuroscience*, *9*, 917–924.

Carlisle, S. J., Marciano-Cabral, F., Staab, A., Ludwick, C., & Cabral, G. A. (2002). Differential expression of the CB2 cannabinoid receptor by rodent macrophages and macrophage-like cells in relation to cell activation. *International Immunopharmacology*, *2*, 69–82.

Carrier, E. J., Kearn, C. S., Barkmeier, A. J., Breese, N. M., Yang, W., Nithipatikom, K., Pfister, S. L., Campbell, W. B., & Hillard, C. J. (2004). Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonylglycerol, which increases proliferation via a CB2 receptor-dependent mechanism. *Molecular Pharmacology*, *65*, 999–1007.

Cuadros, M. A., & Navascués, J. (1998). The origin and differentiation of microglial cells during development. *Progress in Neurobiology*, *56*, 173–189.

Cutando, L., Busquets-Garcia, A., Puighermanal, E., Gomis-González, M., Delgado-García, J. M., Gruart, A., Maldonado, R., & Ozaita, A. (2013). Microglial activation underlies cerebellar deficits produced by repeated cannabis exposure. *Journal of Clinical Investigation*, *123*, 2816–2831.

Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., Littman, D. R., Dustin, M. L., & Gan, W. B. (2005). ATP mediates rapid microglial response to local brain injury in vivo. *Nature Neuroscience*, *8*, 752–758.

Dentesano, G., Serratosa, J., Tusell, J. M., Ramón, P., Valente, T., Saura, J., & Solà, C. (2014). CD200R1 and CD200 expression are regulated by PPAR-γ in activated glial cells. *Glia*, *62*, 982–998.

Franklin, A., & Stella, N. (2003). Arachidonylcyclopropylamide increases microglial cell migration through cannabinoid CB2 and abnormal-cannabidiol-sensitive receptors. *European Journal of Pharmacology*, *474*, 195–198.

Hanisch, U. K., & Kettenmann, H. (2007). Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nature Neuroscience*, *10*, 1387–1394.

Hassan, S., Eldeeb, K., Millns, P. J., Bennett, A. J., Alexander, S. P., & Kendall, D. A. (2014). Cannabidiol enhances microglial phagocytosis via transient receptor potential (TRP) channel activation. *British Journal of Pharmacology*, *171*, 2426–2439.

Haynes, S. E., Hollopeter, G., Yang, G., Kurpius, D., Dailey, M. E., Gan, W. B., & Julius, D. (2006). The P2Y12 receptor regulates microglial activation by extracellular nucleotides. *Nature Neuroscience*, *9*, 1512–1519.

Inoue, K., & Tsuda, M. (2012). Purinergic systems, neuropathic pain and the role of microglia. *Experimental Neurology*, *234*, 293–301.

Kallendrusch, S., Kremzow, S., Nowicki, M., Grabiec, U., Winkelmann, R., Benz, A., Kraft, R., Bechmann, I., Dehghani, F., & Koch, M. (2013). The G protein-coupled receptor 55 ligand l-α-lysophosphatidylinositol exerts microglia-dependent neuroprotection after excitotoxic lesion. *Glia*, *61*, 1822–1831.

Kettenmann, H., Hanisch, U. K., Noda, M., & Verkhratsky, A. (2011). Physiology of microglia. *Physiological Review*, *91*, 461–553.

Kettenmann, H., Kirchhoff, F., & Verkhratsky, A. (2013). Microglia: new roles for the synaptic stripper. *Neuron*, *77*, 10–18.

Kierdorf, K., & Prinz, M. (2013). Factors regulating microglia activation. *Frontiers in Cellular Neuroscience*, *7*, 1–8.

Kim, H. J., Shin, A. H., & Thayer, S. A. (2011). Activation of cannabinoid type 2 receptors inhibits HIV-1 envelope glycoprotein gp120-induced synapse loss. *Molecular Pharmacology*, *80*, 357–366.

Koch, M., Kreutz, S., Böttger, C., Benz, A., Maronde, E., Ghadban, C., Korf, H. W., & Dehghani, F. (2011). Palmitoylethanolamide protects dentate gyrus granule cells via peroxisome proliferator-activated receptor-α. *Neurotoxicity Research*, *19*, 330–340.

Kofler, J., & Wiley, C. A. (2011). Microglia: key innate immune cells of the brain. *Toxicology and Pathology*, *39*, 103–114.

Konno, M., Shirakawa, H., Iida, S., Sakimoto, S., Matsutani, I., Miyake, T., Kageyama, K., Nakagawa, T., Shibasaki, K., & Kaneko, S. (2012). Stimulation of transient receptor potential vanilloid 4 channel suppresses abnormal activation of microglia induced by lipopolysaccharide. *Glia*, *60*, 761–770.

Kreutz, S., Koch, M., Böttger, C., Ghadban, C., Korf, H. W., & Dehghani, F. (2009). 2-Arachidonoylglycerol elicits neuroprotective effects on excitotoxically lesioned dentate gyrus granule cells via abnormal-cannabidiol-sensitive receptors on microglial cells. *Glia*, *57*, 286–294.

Lawson, L. J., Perry, V. H., Dri, P., & Gordon, S. (1990). Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience*, *39*, 151–170.

Lee, C. H., & Won, M. H. (2014). Change of peroxisome proliferator-activated receptor γ expression pattern in the gerbil dentate gyrus after transient global cerebral ischemia. *Anatomy and Cell Biology*, *47*, 111–116.

McHugh, D., Wager-Miller, J., Page, J., & Bradshaw, H. B. (2012). siRNA knockdown of GPR18 receptors in BV-2 microglia attenuates N-arachidonoyl glycine-induced cell migration. *Journal of Molecular Signaling*, *7*, 10.

Mittelbronn, M., Dietz, K., Schluesener, H. J., & Meyermann, R. (2001). Local distribution of microglia in the normal adult human central nervous system differs by up to one order of magnitude. *Acta Neuropathologica*, *101*, 249–255.

Nimmerjahn, A., Kirchhoff, F., & Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science*, *308*, 1314–1318.

Ohsawa, K., Irino, Y., Nakamura, Y., Akazawa, C., Inoue, K., & Kohsaka, S. (2007). Involvement of P2X4 and P2Y12 receptors in ATP-induced microglial chemotaxis. *Glia*, *55*, 604–616.

Palazuelos, J., Aguado, T., Pazos, M. R., Julien, B., Carrasco, C., Resel, E., Sagredo, O., Benito, C., Romero, J., Azcoitia, I., Fernández-Ruiz, J., Guzmán, M., & Galve-Roperh, I. (2009). Microglial CB2 cannabinoid receptors are neuroprotective in Huntington's disease excitotoxicity. *Brain*, *132*, 3152–3164.

Pietr, M., Kozela, E., Levy, R., Rimmerman, N., Lin, Y. H., Stella, N., Vogel, Z., & Juknat, A. (2009). Differential changes in GPR55 during microglial cell activation. *FEBS Letters*, *583*, 2071–2076.

Puffenbarger, R. A., Boothe, A. C., & Cabral, G. A. (2000). Cannabinoids inhibit LPS-inducible cytokine mRNA expression in rat microglial cells. *Glia*, *29*, 58–69.

Raboune, S., Stuart, J. M., Leishman, E., Takacs, S. M., Rhodes, B., Basnet, A., Jameyfield, E., McHugh, D., Widlanski, T., & Bradshaw, H. B. (2014). Novel endogenous *N*-acyl amides activate TRPV1-4 receptors, BV-2 microglia, and are regulated in brain in an acute model of inflammation. *Frontiers in Cellular Neuroscience*, *8*, 195.

Ramírez, B. G., Blázquez, C., Gómez del Pulgar, T., Guzmán, M., & de Ceballos, M. L. (2005). Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *Journal of Neuroscience*, *25*, 1904–1913.

Rinaldi-Carmona, M., Barth, F., Héaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, S., Maruani, J., Néliat, G., Caput, D., et al. (1994). SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Letters*, *350*, 240–244.

Saijo, K., & Glass, C. K. (2011). Microglial cell origin and phenotypes in health and disease. *Nature Reviews Immunology*, *11*, 775–787.

Sheridan, G. K., & Murphy, K. J. (2013). Neuron-glia crosstalk in health and disease: fractalkine and CX3CR1 take centre stage. *Open Biology*, *3*, 130181.

Stella, N. (2009). Endocannabinoid signaling in microglial cells. *Neuropharmacology*, *1*, 244–253.

Stella, N. (2010). Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. *Glia*, *58*, 1017–1030.

Suzuki, T., Hide, I., Ido, K., Kohsaka, S., Inoue, K., & Nakata, Y. (2004). Production and release of neuroprotective tumor necrosis factor by P2X7 receptor-activated microglia. *Journal of Neuroscience*, *24*, 1–7.

Takahashi, K., Prinz, M., Stagi, M., Chechneva, O., & Neumann, H. (2007). TREM2-transduced myeloid precursors mediate nervous tissue debris clearance and facilitate recovery in an animal model of multiple sclerosis. *PLoS Medicine*, *4*(4), e124.

Waksman, Y., Olson, J. M., Carlisle, S. J., & Cabral, G. A. (1999). The central cannabinoid receptor (CB1) mediates inhibition of nitric oxide production by rat microglial cells. *Journal of Pharmacology and Experimental Therapeutics*, *288*, 1357–1366.

Walter, L., Franklin, A., Witting, A., Wade, C., Xie, Y., Kunos, G., Mackie, K., & Stella, N. (2003). Nonpsychotropic cannabinoid receptors regulate microglial cell migration. *Journal of Neuroscience*, *23*, 1398–1405.

Yiangou, Y., Facer, P., Durrenberger, P., Chessell, I. P., Naylor, A., Bountra, C., Banati, R. R., & Anand, P. (2006). COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord. *BMC Neurology*, *6*, 12.

Zhang, J., Hoffert, C., Vu, H. K., Groblewski, T., Ahmad, S., & O'Donnell, D. (2003). Induction of CB2 receptor expression in the rat spinal cord of neuropathic but not inflammatory chronic pain models. *European Journal Neuroscience*, *17*, 2750–2754.

CHAPTER

42

Cannabis and Psychosis: Correlation, Causality, and Consequences

D. Basu, P. Parakh***

***Drug De-addiction and Treatment Centre, Department of Psychiatry, Postgraduate Institute of Medical Education and Research, Chandigarh, India**

****Department of Psychiatry, Ruby General Hospital, Kolkata, India**

SUMMARY POINTS

- Epidemiological studies have shown that cannabis use is associated with a higher risk of developing psychosis.
- The association is dose-dependent, with heavy users having a higher risk.
- Exposure to cannabis in early adolescence is associated with a higher risk of developing psychosis.
- Since only a small proportion of cannabis users go on to develop psychosis, gene–environment interactions are likely to be responsible.
- People with stronger genetic liability for schizophrenia are more susceptible to the psychosis-inducing effects of cannabis.
- Exposure to environmental factors like childhood sexual trauma and urban upbringing has also been implicated in the link between cannabis and psychosis.
- Cannabis acts as a component cause of psychosis. That is, cannabis is neither a necessary nor a sufficient cause of psychosis. Presence of some other factor or factors is required for cannabis to have a causal influence on the risk for psychosis.

KEY FACTS OF GENE–ENVIRONMENT INTERACTION

- It is a type of interplay between genetic and environmental factors.
- When people with different genotypes show different responses to the same environmental factor, it is said to be a gene–environment interaction.
- For example, people with different genotypes may differ in their response to a medicine.
- In gene–environment interaction, either the genotype can affect the response to the environmental factor, or the environmental factor can modulate the expression of the genotype.
- Gene–environment interaction is different from gene–environment correlation where the genotype moderates the exposure to an environmental factor.
- Gene–environment interactions have been implicated in many common diseases like diabetes, obesity, cancer, etc., as well as mental illnesses like schizophrenia, and affective disorders.

LIST OF ABBREVIATIONS

CB1	Cannabinoid-1 receptor
CB2	Cannabinoid-2 receptor
CBD	Cannabidiol
COMT	Catechol-O-methyltransferase
DAT1	Dopamine transporter-1 gene
THC	Tetrahydrocannabinol

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00048-X
Copyright © 2017 Elsevier Inc. All rights reserved.

INTRODUCTION

Cannabis has been used by man since time immemorial for its psychotropic effects. However, its widespread use has often made people wonder if it has any detrimental effects on the human body. This was a matter of debate even as early as in the 19th century, when the British government established the Indian Hemp Drugs Commission to investigate the effects of cannabis. The Commission submitted its seven-volume report in 1893, and concluded that there was no evidence of any weight regarding mental and moral injuries from moderate use of cannabis (Kaplan, 1969). Over the next century, in spite of reports of acute psychotic symptoms following heavy cannabis use (Chopra & Smith, 1974; Talbott & Teague, 1969), cannabis gradually came to be perceived as a harmless recreational drug and, being widely available and inexpensive, became very popular amongst the youth.

CORRELATION

There is ample research to show that regular cannabis use and psychotic disorders such as schizophrenia are associated in the general population (Degenhardt & Hall, 2001; Tien & Anthony, 1990). It is also well known that heavy cannabis users are overrepresented among new cases of schizophrenia (Barbee, Clark, Crapanzo, Heintz, & Kehoe, 1989). However, because of the large number of confounding factors that could have had a bearing on these results, these findings have to be interpreted with caution. Stronger evidence in favor of an association between cannabis and psychosis comes from longitudinal studies of large representative samples of the population who have been followed-up over time to see if cannabis users are at a higher risk of developing psychosis.

The earliest such evidence was from a 15-year prospective study of the Swedish conscript cohort (Andreasson, Allebeck, Engstrom, & Rydberg, 1987). Around 50,000 Swedish conscripts were followed-up, and it was found that those who had tried cannabis by age 18 were 2.4 times more likely to be diagnosed with schizophrenia 15 years later than those who had not. The risk increased to around six times with higher frequency of cannabis use. The risk was substantially reduced but still significant after statistical adjustment for variables that were related to the risk of developing schizophrenia. The same cohort was followed-up over 27 years (Zammit, Allebeck, Andreasson, Lundberg, & Lewis, 2002), and this study also found a dose–response relationship between frequency of cannabis use at baseline and risk of schizophrenia during the follow-up. Again, the relationship between cannabis use and schizophrenia persisted, even after statistically controlling for the effects of other substance use, and other potential confounding factors, including a history of psychiatric symptoms at baseline. Assuming a causal relationship, and given current patterns of use, the authors estimated that 13% of cases of schizophrenia could be averted if all cannabis use was prevented. The recently published 35-year follow-up of the same cohort also supported these results (Manrique-Garcia et al., 2012).

In another study, a birth cohort of 1034 children born in Dunedin, New Zealand, were asked about their drug consumption at the ages of 15 and 18 (Arseneault et al., 2002). At 26 years of age, the sample was interviewed using a standardized psychiatric assessment. It was found that those who had used cannabis by the age of 15 or 18 reported significantly more psychotic symptoms at 26 years of age, as compared with nonusers. In addition, 10% of those who used cannabis by the age of 15 were diagnosed with schizophreniform psychosis when they were 26 years old, as compared with 3% of those who had not used cannabis. Similar results, as summarized in Table 42.1, have been reported by other prospective cohort studies from the Netherlands, New

TABLE 42.1 General Population Studies of the Effect of Cannabis Use on the Risk of Psychosis

Authors	Sample	Follow-up	Odds ratio (95% confidence interval)
Andreasson et al. (1987)	45,570 Swedish male conscripts	15 years	6.0 (4.0–8.9)
Zammit et al. (2002)	50,087 Swedish male conscripts	27 years	3.1 (1.7–5.5)
Arseneault et al. (2002)	1037 New Zealander males and females from Dunedin birth cohort	15 years	3.1 (0.7–13.3)
van Os et al. (2002)	4104 Dutch males and females from population sample, aged 18–64 (NEMESIS)	3 years	2.8 (1.2–6.5)
Fergusson et al. (2003)	1265 New Zealander males and females from Christchurch birth cohort (CHDS)	3 years	1.8 (1.2–2.6)
Henquet et al. (2005a)	2437 German males and females from population sample, aged 14–24 (EDSP)	4 years	1.7 (1.1–2.5)
Manrique-Garcia et al. (2012)	50,087 Swedish male conscripts	35 years	3.7 (2.3–5.8)

This table shows some of the large-scale epidemiological follow-up studies, which have found that cannabis users have a higher risk of developing psychosis later in life.
From Parakh, P., & Basu, D. (2013). Cannabis and psychosis: have we found the missing links? Asian Journal of Psychiatry, *6, 281–287.*

Zealand, Germany, and elsewhere (Fergusson, Horwood, & Swain-Campbell, 2003; Henquet et al., 2005a; van Os et al., 2002).

Cannabis is known to improve negative and depressive symptoms, and may be used to self-medicate symptoms of schizophrenia (Schneier & Siris, 1987). Therefore, a possible explanation of the association between cannabis and psychosis is that cannabis use is a consequence, rather than a cause, of psychosis. In order to control for this, some longitudinal studies excluded patients with psychotic symptoms at baseline, or made statistical adjustments for the same, and yet found an increased risk of psychosis in cannabis users (Arseneault et al., 2002; van Os et al., 2002).

In a study on another birth cohort from New Zealand, data on both cannabis use and psychotic symptoms were collected from the cohort at the ages of 18, 21, and 25 years (Fergusson et al., 2003). The investigators were therefore able to study both the effects of cannabis use on later psychotic symptoms, and also the effect of psychotic symptoms on later cannabis use. Like the other studies discussed earlier, this study also found that cannabis use at the age of 18 was associated with more psychotic symptoms 3 and 7 years later. Moreover, in contradiction of the self-medication hypothesis, the presence of psychotic symptoms at the age of 18 appeared to inhibit, rather than encourage, subsequent cannabis use. However, conflicting results were reported by a 10 year follow-up study (Foti, Kotov, Guey, & Bromet, 2010). The authors concluded that the relationship of cannabis use with psychotic symptoms was bidirectional; cannabis exposure predicted severity of psychosis, and individuals with more severe psychotic symptoms were more likely to use cannabis in the future. In view of the mixed results, it is perhaps too early to discard the self-medication hypothesis altogether.

It has been argued that the association between cannabis and psychosis that many epidemiological studies have found can be due to confounding factors like other substance use. A systematic review of seven longitudinal studies on the relationship between cannabis and psychosis, which adjusted for about 60 confounding factors, including other substance use, personality traits, sociodemographic markers, intellectual ability, other mental health problems, etc., reported an increased risk (odds ratio = 1.4) of any psychotic outcome in individuals who had ever used cannabis (Moore et al., 2007). Findings were consistent with a dose-response effect, with greater risk in people who used cannabis more frequently. Another study attempted to exclude the possibility of unmeasured confounding variables by conducting a sibling pair analysis, nested within a prospective birth cohort, and still found that early cannabis use is associated with psychosis-related outcomes in young adults (McGrath et al., 2010).

Indirect evidence in favor of an association between cannabis and psychosis came from a metaanalysis which found that the age at onset of psychosis for cannabis users was 2.70 years younger than for nonusers, while alcohol use was not associated with a significantly earlier age at onset of psychosis (Large, Sharma, Compton, Slade, & Nielssen, 2011). Another study of heavy cannabis users who went on to develop psychosis found that age at onset of cannabis use was directly associated with age at onset of psychosis, and age at first hospitalization (Galvez-Buccollini et al., 2012). That the association remained significant even after adjusting for potential confounding factors is, according to the authors, consistent with the hypothesis that cannabis can cause or precipitate the onset of psychosis after a prolonged period of time. Similar results were reported by another study, which found that age at initiation of cannabis use predicts the age at onset of psychosis, with the gap being approximately 7–8 years (Stefanis et al., 2013). A study of 410 first-episode psychosis patients designed to investigate the association between gender, patterns of cannabis use, and age at onset of psychosis reported that a younger age at first cannabis use (≤15 years) is associated with a younger age at onset of psychosis, but only in those who had used cannabis daily (Di Forti et al., 2014b). The authors concluded that daily use, especially of high-potency cannabis, drives the earlier onset of psychosis in cannabis users, irrespective of gender.

The epidemiological evidence in favor of an association between cannabis and psychosis is buttressed by studies showing that the association is biologically plausible. The endocannabinoid system (briefly described in Table 42.2) has been implicated in the causation of schizophrenia. Anandamide levels are significantly elevated in schizophrenia patients, both in the cerebrospinal fluid and in the blood (De Marchi et al., 2003; Giuffrida et al., 2004; Koethe et al., 2009; Leweke, Giuffrida, Wurster, Emrich, & Piomelli, 1999). Studies on schizophrenic patients have shown upregulation of cannabinoid-1 (CB1) receptors in cortical brain regions such as the dorsolateral prefrontal cortex (Dalton, Long, Weickert, & Zavitsanou, 2011; Dean, Sundram, Bradbury, Scarr, & Copolov, 2001) and in cingulate cortex (Newell, Deng, & Huang, 2006; Wong et al., 2010; Zavitsanou, Garrick, & Huang, 2004). Cannabinoid-2 (CB2) receptors have also been implicated in schizophrenia. Clinical remission of schizophrenia has been shown to be accompanied by a significant decrease in CB2 receptor mRNA levels in the blood (De Marchi et al., 2003). An increased risk of schizophrenia has been reported for people with low CB2 receptor function (Ishiguro et al., 2010).

There is also direct evidence of cannabis leading to structural, neurophysiological, and neurochemical changes in the brain, which are similar to those seen in schizophrenia. A systematic review combining in vivo

TABLE 42.2 The Endocannabinoid System

Major constituents	Role
Cannabinoid receptors	
• Cannabinoid-1 (CB1) receptors	Found mainly in the central nervous system, in areas such as globus pallidus, hippocampus, cerebral cortex, hypothalamus, cerebellum, and striatum Mostly located in the presynaptic neurons, and control release of other neurotransmitters like dopamine, GABA, acetylcholine, and glutamate Involved in brain functions such as pain, emotion, cognition, and motivation Also found in the periphery in reproductive, metabolic, cardio-vascular, and gastrointestinal systems
• Cannabinoid-2 (CB2) receptors	Mostly located in the periphery, mainly in immune cells Also found in the brain on glial cells, and neurons in brainstem and cerebellum Modulate immune cell migration and cytokine release, in periphery and in brain
Endocannabinoid ligands	
• Anandamide	Binds to both CB1 and CB2 receptors Also activates some noncannabinoid receptors
• 2-arachidonoyglycerol	Selectively binds to CB1 receptors

This table briefly describes the major constituents of the endocannabinoid system, which modulate many central and peripheral cellular processes involved in neuronal development, neurotransmission, inflammation, energy metabolism, pain relief, and efficient functioning of cardiovascular, respiratory, reproductive, and hormonal systems (Galve-Roperh, Aguado, Palazuelos, & Guzman, 2007).

structural neuroimaging and post mortem studies on cannabis-using patients with psychosis, as well as individuals at high risk for psychosis, found evidence for consistent brain structural abnormalities in CB1 receptor-enhanced brain areas such as the cingulate and prefrontal cortices and the cerebellum, which have not been seen in healthy samples (Rapp, Bugra, Riecher-Rössler, Tamagni, & Borgwardt, 2012). Tetrahydrocannabinol (THC) has been shown to decrease theta coherence between the right and left frontal lobes and, since the reduction in coherence was strongly associated with positive psychotic symptoms, this has been proposed as one of the mechanisms for the psychotogenic property of cannabis (Morrison et al., 2011). Long-term cannabis use has been shown to be associated with deficits in working memory and abnormalities in basalganglio-thalamic morphology, similar to that seen in schizophrenia (Smith et al., 2014).

It has been hypothesized that cannabis increases the risk of psychotic disorders by inducing the same dopaminergic alterations seen in schizophrenia. Dopamine neuronal firing in the ventral tegmental area of midbrain is modulated by retrograde endocannabinoid signaling, which is disrupted by exogenous cannabinoids, resulting in excess dopaminergic transmission in mesolimbic tract (Cheer, Wassum, Heien, Phillips, & Wightman, 2004; French, Dillon, & Wu, 1997; Riegel & Lupica, 2004). However, conflicting results were reported by a recent study, which found that chronic cannabis use is associated with reduced dopamine synthesis capacity (Bloomfield et al., 2014). Some researchers have hypothesized that psychosis results from aberrant reward prediction, and abnormal attribution of salience caused by disordered dopamine transmission (Kapur, Mizrahi, & Li, 2005). This may be one of the ways by which cannabis can lead to psychosis as THC has been shown to increase the aberrant attribution of salience (Bhattacharyya et al., 2012b).

Endocannabinoid signaling is an important determinant of maturation of the adult brain, and plays a critical role in neuronal proliferation, migration, axonal guidance, positioning of cortical interneurons, and synaptogenesis (Galve-Roperh et al., 2007). Therefore, disruption of normative endocannabinoid signaling during adolescence may have longstanding consequences on adult brain function (Malone, Hill, & Rubino, 2010). Exposure to exogenous cannabinoids in adolescence can lead to a disturbance of the endocannabinoid system in the brain, and interfere with neurodevelopmental processes. Epidemiological studies have also found that exposure to cannabis in early adolescence is associated with a higher risk of psychosis (Arseneault et al., 2002; Konings, Henquet, Maharajh, Hutchinson, & van Os, 2008; Stefanis et al., 2004).

CAUSALITY

Although the evidence, largely from prospective epidemiological studies, strongly suggests that heavy cannabis use is associated with an increased risk of developing psychosis, the fact remains that a vast majority of cannabis users never go on to develop psychosis. At the same time, it also cannot be denied that not all patients with psychosis have been exposed to cannabis. Thus, cannabis is neither a necessary nor a sufficient cause; rather it must be a component cause of psychosis (Henquet, Murray, Linszen, & van Os, 2005b; Murray, Morrison, Henquet, & Di Forti, 2007; Parakh & Basu, 2013). In other

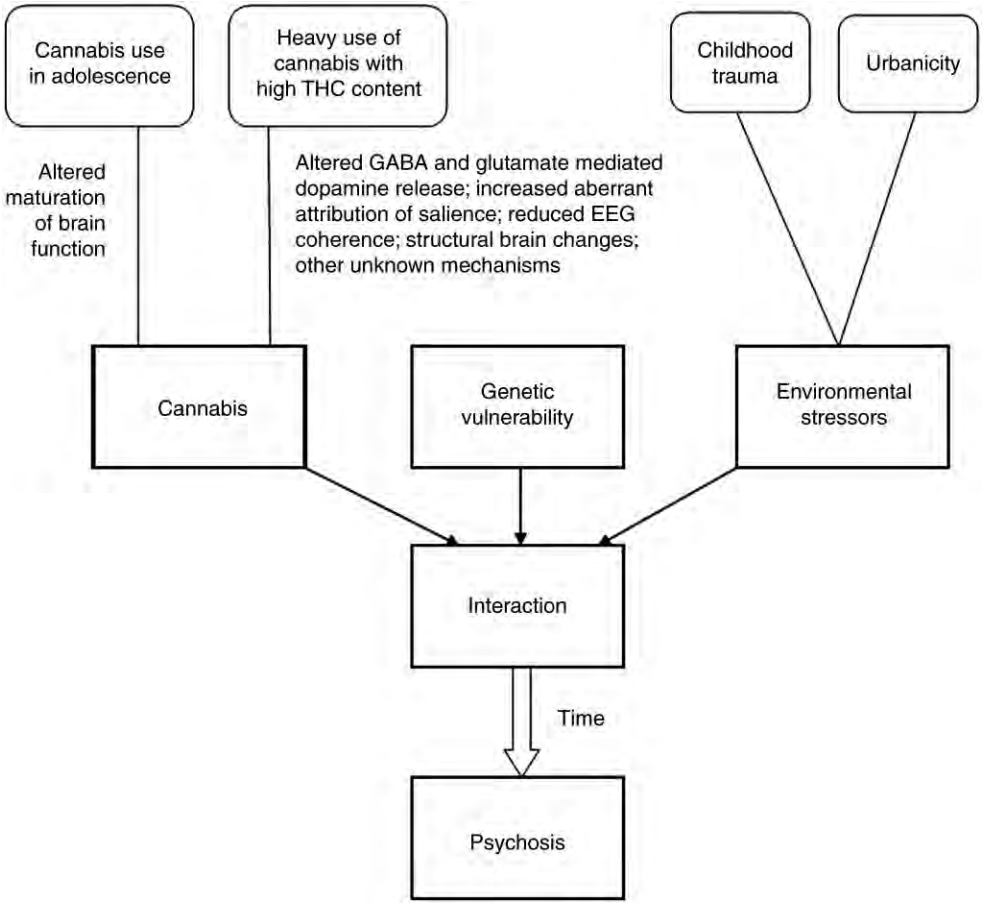


FIGURE 42.1 **Cannabis is a component cause of psychosis.** Cannabis can cause psychosis only in the presence of other susceptibility factors, which may be genetic or environmental, thus acting as a component cause. *THC*, Tetrahydrocannabinol; *GABA*, gamma-amino butyric acid; *EEG*, electroencephalography. *Source: From Parakh, P. & Basu, D. (2013). Cannabis and psychosis: Have we found the missing links?* Asian Journal of Psychiatry, 6, 281–287.

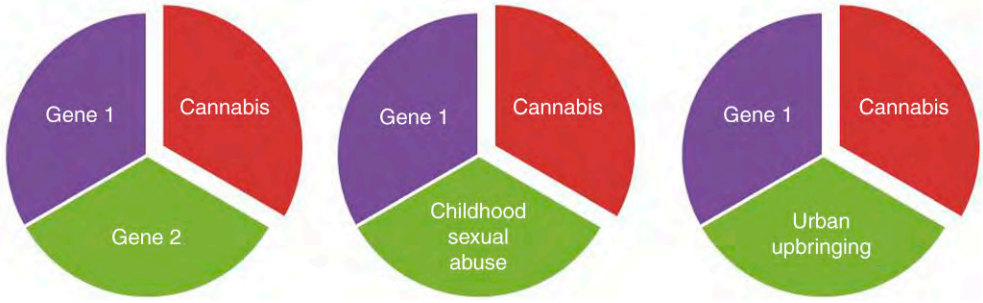


FIGURE 42.2 **Cannabis, by itself, is not a sufficient cause of psychosis.** These are some possible examples of how the presence of other factors is needed for cannabis to cause psychosis.

words, presence of some other factor or factors is required for cannabis to have a causal influence on the risk for psychosis (Figs. 42.1 and 42.2).

Genetic vulnerability to psychosis is one of the factors that make one susceptible to the psychosis inducing potential of cannabis. That is, gene–environment interactions (Fig. 42.3) that explain how one's genotype moderates one's response to cannabis form the basis of the link between cannabis and psychosis. A case-control study of patients with cannabis-associated psychosis found that the risk of schizophrenia was ten times higher for relatives of patients who developed psychosis using cannabis than for the relatives of patients who had not used cannabis and suggested that individuals who have a stronger genetic liability for schizophrenia, indicated by the higher prevalence of schizophrenia in their family, are more vulnerable for the psychotogenic effect of cannabis (McGuire et al., 1995).

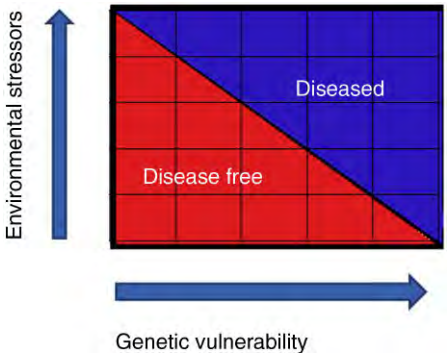


FIGURE 42.3 Genes and environment interact to cause diseases. The figure shows how the interaction between a person's genes and the environment influences the chance of developing a disease. Environmental insults are more likely to cause a disease in people who are inherently susceptible because of their genetic makeup.

Direct evidence for the role of gene–environment interactions comes from studies on genes and their patterns of expression. The first such evidence came from a study on the functional polymorphism in the catechol-O-methyltransferase (*COMT*) gene (Caspi et al., 2005). COMT is the enzyme that degrades dopamine, epinephrine, and norepinephrine. A functional polymorphism of the *COMT* gene results in two common allelic variants, the valine (Val), and the methionine (Met) allele, associated with high versus low enzyme activity, respectively. The authors reported that for individuals homozygous for the Val allele, the relative risk of developing psychotic illness after adolescent cannabis exposure was 10.9, while in individuals homozygous for the methionine allele, the risk was only 1.1. This effect was observed only in those with adolescent-onset cannabis use, and not in those with adult onset cannabis use. However, these findings could not be replicated in a later study (Zammit et al., 2007), while a more recent study reported that cannabis use increased the likelihood to report positive psychotic experiences in Val carriers only, when they were exposed to childhood abuse (Alemany et al., 2014). Another study examined interactions between cannabis use and 152 single-nucleotide polymorphisms in 42 candidate genes, and found that variation in the AKT1 rs2494732 single nucleotide polymorphism may mediate both short-term as well as longer-term effects on psychosis expression associated with use of cannabis (van Winkel & Genetic Risk and Outcome of Psychosis (GROUP) Investigators, 2011). The role of genetic variation at rs2494732 of *AKT1* gene in influencing the risk of developing a psychotic disorder in cannabis users was confirmed in a subsequent case-control study (Di Forti et al., 2012). Another study reported that people using high potency cannabis daily, and having susceptible genetic variants in three candidate genes that affect dopamine signaling (*AKT1*, *DAT1*, and *COMT*) are far more likely to develop psychosis than others (Di Forti, Iyegbe, Falcone, Powell, & Murray, 2014a).

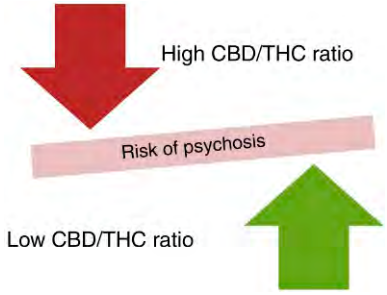


FIGURE 42.4 CBD/THC ratio and the risk of developing psychosis. Use of cannabis strains with low CBD/THC ratio is associated with a higher risk of psychosis. *CBD*, Cannabidiol; *THC*, tetrahydrocannabinol.

Given the complexity of the relationship between cannabis and psychosis, it seems unreasonable to expect that variation in a single gene can explain the differential effects of cannabis. It is more likely that two or more genes are interacting to cause psychosis in cannabis users, or perhaps other environmental factors interact with cannabis to cause psychosis in genetically vulnerable persons (Henquet, Di Forti, Morrison, Kuepper, & Murray, 2008). For example, people with specific genotypes of both *DAT1* and *AKT1* genes have been found to be more sensitive to the psychotogenic effects of cannabis than people with only one of those, and this effect involves an alteration in the neural response to Δ^9-THC in the dopamine-rich regions of striatum and midbrain, consistent with evidence that the psychotic effects of cannabis are mediated by dopamine (Bhattacharyya et al., 2012a).

Environmental factors like childhood sexual trauma (Houston, Murphy, Adamson, Stringer, & Shevlin, 2008) and urban upbringing (Kuepper, van Os, Lieb, Wittchen, & Henquet, 2011) have also been implicated in the link between cannabis and psychosis. While the exact biological basis of this is not known, it has been proposed that repeated exposure to environmental stressors, especially in young age, sensitizes one to the psychotogenic effects of Δ^9-THC (Henquet et al., 2008), and this cross-sensitization process is likely to be mediated by dopamine (Kuepper et al., 2010).

Another factor that has a bearing not only on the relationship between cannabis and psychosis, but also creates methodological hurdles in scientific research on the subject, is the high variability of Δ^9-THC content in the cannabis available on the street. Δ^9-THC is the main psychoactive ingredient in cannabis, and is thought to be the ingredient responsible for the increased risk of developing psychosis following regular cannabis use. Cannabidiol (CBD) is the other major constituent found in cannabis. It has been shown to have opposite effects to Δ^9-THC, and may even have antipsychotic properties (Bhattacharyya et al., 2010). The relative content of Δ^9-THC and CBD, which varies widely between different places and different products, has a bearing on the risk of progression to psychosis (Fig. 42.4). But it is of concern that use

of sinsemilla or skunk, which contains high levels of THC and almost no CBD, is gradually increasing, and may lead to a spate in the incidence of psychosis (Henquet & Kuepper, 2010; Potter, Clark, & Brown, 2008).

CONSEQUENCES

With support for decriminalization of cannabis growing all over the world, the consequences of an association between cannabis and psychosis cannot be overemphasized. Both the public and the policy makers need to be made aware of the scientific evidence available, and the implications. A metaanalysis estimated that about 14% of psychotic outcomes in young adults currently in the United Kingdom would not occur if cannabis were not consumed, considering that around 40% of adolescents and young adults in the United Kingdom have ever used cannabis (Moore et al., 2007). Although individual lifetime risk of chronic psychotic disorders such as schizophrenia, even in people who use cannabis regularly, is likely to be less than 3%, cannabis use is so common that it can be expected to have a substantial effect on psychotic disorders at a population level (Moore et al., 2007). Even if the actual figures turn out to be less, and reducing exposure to cannabis can avert psychotic outcome in only a few people, it should still be of utmost priority, considering how debilitating a psychotic illness can be. This is especially applicable for young adults, for whom a psychotic breakdown can have lifelong consequences, both in the personal and professional spheres.

With interest in prevention of schizophrenia rising all over the world amongst both researchers and policy makers, elucidation of the link between cannabis and psychosis is highly relevant. Unlike genetic vulnerability and other similar factors, which cannot be modified, cannabis use can be regulated. Public health interventions like education campaigns to alert people to the possible risks associated with cannabis, and to warn young people that using cannabis could increase their risk of developing a psychotic illness later in life, can be expected to yield positive results. This becomes even more pertinent in the current scenario as, when governments legalize cannabis, the public is indirectly conveyed the impression that cannabis is quite harmless and people can safely consume it. Since there is no doubt of the link between cannabis and psychosis, as well as a broad understanding of the biological basis of the association, there is no reason why this information should not be disseminated among the masses. While policy makers dither and dally, young lives are being lost to psychosis. The other thing that must be encouraged is further research to unravel the exact biological mechanism of how cannabis leads to psychosis. Once we have a precise knowledge of how cannabis leads to psychosis, it can be expected that newer and more efficacious antipsychotic agents, especially those that can reverse the detrimental effects of cannabis, can be developed.

MINI-DICTIONARY

Cannabidiol It is one of the chemical constituents of the cannabis plant. It is believed to have opposite effects to tetrahydrocannabinol (THC). While THC can cause anxiety and psychotic symptoms, CBD has anxiolytic and possibly antipsychotic activity.

Endocannabinoids Endocannabinoids are the endogenous ligands that bind to the cannabinoid receptors present in the body. Anandamide is the most well-known endocannabinoid. The endocannabinoid system modulates neurotransmission at both inhibitory and excitatory synapses in brain regions, which have a role in the regulation of pain, emotion, motivation, and cognition.

Genetic polymorphism It refers to natural variations in genes that occur with fairly high frequency in the general population. Single nucleotide polymorphisms are the most common type of genetic polymorphism among people, and are produced due to a variation in a single nucleotide. A polymorphism is said to be functional if it can alter the function of a gene.

Sinsemilla It refers to a technique of growing cannabis where only the female plant is allowed to blossom. Without pollen from the male plant, the female blossoms produce no seeds, and have a high content of tetrahydrocannabinol (THC).

Skunk A cannabis strain produced by cross-breeding *Cannabis sativa* and *Cannabis indica* plants. It is very potent and has a high THC content.

Tetrahydrocannabinol (THC) THC or, more precisely, Δ^9-THC, is one of the principal chemical constituents of the cannabis plant. The psychoactive and addictive properties of cannabis are believed to be due to its THC content.

References

Alemany, S., Arias, B., Fatjo-Vilas, M., Villa, H., Moya, J., Ibanez, M. I., Ortet, G., Gasto, C., & Fananas, L. (2014). Psychosis-inducing effects of cannabis are related to both childhood abuse and COMT genotypes. *Acta Psychiatrica Scandinavica*, *129*, 54–62.

Andreasson, S., Allebeck, P., Engstrom, A., & Rydberg, U. (1987). Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. *Lancet*, *330*, 1483–1486.

Arseneault, L., Cannon, M., Poulton, R., Murray, R., Caspi, A., & Moffitt, T. E. (2002). Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. *British Medical Journal*, *325*, 1212–1213.

Barbee, J. G., Clark, P. D., Crapanzo, M. S., Heintz, G. C., & Kehoe, C. E. (1989). Alcohol and substance abuse among schizophrenic patients presenting to an emergency psychiatry service. *Journal of Nervous and Mental Disease*, *177*, 400–417.

Bhattacharyya, S., Morrison, P., Fusar-Poli, P., Martin-Santos, R., Borgwardt, S., Winton-brown, T., Nosarti, C., O'Carroll, C. M., Seal, M., Allen, P., Mehta, M. A., Stone, J. M., Tunstall, N., Giampietro, V., Kapur, S., Murray, R. M., Zuardi, A. W., Crippa, J. A., Atakan, Z., & McGuire, P. K. (2010). Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology*, *35*, 764–774.

Bhattacharyya, S., Atakan, Z., Martin-Santos, R., Crippa, J. A., Kambeitz, J., Prata, D., Williams, S., Brammer, M., Collier, D. A., & McGuire, P. K. (2012a). Preliminary report of biological basis of sensitivity to the effects of cannabis on psychosis: AKT1 and DAT1 genotype modulates the effects of delta-9-tetrahydrocannabinol on midbrain and striatal function. *Molecular Psychiatry*, *17*, 1152–1155.

Bhattacharyya, S., Crippa, J. A., Allen, P., Martin-Santos, R., Borgwardt, S., Fusar-Poli, P., Rubia, K., Kambeitz, J., O'Carroll, C., Seal, M. L., Giampietro, V., Brammer, M., Zuardi, A. W., Atakan, Z., & McGuire, P. K. (2012b). Induction of psychosis by {delta}9-tetrahydrocannabinol reflects modulation of prefrontal and striatal function during attentional salience processing. *Archives of General Psychiatry*, *69*, 27–36.

Bloomfield, M. A. P., Morgan, C. J. A., Egerton, A., Kapur, S., Curran, H. V., & Howes, O. D. (2014). Dopaminergic function in cannabis users and its relationship to cannabis-induced psychotic symptoms. *Biological Psychiatry*, *75*, 470–478.

Caspi, A., Moffitt, T. E., Cannon, M., McClay, J., Murray, R., Harrington, H., Taylor, A., Arseneault, L., Williams, B., Braithwaite, A., Poulton, R., & Craig, I. W. (2005). Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biological Psychiatry*, *57*, 1117–1127.

Cheer, J. F., Wassum, K. M., Heien, M. L., Phillips, P. E., & Wightman, R. M. (2004). Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. *Journal of Neuroscience*, *24*, 4393–4400.

Chopra, G. S., & Smith, J. W. (1974). Psychotic reactions following cannabis use in East Indians. *Archives of General Psychiatry*, *30*, 24–27.

Dalton, V. S., Long, L. E., Weickert, C. S., & Zavitsanou, K. (2011). Paranoid schizophrenia is characterized by increased CB1 receptor binding in the dorsolateral prefrontal cortex. *Neuropsychopharmacology*, *36*, 1620–1630.

De Marchi, N., De Petrocellis, L., Orlando, P., Daniele, F., Fezza, F., & Di Marzo, V. (2003). Endocannabinoid signalling in the blood of patients with schizophrenia. *Lipids in Health and Disease*, *2*, 5.

Dean, B., Sundram, S., Bradbury, R., Scarr, E., & Copolov, D. (2001). Studies on [3H] CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. *Neuroscience*, *103*, 9–15.

Degenhardt, L., & Hall, W. D. (2001). The association between psychosis and problematical drug use among Australian adults: findings from the National Survey of Mental Health and Well-being. *Psychological Medicine*, *31*, 659–668.

Di Forti, M., Iyegbe, C., Sallis, H., Kolliakou, A., Falcone, M. A., Paparelli, A., Sirianni, M., La Cascia, C., Stilo, S. A., Marques, T. R., Handley, R., Mondelli, V., Dazzan, P., Pariante, C., David, A. S., Morgan, C., Powell, J., & Murray, R. M. (2012). Confirmation that the AKT1 (rs2494732) genotype influences the risk of psychosis in cannabis users. *Biological Psychiatry*, *72*, 811–816.

Di Forti, M., Iyegbe, C., Falcone, A., Powell, J., & Murray, R. (2014a). Vulnerability to cannabis-related psychosis: association with frequency and potency of cannabis use, and interaction with genes regulating dopamine signalling. *Lancet*, *383*, S41.

Di Forti, M., Sallis, H., Allegri, F., Trotta, A., Ferraro, L., Stilo, S. A., Marconi, A., La Cascia, C., Marques, T. R., Pariante, C., Dazzan, P., Mondelli, V., Paparelli, A., Kolliakou, A., Prata, D., Gaughran, F., David, A. S., Morgan, C., Stahl, D., Khondoker, M., MacCabe, J. H., & Murray, R. M. (2014b). Daily use, especially of high-potency cannabis, drives the earlier onset of psychosis in cannabis users. *Schizophrenia Bulletin*, *40* (6), 1509–1517.

Fergusson, D. M., Horwood, J. L., & Swain-Campbell, N. R. (2003). Cannabis dependence and psychotic symptoms in young people. *Psychological Medicine*, *33*, 15–21.

Foti, D. J., Kotov, R., Guey, L. T., & Bromet, E. J. (2010). Cannabis use and the course of schizophrenia: 10-year follow-up after first hospitalization. *American Journal of Psychiatry*, *167*, 987–993.

French, E. D., Dillon, K., & Wu, X. (1997). Cannabinoids excite dopamine neurons in the ventral tegmentum and substantia nigra. *Neuroreport*, *8*, 649–652.

Galve-Roperh, I., Aguado, T., Palazuelos, J., & Guzman, M. (2007). The endocannabinoid system and neurogenesis in health and disease. *Neuroscientist*, *13*, 109–114.

Galvez-Buccollini, J. A., Proal, A. C., Tomaselli, V., Trachtenberg, M., Coconcea, C., Chun, J., Manschreck, T., Fleming, J., & Delisi, L. E. (2012). Association between age at onset of psychosis and age at onset of cannabis use in non-affective psychosis. *Schizophrenia Research*, *139*, 157–160.

Giuffrida, A., Leweke, F. M., Gerth, C. W., Schreiber, D., Koethe, D., Faulhaber, J., Klosterkotter, J., & Piomelli, D. (2004). Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. *Neuropsychopharmacology*, *29*, 2108–2114.

Henquet, C., & Kuepper, R. (2010). Does cannabidiol protect against the negative effects of THC? *British Journal of Psychiatry*, *197*, 259–260.

Henquet, C., Krabbendam, L., Spauwen, J., Kaplan, C., Lieb, R., Wittchen, H. U., & van Os, J. (2005a). Prospective cohort study of cannabis use, predisposition for psychosis, and psychotic symptoms in young people. *British Medical Journal*, *330*, 11.

Henquet, C., Murray, R., Linszen, D., & van Os, J. (2005b). The environment and schizophrenia: the role of cannabis use. *Schizophrenia Bulletin*, *31*, 608–612.

Henquet, C., Di Forti, M., Morrison, P., Kuepper, R., & Murray, R. M. (2008). Gene-environment interplay between cannabis and psychosis. *Schizophrenia Bulletin*, *34*, 1111–1121.

Houston, J. E., Murphy, J., Adamson, G., Stringer, M., & Shevlin, M. (2008). Childhood sexual abuse, early cannabis use, and psychosis: testing an interaction model based on the National Comorbidity Survey. *Schizophrenia Bulletin*, *34*, 580–585.

Ishiguro, H., Horiuchi, Y., Ishikawa, M., Koga, M., Imai, K., Suzuki, Y., Morikawa, M., Inada, T., Watanabe, Y., Takahashi, M., Someya, T., Ujike, H., Iwata, N., Ozaki, N., Onaivi, E. S., Kunugi, H., Sasaki, T., Itokawa, M., Arai, M., Niizato, K., Iritani, S., Naka, I., Ohashi, J., Kakita, A., Takahashi, H., Nawa, H., & Arinami, T. (2010). Brain cannabinoid CB2 receptor in schizophrenia. *Biological Psychiatry*, *67*, 974–982.

Kaplan, J. (1969). *Marijuana: report of the Indian Hemp Drugs Commission, 1893–1894*. Silver Springs, MD: Thomas Jefferson Co.

Kapur, S., Mizrahi, R., & Li, M. (2005). From dopamine to salience to psychosis—linking biology, pharmacology and phenomenology of psychosis. *Schizophrenia Research*, *79*, 59–68.

Koethe, D., Giuffrida, A., Schreiber, D., Hellmich, M., Schultze-Lutter, F., Ruhrmann, S., Klosterkotter, J., Piomelli, D., & Leweke, F. M. (2009). Anandamide elevation in cerebrospinal fluid in initial prodromal states of psychosis. *British Journal of Psychiatry*, *194*, 371–372.

Konings, M., Henquet, C., Maharajh, H. D., Hutchinson, G., & van Os, J. (2008). Early exposure to cannabis and risk for psychosis in young adolescents in Trinidad. *Acta Psychiatrica Scandinavica*, *118*, 209–213.

Kuepper, R., Morrison, P. D., van Os, J., Murray, R. M., Kenis, G., & Henquet, C. (2010). Does dopamine mediate the psychosis-inducing effects of cannabis? A review and integration of findings across disciplines. *Schizophrenia Research*, *121*, 107–117.

Kuepper, R., van Os, J., Lieb, R., Wittchen, H. -U., & Henquet, C. (2011). Do cannabis and urbanicity co-participate in causing psychosis? Evidence from a 10-year follow-up cohort study. *Psychological Medicine*, *41*, 2121–2129.

Large, M., Sharma, S., Compton, M. T., Slade, T., & Nielssen, O. (2011). Cannabis use and early onset psychosis: a systematic meta-analysis. *Archives of General Psychiatry*, *68*, 555–561.

Leweke, F. M., Giuffrida, A., Wurster, U., Emrich, H. M., & Piomelli, D. (1999). Elevated endogenous cannabinoids in schizophrenia. *Neuroreport*, *10*, 1665–1669.

Malone, D. T., Hill, M. N., & Rubino, T. (2010). Adolescent cannabis use and psychosis: epidemiology and neurodevelopmental models. *British Journal of Pharmacology*, *160*, 511–522.

Manrique-Garcia, E., Zammit, S., Dalman, C., Hemmingsson, T., Andreasson, S., & Allebeck, P. (2012). Cannabis, schizophrenia and other non-affective psychoses: 35 years of follow-up of a population-based cohort. *Psychological Medicine*, *42*, 1321–1328.

McGrath, J., Welham, J., Scott, J., Varghese, D., Degenhardt, L., Hayatbakhsh, M. R., Alati, R., Williams, G. M., Bor, W., & Najman, J. M. (2010). Association between cannabis use and psychosis-related outcomes using sibling pair analysis in a cohort of young adults. *Archives of General Psychiatry*, *67*, 440–447.

McGuire, P. K., Jones, P., Harvey, I., Williams, M., McGuffin, P., & Murray, R. M. (1995). Morbid risk of schizophrenia for relatives of patients with cannabis associated psychosis. *Schizophrenia Research*, *15*, 277–281.

Moore, T. H. M., Zammit, S., Lingford-Hughes, A., Barnes, T. R. E., Jones, P. B., Burke, M., & Lewis, G. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet*, *370*, 319–328.

Morrison, P. D., Nottage, J., Stone, J. M., Bhattacharyya, S., Tunstall, N., Brenneisen, R., Holt, D., Wilson, D., Sumich, A., McGuire, P., Murray, R. M., Kapur, S., & ffytche, D. H. (2011). Disruption of frontal theta coherence by Delta(9) tetrahydrocannabinol is associated with positive psychotic symptoms. *Neuropsychopharmacology*, *36*, 827–836.

Murray, R. M., Morrison, P. D., Henquet, C., & Di Forti, M. (2007). Cannabis, the mind and society: the hash realities. *Nature Reviews Neuroscience*, *8*, 885–895.

Newell, K. A., Deng, C., & Huang, X. F. (2006). Increased cannabinoid receptor density in the posterior cingulate cortex in schizophrenia. *Experimental Brain Research*, *172*, 556–560.

Parakh, P., & Basu, D. (2013). Cannabis and psychosis: have we found the missing links? *Asian Journal of Psychiatry*, *6*, 281–287.

Potter, D. J., Clark, P., & Brown, M. B. (2008). Potency of delta-9-THC and other cannabinoids in cannabis in England in 2005: implications for psychoactivity and pharmacology. *Journal of Forensic Science*, *53*, 90–94.

Rapp, C., Bugra, H., Riecher-Rössler, A., Tamagni, C., & Borgwardt, S. (2012). Effects of cannabis use on human brain structure in psychosis: a systematic review combining in vivo structural neuroimaging and post mortem studies. *Current Pharmaceutical Design*, *18*, 5070–5080.

Riegel, A. C., & Lupica, C. R. (2004). Independent presynaptic and postsynaptic mechanisms regulate endocannabinoid signaling at multiple synapses in the ventral tegmental area. *Journal of Neuroscience*, *24*, 11070–11078.

Schneier, F. R., & Siris, S. G. (1987). A review of psychoactive substance use and abuse in schizophrenia: patterns of drug choice. *Journal of Nervous and Mental Disease*, *175*, 641–652.

Smith, M. J., Cobia, D. J., Wang, L., Alpert, K. I., Cronenwett, W. J., Goldman, M. B., Mamah, D., Barch, D. M., Breiter, H. C., & Csernansky, J. G. (2014). Cannabis-related working memory deficits and associated subcortical morphological differences in healthy individuals and schizophrenia subjects. *Schizophrenia Bulletin*, *40*, 287–299.

Stefanis, N. C., Delespaul, P., Henquet, C., Bakoula, C., Stefanis, C. N., & van Os, J. (2004). Early adolescent cannabis exposure and positive and negative dimensions of psychosis. *Addiction*, *99*, 1333–1341.

Stefanis, N. C., Dragovic, M., Power, B. D., Jablensky, A., Castle, D., & Morgan, V. A. (2013). Age at initiation of cannabis use predicts age at onset of psychosis: the 7-to 8-year trend. *Schizophrenia Bulletin*, *39*, 251–254.

Talbott, J. A., & Teague, J. W. (1969). Marihuana psychosis. Acute toxic psychosis associated with the use of Cannabis derivatives. *JAMA*, *210*, 299–302.

Tien, A. Y., & Anthony, J. C. (1990). Epidemiological analysis of alcohol and drug use as risk factors for psychotic experiences. *Journal of Nervous and Mental Disease*, *178*, 473–480.

van Os, J., Bak, M., Hanssen, M., Bijl, R. V., de Graaf, R., & Verdoux, H. (2002). Cannabis use and psychosis: a longitudinal population-based study. *American Journal of Epidemiology*, *156*, 319–327.

van Winkel, R. Genetic Risk and Outcome of Psychosis (GROUP) Investigators. (2011). Family-based analysis of genetic variation underlying psychosis-inducing effects of cannabis: sibling analysis and proband follow-up. *Archives of General Psychiatry*, *68*, 148–157.

Wong, D. F., Kuwabara, H., Horti, A. G., Raymont, V., Brasic, J., Guevara, M., Ye, W., Dannals, R. F., Ravert, H. T., Nandi, A., Rahmima, A., Ming, J. E., Grachev, I., Roy, C., & Cascella, N. (2010). Quantification of cerebral cannabinoid receptors subtype 1 (CB1) in healthy subjects and schizophrenia by the novel PET radioligand [11C] OMAR. *Neuroimage*, *52*, 1505–1513.

Zammit, S., Allebeck, P., Andreasson, S., Lundberg, I., & Lewis, G. (2002). Self reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: historical cohort study. *British Medical Journal*, *325*, 1199–1201.

Zammit, S., Spurlock, G., Williams, H., Norton, N., Williams, N., O'Donovan, M. C., & Owen, M. J. (2007). Genotype effects of CHRNA7, CNR1 and COMT in schizophrenia: interactions with tobacco and cannabis use. *British Journal of Psychiatry*, *191*, 402–407.

Zavitsanou, K., Garrick, T., & Huang, X. F. (2004). Selective antagonist [3H] SR 141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulate cortex in schizophrenia. *Progress in Neuropsychopharmacology, Biology and Psychiatry*, *28*, 355–360.

CHAPTER

43

Cannabis Use in Bipolar Disorder

T.V. Lagerberg

NORMENT KG Jebsen Centre for Psychosis Research, Institute of Clinical Medicine, University of Oslo, Oslo, Norway; Division of Mental Health and Addiction, Oslo University Hospital, Oslo, Norway

SUMMARY POINTS

- Cannabis use and use disorders are common in bipolar disorder.
- There are three main hypotheses regarding the association between cannabis use and bipolar disorder: cannabis use triggers bipolar disorder, patients with bipolar disorder self-medicate with cannabis, and common third factors, such as genes and traumas, confound the relationship.
- Evidence that cannabis use may trigger bipolar disorder in vulnerable individuals is growing.
- Cannabis use disorders are associated with a more severe course of the bipolar disorder, including increased symptom levels, and frequency of episodes.
- Cannabis use disorders in bipolar disorder are associated with additional substance use disorders, as well as additional psychiatric comorbidity.
- There are some indications that cognitive functioning is better in subjects with bipolar disorder and cannabis use, compared to subjects with bipolar disorder only.

KEY FACTS OF BIPOLAR DISORDER

Bipolar disorder, previously referred to as manic-depressive disorder, is a severe mental disorder. Descriptions of the disorder date back to the 1st century AD. Bipolar disorder comprises periods with elevated and depressed mood, in between periods of normal mood. The pathophysiology of the disorder is not fully known, but the disorder is highly heritable, and involves disturbances in several brain functions. Bipolar disorder also appears to be linked to creativity. The main treatment for the disorder consists of medication with mood stabilizing or antipsychotic properties, with psychosocial treatments as an important supplement. The propensity for substance abuse in bipolar disorder was observed more than 200 years ago, and has been a focus of research in the last decades.

LIST OF ABBREVIATIONS

BD	Bipolar disorder
CBD	Cannabidiol
CUD	Cannabis use disorder
THC	Tetrahydrocannabinol

INTRODUCTION

Bipolar Disorder

Bipolar disorder (BD) is a severe mental illness characterized by periods of depressive and elevated mood, with normal mood in between. There are two main types of disorder, distinguished by a greater severity of the manic episodes in bipolar I disorder, compared to the less severe hypomanic episodes in bipolar II disorder. Hypomania and mania share features such as heightened mood, irritability, increased self-esteem, energy, and activity levels, in addition to reduced inhibition, concentration, and need for sleep. Mania, as opposed to hypomania, is also characterized by a marked reduction in daily functioning, and often accompanied by psychotic symptoms such as delusions and hallucinations, and with a need for hospitalization. The vast majority of patients with BD suffer from longer periods of depression than of elevated mood (Judd et al., 2003), and the

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00049-1
Copyright © 2017 Elsevier Inc. All rights reserved.

disorder is associated with a substantially increased risk for suicide (Tondo, Isacsson, & Baldessarini, 2003). The lifetime prevalence is approximately 1% for bipolar I and 1% for bipolar II disorder, although rates vary somewhat across studies. Due to its profound impact on ability to work and function, and its association with premature mortality, BD, together with other mental disorders and substance use disorders, is highly ranked in the WHO's global burden of disease list (Whiteford et al., 2013).

Cannabis Use Disorders

Cannabis use disorders (CUD) refer to the abuse of or dependence on cannabis, according to diagnostic criteria. Most often in clinical studies, the criteria of the *Diagnostic and Statistical Manual* are used (APA, 1994), but a range of different use levels and criteria have been used, creating challenges when comparing results in different studies.

Prevalence of Cannabis Use and Use Disorders in BD

In BD, as in other severe mental disorders, the rates of substance use and use disorders are high, that is, 3–11 times that of the general population (Hartz et al., 2014; Regier et al., 1990). As in the general population, cannabis is the most commonly used illegal drug (Cerullo & Strakowski, 2007). It is estimated that individuals with mental illnesses consume approximately 83% of all cannabis consumed in the United States (Lev-Ran, Le Foll, McKenzie, George, & Rehm, 2013b). Individuals with BD have been reported to be approximately 7 times more likely to use cannabis than healthy subjects (Agrawal, Nurnberger, Jr., & Lynskey, 2011), and have lifetime rates of CUDs (abuse and dependence) in the range of 10–50% (Agrawal et al., 2011; Lagerberg et al., 2014) (Fig. 43.1). The prevalence is higher in the United States, compared to Europe and other parts of the world, which also reflects the level of use in the general populations (Degenhardt et al., 2013). Rates also seem to be higher in bipolar I compared to bipolar II disorder (Lev-Ran, Le Foll, McKenzie, George, & Rehm, 2013a), in first episode compared to multiepisode samples (Tohen, Vieta, Gonzalez-Pinto, Reed, & Lin, 2010) in younger compared to older subjects (Dell'Osso et al., 2011), and in males compared to females (Cotton et al., 2013). Interestingly, cannabis use peaks at the age when BD usually develops, around 20–24 years (Degenhardt et al., 2013).

There are also indications that cannabis is more commonly used in BD than in schizophrenia and other mood disorders (Lev-Ran et al., 2013b; Hartz et al., 2014). In line with this, patients with BD seem to be at greater risk for continuing their cannabis use after hospitalization, compared to patients with schizophrenia and depression (Bahorik, Newhill, & Eack, 2013).

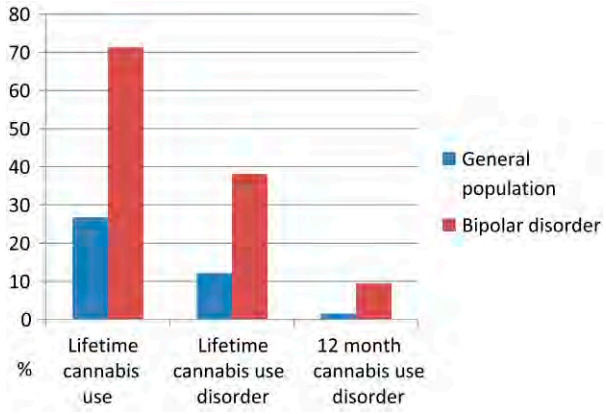


FIGURE 43.1 Prevalence of cannabis use and use disorders in bipolar disorder and the general population in the United States. Lifetime and 12 month prevalence of cannabis use, and use disorders, in bipolar disorder and the general population, according to epidemiological and clinical studies from the United States, published recently. *Source: Agrawal et al. (2011),* Psychiatry Research*; Etain et al. (2012),* Journal of Clinical Psychiatry*; Lev-Ran et al. (2013),* Comprehensive Psychiatry*; Haberstick et al. (2014),* Drug and Alcohol Dependence.

WHY DO BIPOLAR DISORDER AND CANNABIS USE DISORDERS OFTEN COOCCUR?

Hypotheses for the Strong Association

There are several models that may explain the relationship between psychotic disorders and cannabis use. These models are also the most relevant ones for the relationship between BD and CUDs, and can be described as follows (van Winkel & Kuepper, 2014; Mueser, Drake, & Wallach, 1998) (Fig. 43.2):

1. BD causes cannabis use;
2. Cannabis use causes BD;
3. Genetic vulnerability models: gene-environment interaction, genetic confounding, and environmental mediation of genetic influences;
4. Other common factor models.

Does Bipolar Disorder Cause Cannabis Use Disorders?

The first hypothesis implies that BD leads to an increased risk for using cannabis. This can happen in a "direct" way: that is, in an elevated mood state, one of the symptoms is lack of inhibition, or an increased tendency to involve oneself in pleasurable activities. In this state, the barriers to using drugs may be reduced. While this may be the case for the *use* of cannabis, it is unlikely to be true for *use disorders*, which usually need a longer period of time to develop than the duration of a manic episode. To the author's knowledge, there are no empirical studies directly addressing this hypothesis; that is, in

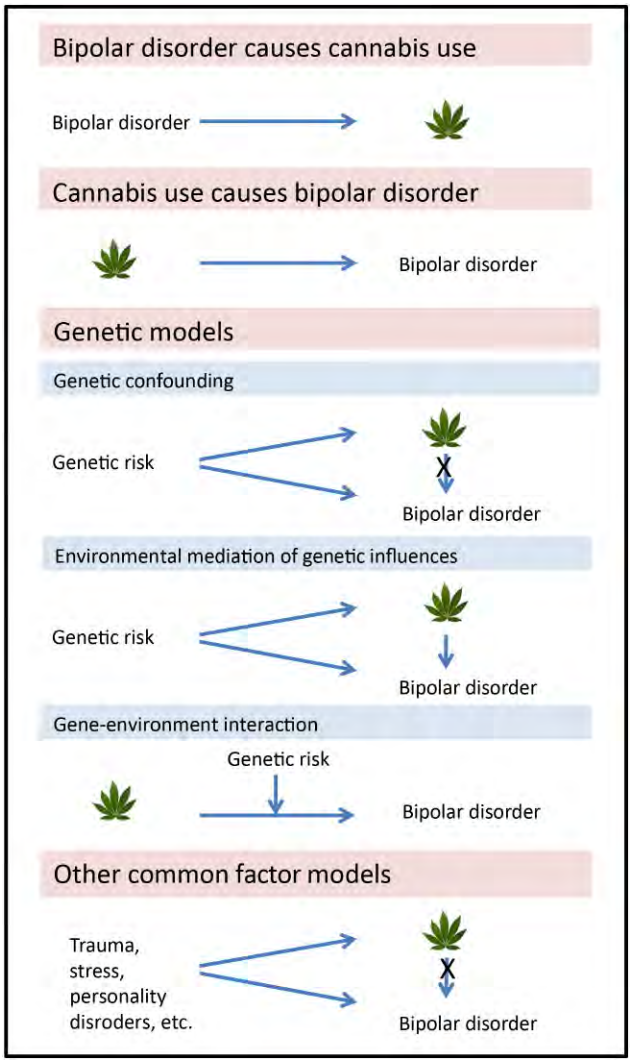


FIGURE 43.2 Representations of the relationship between cannabis use disorders and bipolar disorder. Fig. 43.2 shows a representation of the possible relationships between cannabis use and bipolar disorder.

which phase of BD is cannabis use usually initiated, and whether it is associated with inhibition.

BD could also lead to cannabis use, if the drug is used as an attempt to deal with the symptoms of the disorder, usually referred to as the "self-medication hypothesis" (Khantzian, 1997). This hypothesis does not aim at explaining the specific link between cannabis use and BD, but rather the link between dysphoria and other negative psychological experiences, and substance use in general. To date, there is mixed evidence regarding the self-medication hypothesis from two prospective epidemiological studies investigating BD and cannabis use. Manic/hypomanic symptoms predicted subsequent onset of cannabis use, and use disorders in one study (Wittchen et al., 2007), but not in another (Henquet, Krabbendam, de Graaf, Ten Have, & van Os, 2006). Research is also limited regarding clinical observations of cannabis use as an attempt to self-medicate symptoms of BD. Only a few studies specifically address reasons for cannabis use in BD. One qualitative study on 15 patients with BD and different substance use disorders reported that the subjects used cannabis to curb elevated mood, and manage anxiety and depressive symptoms (Healey, Peters, Kinderman, McCracken, & Morriss, 2009). Furthermore, a few anecdotal reports indicate that patients with BDs use cannabis to stabilize their mood (Grinspoon & Bakalar, 1998; Ashton, Moore, Gallagher, & Young, 2005). While smoking marijuana was found to reduce depressive symptoms and tension, and increase vigor in subjects with BD, in a pilot study investigating whether attempts to self-medicate may be successful, it increased depressive symptoms, confusion, and fatigue in cannabis smokers without psychiatric morbidity (Gruber et al., 2012). Based on these and other findings, some have argued that the cannabis compound cannabidiol (CBD) may have therapeutic potential in psychiatric disorders, including BD (Campos, Moreira, Gomes, Del Bel, & Guimaraes, 2012; Ashton et al., 2005). There is, however, no evidence suggesting relief of the whole *syndrome* of BD from cannabis. On the contrary, cannabis use appears to have a negative impact on the symptoms and course of BD. This will be addressed later in this chapter. However, since a substantial proportion of patients start using cannabis after the onset of their BD, and patients report using substances to alleviate affective symptoms, and seem to experience instant relief after cannabis use, further exploration of the self-medication hypothesis is warranted.

Does Cannabis Use Cause Bipolar Disorder?

When investigating the causal relationship between BD and cannabis use, a focus has been put on sequencing, that is, which of the phenomena most commonly occurs first. Results have been somewhat mixed, with the sequence of the onsets proving difficult to determine. In two studies, the first hypomanic/manic episode more often occurred before the onset of the CUD than the opposite (Agrawal et al., 2011; Heffner et al., 2012). In another study, the proportions with cannabis use or BD onset first was approximately the same (Strakowski et al., 2007), and in a study on the prodromal phase of BD, 68% of the subjects had substance use disorders (of which 82% had a CUD) prior to the onset (Conus et al., 2010). In a prospective study on established BD, it was found that cannabis use tended to precede or coincide with, rather than follow, manic symptoms (Baethge et al., 2008). From these findings, we can conclude that CUDs appear to both precede and follow the development of BD. In established BD, however, cannabis use does not seem to follow manic episodes.

This leads us to the second main hypothesis of the relationship between cannabis use and BD, namely that cannabis use can lead to or trigger BD. The onset

TABLE 43.1 Age at Onset, Medication Use, and Suicide Attempts in BD Patients With and Without Recent Cannabis Use

	No cannabis use last 6 months			Cannabis use last 6 months			
	N = 77			*N* = 24			
	N	**Mean**	**SD**	***N***	**Mean**	**SD**	***P***
Age at onset first psychotic episode	50[a]	29.5	10.4	15[a]	22.1	3.5	0.000
Age at onset first manic episode	74[a]	28.9	11.2	21[a]	23.3	7.0	0.008
Age at onset first depressive episode	57[a]	24.9	11.0	21[a]	19.8	6.2	0.013
Age at onset first psychotic episode	50[a]	29.5	10.4	15[a]	22.1	3.5	0.000
Age at onset BD I regardless of episode type[b]	77	24.5	10.4	24	20.1	6.6	0.018
	N	**%**		***N***	**%**		***P***
Current use of antipsychotics	57	74.0		10	41.7		0.006
Current use of mood stabilizers	19	24.7		4	16.7		0.579
One or more suicide attempts, lifetime	16	28.1		12	63.2		0.012

[a] *Lower N because not all patients experienced this episode.*
[b] *Psychotic, manic, depressive or mixed onset.*
Patients early in the course of bipolar disorder with recent cannabis use showed an earlier onset, and a higher prevalence of lifetime suicide attempt, compared to patients without recent cannabis use. The results remained significant after controlling for possible confounders, such as use of medication. Abbreviations: *BD I*, bipolar I disorder; *SD*, standard deviation.
Source: Reprinted from Kvitland, L. R., Melle, I., Aminoff, S. R., Lagerberg, T. V., Andreassen, O. A., and Ringen, P. A. (2014). Cannabis use in first-treatment bipolar I disorder: relations to clinical characteristics. Early Intervention in Psychiatry, 8*(2); with permission from Elsevier.*

of mania following excessive cannabis use has been noted by clinicians, and described in case reports (Iskandar, Griffeth, & Sharma, 2011). There are strong indications that cannabis use increases the risk for developing schizophrenia and other psychotic disorders (van Winkel & Kuepper, 2014). As BD and schizophrenia are closely related disorders, with shared pathophysiology and genetic risk, there is a possibility that cannabis may be a risk factor in BD, as well. Growing evidence from both clinical and epidemiological studies supports this. Prospective population studies have shown that cannabis use is associated with increased rates of manic symptoms (Henquet et al., 2006; Tijssen et al., 2010), and also with BD (van Laar, van Dorsselaer, Monshouwer, & de Graaf, 2007; Wittchen et al., 2007), following the use, with odds ratios in the range of 2.5–5 after controlling for possible confounders such as psychotic symptoms, and previous manic symptoms.

Furthermore, clinical studies have identified a relationship between cannabis use and earlier onsets of BD (Etain et al., 2012; Lagerberg et al., 2011; de Hert et al., 2011), also pointing toward cannabis use as a possible trigger of the disorder. This association has also been found in early phases of BD (Kvitland et al., 2014) (Table 43.1), reducing the possibility that the association is confounded by a recall-bias regarding the sequencing of the disorders. Furthermore, there are indications of a dose-response relationship, that is, the higher levels of cannabis use, the higher the risk of developing the disorder (van Laar et al., 2007), and the earlier the onset of the BD. Earlier age at onset has also been found in relation to cannabis use, regardless of whether the onset episode was depression or (hypo)mania, suggesting that cannabis may trigger the whole syndrome of BD, and not only manic *or* depressive episodes (Fig. 43.3) (Lagerberg et al., 2014). However, there are contradictory findings. One study found a later onset in patients with cannabis use preceding the BD, compared to patients with the opposite sequencing and patients with no cannabis use (Strakowski et al., 2007). In this study, the patients with BD first also had a faster recovery. As the authors point out, these findings may point toward cannabis as a possible trigger, since cannabis could have precipitated the BD at a later age in individuals with a lower constitutional vulnerability for the disorder.

IN SEARCH OF MECHANISMS: THE NEUROBIOLOGY OF CANNABIS IN BIPOLAR DISORDER

What could be the neurobiological mechanisms by which cannabis may trigger manic episodes and BD? The causes of BD are not fully identified, and the pathophysiology is still largely unknown. We do know, however, that the levels and actions of several neurotransmitters, such as dopamine and glutamate, are disturbed in the brain of subjects with BD, that is, hyperactivity in mesolimbic dopaminergic pathways has been implicated in mania. Cannabinoids affect such transmitter systems involved in affect regulation (Moreira & Lutz, 2008). For instance, THC, one of the psychoactive compounds of cannabis, is known to enhance activity in dopaminergic

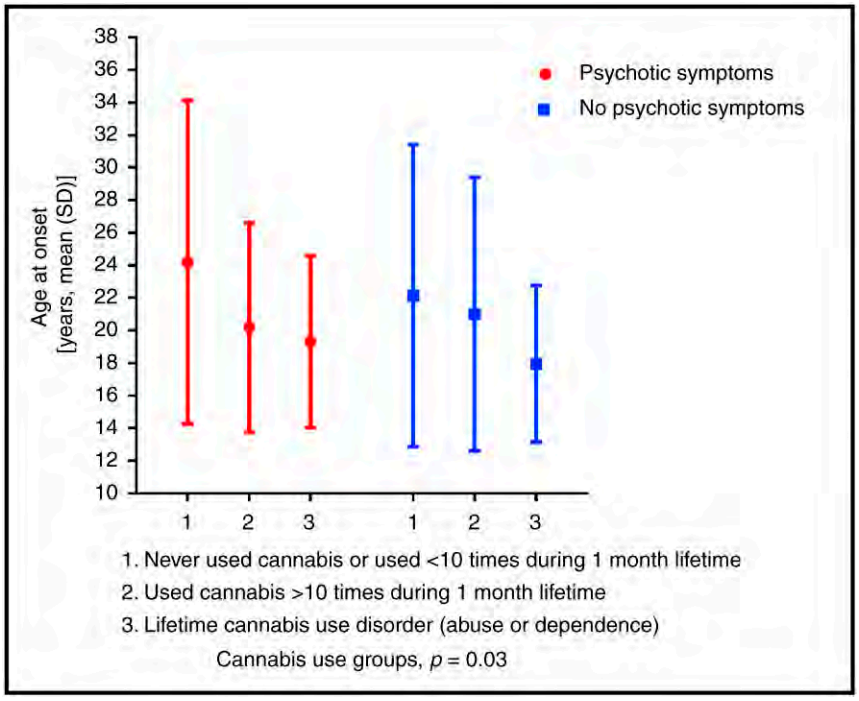


FIGURE 43.3 **Indications of a dose-response relationship between cannabis use and age at onset in bipolar disorder.** **Fig. 43.3** shows a decreasing age at onset of bipolar disorder with increasing levels of cannabis use. It also shows that the pattern is the same, regardless of presenting polarity: age at onset decreases as levels of cannabis use increase for patients presenting with both depressive and (hypo)manic episodes. *Source: Reprinted from Lagerberg, T. V., Kvitland, L. R., Aminoff, S. R., Aas, M., Ringen, P. A., Andreassen, O. A., and Melle, I. (2014). Indications of a dose-response relationship between cannabis use and age at onset in bipolar disorder.* Psychiatry Research, 215*(1), 101–104; with permission from Elsevier.*

pathways (D'Souza et al., 2005). Furthermore, cortical glutamate levels seem to play a significant role in acute mania (Öngur et al., 2008), and have also been found to be increased by THC, in rats (Tomasini et al., 2002). Also, the potential negative impact of cannabis on mental health is most likely related to the concentration ratio of THC/CBD (Englund et al., 2013), which has been increasing the last decade. Furthermore, there are indications of certain critical periods of brain development that are vulnerable for the use of cannabis, and it has been argued that cannabis may shift the brain's development trajectory toward a disease-vulnerable state (Chadwick, Miller, & Hurd, 2013).

Genetic Vulnerability Models

Since the majority of subjects using cannabis do not develop BD or other psychotic illnesses, a genetic vulnerability for the disorder is likely to be a prerequisite. This would imply a *gene-environment interaction*, in which cannabis use elicits BD only in individuals with a genetic predisposition. So far, genetic vulnerability has been difficult to measure directly, but it has been measured indirectly through the subject's family history of relevant disorder(s). In a prospective study investigating individuals with familial risk (offspring of individuals with BD), those who developed a substance use disorder (in most cases a CUD) were at greater risk for developing BD, suggesting a gene-environment interaction (Duffy et al., 2012). There is also the possibility of an interaction between several environmental factors, increasing the risk for BD only when occurring in combination. This has, to the author's knowledge, not been investigated specifically. Relevant to this, however, are findings from a study investigating cannabis use and childhood trauma in BD. Here, both factors were independently associated with earlier onsets and other BD severity indicators (Aas et al., 2013).

A matter of debate is whether the comorbidity of CUDs and psychotic disorders (including BD) could be due to *genetic confounding*, meaning that the relationship is explained by a common genetic diathesis between the two disorders, and not by a causal relationship between them. The finding that CUDs increase the risk of developing BD in high risk individuals would, however, not be expected if a common genetic diathesis was the only basis for the association (Duffy et al., 2012). A third possibility, *environmental mediation of genetic influences*, implies that, in addition to a common genetic diathesis, cannabis use additionally increases the risk for BD. This has not yet been investigated empirically. As modern genetic studies (genome-wide association studies) are starting to identify the genetic basis for severe mental disorders such as BD, the possibility to investigate these genetic models will increase substantially.

Other Common Factor Models

Other common factors, such as sociodemographic factors, trauma, stress, or personality traits, could also contribute to explaining the high prevalence of CUDs in BD. For instance, childhood trauma appears to be highly prevalent in both BD and substance use disorders (Larsson et al., 2013; Enoch, 2011). Childhood trauma is also associated with CUD comorbidity in BD (Aas et al., 2013). Furthermore, stressful life events have been suggested as risk factors for BD (Post, 1992), as well as for CUDs (Hyman & Sinha, 2009). Temperament or personality traits have also been suggested as common factors. Impulsivity appears to be associated with both BD and substance use disorders (Swann, 2010), and is found to be additionally increased in the comorbid subjects (Etain et al., 2013). Personality disorders are also more prevalent in comorbid subjects (Lev-Ran et al., 2013a).

In conclusion, research on the different hypotheses is sparse, so far, and it is premature to draw any firm conclusions regarding their validity. There are several indications that cannabis may trigger BD in genetically vulnerable individuals. However, competing explanations such as a common genetic diathesis and the self-medication hypothesis have not been ruled out. Also, the relationship may be bidirectional; cannabis use may elicit BD in vulnerable individuals, who also may use cannabis as an attempt to self-medicate the symptoms of the disorder, once it is established. Indications for a bidirectional relationship between the two disorders was found in a prospective study on subjects with familial risk (Duffy et al., 2012), but not in a prospective epidemiological study (Henquet et al., 2006). Most likely, no single model can explain all cases of comorbidity, thus several models may be needed in order to fully account for the relationship.

THE CONSEQUENCES OF CANNABIS USE IN BIPOLAR DISORDER

Cannabis Use Disorders and the Symptoms and Course of BD

CUDs have been associated with more severe clinical characteristics in BD. From cross-sectional studies, we know that comorbid patients have a higher number of illness episodes (Lev-Ran et al., 2013a), an increased prevalence of mixed episodes (Agrawal et al., 2011), and psychotic symptoms (Braga, Burdick, Derosse, & Malhotra, 2012), as well as higher rates of suicide attempts (Aas et al., 2013; Bellivier et al., 2011), compared to patients without a CUD. The duration of cannabis use has also been found to be associated with the duration of manic episodes (Cerullo & Strakowski, 2007). However, since these studies are cross-sectional, one cannot conclude whether subjects with a greater illness severity use cannabis because of the increased symptoms (eg, self-medication), or whether the increased severity is a consequence of cannabis use.

Findings from a few longitudinal studies, in which causal relationships can be explored, indicate that there is a negative effect from CUDs on the response to treatment and the course of the BD. In these studies, CUDs were associated with reduced compliance with medication, higher levels of manic and psychotic symptoms, and higher overall illness severity (Van Rossum, Boomsma, Tenback, Reed, & van Os, 2009; Bahorik et al., 2013). Also, there appears to be an increased risk for rapid cycling and prolonged duration of manic episodes (Strakowski et al., 2007), and for switching into mania in depressed individuals (Ostacher et al., 2010), in the presence of a CUD. In a study on subjects with a first psychotic episode, manic symptoms improved more in subjects that stopped their cannabis use during follow-up, compared to subjects that continued their use (Stone et al., 2014).

Cannabis Use, and Brain Structure and Functioning

To date, this area has only been studied in one research group focusing on adolescent subjects after a first manic episode with psychosis. The comorbid adolescents showed greater structural abnormalities in frontal and temporal cortical regions, as well as in subcortical areas linked with emotion and motivational regulation, compared to adolescents with BD alone (Jarvis et al., 2008). In a functional brain imaging study from the same research group, the comorbid adolescents did not show the same overactivation of regions involved in emotional processing seen in adolescents with BD alone, when compared to healthy controls (Bitter et al., 2014). These findings could imply either a different neuropathology in the cannabis using group, or that cannabis use influences brain structure, and reduces the overactivation in the brain regions found in subjects with BD only.

Cannabis Use and Cognition

Although research so far is sparse, in a few studies, cognitive functioning was somewhat surprisingly found to be better in comorbid subjects, compared to subjects with BD alone (Braga et al., 2012; Ringen et al., 2010). This has been interpreted in several ways: as supporting the notion that cannabis may trigger the disorder in less vulnerable individuals, that cannabis may improve cognitive functions related to dopamine, or that these subjects constitute a selected group, since regular cannabis consumption requires certain cognitive skills. Further replications and investigations are needed to clarify this issue.

Cannabis Use, Daily Functioning, and Quality of Life

The results are somewhat mixed regarding CUD and daily functioning, and quality of life. CUD was associated with reduced social functioning in a cross-sectional study (Hellvin, Sundet, Aminoff, Andreassen, & Melle, 2013). In a longitudinal study, cannabis was associated with not being in a relationship (Van Rossum et al., 2009). General life functioning appears to be reduced (Agrawal et al., 2011), also confirmed by a longitudinal study (Bahorik et al., 2013). Work functioning, however, has not been found to be associated with CUDs (Goetz, Tohen, Reed, Lorenzo, & Vieta, 2007; Van Rossum et al., 2009). Furthermore, results are mixed regarding whether or not life satisfaction is reduced in comorbid subjects, compared to subjects with BD only (Van Rossum et al., 2009; Lev-Ran et al., 2013a). The lack of additional reductions in measures of functioning and quality of life in comorbid subjects, found in some studies, could be due to the already low levels associated with BD itself.

The Challenge of Additional Comorbidities

CUDs are often accompanied by excessive use of other substances such as nicotine, alcohol, and other drugs (Lev-Ran et al., 2013a; Wittchen et al., 2007; Heffner et al., 2012). Furthermore, individuals with BD and CUD often also suffer from additional psychiatric disorders, such as personality disorders (Lev-Ran et al., 2013a). Many of these comorbidities contribute independently to more severe outcomes. In addition to causing major challenges in the lives of the patients, and in the treatment of the disorders, it causes difficulties in disentangling the effect of cannabis from effects of other disorders on the BD course. Many studies have failed to control for the presence of other substance use disorders. Thus, further research is warranted to establish independent effects from cannabis use on the symptoms and course of BD. However, knowledge is sufficient to conclude that cannabis use should be prevented in individuals at risk, and treated when present in established BD.

MINI-DICTIONARY

Bipolar disorder A severe affective disorder comprising elevated and depressed mood episodes in between periods of normal mood.
Cannabis use disorders A maladaptive pattern of cannabis use which leads to substantial clinical discomfort or functional loss.
Comorbidity When two or more diseases cooccur.
Depression (severe) A period of severe sadness and loss of pleasure or interest, in addition to symptoms such as loss of energy, appetite, sleep, and suicidal thoughts.
Hypomania A less severe form of mania in which symptoms are similar, but does not include psychotic symptoms, and does not cause marked functional loss.
Mania Periods of elevated or irritable mood, and additional symptoms such as increased self-esteem, activity levels and, in some cases, psychotic symptoms, causing marked functional loss.
Mixed episode A period of cooccurring manic and depressive symptoms.
Neurotransmitters Endogenous chemicals transmitting signals between neurons across a synapse.
Pathophysiology The mechanisms whereby a disease or condition develops and progresses.
Psychotic symptoms Delusions, hallucinations, and disorganized thought or behavior.
Rapid cycling Having four or more bipolar episodes within 1 year.
Schizophrenia Severe psychotic disorder comprising delusions, hallucinations, disorganized behavior, apathy, and social withdrawal.

References

Aas, M., Etain, B., Bellivier, F., Henry, C., Lagerberg, T., Ringen, A., et al. (2013). Additive effects of childhood abuse and cannabis abuse on clinical expressions of bipolar disorders. *Psychological Medicine, 44,* 1653–1662.

Agrawal, A., Nurnberger, J. I., Jr., & Lynskey, M. T. (2011). Cannabis involvement in individuals with bipolar disorder. *Psychiatry Research, 185,* 459–461.

APA. (1994). *Diagnostic and statistical manual of mental disorders* (4th ed.). Washington, DC: American Psychiatric Association.

Ashton, C. H., Moore, P. B., Gallagher, P., & Young, A. H. (2005). Cannabinoids in bipolar affective disorder: a review and discussion of their therapeutic potential. *Journal of Psychopharmacology, 19,* 293–300.

Baethge, C., Hennen, J., Khalsa, H. M., Salvatore, P., Tohen, M., & Baldessarini, R. J. (2008). Sequencing of substance use and affective morbidity in 166 first-episode bipolar I disorder patients. *Bipolar Disorders, 10,* 738–741.

Bahorik, A. L., Newhill, C. E., & Eack, S. M. (2013). Characterizing the longitudinal patterns of substance use among individuals diagnosed with serious mental illness after psychiatric hospitalization. *Addiction, 108,* 1259–1269.

Bellivier, F., Yon, L., Luquiens, A., Azorin, J. M., Bertsch, J., Gerard, S., et al. (2011). Suicidal attempts in bipolar disorder: results from an observational study (EMBLEM). *Bipolar Disorders, 13,* 377–386.

Bitter, S. M., Adler, C. M., Eliassen, J. C., Weber, W. A., Welge, J. A., Burciaga, J., et al. (2014). Neurofunctional changes in adolescent cannabis users with and without bipolar disorder. *Addiction, 109,* 1901–1909.

Braga, R. J., Burdick, K. E., Derosse, P., & Malhotra, A. K. (2012). Cognitive and clinical outcomes associated with cannabis use in patients with bipolar I disorder. *Psychiatry Research, 200,* 242–245.

Campos, A. C., Moreira, F. A., Gomes, F. V., Del Bel, E. A., & Guimaraes, F. S. (2012). Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. *Philosophical Transactions of the Royal Society London Series B, 367,* 3364–3378.

Cerullo, M. A., & Strakowski, S. M. (2007). The prevalence and significance of substance use disorders in bipolar type I and II disorder. *Substance Abuse Treatment and Prevention Policy, 2,* 29.

Chadwick, B., Miller, M. L., & Hurd, Y. L. (2013). Cannabis use during adolescent development: susceptibility to psychiatric illness. *Frontiers in Psychiatry, 4,* 129.

Conus, P., Ward, J., Lucas, N., Cotton, S., Yung, A. R., Berk, M., et al. (2010). Characterisation of the prodrome to a first episode of psychotic mania: results of a retrospective study. *Journal of Affective Disorders, 124,* 341–345.

Cotton, S. M., Lambert, M., Berk, M., Schimmelmann, B. G., Butselaar, F. J., McGorry, P. D., et al. (2013). Gender differences in first episode psychotic mania. *BMC Psychiatry, 13,* 82.

D'Souza, D. C., Abi-Saab, W. M., Madonick, S., Forselius-Bielen, K., Doersch, A., Braley, G., et al. (2005). Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biological Psychiatry*, *57*, 594–608.

de Hert, M., Wampers, M., Jendricko, T., Franic, T., Vidovic, D., De Vriendt, N., et al. (2011). Effects of cannabis use on age at onset in schizophrenia and bipolar disorder. *Schizophrenia Research*, *126*, 270–276.

Degenhardt, L., Ferrari, A. J., Calabria, B., Hall, W. D., Norman, R. E., McGrath, J., et al. (2013). The global epidemiology and contribution of cannabis use and dependence to the global burden of disease: results from the GBD 2010 study. *PLoS One*, *8*, e76635.

Dell'Osso, B., Buoli, M., Bortolussi, S., Camuri, G., Vecchi, V., & Altamura, A. C. (2011). Patterns of Axis I comorbidity in relation to age in patients with bipolar disorder: a cross-sectional analysis. *Journal of Affective Disorders*, *130*, 318–322.

Duffy, A., Horrocks, J., Milin, R., Doucette, S., Persson, G., & Grof, P. (2012). Adolescent substance use disorder during the early stages of bipolar disorder: a prospective high-risk study. *Journal of Affective Disorders*, *142*, 57–64.

Englund, A., Morrison, P. D., Nottage, J., Hague, D., Kane, F., Bonaccorso, S., et al. (2013). Cannabidiol inhibits THC-elicited paranoid symptoms and hippocampal-dependent memory impairment. *Journal of Psychopharmacology*, *27*, 19–27.

Enoch, M. A. (2011). The role of early life stress as a predictor for alcohol and drug dependence. *Psychopharmacology*, *214*, 17–31.

Etain, B., Lajnef, M., Bellivier, F., Mathieu, F., Raust, A., Cochet, B., et al. (2012). Clinical expression of bipolar disorder type I as a function of age and polarity at onset: convergent findings in samples from France and the United States. *Journal of Clinical Psychiatry*, *73*, e561–e566.

Etain, B., Mathieu, F., Liquet, S., Raust, A., Cochet, B., Richard, J. R., et al. (2013). Clinical features associated with trait-impulsiveness in euthymic bipolar disorder patients. *Journal of Affective Disorders*, *144*, 240–247.

Goetz, I., Tohen, M., Reed, C., Lorenzo, M., & Vieta, E. (2007). Functional impairment in patients with mania: baseline results of the EMBLEM study. *Bipolar Disorders*, *9*, 45–52.

Grinspoon, L., & Bakalar, J. B. (1998). The use of cannabis as a mood stabilizer in bipolar disorder: anecdotal evidence and the need for clinical research. *Journal of Psychoactive Drugs*, *30*, 171–177.

Gruber, S. A., Sagar, K. A., Dahlgren, M. K., Olson, D. P., Centorrino, F., & Scott, E. L. (2012). Marijuana impacts mood in bipolar disorder: a pilot study. *Mental Health and Substance Use*, *5*, 228–239.

Hartz, S. M., Pato, C. N., Medeiros, H., Cavazos-Rehg, P., Sobell, J. L., Knowles, J. A., et al. (2014). Comorbidity of severe psychotic disorders with measures of substance use. *JAMA Psychiatry*, *71*, 248–254.

Healey, C., Peters, S., Kinderman, P., McCracken, C., & Morriss, R. (2009). Reasons for substance use in dual diagnosis bipolar disorder and substance use disorders: a qualitative study. *Journal of Affective Disorders*, *113*, 118–126.

Heffner, J. L., DelBello, M. P., Anthenelli, R. M., Fleck, D. E., Adler, C. M., & Strakowski, S. M. (2012). Cigarette smoking and its relationship to mood disorder symptoms and co-occurring alcohol and cannabis use disorders following first hospitalization for bipolar disorder. *Bipolar Disorders*, *14*, 99–108.

Hellvin, T., Sundet, K., Aminoff, S. R., Andreassen, O. A., & Melle, I. (2013). Social functioning in first contact mania: clinical and neurocognitive correlates. *Comprehensive Psychiatry*, *54*, 432–438.

Henquet, C., Krabbendam, L., de Graaf, R., Ten Have, M., & van Os, J. (2006). Cannabis use and expression of mania in the general population. *Journal of Affective Disorders*, *95*, 103–110.

Hyman, S. M., & Sinha, R. (2009). Stress-related factors in cannabis use and misuse: implications for prevention and treatment. *Journal of Substance Abuse and Treatment*, *36*, 400–413.

Iskandar, J. W., Griffeth, B., & Sharma, T. (2011). Marijuana-induced mania in a healthy adolescent: a case report. *General Hospital Psychiatry*, *33*, 640–644.

Jarvis, K., DelBello, M. P., Mills, N., Elman, I., Strakowski, S. M., & Adler, C. M. (2008). Neuroanatomic comparison of bipolar adolescents with and without cannabis use disorders. *Journal of Child and Adolescent Psychopharmacology*, *18*, 557–563.

Judd, L. L., Akiskal, H. S., Schettler, P. J., Coryell, W., Maser, J., Rice, J. A., et al. (2003). The comparative clinical phenotype and long term longitudinal episode course of bipolar I and II: a clinical spectrum or distinct disorders? *Journal of Affective Disorders*, *73*, 19–32.

Khantzian, E. J. (1997). The self-medication hypothesis of substance use disorders: a reconsideration and recent applications. *Harvard Review of Psychiatry*, *4*, 231–244.

Kvitland, L. R., Melle, I., Aminoff, S. R., Lagerberg, T. V., Andreassen, O. A., & Ringen, P. A. (2014). Cannabis use in first-treatment bipolar I disorder: relations to clinical characteristics. *Early Intervention Psychiatry*, *10*, 36–44.

Lagerberg, T. V., Kvitland, L. R., Aminoff, S. R., Aas, M., Ringen, P. A., Andreassen, O. A., et al. (2014). Indications of a dose-response relationship between cannabis use and age at onset in bipolar disorder. *Psychiatry Research*, *215*, 101–104.

Lagerberg, T. V., Sundet, K., Aminoff, S. R., Berg, A. O., Ringen, P. A., Andreassen, O. A., et al. (2011). Excessive cannabis use is associated with earlier age at onset in bipolar disorder. *European Archives of Psychiatry and Clinical Neurosciences*, *261*, 397–405.

Larsson, S., Andreassen, O. A., Aas, M., Rossberg, J. I., Mork, E., Steen, N. E., et al. (2013). High prevalence of childhood trauma in patients with schizophrenia spectrum and affective disorder. *Comprehensive Psychiatry*, *54*, 123–127.

Lev-Ran, S., Le Foll, B., McKenzie, K., George, T. P., & Rehm, J. (2013a). Bipolar disorder and co-occurring cannabis use disorders: characteristics, co-morbidities and clinical correlates. *Psychiatry Research*, *209*, 459–465.

Lev-Ran, S., Le Foll, B., McKenzie, K., George, T. P., & Rehm, J. (2013b). Cannabis use and cannabis use disorders among individuals with mental illness. *Comprehensive Psychiatry*, *54*, 589–598.

Moreira, F. A., & Lutz, B. (2008). The endocannabinoid system: emotion, learning and addiction. *Addiction Biology*, *13*, 196–212.

Mueser, K. T., Drake, R. E., & Wallach, M. A. (1998). Dual diagnosis: a review of etiological theories. *Addictive Behaviors*, *23*, 717–734.

Öngur, D., Jensen, J. E., Prescot, A. P., Stork, C., Lundy, M., Cohen, B. M., et al. (2008). Abnormal glutamatergic neurotransmission and neuronal-glial interactions in acute mania. *Biological Psychiatry*, *64*, 718–726.

Ostacher, M. J., Perlis, R. H., Nierenberg, A. A., Calabrese, J., Stange, J. P., Salloum, I., et al. (2010). Impact of substance use disorders on recovery from episodes of depression in bipolar disorder patients: prospective data from the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD). *American Journal of Psychiatry*, *167*, 289–297.

Post, R. M. (1992). Transduction of psychosocial stress into the neurobiology of recurrent affective disorder. *American Journal of Psychiatry*, *149*, 999–1010.

Regier, D. A., Farmer, M. E., Rae, D. S., Locke, B. Z., Keith, S. J., Judd, L. L., et al. (1990). Comorbidity of mental disorders with alcohol and other drug abuse. Results from the Epidemiologic Catchment Area (ECA) Study. *JAMA*, *264*, 2511–2518.

Ringen, P. A., Vaskinn, A., Sundet, K., Engh, J. A., Jonsdottir, H., Simonsen, C., et al. (2010). Opposite relationships between cannabis use and neurocognitive functioning in bipolar disorder and schizophrenia. *Psychological Medicine*, *40*, 1337–1347.

Stone, J. M., Fisher, H. L., Major, B., Chisholm, B., Woolley, J., & Lawrence, J. (2014). Cannabis use and first-episode psychosis: relationship with manic and psychotic symptoms, and with age at presentation. *Psychological Medicine*, *44*, 499–506.

Strakowski, S. M., DelBello, M. P., Fleck, D. E., Adler, C. M., Anthenelli, R. M., Keck, P. E. J., et al. (2007). Effects of co-occurring cannabis use disorders on the course of bipolar disorder after a first hospitalization for mania. *Archives of General Psychiatry*, *64*, 57–64.

Swann, A. C. (2010). Mechanisms of impulsivity in bipolar disorder and related illness. *Epidemiologia e Psichiatria Sociale*, *19*, 120–130.

Tijssen, M. J., van, O. J., Wittchen, H. U., Lieb, R., Beesdo, K., & Wichers, M. (2010). Risk factors predicting onset and persistence of subthreshold expression of bipolar psychopathology among youth from the community. *Acta Psychiatrica Scandinavica*, *122*, 255–266.

Tohen, M., Vieta, E., Gonzalez-Pinto, A., Reed, C., & Lin, D. (2010). Baseline characteristics and outcomes in patients with first episode or multiple episodes of acute mania. *Journal of Clinical Psychiatry*, *71*, 255–261.

Tomasini, M. C., Ferraro, L., Bebe, B. W., Tanganelli, S., Cassano, T., Cuomo, V., et al. (2002). Delta(9)-tetrahydrocannabinol increases endogenous extracellular glutamate levels in primary cultures of rat cerebral cortex neurons: involvement of CB(1) receptors. *Journal of Neuroscience Research*, *68*, 449–453.

Tondo, L., Isacsson, G., & Baldessarini, R. (2003). Suicidal behaviour in bipolar disorder: risk and prevention. *CNS Drugs*, *17*, 491–511.

van Laar, M., van Dorsselaer, S., Monshouwer, K., & de Graaf, R. (2007). Does cannabis use predict the first incidence of mood and anxiety disorders in the adult population? *Addiction*, *102*, 1251–1260.

Van Rossum, I., Boomsma, M., Tenback, D., Reed, C., & van Os, J. (2009). Does cannabis use affect treatment outcome in bipolar disorder? A longitudinal analysis. *Journal of Nervous and Mental Disease*, *197*, 35–40.

van Winkel, R., & Kuepper, R. (2014). Epidemiological, neurobiological, and genetic clues to the mechanisms linking cannabis use to risk for nonaffective psychosis. *Annual Review of Clinical Psychology*, *10*, 767–791.

Whiteford, H. A., Degenhardt, L., Rehm, J., Baxter, A. J., Ferrari, A. J., Erskine, H. E., et al. (2013). Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *Lancet*, *382*, 1575–1586.

Wittchen, H. U., Frohlich, C., Behrendt, S., Gunther, A., Rehm, J., Zimmermann, P., et al. (2007). Cannabis use and cannabis use disorders and their relationship to mental disorders: a 10-year prospective-longitudinal community study in adolescents. *Drug and Alcohol Dependence*, *88*(Suppl. 1), S60–S70.

CHAPTER

44

Cannabis Use in Epilepsy—Risks and Benefits

*M. Holtkamp**, M. Hamerle***

***Epilepsy-Center Berlin-Brandenburg, Department of Neurology, Charité – Universitätsmedizin Berlin, Berlin, Germany**

****Department of Psychiatry and Psychotherapy, Ludwig-Maximilian-University Hospital, Munich, Germany**

SUMMARY POINTS

- This chapter focuses on cannabis and some of its well characterized compounds in the treatment of epilepsy.
- In one-third of patients with epilepsy, seizures cannot be adequately controlled with antiepileptic drugs.
- Therefore, novel treatment options are needed.
- Animal data suggest that cannabinoids have anticonvulsant properties.
- Available human data give hints for some seizure-preventing properties of cannabinoids, but the quality of those studies is poor.
- Clinically significant side effects of cannabinoids, beyond the psychoactive properties of Δ^9-tetrahydrocannabinol, have not been described.
- A high-quality, placebo-controlled, randomized controlled trial on efficacy and safety of cannabidiol in patients with intractable epilepsy is warranted.

KEY FACTS OF SEIZURES AND EPILEPSY

- An epileptic seizure is a clinical symptom, the pathophysiological correlate are excessive synchronized neuronal discharges in the brain.
- Depending on the brain region in which the neuronal discharges occur, clinical symptoms and signs vary.
- Subjective epileptic symptoms comprise visual, auditory, somatosensory, mnestic, and psychic perceptions, and speech arrest; motor signs include circumscribed or generalized tonic, clonic, or tonic clonic features.
- An epileptic seizure may occur due to some acute brain disorders, such as stroke or trauma; conceptually, this seizure is provoked, or of acute-sympomatic origin.
- Unprovoked epileptic seizures define epilepsy if more than two seizures have occurred, or if an MRI or EEG reveal an enduring condition of the brain that indicates an increased risk for further seizures, after one has occurred.

KEY FACTS ON TREATMENT OF EPILEPSY

- The hallmarks of treatment of epilepsy are pharmacological substances that aim to prevent the occurrence of further unprovoked epileptic seizures.
- Thus, conceptually, epilepsy treatment is secondary prophylaxis; the underlying condition "epilepsy" is not affected by antiepileptic drugs.
- Antiepileptic monotherapy results in seizure freedom, that is, no seizures in the last 12 months, in 50–60% of patients.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00050-8
Copyright © 2017 Elsevier Inc. All rights reserved.

- Antiepileptic polytherapy is initiated after failure of two trials of monotherapy; polytherapy adds another 10% of seizure-free patients.
- If two antiepileptic drugs have failed, epilepsy is pharmacoresistant by definition; this applies to one-third of all patients with epilepsy.
- Patients with pharmacoresistant epilepsy should be assessed whether they are eligible for epilepsy surgery, that is, the operative removal of the seizure focus; less than 10% of all patients undergoing presurgical assessment are eventually resected.
- Thus, a large number of epilepsy patients urgently need novel—pharmacological or nonpharmacological—treatment approaches.

KEY FACTS ON CANNABIDIOL USE IN EPILEPSY

- The two most studied active compounds of the plant *Cannabis sativa* are cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC); the latter is psychoactive, the former is not.
- In animal models, both compounds have anticonvulsant properties; in some species, THC turned out to be proconvulsant.
- Human data on efficacy of CBD in epilepsy are scarce, but they give some first hints for an anticonvulsant potential.
- High-level evidence for efficacy and safety of CBD in epilepsy is so far lacking, accordant randomized controlled trials are urgently needed and currently planned.
- Until reliable data on CBD in epilepsy are available, treatment trials in individual patients with various burdens of difficult-to-treat epilepsy are explicitly warranted.

LIST OF ABBREVIATIONS

AED Antiepileptic drug
CBD Cannabidiol
EEG Electroencephalogram
OR Odds ratio
THC Δ^9-Tetrahydrocannabinol

INTRODUCTION

Cannabis has been used for millennia, and across various cultures, to treat a plethora of chronic medical conditions, including epilepsy. In modern medical research, physicians have been discussing for decades whether cannabis or its compounds cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC) facilitate or prevent epileptic seizures, in people with and without epilepsy. Recently, interest in this topic has intensified (Mathern, Nehlig, & Sperling, 2014).

One in three patients with epilepsy continues to have epileptic seizures, despite polypharmacological treatment regimen. Some of these patients have dozens of convulsive seizures per day. They or their proxies are looking desperately for novel adjunctive treatment options, one of which may be cannabis or its compounds.

The plant *Cannabis sativa* produces more than 80 different cannabinoid compounds (Devinsky et al., 2014) that are highly lipophilic and, therefore, capable to easily cross the blood–brain barrier. The two most studied compounds are THC, which has prominent psychoactive properties, and CBD, which lacks psychoactive effects. In experimental models, THC has been shown to be pro- (Martin & Consroe, 1976) and anticonvulsant (Hill et al., 2010), while CBD had only anticonvulsant properties (Izquierdo & Tannhauser, 1973; Jones et al., 2010, 2012).

The aim of this review is to discuss seizure facilitating or preventing characteristics of cannabis, and its two clinically relevant compounds, CBD and THC. We assess currently available data on people with and without prior seizures or established epilepsy. We further discuss the safety of cannabis use for medical purposes. A short overview on allocation, classification, and treatment of epileptic seizures and epilepsy will be introduced into the field.

SOME FACTS ON SEIZURES AND EPILEPSY

The pathophysiological basis of an epileptic seizure is constituted by paroxysmal synchronized depolarization of neuronal networks. Clinical signs and symptoms of an epileptic seizure depend on the functional properties of the brain region involved in the onset and propagation of epileptic activity. Following the seizure classification of the International League against Epilepsy, seizures are dichotomized into partial onset and generalized forms (Commission on Classification and Terminology of the International League Against Epilepsy, 1981). The former comprises seizures that commence in a circumscribed area of the brain, and comprehends simple partial (consciousness maintained), complex partial (consciousness impaired), and secondary generalized tonic clonic (consciousness lost) seizures. Common generalized seizure types are absences, myoclonus, and "primary" generalized tonic clonic seizures. These seizure types are characterised by simultaneous onset in widespread bilateral neuronal networks.

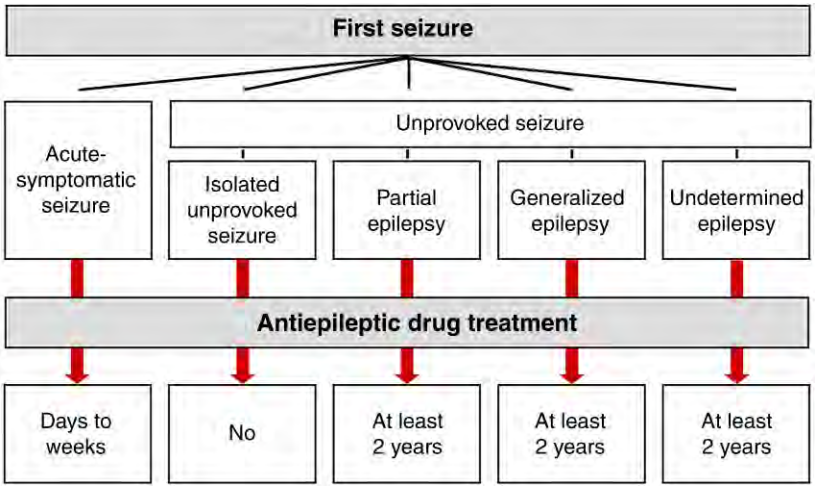


FIGURE 44.1 **Stratification of first seizures.** The first epileptic seizure has to be stratified dichotomously into either an acute symptomatic seizure or an unprovoked seizure. The former occurs in close temporal relation to acute systemic disturbances or acute brain disorders; acute symptomatic seizures are provoked by these transient conditions. A first unprovoked seizure may be associated with no clinically relevant recurrence risk; conceptually, this condition is accordant to an isolated unprovoked seizure. If pathological findings in cranial MRI and/or EEG suggest an enduring increased risk for further unprovoked seizures, the patient suffers by definition from epilepsy, that syndromatically may be partial (60% of cases), generalized (20%), or undetermined (20%). Indication for and initially presumed duration of antiepileptic drug treatment depends on seizure recurrence risk. This is why correct stratification to one of the groups as given in this figure is paramount.

The proper etiological allocation of an epileptic seizure is the basis for further antiepileptic drug (AED) treatment. On the one hand, epileptic seizures can be the consequence of an acute systemic medical disturbance, or of an acute brain disorder. In this case, epileptic seizures are of acute symptomatic origin. On the other hand, epileptic seizures can be unprovoked when they are not timely related to such acute conditions.

Even in otherwise healthy subjects, acute medical disturbances may facilitate occurrence of epileptic seizures. These comprise intoxications (eg, with lithium or theophylline), abrupt detoxications (eg, from alcohol or benzodiazepines), metabolic disturbances (eg, hyponatremia or hypoglycemia), and systemic infections with elevated body temperature. Within the first week, brain disorders, such as stroke, trauma, or infection can cause acute symptomatic epileptic seizures. Risk of seizure recurrence is elevated as long as the predisposing condition is prevalent; a couple of days in systemic disturbances, and a couple of weeks in acute brain disorders. These are the specific time frames patients with acute symptomatic seizure should be treated for, either with oral benzodiazepines (seizures caused by systemic disturbances), or with AEDs that can be titrated rapidly and, ideally, can be administered intravenously, for example, levetiracetam or valproic acid (seizures caused by acute brain disorders).

A first unprovoked seizure is either isolated, that is, brain magnetic resonance imaging and electroencephalogram (EEG) are normal; or can already be defined as epilepsy if neuroimaging or EEG are pathologic, and indicate an enduring increased risk for further seizures (Fisher et al., 2014). Epilepsy is also defined by two or more unprovoked epileptic seizures. If seizure recurrence risk is increased, patients should be recommended to take AEDs regularly to prevent occurrence of further seizures, at least for the next 2 years, and in most cases even longer (Fig. 44.1). At epilepsy onset, the overall aim of AED treatment is seizure freedom, and occurrence of no, or at most minimal, side effects. Two-thirds of patients with new-onset epilepsy remain seizure-free upon the first or second AED administered in monotherapy. The other patients require AED polytherapy, and most of these patients are pharmacoresistant, that is, they continue to suffer from epileptic seizures despite treatment with AEDs. Patients with pharmacoresistant epilepsy should be thoroughly assessed if they are eligible for epilepsy surgery, that is, for resection of the epileptogenic focus. If not, other nonpharmacological and, up to now, "experimental" pharmacological treatment options are urgently needed. A treatment algorithm for treatment of epilepsy is shown in Fig. 44.2.

The main burden and danger of epileptic seizures is the unexpected occurrence that implies the risk of falls, drowning, and other injuries. Half of the patients report rather severe headache following an epileptic seizure (Duchaczek, Ghaeni, Matzen, & Holtkamp, 2013), and generalized strong muscle soreness is common following generalized tonic clonic seizures.

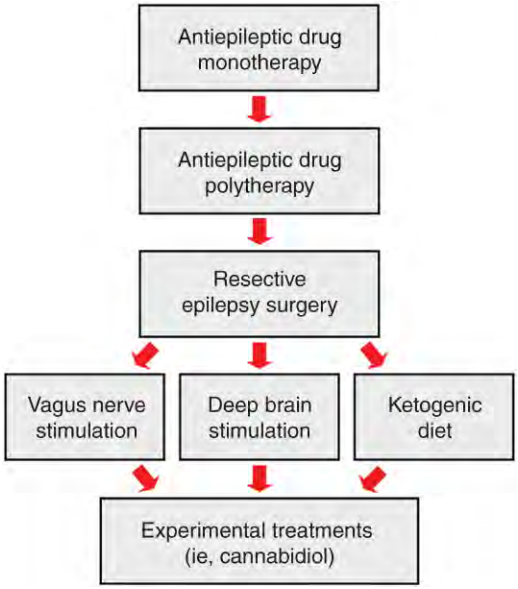


FIGURE 44.2 Treatment algorithm in epilepsy. The mainstay of treatment in epilepsy is pharmacotherapy. This is not a real "antiepileptic" treatment, but the drugs aim to prevent the occurrence of the next seizure. Conceptually, this is a secondary prophylaxis, or an "antiseizure" treatment. Usually, treatment starts with a substance in monotherapy. When seizures persist even in high doses of this drug, this is either changed to another substance, or a second drug is added to the first one. If two drugs in sufficiently high dose—either administered subsequently or in combination—fail, epilepsy is by definition pharmacoresistant. In this constellation, it is highly unlikely that the patient becomes seizure-free with further antiepileptic drugs. There is common sense among experts that patients with pharmacoresistant epilepsy should be assessed by continuous video-EEG-recording and further tests, if they are eligible for epilepsy surgery. If the seizure focus can clearly be identified, and eventually resected, epilepsy has a high chance of 50–70% to become resolved. However, only a minority of patients qualify for resective surgery and, in this group of patients, further treatment options may be tried. At this stage, the realistic aim is not seizure-freedom, but some kind of control of seizures in regard of frequency and severity of events. Randomized controlled trials have demonstrated efficacy of vagus nerve and deep brain stimulation, as well as ketogenic diet. If these treatments do not have a sustained effect, experimental treatment approaches, which so far have not been assessed in controlled trials, may be applied. One of these is cannabidiol.

DOES CANNABIS USE AFFECT SEIZURES AND EPILEPSY?

Pro- and anticonvulsant effects of cannabis have to be assessed in two entirely different clinical constellations. On the one hand, cannabis or its major medically used compound, CBD, may provoke epileptic seizures in people without history of epilepsy; on the other hand, the substance may prevent or facilitate seizures in patients with prior seizures, or established epilepsy.

Cannabis in People Without Epilepsy

Proconvulsant effects of cannabis in people without epilepsy may be seen following habitual recreational use, or in patients treated with CBD for conditions other than epilepsy.

The former situation, so far, has not been assessed in epidemiological studies of regular cannabis users, so no conclusions on proconvulsant properties in otherwise healthy individuals can be drawn. However, one retrospective study gives some indirect hints on preventive properties of cannabis consumption regarding occurrence of new-onset seizures (Brust, Ng, Hauser, & Susser, 1992). In 308 subjects who were admitted to the emergency department of the Harlem Hospital, New York, with new-onset seizures, male patients had consumed cannabis significantly less often in their life time (adjusted OR 0.42) or in the last 3 months (adjusted OR 0.36), compared to a control group that had presented with other clinical conditions. In addition, male subjects with acute symptomatic seizures had also used cannabis less often in the last 3 months (OR 0.8). Neither of these effects was observed in women. These data may suggest possible protective effects of cannabis use regarding manifestation of acute symptomatic and new-onset unprovoked seizures.

Proconvulsant effects of medically administered CBD or THC, in people without epilepsy, were assessed in randomized controlled trials, as performed in patients with social phobia (Bergamaschi et al., 2011), Huntington Disease (Curtis, Mitchell, Patel, Ives, & Rickards, 2009), and other movement disorders (Carroll et al., 2004; Muller-Vahl et al., 2003). In all these trials, occurrence of new-onset seizures has either not been described, or no differences between the study and the control group (treated with placebo or another active comparator) were reported. However, most studies were underpowered and, therefore, available data do not suggest that the use of cannabis, or administration of CBD or THC, facilitates the occurrence of epileptic seizures in people without prior seizures, or established epilepsy.

Cannabis and its Compounds in Established Epilepsy

In patients with difficult-to-treat epilepsy, no high-quality, adequately powered randomized controlled trial has been performed so far comparing add-on CBD or THC with placebo, or an active compound. Most available data is based on case reports, surveys, and four small prospective controlled trials.

Though case reports on efficacy of therapeutic interventions are always biased by possible placebo effects, they may be helpful to highlight clinical problems, and may give first hints for novel treatment approaches. A 5-year-old girl, suffering from Dravet syndrome, a condition based on an *SCN1A* gene mutation, and characterized by frequent daily tonic, tonic clonic, and myoclonic seizures not responding to AEDs, was treated

with adjunctive CDB/THC (Maa & Figi, 2014). Starting with more than 300 generalized tonic clonic seizures per week, after 3 months of treatment with CBD extract, seizure frequency was reduced by more than 90%. CBD was tapered at three different time points, and seizure frequency returned to baseline each time. This gives at least some proof for efficacy; however, the results may be limited to the particular case, and may not be generalized to other patients with severe forms of epilepsy. Other individual case reports on cannabis in epilepsy provide conflicting results. In some cases, cannabis use helped to control epileptic seizures as add-on therapy to conventional AEDs (Consroe, Wood, & Buchsbaum, 1975). In other epilepsy patients, occurrence of tonic clonic generalized seizures associated with acute cannabis use has also been reported (Keeler, 1967).

Porter and Jacobson (2013) surveyed 19 parents whose children were suffering from intractable severe childhood epilepsies, and who had used CBD as additional therapy. Most of the children suffered from Dravet syndrome, and the average number of antiepileptic drugs tried before was 12. Of the 19 parents, 16 (84%) reported reduced seizure frequency in their child, after beginning treatment with CBD, 8 even stated a reduction of seizure frequency of more than 80%. Other beneficial effects of CBD treatment as reported by parents were stabilized mood (79%), increased alertness (74%), and improved sleep (68%). As the authors have mentioned, it is important to notice that the interviewed parents strongly supported medical use of cannabis, and that the reports on beneficial effects on the children's condition may therefore be biased. Also, no experienced clinicians were involved in validating seizure improvement professionally. However, the results of this survey at least support the conclusion that some forms of pharmacoresistant childhood epilepsy may benefit from CBD treatment.

In a Canadian phone survey of 136 adult patients with epilepsy, 48% had consumed cannabis at least once in their lifetime. Moreover, 21% had used cannabis in the last 12 months (= active use), and 68/54% of these reported decreased seizure severity/frequency (Fig. 44.3). 43% of the interviewed subjects stated medical reasons for regular cannabis consumption (Gross, Hamm, Ashworth, & Quigley, 2004).

In an epidemiologic study of 310 adult epilepsy patients, performed by the authors of this chapter, only 28.1% patients had ever consumed cannabis in their lifetime (20.3% after onset of epilepsy), and 4.2% (n = 13) had been active users (Hamerle, Ghaeni, Kowski, Weissinger, & Holtkamp, 2014). In 84.1% of subjects who had used cannabis after the diagnosis of epilepsy had been made (including 11 of 13 active users), cannabis was not reported to affect seizure frequency or severity in either direction. Seven patients stated worsening of seizure frequency associated with cannabis use (repeated seizure manifestation within 24 h after cannabis use). Two active cannabis users stated beneficial effects of cannabis on epilepsy. However, these more likely tended to be secondary beneficial effects, for example, reduction of postictal headache, and did not seem to affect seizure frequency or severity directly (Fig. 44.4).

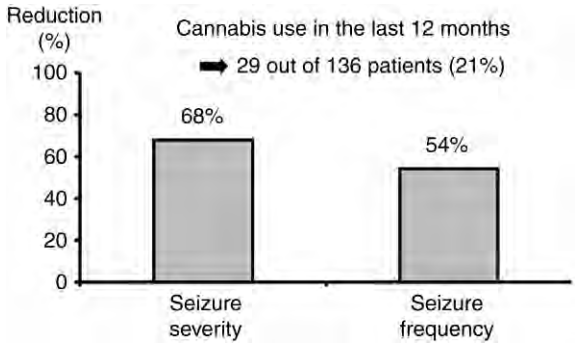


FIGURE 44.3 **Patient-reported effects of cannabis consumption.** In a Canadian telephone survey on 136 patients with epilepsy, 29 (= 21%) reported to have used cannabis in the previous 12 months. Of those, 68% reported decreased seizure severity, and, in 54%, frequency of seizures was reduced.

These results are in line with another survey of 215 adult epilepsy patients, the majority of which (90.2%) did not identify any effect of cannabis use on either seizure frequency or severity (Gordon & Devinsky, 2001). In a further study of 42 patients with epilepsy, 12 subjects reported cannabis use in the past. Only two patients reported effects on epilepsy: one subject stated beneficial effects, the other reported seizure worsening (Feeney, 1976).

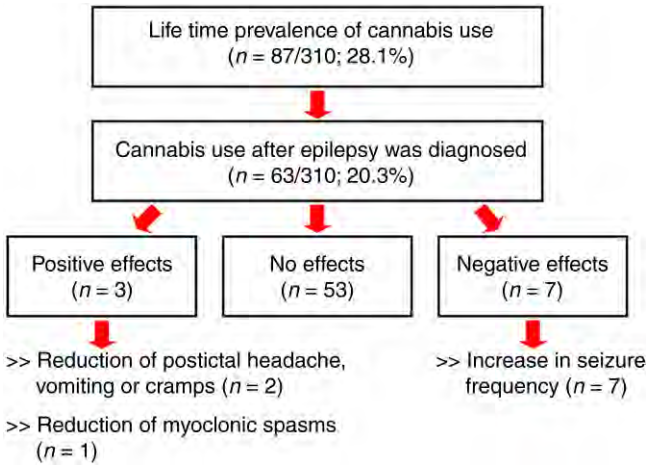


FIGURE 44.4 **Cannabis use and effects in epilepsy.** Findings of a survey study on use and effect of cannabis in 310 adult people with diverse forms of epilepsy, performed by the authors of this article. One in five patients had used cannabis after the diagnosis of epilepsy has been made. The majority of those patients did not report any positive or negative effect on epilepsy. More than 10% of the patients with cannabis use reported some increase in seizure frequency, while three patients reported some benefit.

TABLE 44.1 Clinical Trial on Cannabidiol in Patients With Dravet Syndrome

Primary outcome measures
- Genetic differences between patients with Dravet syndrome (*SCN1A* gene mutation) who appear to respond to high concentration cannabidiol oil therapy versus those who do not
- Patients completing 3 months of therapy with a seizure reduction of >50% will be designated a "responder," and patients who discontinue therapy for any reason prior to 3 months or have a ≤50% seizure reduction will be designated "nonresponder;" between-groups genetic analysis will be performed

Inclusion criteria
- Age 1–50 years with gene-confirmed Dravet syndrome
- Uncontrolled epilepsy, having failed at least two prior treatments (including appropriate antiepileptic drugs, ketogenic diet, vagus nerve stimulation therapy)
- Seizure frequency should be more than two visually countable motor seizures per week (tonic clonic, tonic, clonic, myoclonic, or astatic)
- Care providers able to identify convulsive seizures, drop attacks, or tonic seizures, or any combination of countable seizure types
- Self-seeking therapy with Charlotte Web strain of medical marijuana with the assistance of a medical marijuana doctor, but are still naive to therapy
- Care provider able to complete seizure count diary
- Able to obtain oral swab sample from subject with Dravet syndrome
- Agree to forfeit any right to results of genetic analysis

Exclusion criteria
- Subjects having nonepileptic seizures that are not distinguishable from epileptic seizures by the care provider
- Prisoners
- Pregnant women
- Failure to forfeit right to results of genetic analysis

www.clinicaltrial.gov; identifier: NCT02229032.

A randomized controlled trial of 15 subjects suffering from uncontrolled partial epilepsy with secondary generalized tonic clonic seizures has investigated the anticonvulsant efficacy of CBD versus placebo in addition to conventional AEDs that were kept stable (Cunha et al., 1980). Eight patients received oral CBD (200–300 mg/day), and seven patients were randomized to placebo; all were treated for 3–18 weeks. Seven of eight subjects treated with CBD reported considerable clinical improvement, whereas the condition of six out of seven patients of the placebo group remained unchanged. This study is limited, as one subject changed groups (not clearly blinded), and CBD dose adjustment was possible. Moreover, average treatment time of CBD subjects was longer than in the placebo group.

In another trial by Mechoulam and Carlini (1978), nine patients with refractory epilepsy were randomized to either CBD (200 mg) or placebo, for a period of 3 months, while conventional AED treatment was continued. Two out of four subjects of the CBD group achieved seizure freedom, while improvement of seizures was not observed in any of the five patients of the placebo group.

In these two and two further randomized controlled trials, a total of 48 patients were included (Ames & Cridland, 1986; Trembly & Sherman, 1990). A recent Cochrane Review on cannabinoids for epilepsy criticizes that details of randomization were not included in any of the study reports, and that it was unclear whether baseline parameters of treatment and control groups were same or different (Gloss & Vickrey, 2014). Thus, there is an urgent need for better randomized controlled trials on efficacy and safety of CBD in epilepsy.

In the last years, several Western countries have changed their attitude toward the use of cannabis for medical purposes, and have liberalized their laws accordingly. Therefore, funding for and development of clinical trials have become less complicated. In the United States, randomized, placebo-controlled, double-blind trials on patients with two very severe epilepsy syndromes, Dravet syndrome and Lennox–Gastaut syndrome, are currently planned (Devinsky et al., 2014). Patients with these syndromes are eligible for such trials in particular, as they have an extraordinary high frequency of seizures and, thus, an urgent need for new treatment options, and they have relatively homogeneous epilepsy syndromes making comparison more favorable. For reasons of data accuracy, long-term video-EEG is planned for correct seizure count. In a first step, a currently ongoing study on patients with Dravet syndrome genetically compares CBD responders (>50% reduction in seizure frequency in 3 months of treatment) to nonresponders (www.clinicaltrials.gov; identifier: NCT02229032); for details see Table 44.1.

In summary, efficacy of cannabis in epilepsy patients, regarding reduction of seizure frequency and severity, so far has not been investigated comprehensively. Therefore, the previously mentioned randomized controlled trial planned in the United States is a step in the right direction. So far, only limited data exists on subjective effects of cannabis use on ancillary symptoms associated with epileptic seizures, such as postictal headache and muscle soreness.

IS CANNABIS SAFE AND WELL TOLERATED IN EPILEPSY PATIENTS?

Neither controlled trials nor case reports on cannabis use in epilepsy patients indicate clinically relevant medical or neurological side effects (Ames & Cridland, 1986; Cunha et al., 1980; Mechoulam & Carlini, 1978; Trembly & Sherman, 1990). A 2014 Cochrane Review on cannabinoids for epilepsy summarizes the available data as follows: "The dose of 200 to 300 mg daily of cannabidiol was safely administered to small numbers of patients generally for short periods of time, and so the safety of long term cannabidiol treatment cannot be reliably assessed." (Gloss & Vickrey, 2014).

Safety data on adult subjects using CBD for other indications than epilepsy, or for recreational purposes, also demonstrate favorable tolerability within a dose up to 1500 mg cannabinoid per day (Bergamaschi et al., 2011; Devinsky et al., 2014). As is the case with efficacy, side effects as a marker for tolerability that are specific to CBD can only be demonstrated or excluded by a double-blind randomized controlled trial. Opposite to CBD, THC is well-known to produce psychoactive adverse effects (Cilio, Thiele, & Devinsky, 2014). In children younger than 15 years of age, risk of psychosis (Griffith-Lendering et al., 2013) and long-term impairment of executive functions (Fontes et al., 2011) have been described.

In a recent review on cannabidiol in epilepsy, Devinsky et al. (2014) discuss the potential risk of immunosuppression, as interleukin 8 and 10 production may be suppressed (Srivastava, Srivastava, & Brouhard, 1998; Wu et al., 2008). The clinical relevance of these finding so far is undetermined.

In patients with epilepsy, drug interactions between cannabidiol and AED have not been assessed thoroughly. Cannabidiol is an inhibitor of hepatic CYP isozymes, and is metabolized itself by CYP3A4. Therefore, metabolism of AED may be affected, and enzyme inducers, such as the standard AED carbamazepine may reduce serum concentrations of cannabidiol (Devinsky et al., 2014).

In summary, CBD for medical use presumably is well tolerated and safe; whether immunosuppression or interaction with specific AED are of clinical significance requires further research.

PRACTICAL ISSUES OF CANNABIS USE FOR MEDICAL PURPOSES

Practical issues of CBD/cannabis use for medical purpose in patients with epilepsy

- CBD has been administered in several smaller studies as augmentation therapy in a dosage of 200–300 mg/day with favorable tolerability
- CBD augmentation has been shown to be usually well tolerated in dosages up to 1500 mg/day, even with chronic administration (Bergamaschi et al., 2011)
- In children, cannabis use may impair cognitive development or may increase the risk for psychosis (Fontes et al., 2011; Griffith-Lendering et al., 2013)
- CBD may theoretically induce immunosuppression by suppressing interleukin-8 and interleukin-10. However, clinical relevance has not been clearly demonstrated (Srivastava et al., 1998; Wu et al., 2008)

MINI-DICTIONARY

Anti- and proconvulsant effects Any therapeutic intervention in patients with epilepsy may suppress or prevent seizures (anticonvulsant effect), or facilitate their occurrence (proconvulsant effect).
Antiepileptic drugs (AED) Pharmacological substances that have the propensity to prevent the occurrence of further epileptic seizures but do not interfere with the underlying chronic condition of epilepsy; therefore a more precise term would be antiseizure or anti-ictal drug.
Dravet syndrome Genetically determined epilepsy syndrome described by Charlotte Dravet; it is based on an SCN1A gene mutation, characterized clinically by frequent and often daily tonic, tonic clonic, and myoclonic seizures. It is commonly pharmacoresistant.
Electroencephalogram (EEG) A method to record physiological and pathological brain activity by electrodes commonly placed on the scalp; intracranial recording with special electrodes is also possible.
Epileptic seizure Clinical correlate of excessive synchronized neuronal discharges in the brain.
Epilepsy Chronic condition of the brain which facilitates the occurrence of further unprovoked seizures after at least one seizure had already occurred.
Lennox–Gastaut syndrome Etiologically heterogeneous epilepsy syndrome described by William Lennox and Henri Gastaut; commonly childhood-onset, frequent and various seizures. It is often associated with developmental delay.
Long-term video-electroencephalography Simultaneous video recording of patient's behavior and scalp, EEG recording of brain activity for the purpose of detecting and analyzing epileptic seizures; duration of recording is commonly at least 24 h, and may last several days.
Magnetic resonance imaging (MRI) Noninvasive technique to visualize morphology and on occasion functional properties of the brain; essential to help clarify the etiology of epilepsy.
Randomized controlled trial Clinical study design that is characterized by treatment with intervention A, or a control intervention B (that in pharmacological studies may be an inactive placebo) by chance; generally, the patient (single blind), or the patient and the treating physician (double blind) are unaware of which treatment is given to the patient.

References

Ames, F. R., & Cridland, S. (1986). Anticonvulsant effect of cannabidiol. *South African Medical Journal*, *69*, 14.
Bergamaschi, M. M., Queiroz, R. H., Chagas, M. H., de Oliveira, D. C., De Martinis, B. S., Kapczinski, F., Quevedo, J., Roesler, R., Schroder,

N., Nardi, A. E., Martin-Santos, R., Hallak, J. E., Zuardi, A. W., & Crippa, J. A. (2011). Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naive social phobia patients. *Neuropsychopharmacology*, *36*, 1219–1226.

Brust, J. C., Ng, S. K., Hauser, A. W., & Susser, M. (1992). Marijuana use and the risk of new onset seizures. *Transactions of the American Clinical and Climatological Association*, *103*, 176–181.

Carroll, C. B., Bain, P. G., Teare, L., Liu, X., Joint, C., Wroath, C., Parkin, S. G., Fox, P., Wright, D., Hobart, J., & Zajicek, J. P. (2004). Cannabis for dyskinesia in Parkinson disease: a randomized double-blind crossover study. *Neurology*, *63*, 1245–1250.

Cilio, M. R., Thiele, E. A., & Devinsky, O. (2014). The case for assessing cannabidiol in epilepsy. *Epilepsia*, *55*, 787–790.

Commission on Classification and Terminology of the International League against Epilepsy. (1981). Proposal for revised clinical and electroencephalographic classification of epileptic seizures. *Epilepsia*, *22*, 489–501.

Consroe, P. F., Wood, G. C., & Buchsbaum, H. (1975). Anticonvulsant nature of marihuana smoking. *JAMA*, *234*, 306–307.

Cunha, J. M., Carlini, E. A., Pereira, A. E., Ramos, O. L., Pimentel, C., Gagliardi, R., Sanvito, W. L., Lander, N., & Mechoulam, R. (1980). Chronic administration of cannabidiol to healthy volunteers and epileptic patients. *Pharmacology*, *21*, 175–185.

Curtis, A., Mitchell, I., Patel, S., Ives, N., & Rickards, H. (2009). A pilot study using nabilone for symptomatic treatment in Huntington's disease. *Movement Disorders*, *24*, 2254–2259.

Devinsky, O., Cilio, M. R., Cross, H., Fernandez-Ruiz, J., French, J., Hill, C., Katz, R., Di Marzo, V., Jutras-Aswad, D., Notcutt, W. G., Martinez-Orgado, J., Robson, P. J., Rohrback, B. G., Thiele, E., Whalley, B., & Friedman, D. (2014). Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. *Epilepsia*, *55*, 791–802.

Duchaczek, B., Ghaeni, L., Matzen, J., & Holtkamp, M. (2013). Interictal and periictal headache in patients with epilepsy. *European Journal of Neurology*, *20*, 1360–1366.

Feeney, D. M. (1976). Letter: marihuana use among epileptics. *JAMA*, *235*, 1105.

Fisher, R. S., Acevedo, C., Arzimanoglou, A., Bogacz, A., Cross, J. H., Elger, C. E., Engel, J., Jr., Forsgren, L., French, J. A., Glynn, M., Hesdorffer, D. C., Lee, B. I., Mathern, G. W., Moshe, S. L., Perucca, E., Scheffer, I. E., Tomson, T., Watanabe, M., & Wiebe, S. (2014). ILAE official report: a practical clinical definition of epilepsy. *Epilepsia*, *55*, 475–482.

Fontes, M. A., Bolla, K. I., Cunha, P. J., Almeida, P. P., Jungerman, F., Laranjeira, R. R., Bressan, R. A., & Lacerda, A. L. (2011). Cannabis use before age 15 and subsequent executive functioning. *British Journal of Psychiatry*, *198*, 442–447.

Gloss, D., & Vickrey, B. (2014). Cannabinoids for epilepsy. *Cochrane Database Systematic Reviews*, *3*, CD009270.

Gordon, E., & Devinsky, O. (2001). Alcohol and marijuana: effects on epilepsy and use by patients with epilepsy. *Epilepsia*, *42*, 1266–1272.

Griffith-Lendering, M. F., Wigman, J. T., Prince van Leeuwen, A., Huijbregts, S. C., Huizink, A. C., Ormel, J., Verhulst, F. C., van Os, J., Swaab, H., & Vollebergh, W. A. (2013). Cannabis use and vulnerability for psychosis in early adolescence—a TRAILS study. *Addiction*, *108*, 733–740.

Gross, D. W., Hamm, J., Ashworth, N. L., & Quigley, D. (2004). Marijuana use and epilepsy: prevalence in patients of a tertiary care epilepsy center. *Neurology*, *62*, 2095–2097.

Hamerle, M., Ghaeni, L., Kowski, A., Weissinger, F., & Holtkamp, M. (2014). Cannabis and other illicit drug use in epilepsy patients. *European Journal of Neurology*, *21*, 167–170.

Hill, A. J., Weston, S. E., Jones, N. A., Smith, I., Bevan, S. A., Williamson, E. M., Stephens, G. J., Williams, C. M., & Whalley, B. J. (2010). Delta(9)-tetrahydrocannabivarin suppresses in vitro epileptiform and in vivo seizure activity in adult rats. *Epilepsia*, *51*, 1522–1532.

Izquierdo, I., & Tannhauser, M. (1973). Letter: the effect of cannabidiol on maximal electroshock seizures in rats. *Journal of Pharmacy and Pharmacology*, *25*, 916–917.

Jones, N. A., Glyn, S. E., Akiyama, S., Hill, T. D., Hill, A. J., Weston, S. E., Burnett, M. D., Yamasaki, Y., Stephens, G. J., Whalley, B. J., & Williams, C. M. (2012). Cannabidiol exerts anti-convulsant effects in animal models of temporal lobe and partial seizures. *Seizure*, *21*, 344–352.

Jones, N. A., Hill, A. J., Smith, I., Bevan, S. A., Williams, C. M., Whalley, B. J., & Stephens, G. J. (2010). Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo. *Journal of Pharmacology and Experimental Therapy*, *332*, 569–577.

Keeler, M. H. (1967). Adverse reaction to marihuana. *American Journal of Psychiatry*, *124*, 674–677.

Maa, E., & Figi, P. (2014). The case for medical marijuana in epilepsy. *Epilepsia*, *55*, 783–786.

Martin, P., & Consroe, P. (1976). Cannabinoid induced behavioral convulsions in rabbits. *Science*, *194*, 965–967.

Mathern, G., Nehlig, A., & Sperling, M. (2014). Cannabidiol and medical marijuana for the treatment of epilepsy. *Epilepsia*, *55*, 781–782.

Mechoulam, R., & Carlini, E. A. (1978). Toward drugs derived from cannabis. *Naturwissenschaften*, *65*, 174–179.

Muller-Vahl, K. R., Schneider, U., Prevedel, H., Theloe, K., Kolbe, H., Daldrup, T., & Emrich, H. M. (2003). Delta 9-tetrahydrocannabinol (THC) is effective in the treatment of tics in Tourette syndrome: a 6-week randomized trial. *Journal of Clinical Psychiatry*, *64*, 459–465.

Porter, B. E., & Jacobson, C. (2013). Report of a parent survey of cannabidiol-enriched cannabis use in pediatric treatment-resistant epilepsy. *Epilepsy Behavior*, *29*, 574–577.

Srivastava, M. D., Srivastava, B. I., & Brouhard, B. (1998). Delta9 tetrahydrocannabinol and cannabidiol alter cytokine production by human immune cells. *Immunopharmacology*, *40*, 179–185.

Trembly, B., & Sherman, M. (1990). Double-blind clinical study of cannabidiol as a secondary anticonvulsant. *International Association for Cannabinoid Medicines*, Abstract Book, 5.

Wu, H. Y., Chu, R. M., Wang, C. C., Lee, C. Y., Lin, S. H., & Jan, T. R. (2008). Cannabidiol-induced apoptosis in primary lymphocytes is associated with oxidative stress-dependent activation of caspase-8. *Toxicology and Applied Pharmacology*, *226*, 260–270.

CHAPTER

45

Cannabis, Cannabinoids, and Visceral Pain

R. Abalo, M. Isabel Martín-Fontelles

Area of Pharmacology and Nutrition, Faculty of Health Sciences, University Rey Juan Carlos, Alcorcón, Madrid, Spain; Associated Unit I+D+i of the Institute of Medicinal Chemistry (IQM) and of the Institute of Research in Food Sciences (CIAL), Spanish National Research Council (CSIC), Madrid, Spain

SUMMARY POINTS

- This chapter focuses on pain originating in internal organs (visceral pain).
- Visceral pain is characterized by distinct anatomical and functional features.
- The endocannabinoid system (ECS) is found throughout the different viscera and their innervation.
- In some organs, expression of the ECS components is low in nonpathological conditions, but increases under inflammation and/or cancer, with effective reduction of visceral pain by cannabinoid agonists (particularly CB2R agonists), and inhibitors of endocannabinoids degradation.
- The involvement of the ECS, and possible usefulness of cannabinoids, has not been tested yet in some painful visceral syndromes such as biliary or renal colic pain, orchialgia, or polycystic ovary.
- The major limitations of cannabinoids for treatment of visceral pain are their central side effects.
- Acute heavy cannabinoid use may induce visceral pain associated to visceral toxicity.

KEY FACTS OF VISCERAL PAIN

- It is poorly localized with referral to somatic structures.
- It produces nonspecific regional or whole-body motor responses, strong autonomic responses, and strong affective responses.
- It leads to sensitization of somatic tissues and visceral organs.
- Visceral afferents travel together with autonomic efferents.
- The components of the endocannabinoid system (ECS) have been demonstrated in visceral tissues, and also along the afferent pathways up to the brain.

LIST OF ABBREVIATIONS

Δ^9-THC	Δ^9-Tetrahydrocannabinol
CB1R	Cannabinoid receptor type 1
CB2R	Cannabinoid receptor type 2
ECS	Endogenous cannabinoid (or endocannabinoid) system
FAAH	Fatty acid amide hydrolase
GPR55	G-protein coupled receptor 55
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
LUTS	Lower urinary tract symptoms
MAGL	Monoacyl glycerol lipase

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00051-X
Copyright © 2017 Elsevier Inc. All rights reserved.

mRNA Messenger ribonucleic acid
NSAID Nonsteroidal antiinflammatory drugs
PPAR Peroxisome proliferator-activated receptor
TRPA1 Ion channel transient receptor potential A1
TRPV1 Transient receptor potential vanilloid-1 channels

INTRODUCTION

Marijuana, obtained from the plant *Cannabis sativa*, is the most widely used recreational drug in the Western world for its psychotropic properties, mainly exerted by its main component, Δ^9-tetrahydrocannabinol (Δ^9-THC). Cannabis and cannabinoids (both natural and synthetic) exert their effects mainly through cannabinoid receptors type 1 and 2 (CB1R and CB2R). In addition to these receptors, the endogenous cannabinoid system (ECS) include endogenous ligands (anandamide, 2-arachidonoylglycerol, …), their biosynthetic and degradative enzymes (fatty acid amide hydrolase, FAAH; monoacyl glycerol lipase, MAGL), and other cannabinoid-related receptors (G-protein coupled receptor 55, GPR55; peroxisome proliferator-activated receptors, PPAR α and γ; transient receptor potential vanilloid-1 channels, TRPV1), as well as a hypothetical membrane transporter not yet cloned (Di Marzo, 2009).

The ECS is one of the most ubiquitous neural systems in the body. All the components of the ECS have been found in the viscera, including their sensory innervation (Fig. 45.1), and may participate in the generation/relief of visceral pain. In this chapter, we will first briefly describe the basics of visceral pain, and then we will focus on the role cannabinoids may play in this context.

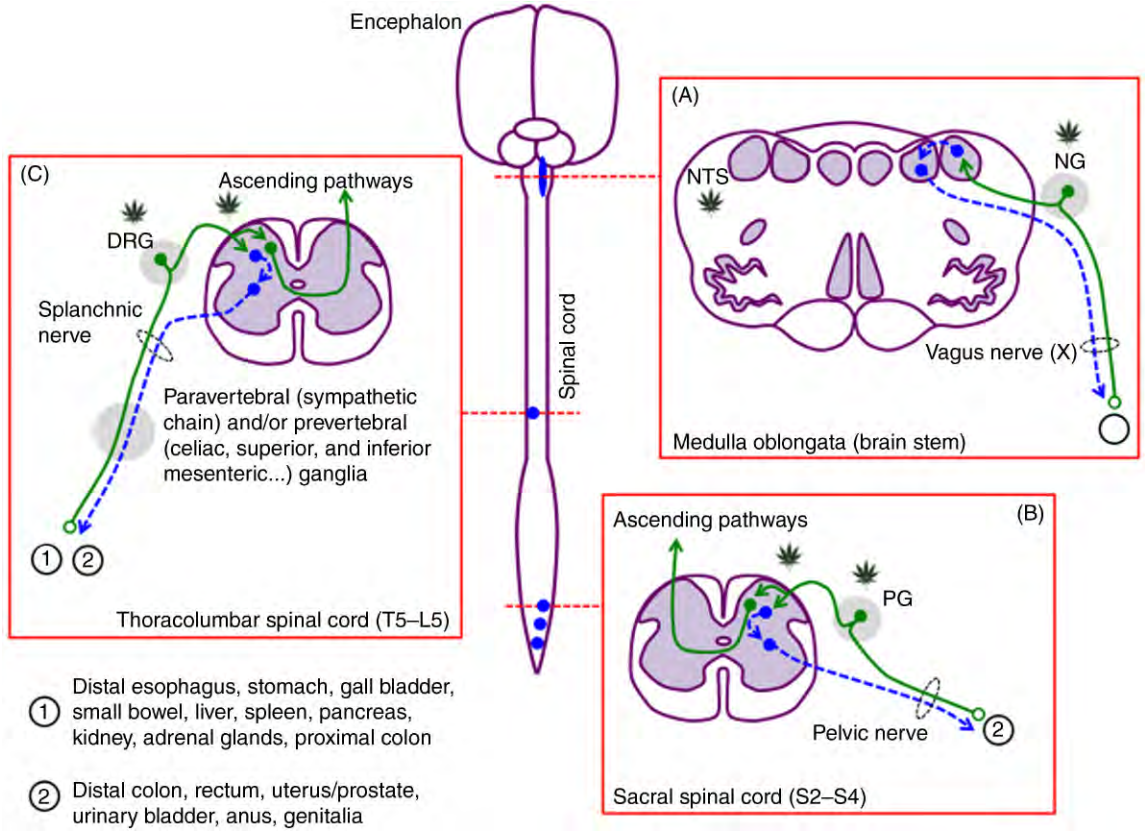


FIGURE 45.1 Sensory innervation of abdominal and pelvic viscera and the endocannabinoid system. Abdominal and pelvic viscera can be classified into two main categories according to their sensory innervation: distal esophagus, stomach… (1); distal colon, rectum… (2). Afferents from the vagus nerve (X) have their somata in the nodose ganglion (NG), and innervate the first category; afferents from the pelvic nerve have their somata in the pelvic ganglia (PG) and innervate the second category. Both have a role in transmitting mainly physiological signals to the central nervous system: the vagus nerve to the nucleus of the solitary tract (NTS) (A: cross-section of medulla oblongata in the brain stem at this level); the pelvic nerve to the dorsal horn of the sacral spinal cord (B: cross-section of the spinal cord, level S2–S4). Additionally, both categories of viscera are innervated by splanchnic afferents with their somata in the dorsal root ganglia (DRG), and connections with neurons lying in the paravertebral and/or prevertebral ganglia. The splanchnic nerves transmit signals generated by painful stimuli to the thoracolumbar spinal cord (C: cross-section of the spinal cord, level T5–L5). All these signals (vagal, pelvic, and splanchnic) travel to higher brain nuclei within different ascending pathways and are modulated by central information traveling downward within descending pathways (not shown). CB1R (marijuana leaf symbol) have been demonstrated immunohistochemically in NTS, NG, PG, DRG, and the dorsal horn of the spinal cord. *Green lines*: afferents from the abdominal and pelvic viscera. *Blue dotted lines*: parasympathetic (A,B) and sympathetic (C) efferents to the gut.

VISCERAL PAIN

Visceral pain can be defined as the pain originating from internal organs within the thoracic, abdominal, or pelvic cavities. It is a common cause for seeking medical attention. Thus, a recent review and metaanalysis (Viniol et al., 2014) showed that mean consultation prevalence for abdominal pain is 2.8%, with an unknown underlying cause in about one-third of patients. The most common declared etiologies were: gastroenteritis (7.2–18.7%), inflammatory bowel disease (IBD) (2.6–13.2%), urological cause (5.3%), and gastritis (5.2%). About 1 in 10 abdominal pain patients suffers from an acute disease like appendicitis (1.9%), diverticulitis (3%), biliary/pancreatic (4%) or neoplastic (1%) diseases needing immediate therapy.

In the case of visceral cancer pain, the growth of a tumor may lead to many activating noxious stimuli, ranging from chemicals released by cancer cells, immune cells, distension or obstruction of luminal organs, and/or neuropathic events such as denervation and/or nerve sprouting, and other changes in neuronal function (Sikandar & Dickenson, 2012). Furthermore, as demonstrated in experimental animals, use of antineoplastic drugs may also alter visceral innervation and function (Vera et al., 2011; Cabezos, Vera, Martín-Fontelles, Fernández-Pujol, & Abalo, 2010; Wafai, Taher, Jovanovska, Bornstein, Dass, & Nurgali, 2013), probably contributing also to altered pain responses.

Importantly, common painkillers such as nonsteroidal antiinflammatory drugs (NSAID) or opioids often adversely affect visceral (gastrointestinal) function or even integrity, thus limiting their clinical utility, and prompting a search for alternatives (Christianson et al., 2009). Thus, "visceral-specific" analgesics would be most useful; among them, some molecules targeting the ECS have shown potential therapeutic profiles (see next section: "Cannabinoids and visceral pain").

Different recent reviews describe how visceral pain is generated, its features, differences with somatic pain, etiology, management, etc. (Sengupta, 2009; Sikandar & Dickenson, 2012; de Carvalho Rocha, Dantas, Rolim, Costa, & de Medeiros, 2014; Vermeulen, De Man, Pelckmans, & De Winter, 2014; González, Merrill, & Vizzard, 2014; Larauche, Mulak, & Taché, 2012; Davis, 2012). As shown in Fig. 45.2, visceral pain is poorly localized with referral to somatic structures, produces nonspecific regional or whole-body motor responses, as well as strong autonomic responses, and strong affective responses. Therefore, visceral pain can be reinforced by anxiety and depression (Sikandar & Dickenson, 2012). It also leads to sensitization of somatic tissues and visceral organs. Visceral hypersensitivity can occur due to: sensitization of primary sensory afferents innervating the viscera; hyperexcitability of spinal ascending neurons (central sensitization) receiving synaptic input from the viscera; and dysregulation of descending pathways that modulate spinal nociceptive transmission (Sengupta, 2009). Interestingly, some of the general features of visceral pain (namely referral to somatic structures and autonomic changes) can also be observed in animal models upon which new drugs may be tested (Sengupta, 2009).

Frequently, several pathologic processes and targets are involved in visceral diseases. One example might be the lower urinary tract symptoms (LUTS). In male LUTS, many factors are involved: androgen signaling, inflammation and metabolic factors, the urothelium, prostate, interstitial cells, detrusor, neurotransmitters, neuromodulators… Therefore, the potential therapeutic approaches are complex, and must take into account both the functional and sensory disorders.

CANNABINOIDS AND VISCERAL PAIN

The palliative effects of *Cannabis sativa* (marijuana) and its main active ingredient (Δ^9-THC), include inhibition of nausea and emesis associated with chemo- or radiotherapy, appetite stimulation, pain relief, mood elevation, and relief from insomnia in cancer patients (Pacher, Batkai, & Kunos, 2006). Synthetic Δ^9-THC (dronabinol, in Marinol) and its derivative nabilone (Cesamet), as well as plant-derived extracts containing controlled amounts of Δ^9-THC and other plant-derived cannabinoids (ie, nabiximols = Δ^9-THC + cannabidiol, in Sativex), have been approved in several countries to control nausea in cancer patients undergoing chemotherapy, to stimulate appetite and/or to attenuate cancer-related pain (Pacher et al., 2006; Velasco, Sánchez, & Guzmán, 2012).

In animal models of nonspecific, noncancerous visceral pain, such as intraperitoneal administration of acetic acid, Δ^9-THC reduced abdominal pain behavior. Cannabinol also reduced pain behavior, whereas tetrahydrocannabivarin antagonized Δ^9-THC (Booker, Naidu, Razdan, Mahadevan, & Lichtman, 2009). In addition, inhibitors of FAAH alone, or in combination with NSAID, also reduced acetic acid-induced abdominal stretching in mice (Naidu, Booker, Cravatt, & Lichtman, 2009).

Recent literature demonstrating the presence of the ECS and their hypothetical use in different viscera will be discussed below (Table 45.1).

Heart

In the last few years, some cases of acute chest pain associated to myocardial infarction have been reported in young consumers of designer drugs like K2/Spice, which contain cannabinoid agonists with higher affinity

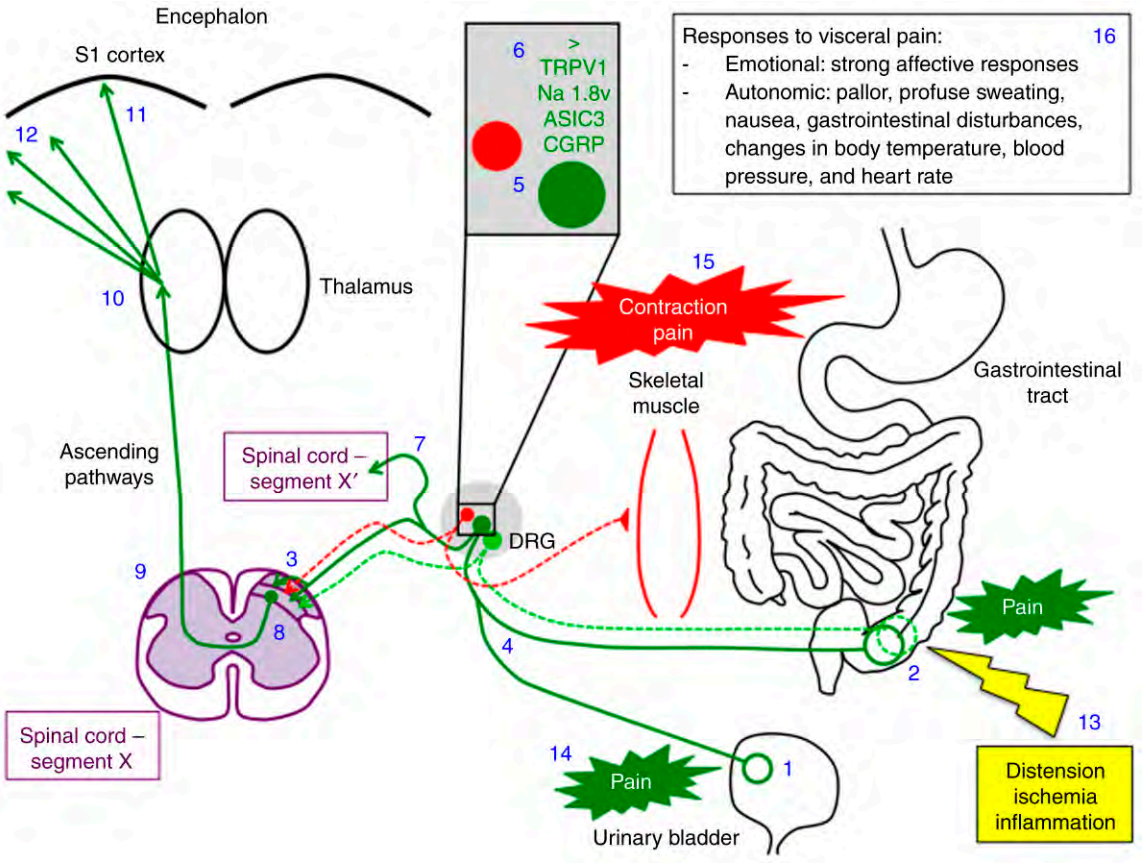


FIGURE 45.2 **Anatomic and functional features of visceral pain.** In this figure, viscera are represented by the gastrointestinal tract and the urinary bladder. Cell bodies of primary visceral *(green)* and somatic *(red)* afferents reside within the dorsal root ganglia (DRG). Ascending pathways include second order sensory neurons (projecting from the dorsal horn of the spinal cord to the thalamus), and third order sensory neurons (projecting from the thalamus to the brain cortex). Anatomic features of visceral pain are: lack of specialized nerve terminals (1); widely overlapping receptive fields (2); dual extrinsic afferent system—afferents ascend with parasympathetic and sympathetic neuronal projections (see Fig. 45.1); convergence of afferents on somatic and other visceral afferents within the spinal cord (3); dichotomization of fibers where a single neuron innervates two viscera (4); cell bodies within DRG *(green circle)* are larger than somatic ones *(red circle)* (5), and with greater expression of TRPV1, Na 1.8 and ASIC3 ion channels, as well as CGRP (6); first-order afferents arborize over several spinal segments (X and X′) (7); discrete location of first-order terminals within the spinal cord (superficial dorsal horn, lamina V, and central) (8); second-order afferents ascend in the dorsal column (9); nonsomatotopically arranged input in dorsal column and central lateral thalamus (unlike the lateral spinothalamics) (10); poor representation in S1 cortex (11); extensive divergence within the central nervous system (12). Functional features of visceral pain are: limited number of stimulus responses (distension, ischemia, and inflammation) (13); viscerovisceral hyperalgesia and hypersensitivity *(green stars)* (14); visceromotor responses and referred pain to somatic sites within the same metameric field *(red star)* (15); greater emotional and autonomic responses to noxious stimuli than somatic pain (16). *TRPV1*, Transient receptor potential vanilloid 1; *Na 1.8v*, sodium channel 1.8; *ASIC3*, acid-sensing ion channel 3; *CGRP*, calcitonin gene-related peptide.

and selectivity to CB1R than Δ^9-THC. These synthetic cannabinoids are 2–100 times more potent than Δ^9-THC (Castaneto et al., 2014). In long-term marijuana users, adverse cardiovascular events, including tachyarrhythmias, acute coronary syndrome, vascular complications, and even congenital heart defects may also occur (Aryana & Williams, 2007), and smoking marijuana increased myocardial oxygen demand in anginal patients (Gottschalk, Aronow, & Prakash, 1977). In more recent research, patients with coronary artery disease had elevated blood levels of endocannabinoids, and increased expression of CB1R in coronary atheroma. CB1R blockade reduced the production of proinflammatory mediators by macrophages, which might provide beneficial effects on atherogenesis (Sugamura et al., 2009).

Gastrointestinal Tract

In the gastrointestinal tract, cannabinoids reduce motility and pain sensitivity (Izzo & Camilleri, 2008). The cannabinoid inhibitory effects on gastrointestinal motility are generally mediated via the CB1R, while actions via the CB2R have been associated to resolution of inflammation (Wright et al., 2008). The role of cannabis and cannabinoids on gastrointestinal motility has been described in detail in another Chapter 98 of this book

TABLE 45.1 Cannabinoid Involvement in Visceral Pain of Nongastrointestinal and Gastrointestinal Origin

Nongastrointestinal organ	Species	Drug	Effect	References
Heart	Human	Synthetic cannabinoids (smoked)	Acute chest pain associated to myocardial infarction	Castaneto et al. (2014)
	Human	CB1R antagonists	Reduced production of proinflammatory mediators	Sugamura et al. (2009)
Kidney	Human	Synthetic cannabinoids (smoked)	Acute kidney injury with flank/abdominal pain	Buser et al. (2014)
	Mice	CB2R agonists, cannabidiol	Protection against cisplatin-induced nephrotoxicity	Pan et al. (2009)
Urinary bladder	Rats, mice	CB1R agonists, FAAH inhibitors. TRPV1 agonists	Reduced sensory activity of isolated tissues, antihyperalgesia in animal models of bladder inflammation	Hedlund (2014); Walczak and Cerveró (2011)
Urethra	Human (isolated tissues)	CB1R agonists	Reduced the relaxing effect of TRPA1 agonists	Weinhold et al. (2010)
		TRFV1 agonists	Enhanced the relaxing effect of TRPA1 agonists	Weinhold et al (2010)
Ovary	Human		CB1R gene polymorphisms are involved in the polycystic ovary syndrome	Kuliczkowska Plaksej et al. (2014)
Endometrium	Human	Cannabis	Relieve endometriosis-related pelvic pain, back pain, dysuria, dyschezia, dyspareunia	Dmitrieva et al. (2010)
	Rat	WIN55, 212-2 (through CB1R activation)	Decreased endometriosis-associated hyperalgesia	Dmitrieva et al. (2010)
Testes and scrotum		Cannabinoid agonists	They might alleviate neuropathic pain symptoms associated to orchialgias	NR
Prostate		Cannabinoid agonists	They might relieve chronic prostatitis/chronic pain syndrome	NR
		Cannabinoid agonists	They might relieve pain associated to bone metastatic prostate cancer	NR but suggested by Ramos and Bianco (2012)
Gastrointestinal organ	**Species**	**Drug**	**Effect**	**References**
General	Human, animals	CB1R agonists	Inhibit motility	Izzo and Camilleri (2008); Wright, Duncan, and Sharkey (2008)
		CB2R agonists	Resolve inflammation	
		TRPV1 agonists	Favor visceral hypersensitivity	
Stomach	Rat	Cannabis extract (through CB2R activation)	Reduced gastric-induced pain	Wallace et al. (2013)
	Rat, mice	CB1R agonists, MAGL inhibitors	Were gastroprotective in models of gastric damage induced by NSAID or cold-restraint stress	Wallace et al. (2013); Rutkowska and Fereniec-Goltbiewska (2006); Germanò et al. (2001)
Small and large intestine	Rodents	CB1R agonists, CB2R agonists, FAAH inhibitors, EMT inhibitors	Protect mucosa and reduce visceral sensation and abdominal pain induced by various stimuli modeling IBS or IBD (mustard oil, DNBS, TNBS, DSS, LPS…)	For references, see Chapter 49 by Salaga et al.: "Cannabinoids and the effects on gastroinstestinal functions"
	Human	Dronabinol	Increased pain during colorectal distension	

(Continued)

TABLE 45.1 Cannabinoid Involvement in Visceral Pain of Nongastrointestinal and Gastrointestinal Origin (*cont.*)

Gastrointestinal organ	Species	Drug	Effect	References
Pancreas	Human	CB1R agonists	In pancreatic cancer, high CB1R levels in nerves are associated with less pain but shorter survival	Michalski et al. (2008)
		Cannabinoid agonists	In acute pancreatitis, they might reduce pain and inflammation (endocannabinoid ligands and receptors are upregulated)	Michalski et al. (2007)
	Human	Heavy cannabis use	Case of acute pancreatitis with transfixiant epigastric abdominal pain, nausea, and vomiting	Fatma, Mouna, Leila, Radhouane, and Taoufik (2013)
	Rat	CB2R agonists	Tissue protection and analgesia in a model of chronic pancreatitis	Zhang, Kline, McNearney, Johnson, and Westlund (2014)
Liver	Mammals	CB2R agonists	Endocannabinoids are protective in hepatic inflammatory conditions and other diseases	Pacher and Mechoulam (2011)
Biliary tract and gall bladder	Rat	WIN55-212-2, FAAH inhibitors (through CB1R activation)	Reduced cholestasis-induced pruritus	Gingold and Bergasa (2003); Hasanein, Shahidi, Komaki, and Mirazi (2008)

NR, Not reported.

(*Cannabinoids and the effects on gastrointestinal tract: a focus on motility*). Here we will focus on cannabinoid effects on pain of gastrointestinal origin.

Stomach

In anesthetized rats, a cannabis extract was able to reduce gastric distension-induced pain, measured as bradychardia, via CB2R activation (Wallace et al., 2013).

In models of gastric damage induced by naproxen (Wallace et al., 2013), aspirin (Rutkowska & Fereniec-Goltbiewska, 2006), or cold-restraint stress (Germanò et al., 2001), CB1R stimulation was protective. Interestingly, the gastroprotective effect of the MAGL inhibitor JZL184, used at low doses in a mouse model of NSAID-induced gastric hemorrhages, did not decrease with chronic administration (Kinsey et al., 2013). The brain-impermeant FAAH inhibitor, URB937, which per se exerted peripheral antinociceptive effects in different experimental models of pain, reduced the number and severity of gastric lesions produced by indomethacin, while exerting no ulcerogenic effect when administered alone, suggesting that this kind of drug may be beneficial when NSAID are to be used as painkillers (Sasso et al., 2012).

Small and Large Intestine

The role of the ECS and the potential therapeutic usefulness of cannabinoids in IBS, IBD, and colorectal cancer have been discussed more deeply in another Chapter 49 within this book (*Cannabis, cannabinoids and the effects on gastrointestinal function: an overview*).

Pancreas

Immunohistochemical analyses of normal human pancreas revealed weak immunoreactivity for CB1R and CB2R in pancreatic acini, nerves, blood vessels, and ductal cells, but this profile changes in malignant tissue. Furthermore, high levels of CB1R were associated with less pain, whereas low CB1R levels in nerves were associated with strong pain symptoms, demonstrating the involvement of the ECS in pancreatic pain perception. However, high CB1R levels were associated with shorter survival, a fact that has been attributed to the role of pain as a warning symptom leading to earlier diagnosis (Michalski et al., 2008), although this hypothesis is not fully confirmed.

Acute pancreatitis is also associated with upregulation of endocannabinoid ligands and receptors and, therefore, cannabinoids might be therapeutically useful to reduce pain associated with acute pancreatitis and, perhaps, also inflammation (Michalski et al., 2007). Nevertheless, a case of acute pancreatitis with transfixiant epigastric abdominal pain, nausea, and vomiting induced by heavy cannabis use was recently reported, pointing at the complexity of ECS involvement in this condition (Fatma et al., 2013).

Finally, in a rat model of chronic pancreatitis induced by an alcohol/high fat diet, stimulation of CB2R showed tissue-protective and analgesic properties (Zhang et al., 2014).

Liver

CB1R and CB2R expression is very low in the normal liver. However, the ECS may play a major role in

several liver pathological conditions. As recently reviewed, the ECS seems to be protective in hepatic inflammatory conditions, and other diseases affecting the liver, including fibrogenesis, cirrhosis, hepatic ischemia-reperfusion injury, autoimmune hepatitis, and even hepatic encephalopathy (Lotersztajn et al., 2008; Miller & Devi, 2011; Pacher & Mechoulam, 2011). Although CB1R may as well participate, the role of CB2R upregulation seems to be prominent in all these conditions (Pacher & Mechoulam, 2011). In addition, CB1R, CB2R, and TRPV-1 overexpression was associated to improved prognosis in patients with hepatocellular carcinoma (Xu et al., 2006)

Specific studies addressed at evaluating the role of ECS in pain of liver origin have not been reported. However, in view of its role in hepatic inflammation, and in pain in other sites, it is conceivable that they would also be useful in these conditions.

Regarding disorders of the biliary tract and gall bladder, in rats subjected to bile duct resection, cholestasis-induced nociception (measured by the tail-flick assay) was reduced by previous administration of the nonselective cannabinoid agonist WIN55,212-2, suggesting that cannabinoids could be useful in reducing cholestasis-induced pruritus (Gingold & Bergasa, 2003). More recently, the FAAH inhibitor URB597 was also shown to increase the threshold in the tail-flick test in a CB1R-sensitive manner (Hasanein et al., 2008). No study has been performed on the possible role of cannabinoids in biliary colic pain.

Genitourinary Tract

Kidney and Ureter

The presence of CB1R and CB2R and their physiological role in the kidney has been described in animal models (Barutta et al., 2014). Both inflammatory cells and podocytes express CB2R, and their activation had beneficial effects in experimental diabetic nephropathy.

In human adult and fetal kidney, polymerase chain reaction confirmed the presence of CB1R mRNA, and the absence of CB2R mRNA; the presence of the CB1R protein was confirmed by western-blot and immunohistochemical assays, whereas assays for the CB2R protein were consistently negative (Larrinaga et al., 2010).

Smoking synthetic cannabinoids may induce acute kidney injury with intense nausea, and flank or abdominal pain (Buser et al., 2014). In contrast, cannabidiol (a nonpsychotropic constituent of cannabis, with antioxidant properties) and CB2R agonists (also devoid of psychotropic effects) protected against cisplatin-induced nephrotoxicity in mice, through attenuation of oxidative/nitrosative stress, inflammation, and cell death in the kidney, and improved renal function (Pan et al., 2009; Mukhopadhyay et al., 2010).

No study has been performed on the possible role of cannabinoids in renal colic pain.

Urinary Bladder and Urethra

As recently reviewed (Hedlund, 2014), the ECS is involved in regulation of bladder function, possibly at several levels of the micturition pathway. Components of the ECS (CB1R, CB2R, FAAH, TRPV1) have been located to lower urinary tract tissues of mice, rats, monkeys, and humans. CB receptors are found in urothelium and sensory nerves, and FAAH in the urothelium. CB receptor- and FAAH-related activities have also been reported in the lumbosacral spinal cord. Cannabinoids reduce sensory activity of isolated tissues, cause antihyperalgesia in animal studies of bladder inflammation, affect urodynamics parameters reflecting sensory functions in animal models, and appear to have effects on storage symptoms in humans. FAAH inhibitors affected sensory bladder functions, and reduced bladder overactivity in rat models. Cannabinoids may modify nerve-mediated functions of isolated lower urinary tract tissues. Modulation by the cannabinoid system of bladder hypersensitivity during inflammation may be exerted peripherally, via CB1R and TRPV1 (Walczak & Cerveró, 2011).

Ion channel transient receptor potential A1 (TRPA1), as well as cannabinoid receptors, are involved in mechanoafferent signaling from bladder and urethra. CB1R and CB2R were immunohistochemically detected in nerve fibers of the human urethra. CB1R activation reduced – whereas TRPV1 stimulation enhanced – the relaxing effect of TRPA1 agonists on human urethra preparations in vitro (Weinhold et al., 2010).

Ovary

The effects of cannabinoids on animal reproductive system have been known for decades (Adashi, Jones, & Hsueh, 1983; Bagavandoss & Grimshaw, 2010), and their effects on folliculogenesis have also been studied. The presence of the ECS in the human ovary has recently been analyzed (El-Talatini et al., 2009). Immunostaining studies showed the expression of CB1R and CB2R in the ovarian medulla and cortex, in the corpus luteum, and in the corpus albicans. Regarding the oocytes, only those of tertiary follicles (at the last stage of their development) expressed CB2R. In addition, anandamide was produced in the human ovary probably under hormonal control. Thus, the ECS seems to play an important role in ovarian follicle and oocyte maturity (Di Blasio, Vignali, & Gentilini, 2013).

Concerning ovarian pathologies, CB1R gene polymorphisms are involved in the polycystic ovary syndrome (Kuliczkowska Plaksej et al., 2014). However, no information is available on ECS involvement in sensory or painful neurotransmission in the ovary.

Endometrium

The main components of the ECS (receptors and enzymes) coexist in many cells in the human endometrium and in sensory and sympathetic neurons, and suffer modifications throughout the menstrual cycle (Taylor, Abbas, Habiba, & Konje, 2010). It has also been demonstrated that the ECS affects critical mechanisms (inflammation, cell proliferation and cell survival), underlying the establishment and maintenance of endometriosis (Pertwee, 2009).

Endometriosis is usually associated with pelvic pain such as chronic dysmenorrhea, intermenstrual abdominal and pelvic pain, back pain, dysuria, dyschezia, and dyspareunia that have been traditionally treated with cannabis to provide pain relief. Recently, cannabinoids have been evaluated in basic and clinical trials (Dmitrieva et al., 2010). In an animal model of endometriosis WIN55,212-2 decreased endometriosis-associated hyperalgesia, whereas the CB1R antagonist AM251, but not the CB2R antagonist AM630, increased the symptoms, suggesting that the antihyperalgesic effect is mediated by CB1R (Dmitrieva et al., 2010).

Thus, although the potential of cannabinoids must still be clarified, they seem to be a promising alternative for the development of new therapeutic strategies for endometriosis, and especially for endometriosis-related pain.

Testes and Scrotum

As recently reviewed (Bovolin et al., 2014), endocannabinoids are involved in male vertebrate reproduction, and this function involves regulatory mechanisms at both central and gonadal level. The appropriate endocannabinoid tone in the testis is critical for spermatogenesis progression, for sperm quality, and the acquisition of sperm functions related to fertilization. A specific and significant association between the use of marijuana and the occurrence of nonseminomatous and mixed testicular germ cell tumors has been recently reported in humans (Lacson et al., 2012), suggesting that the recreational and therapeutic use of cannabinoids may represent a risk factor for these cancerous processes. In addition, increased levels of anandamide are associated to reduced sperm motility and fertility. Cannabis reduces testosterone production by Leydig cells, and anandamide induces apoptosis of Sertoli cells.

Acute scrotal pain is related mainly to testicular torsion, epididymitis, or torsion of the appendix testis. Whereas testicular torsion requires urgent surgery, epididymitis and torsion of the appendix testis, which may actually present as chronic conditions, may be managed conservatively (once testicular torsion has been ruled out), including treatment with NSAID (Srinath, 2013).

Chronic orchialgia interferes significantly with work and social life, and has important psychological and financial implications. Management is established according to the underlying cause. Whereas surgery is not always useful to improve orchialgia, due to intrascrotal lesion, pharmacological treatment of different orchialgias may vary from simple analgesics to opioids, and adjuvants are added in case of neuropathic pain symptoms (Kumar, Mehta, & Nargund, 2010).

Although cannabinoids have not been tested in either acute or chronic testicular pain, they might be useful, because they alleviate peripheral neuropathy associated to diabetes, or administration of antineoplastic drugs in experimental animals (Vera, López-Miranda, Herradón, Martín, & Abalo, 2012; Vera, Cabezos, Martín, & Abalo, 2013).

Prostate

The distribution of CB receptors in prostatic tissue suggest a role for these receptors in mechanoafferent signals, epithelial homeostasis, emission, or inflammation of the human prostate (Gratzke et al., 2010).

Chronic prostatitis/chronic pelvic pain syndrome is a heterogeneous condition, and many treatments are largely ineffective. Limited success of clinical trials of this condition is related to placebo and treatment duration (Cohen et al., 2012). To our knowledge, cannabinoids have not been tested in this painful condition.

Interestingly, the association between prostatitis and prostate cancer, a global public health problem, has been found to be significant (Jiang et al., 2013). Experimental evidence shows that prostate tissue possesses increased expression of both CB1R and CB2R, and their stimulation results in decreased cell viability, increased apoptosis, and decreased androgen receptor expression, as well as prostate-specific antigen excretion. The ECS can act as a local regulatory mechanism to keep prostate cancer cells in check. Both activation of CB1R by anandamide, and inhibition of MAGL, the primary metabolizing enzyme of 2-arachydonoylglycerol, have shown efficacy to achieve this. In addition, it has been suggested that cannabinoid agonists might be particularly interesting in men with bone metastatic prostate cancer, who would not only benefit from the possible antiandrogenic effects of cannabinoids, but also from analgesia of bone pain, improving quality of life, while reducing narcotic consumption, and preventing opioid dependence (Ramos & Bianco, 2012).

CONCLUDING REMARKS

Visceral pain is difficult to treat, but may benefit from the use of cannabinoid agonists (particularly CB2R agonists for chronic inflammatory conditions) or inhibitors of endocannabinoid degradation. However, excessive use of natural or synthetic cannabinoids by abusers or patients may lead to chest or abdominal pain related to visceral toxicity. In addition, psychotropic undesirable

effects may occur upon systemic administration of fat-soluble drugs, increasing CB1R activity. Local routes of administration or cannabinoid-related drugs devoid of such effects will have to prove their usefulness in clinical settings. Time will tell which cannabinoid approaches are finally adopted in therapy.

MINI-DICTIONARY

Dorsal root ganglion (spinal ganglion) Ganglion located along the dorsal root of a spinal nerve, containing the cell bodies of afferents innervating the viscera (sympathetic afferents), as well as the skin and skeletal muscle (somatic afferents).
Dyschezia Difficulty in defecating (usually as a consequence of long continued voluntary suppression of the urge to defecate).
Dyspareunia Painful sexual intercourse due to medical or psychological causes.
Dysuria Painful urination.
Endometriosis Hormonal and immune system disease in which cells similar to those lining the uterus (endometrium) grow outside the uterine cavity, most commonly on the peritoneum, leading to pain and infertility. Pain is often worse with the menstrual cycle, and is the most common cause of secondary dysmenorrhea.
Lower urinary tract symptoms Group of medical symptoms affecting the lower urinary tract (storage, voiding, and postmicturition symptoms), affecting approximately 40% of older men.
Nodose ganglion (inferior ganglion of the vagus nerve) Ganglion located along the vagus nerve (X), containing the cell bodies of vagal (cranial parasympathetic) afferents innervating distal esophagus, stomach, gall bladder, small bowel, liver, spleen, pancreas, kidney, adrenal glands, and proximal colon.
Nucleus of the solitary tract Major sensory nucleus in the dorsal medulla that receives cardiovascular, gastrointestinal, respiratory, gustatory, and orotactile information from vagal afferents.
Orchialgia Long-term pain of the testes.
Pelvic ganglion Ganglion located along the pelvic splanchnic nerve, containing the cell bodies of the pelvic (sacral parasympathetic) afferents innervating the distal colon, rectum, uterus/prostate, urinary bladder, anus, and genitalia.
Referred pain Pain induced on somatic structures by the presence of pain in a viscus belonging to the same metameric field.

References

Adashi, E. Y., Jones, P. B., & Hsueh, A. J. (1983). Direct antigonadal activity of cannabinoids: suppression of rat granulosa cell functions. *American Journal of Physiology*, *244*, E177–E185.

Aryana, A., & Williams, M. A. (2007). Marijuana as a trigger of cardiovascular events: speculation or scientific certainty? *International Journal of Cardiology*, *118*, 141–144.

Bagavandoss, P., & Grimshaw, S. (2010). Temporal and spatial distribution of the cannabinoid receptors (CB1, CB2) and fatty acid amide hydroxylase in the rat ovary. *Anatomical Record (Hoboken)*, *293*, 1425–1432.

Barutta, F., Grimaldi, S., Franco, I., Bellini, S., Gambino, R., Pinach, S., Corbelli, A., Bruno, G., Rastaldi, M. P., Aveta, T., Hirsch, E., Di Marzo, V., & Gruden, G. (2014). Deficiency of cannabinoid receptor of type 2 worsens renal functional and structural abnormalities in streptozotocin-induced diabetic mice. *Kidney International*, *86*, 979–990.

Booker, L., Naidu, P. S., Razdan, R. K., Mahadevan, A., & Lichtman, A. H. (2009). Evaluation of prevalent phytocannabinoids in the acetic acid model of visceral nociception. *Drug and Alcohol Dependence*, *105*, 42–47.

Bovolin, P., Cottone, E., Pomatto, V., Fasano, S., Pierantoni, R., Cobellis, G., & Meccariello, R. (2014). Endocannabinoids are involved in male vertebrate reproduction: regulatory mechanisms at central and gonadal level. *Frontiers in Endocrinology (Lausanne)*, *5*, 54.

Buser, G. L., Gerona, R. R., Horowitz, B. Z., Vian, K. P., Troxell, M. L., Hendrickson, R. G., Houghton, D. C., Rozansky, D., Su, S. W., & Leman, R. F. (2014). Acute kidney injury associated with smoking synthetic cannabinoid. *Clinical Toxicology (Philadelphia)*, *52*, 664–673.

Cabezos, P. A., Vera, G., Martín-Fontelles, M. I., Fernández-Pujol, R., & Abalo, R. (2010). Cisplatin-induced gastrointestinal dysmotility is aggravated after chronic administration in the rat. Comparison with pica. *Neurogastroenterology and Motility*, *22*, 797–805, e224–5..

Castaneto, M. S., Gorelick, D. A., Desrosiers, N. A., Hartman, R. L., Pirard, S., & Huestis, M. A. (2014). Synthetic cannabinoids: epidemiology, pharmacodynamics, and clinical implications. *Drug and Alcohol Dependence*, *144*, 12–41.

Christianson, J. A., Bielefeldt, K., Altier, C., Cenac, N., Davis, B. M., Gebhart, G. F., High, K. W., Kollarik, M., Randich, A., Undem, B., & Vergnolle, N. (2009). Development, plasticity and modulation of visceral afferents. *Brain Research Reviews*, *60*, 171–186.

Cohen, J. M., Fagin, A. P., Hariton, E., Niska, J. R., Pierce, M. W., Kuriyama, A., Whelan, J. S., Jackson, J. L., & Dimitrakoff, J. D. (2012). Therapeutic intervention for chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS): a systematic review and meta-analysis. *PLoS One*, *7*(8), e41941.

Davis, M. P. (2012). Drug management of visceral pain: concepts from basic research. *Pain Research and Treatment*, *2012*, 265605.

de Carvalho Rocha, H. A., Dantas, B. P., Rolim, T. L., Costa, B. A., & de Medeiros, A. C. (2014). Main ion channels and receptors associated with visceral hypersensitivity in irritable bowel syndrome. *Annals of Gastroenterology*, *27*, 200–206.

Di Blasio, A. M., Vignali, M., & Gentilini, D. (2013). The endocannabinoid pathway and the female reproductive organs. *Journal of Molecular Endocrinology*, *50*, R1–R9.

Di Marzo, V. (2009). The endocannabinoid system: its general strategy of action, tools for its pharmacological manipulation and potential therapeutic exploitation. *Pharmacologic Research*, *60*, 77–84.

Dmitrieva, N., Nagabukuro, H., Resuehr, D., Zhang, G., McAllister, S. L., McGinty, K. A., Mackie, K., & Berkley, K. J. (2010). Endocannabinoid involvement in endometriosis. *Pain*, *151*, 703–710.

El-Talatini, M. R., Taylor, A. H., Elson, J. C., Brown, L., Davidson, A. C., & Konje, J. C. (2009). Localisation and function of the endocannabinoid system in the human ovary. *PLoS One*, *4*, e4579.

Fatma, H., Mouna, B., Leila, M., Radhouane, D., & Taoufik, N. (2013). Cannabis: a rare cause of acute pancreatitis. *Clinics and Research in Hepatology and Gastroenterology*, *37*, e24–e25.

Germanò, M. P., D'Angelo, V., Mondello, M. R., Pergolizzi, S., Capasso, F., Capasso, R., Izzo, A. A., Mascolo, N., & De Pasquale, R. (2001). Cannabinoid CB1-mediated inhibition of stress-induced gastric ulcers in rats. *Naunyn Schmiedebergs Archives of Pharmacology*, *363*, 241–244.

Gingold, A. R., & Bergasa, N. V. (2003). The cannabinoid agonist WIN 55, 212-2 increases nociception threshold in cholestatic rats: implications for the treatment of the pruritus of cholestasis. *Life Sciences*, *73*, 2741–2747.

González, E. J., Merrill, L., & Vizzard, M. A. (2014). Bladder sensory physiology: neuroactive compounds and receptors, sensory transducers, and target-derived growth factors as targets to improve function. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *306*, R869–R878.

Gottschalk, L. A., Aronow, W. S., & Prakash, R. (1977). Effect of marijuana and placebo-marijuana smoking on psychological state and on psychophysiological cardiovascular functioning in anginal patients. *Biological Psychiatry*, *12*, 255–266.

Gratzke, C., Weinhold, P., Reich, O., Seitz, M., Schlenker, B., Stief, C. G., Andersson, K. E., & Hedlund, P. (2010). Transient receptor potential A1 and cannabinoid receptor activity in human normal and hyperplastic prostate: relation to nerves and interstitial cells. *European Urology*, *57*, 902–910.

Hasanein, P., Shahidi, S., Komaki, A., & Mirazi, N. (2008). Effects of URB597 as an inhibitor of fatty acid amide hydrolase on modulation of nociception in a rat model of cholestasis. *European Journal of Pharmacology*, *591*, 132–135.

Hedlund, P. (2014). Cannabinoids and the endocannabinoid system in lower urinary tract function and dysfunction. *Neurourology and Urodynamics*, *33*, 46–53.

Izzo, A. A., & Camilleri, M. (2008). Emerging role of cannabinoids in gastrointestinal and liver diseases: basic and clinical aspects. *Gut*, *57*, 1140–1155.

Jiang, J., Li, J., Yunxia, Z., Zhu, H., Liu, J., & Pumill, C. (2013). The role of prostatitis in prostate cancer: meta-analysis. *PLoS One*, *8*(12), e85179.

Kinsey, S. G., Wise, L. E., Ramesh, D., Abdullah, R., Selley, D. E., Cravatt, B. F., & Lichtman, A. H. (2013). Repeated low-dose administration of the monoacylglycerol lipase inhibitor JZL184 retains cannabinoid receptor type 1-mediated antinociceptive and gastroprotective effects. *Journal of Pharmacology and Experimental Therapeutics*, *345*, 492–501.

Kuliczkowska Plaksej, J., Laczmanski, L., Milewicz, A., Lenarcik-Kabza, A., Trzmiel-Bira, A., Zaleska-Dorobisz, U., Lwow, F., & Hirnle, L. (2014). Cannabinoid receptor 1 gene polymorphisms and nonalcoholic fatty liver disease in women with polycystic ovary syndrome and in healthy controls. *International Journal of Endocrinology*, *2014*, 232975.

Kumar, P., Mehta, V., & Nargund, V. H. (2010). Clinical management of chronic testicular pain. *Urology International*, *84*, 125–131.

Lacson, J. C., Carroll, J. D., Tuazon, E., Castelao, E. J., Bernstein, L., & Cortessis, V. K. (2012). Population-based case-control study of recreational drug use and testis cancer risk confirms an association between marijuana use and nonseminoma risk. *Cancer*, *118*, 5374–5383.

Larauche, M., Mulak, A., & Taché, Y. (2012). Stress and visceral pain: from animal models to clinical therapies. *Experimental Neurology*, *233*, 49–67.

Larrinaga, G., Varona, A., Pérez, I., Sanz, B., Ugalde, A., Cándenas, M. L., Pinto, F. M., Gil, J., & López, J. I. (2010). Expression of cannabinoid receptors in human kidney. *Histology and Histopathology*, *25*, 1133–1138.

Lotersztajn, S., Teixeira-Clerc, F., Julien, B., Deveaux, V., Ichigotani, Y., Manin, S., Tran-Van-Nhieu, J., Karsak, M., Zimmer, A., & Mallat, A. (2008). CB2 receptors as new therapeutic targets for liver diseases. *British Journal of Pharmacology*, *153*, 286–289.

Michalski, C. W., Laukert, T., Sauliunaite, D., Pacher, P., Bergmann, F., Agarwal, N., Su, Y., Giese, T., Giese, N. A., Bátkai, S., Friess, H., & Kuner, R. (2007). Cannabinoids ameliorate pain and reduce disease pathology in cerulein-induced acute pancreatitis. *Gastroenterology*, *132*, 1968–1978.

Michalski, C. W., Oti, F. E., Erkan, M., Sauliunaite, D., Bergmann, F., Pacher, P., Batkai, S., Müller, M. W., Giese, N. A., Friess, H., & Kleeff, J. (2008). Cannabinoids in pancreatic cancer: correlation with survival and pain. *International Journal of Cancer*, *122*, 742–750.

Miller, L. K., & Devi, L. A. (2011). The highs and lows of cannabinoid receptor expression in disease: mechanisms and their therapeutic implications. *Pharmacological Reviews*, *63*, 461–470.

Mukhopadhyay, P., Rajesh, M., Pan, H., Patel, V., Mukhopadhyay, B., Bátkai, S., Gao, B., Haskó, G., & Pacher, P. (2010). Cannabinoid-2 receptor limits inflammation, oxidative/nitrosative stress, and cell death in nephropathy. *Free Radical Biology and Medicine*, *48*, 457–467.

Naidu, P. S., Booker, L., Cravatt, B. F., & Lichtman, A. H. (2009). Synergy between enzyme inhibitors of fatty acid amide hydrolase and cyclooxygenase in visceral nociception. *Journal of Pharmacology and Experimental Therapeutics*, *329*, 48–56.

Pacher, P., Batkai, S., & Kunos, G. (2006). The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacological Reviews*, *58*, 389–462.

Pacher, P., & Mechoulam, R. (2011). Is lipid signaling through cannabinoid 2 receptors part of a protective system? *Progress in Lipid Research*, *50*, 193–211.

Pan, H., Mukhopadhyay, P., Rajesh, M., Patel, V., Mukhopadhyay, B., Gao, B., Haskó, G., & Pacher, P. (2009). Cannabidiol attenuates cisplatin-induced nephrotoxicity by decreasing oxidative/nitrosative stress, inflammation, and cell death. *Journal of Pharmacology and Experimental Therapeutics*, *328*, 708–714.

Pertwee, R. G. (2009). Emerging strategies for exploiting cannabinoid receptor agonists as medicines. *British Journal of Pharmacology*, *156*, 397–411.

Ramos, J. A., & Bianco, F. J. (2012). The role of cannabinoids in prostate cancer: basic science perspective and potential clinical applications. *Indian Journal of Urology*, *28*, 9–14.

Rutkowska, M., & Fereniec-Goltbiewska, L. (2006). ACEA (arachidonyl-2-chloroethylamide), the selective cannabinoid CB1 receptor agonist, protects against aspirin-induced gastric ulceration. *Pharmazie*, *61*, 341–342.

Sasso, O., Bertorelli, R., Bandiera, T., Scarpelli, R., Colombano, G., Armirotti, A., Moreno-Sanz, G., Reggiani, A., & Piomelli, D. (2012). Peripheral FAAH inhibition causes profound antinociception and protects against indomethacin-induced gastric lesions. *Pharmacological Research*, *65*, 553–563.

Sengupta, J. N. (2009). Visceral pain: the neurophysiological mechanism. *Handbook of Experimental Pharmacology*, *194*, 31–74.

Sikandar, S., & Dickenson, A. H. (2012). Visceral pain: the ins and outs, the ups and downs. *Current Opinion in Supportive and Palliative Care*, *6*, 17–26.

Srinath, H. (2013). Acute scrotal pain. *Australian Family Physician*, *42*, 790–792.

Sugamura, K., Sugiyama, S., Nozaki, T., Matsuzawa, Y., Izumiya, Y., Miyata, K., Nakayama, M., Kaikita, K., Obata, T., Takeya, M., & Ogawa, H. (2009). Activated endocannabinoid system in coronary artery disease and antiinflammatory effects of cannabinoid 1 receptor blockade on macrophages. *Circulation*, *119*, 28–36.

Taylor, A. H., Abbas, M. S., Habiba, M. A., & Konje, J. C. (2010). Histomorphometric evaluation of cannabinoid receptor and anandamide modulating enzyme expression in the human endometrium through the menstrual cycle. *Histochemistry and Cell Biology*, *133*, 557–565.

Velasco, G., Sánchez, C., & Guzmán, M. (2012). Towards the use of cannabinoids as antitumour agents. *Nature Reviews: Cancer*, *12*, 436–444.

Vera, G., Cabezos, P. A., Martín, M. I., & Abalo, R. (2013). Characterization of cannabinoid-induced relief of neuropathic pain in a rat model of cisplatin-induced neuropathy. *Pharmacology Biochemistry and Behavior*, *105*, 205–212.

Vera, G., Castillo, M., Cabezos, P. A., Chiarlone, A., Martín, M. I., Gori, A., Pasquinelli, G., Barbara, G., Stanghellini, V., Corinaldesi, R., De Giorgio, R., & Abalo, R. (2011). Enteric neuropathy evoked by repeated cisplatin in the rat. *Neurogastroenterology and Motility*, *23*, 370–378, e162–3..

Vera, G., López-Miranda, V., Herradón, E., Martín, M. I., & Abalo, R. (2012). Characterization of cannabinoid-induced relief of neuropathic pain in rat models of type 1 and type 2 diabetes. *Pharmacology Biochemistry and Behavior*, *102*, 335–343.

Vermeulen, W., De Man, J. G., Pelckmans, P. A., & De Winter, B. Y. (2014). Neuroanatomy of lower gastrointestinal pain disorders. *World Journal of Gastroenterology*, *20*, 1005–1020.

Viniol, A., Keunecke, C., Biroga, T., Stadje, R., Dornieden, K., Bösner, S., Donner-Banzhoff, N., Haasenritter, J., & Becker, A. (2014). Studies of the symptom abdominal pain—a systematic review and meta-analysis. *Family Practice*, *31*, 517–529.

Wafai, L., Taher, M., Jovanovska, V., Bornstein, J. C., Dass, C. R., & Nurgali, K. (2013). Effects of oxaliplatin on mouse myenteric neurons and colonic motility. *Frontiers in Neuroscience*, *7*, 30.

Walczak, J. S., & Cerveró, F. (2011). Local activation of cannabinoid CB_1 receptors in the urinary bladder reduces the inflammation-induced sensitization of bladder afferents. *Molecular Pain*, *7*, 31.

Wallace, J. L., Flannigan, K. L., McKnight, W., Wang, L., Ferraz, J. G., & Tuitt, D. (2013). Pro-resolution, protective and anti-nociceptive effects of a cannabis extract in the rat gastrointestinal tract. *Journal of Physiology and Pharmacology*, *64*, 167–175.

Weinhold, P., Gratzke, C., Streng, T., Stief, C., Andersson, K. E., & Hedlund, P. (2010). TRPA1 receptor induced relaxation of the human urethra involves TRPV1 and cannabinoid receptor mediated signals, and cyclooxygenase activation. *Journal of Urology*, *183*, 2070–2076.

Wright, K. L., Duncan, M., & Sharkey, K. A. (2008). Cannabinoid CB2 receptors in the gastrointestinal tract: a regulatory system in states of inflammation. *British Journal of Pharmacology*, *153*, 263–270.

Xu, X., Liu, Y., Huang, S., Liu, G., Xie, C., Zhou, J., Fan, W., Li, Q., Wang, Q., Zhong, D., & Miao, X. (2006). Overexpression of cannabinoid receptors CB1 and CB2 correlates with improved prognosis of patients with hepatocellular carcinoma. *Cancer Genetics and Cytogenetics*, *171*, 31–38.

Zhang, L., Kline, R. H., IV, McNearney, T. A., Johnson, M. P., & Westlund, K. N. (2014). Cannabinoid receptor 2 agonist attenuates pain related behavior in rats with chronic alcohol/high fat diet induced pancreatitis. *Molecular Pain*, *10*, 66.

CHAPTER

46

Cannabis and Postoperative Analgesia

S.O Cawich, U. Deonarine*, H.E. Harding**, D. Dan*, V. Naraynsingh**

***Department of Clinical Surgical Sciences, University of the West Indies, St. Augustine Campus, St Augustine, Trinidad and Tobago**

****Department of Surgery, University of the West Indies, Mona Campus, Kingston, Jamaica**

SUMMARY POINTS

- The mechanisms of postoperative pain include peripheral sensitization, central sensitization, inflammation, ischemia, and affective components.
- Multimodal therapy is advocated to target the multidimensional nature of postoperative pain.
- There are two G-protein coupled cannabinoid receptors (CB1 and CB2) that can be stimulated by organic cannabinoids, endocannabinoids, or synthetic cannabinoids.
- Endocannabinoids are released as a natural anesthetic, postoperatively. High concentrations of the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) have been associated with faster resolution of postoperative pain.
- Endocannabinoids may also decrease postoperative inflammation by inhibition of the cyclooxygenase pathway.
- There is substantial theoretical evidence suggesting cannabinols may be useful medicines in the treatment of postoperative pain, but this has not translated uniformly into the clinical research.
- Clinical human studies have not clearly demonstrated cannabinols as effective postoperative analgesia. At doses high enough to produce adequate analgesia, there is also higher incidence of adverse events.
- Experimental research has shown a clear synergistic effect when cannabinoids are used in combination with opioids. This has not translated into human clinical trials.
- Anecdotal reports are emerging concerning the phenomenon of extreme refractory hyperalgesia, and increased postoperative analgesic requirements in the chronic cannabis user.
- Chronic use of cannabis leads to CB1 receptor downregulation that may, in part, account for this extreme hyperalgesia.
- Based on the current research, the authors do not recommend the use of cannabis or cannabinoids to treat postoperative pain.
- It is not ill advised to express heightened vigilance to the analgesic requirements of the cannabis user.

KEY FACTS

- The endogenous opioid system is comprised of several neurons scattered throughout the body that produce endogenous opioids such as endorphins, enkephalins, and dynorphins.
- These naturally produced endogenous opioids are neuromodulators at various synapses along the pain pathways.
- Cannabinoids play a role in decreasing postoperative pain by interacting with the endogenous opioid system.
- Experimental evidence supports a synergistic interaction between opioid and cannabinoid receptors that leads to enhanced analgesia for chronic pain.
- The possibility exists of increased analgesic requirements in the chronic cannabis user.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00052-1
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

Δ^9-THC	Delta-9-tetrahydrocannabinol
2-AG	2-Arachidonoylglycerol
AEA	Anandamide
cAMP	Cyclic adenosine monophosphate
CB1	Cannabinoid receptor type 1
CB2	Cannabinoid receptor type 2
MAPK	Mitogen-activated protein kinases
mRNA	Messenger ribosomal nucleic acid

INTRODUCTION

Medicinal properties of the cannabis plant were described as early as 2700 BC, in the ancient Chinese pharmacopeia. In ancient India, the plant was also prescribed to treat a multitude of ailments, including infections, pain disorders, inflammation, seizures, and anorexia. There are even early reports of the use of cannabis in an anesthetic setting, dating back to the second century, and the work of Hua Tuo (AD 110–207). Hua Tuo was a renowned ancient Chinese surgeon who used a herbal mixture called "mafeisan"—roughly translated as "cannabis boiling powder"—to anesthetize his patients prior to surgery (Zuardi, 2006).

Since this era, our knowledge of the mechanisms of action of cannabis extracts has improved. As it relates specifically to pain, cannabis has been shown to attenuate the perception of chronic pain through effects on the endogenous opioid system (Mackie, 2008). Based on these properties, it was licensed for medicinal use in chronic pain syndromes, and for terminally-ill cancer patients in the Netherlands, Switzerland, Spain (Martínez-Rodríguez et al., 2008), Canada (Spurgeon, 2001), and in 20 states of the United States of America (Morris, TenEyck, Barnes, & Kovandzic, 2014; Sevigny, Pacula, & Heaton, 2014).

Naturally, the question arose whether it may also be of value to alleviate acute postoperative pain. This is important because, even in the 21st century, there are still reports that acute pain continues to be poorly treated after surgery (Wu & Raja, 2011) in up to 39% of patients (Apfelbaum, Chen, Mehta, & Gan, 2003). These poor outcomes are echoed in statistics across the globe (Myles & Power, 2007; Taylor & Stanbury, 2009). This is disappointing, since there are clear benefits to adequate postoperative analgesia, including early ambulation, quicker recovery, reduced morbidity, shorter hospitalization, and better patient satisfaction.

The potential for cannabis to be used in the treatment of acute postoperative pain has sparked much debate, with opponents pointing out that the mechanism of acute pain differs significantly from chronic pain models. Therefore, it is prudent to review the mechanisms of acute and chronic pain.

MECHANISM OF CHRONIC PAIN

In the chronic pain model, there is downregulation of opioid receptors in the dorsal horn of the spinal cord (Mackie, 2008), explaining the reduced efficacy of opioids in chronic pain. However, there is compensatory upregulation of cannabinoid receptors type 1 (CB1) in the thalamus (Mackie, 2008). When stimulated, CB1 sends inhibitory impulses along descending tracts that synapse on the dorsal horn of the spinal cord at the level of origin of pain impulses (Jefferson, Harding, Cawich, & Jackson-Gibson, 2013) to modulate the upward transmission of chronic pain signals. This is the primary mechanism by which cannabis provides analgesia in chronic pain models.

MECHANISM OF ACUTE POSTOPERATIVE PAIN

On the other hand, several different pathophysiologic mechanisms are active to produce postoperative pain. Firstly, acute pain is transmitted mainly by small-diameter Aδ fibers, in contrast to the large-diameter C-fibers that predominate in chronic pain models (Pogatzki, Gebhart, & Brennan, 2002). As early as the first postoperative day, there is primary sensitization at these peripheral myelinated Aδ fibers, as well as the unmyelinated C fibers (Pogatzki et al., 2002). Several mechanisms are involved in primary sensitization, including reduction of receptor threshold potentials, spontaneous depolarization, enlargement of the receptive fields, and greater recruitment of pain receptors to respond to noxious stimuli (Brennan, 2011). Similar mechanisms lead to central sensitization in the dorsal horn of the spinal cord (Dirks, Møiniche, Hilsted, & Dahl, 2002). An inflammatory component also exists, and is due to prostaglandin-induced neuropeptide release (Schug & Manopas, 2007) that, in turn, leads to prolonged stimulatory input to large diameter C-fibers (Grape & Tramèr, 2007). Tissue ischemia contributes as well, as evidenced by increased lactate levels and decreased pH in tissues, after operation (Wu & Raja, 2011). Finally, there is a significant affective excitatory component that contributes to hyperalgesia (Roykulcharoen & Good, 2004). This explains how relaxation techniques alleviate postoperative anxiety, and reduce analgesic requirements (Roykulcharoen & Good, 2004).

A better understanding of the multifaceted nature of acute postsurgical pain has led to the concept of the multimodal approach to postoperative analgesia, as demonstrated in Fig. 46.1 (Myles & Power, 2007). The choice of analgesia, then, is affected by the pain intensity, the surgical procedure, and integrity of the gastrointestinal tract.

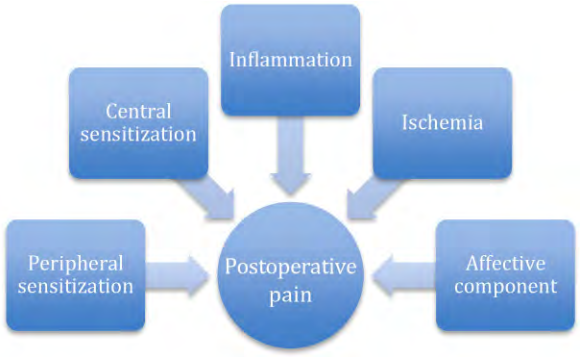


FIGURE 46.1 Multidimensional model for pathophysiology of postoperative pain. The multidimensional nature of postoperative pain. This chart describes the different factors that contribute to the intensity of postoperative pain.

Opioids are still the most commonly utilized drugs to treat moderate-to-severe pain in the acute postoperative setting, but there has been growing interest in multimodal drug therapy which has the potential to reduce opioid dosages, and prevent complications such as respiratory depression (Hamilton & Baskett, 2000).

Many have suggested that cannabis may have a role in the multimodal drug therapy regimens, since CB1 can be located in critical locations, such as the presynaptic cell membranes in the dorsal horn of the spinal cord, the hypothalamus, and periaqueductal gray matter (Mackie, 2008). A detailed understanding of the endocannabinoid system is required to explore the role of cannabis in the treatment of postoperative pain.

THE ENDOCANNABINOID SYSTEM

The crude form of the *Cannabis sativa* plant contains over 100 phytocannabinoids (Hill, Williams, Whalley, & Stephens, 2012). The widely varying chemical composition has made the cannabis plant difficult to work with, from a research perspective. The most active extract that has been isolated is delta-9-tetrahydrocannabinol (Δ^9-THC), which has significant psychotropic effects. The Δ^9-THC extract is the prototype cannabinoid, but there is ongoing research using botanical cannabinoids that have less psychotropic effects, such as cannabidiol and cannabinol (Hill et al., 2012).

Some synthetic cannabinoids have been clinically tested with success. These include synthetic cannabinoids such as nabilone and dronabinol that have been licensed for use since 1985 to treat refractory chemotherapy-induced nausea and vomiting (Borgelt, Franson, Nussbaum & Wang, 2013).

The human body produces several forms of endogenous cannabinoids that are released in response to acute pain (Guindon & Hohmann, 2009). The endocannabinoids, such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG), mediate their effects through the cannabinoid receptors, as outlined in Table 46.1 (Karst, Wippermann, & Ahrens, 2010; Mackie, 2008).

TABLE 46.1 The Distribution of Cannabinoid Receptors

Receptor	Location of the cannabinoid receptors
CB1 (high density)	Basal ganglia (lateral part of the striatum, globus pallidus, entopeduncular nucleus, substantia nigra pars reticulata) Cerebellum Hippocampus Neocortex Hypothalamus Limbic cortex
CB1 (moderate density)	Periaqueductal gray matter Dorsal horn of the spinal cord
CB1 (low density)	Thalamus Brainstem
CB2	Immune cells (peripheral macrophages such as microglia Keratinocytes

This table lists the locations and relative densities of the cannabinoid receptors.

The cannabinoid receptors are transmembrane G-protein coupled receptors (Elikkottil, Gupta, & Gupta, 2009). When activated by cannabinoid binding, there is inhibition of adenylyl cyclase, reduction in cAMP, and closure of voltage-activated calcium channels, leading to reduced calcium conductance across the cell membrane. Simultaneously, there is activation of mitogen-activated protein kinases (MAPK), and opening of potassium channels that lead to increased potassium conductance (Elikkottil et al., 2009). Through these downstream effects, the endocannabinoids attenuate neural transmission of pain impulses, thereby hastening the resolution of postoperative pain in animal models (Alkaitis et al., 2010). These studies have also demonstrated that the levels of AEA and 2-AG are low on the first postoperative day, when pain is most severe, but progressively increase, coinciding with progressive resolution of hyperalgesia (Alkaitis et al., 2010).

Other mechanisms of action are also believed to be active, including inhibition of the cyclooxygenase pathway leading to attenuation of the inflammatory component to acute pain (Costa et al., 2004). Finally, they are also believed to attenuate secondary central sensitization because they are found in moderate concentrations in the dorsal horn of the spinal cord (Alkaitis et al., 2010). In summary, there is theoretical evidence to suggest that cannabinols may be useful in the treatment of postoperative pain, but there are mixed results when tested in clinical trials.

CLINICAL TRIALS USING CANNABINOIDS TO TREAT ACUTE POSTOPERATIVE PAIN

Although animal studies have suggested that cannabinoids have a beneficial effect in treating postoperative pain, the results of clinical trials have not been as clear. To date, 5 randomized blinded clinical trials have been performed in 314 human subjects, in an attempt to determine the effect of cannabinoids.

Two studies actually suggested that cannabinoids *worsened* pain scores in patients with acute pain. Beaulieu (2006) designed a double-blinded trial in which they randomized 44 patients to receive nabilone (a synthetic Δ^9-THC analog), ketoprofen, or placebo at 8-h dosing intervals, after undergoing a range of operative procedures. The patients receiving nabilone reported significantly greater pain scores at rest and during movement, when compared to ketoprofen or placebo. There was also no difference in rescue morphine consumption, quality of sleep, or side effects. Naef et al. (2003) exposed 12 healthy volunteers to thermal, pressure, and electrical stimuli to produce acute pain. The volunteers were randomized either to a placebo, 20 mg THC, 30 mg morphine, or a combination of 30 mg morphine + 20 mg THC. There were worsened pain scores reported in response to thermal stimuli, and no significant analgesic effect with pressure and electrical stimuli.

Two studies suggested that the cannabinoids provided no useful analgesic effect in humans, in the postoperative period. Buggy et al. (2003) investigated a cohort of 40 patients who were scheduled to undergo elective hysterectomies. They were routinely placed on patient-controlled analgesia, in the immediate postoperative phase. Once the PCA was discontinued on the second postoperative day, the patients were prospectively randomized to identical tablets containing 5 mg of Δ^9-THC or placebo. Buggy et al. (2003) found no difference in visual pain scores, summed pain intensity difference, time to rescue analgesia, or adverse events between Δ^9-THC and placebo. Seeling et al. (2006) then studied a cohort of 100 patients scheduled for radical prostatectomy. The patients were randomized to receive either placebo or 5 mg drobanibol (Δ^9-THC analog), starting from the evening before operation. They found no analgesic effect, and no difference in the amount of morphine used as rescue analgesia by patients who received drobanibol.

One prospective blinded randomized trial suggested that cannabinoids were beneficial in controlling postoperative pain. Jain, Ryan, McMahon, and Smith (1981) studied a group of 56 patients who had acute pain after trauma or operation. They prospectively randomized the patients to receive intramuscular injections of levonantradol (Δ^9-THC analog) or placebo. The patients receiving levonantradol reported significant reductions in pain intensity, but significantly more side effects. Approximately 95% of patients taking levonantradol experienced unwanted side effects, most notably CNS sedation and dizziness (Jain et al., 1981).

An interesting study supporting cannabinoid use was reported by Holdcroft, Maze, Dore, Tebbs, and Thompson (2006). They evaluated an extract of cannabis called cannador that contained a mixture of Δ^9-THC and cannabinol in a ratio of 1:0.3 or 1:0.5. A dose of 5 mg of cannador was commenced after discontinuation of patient controlled analgesia, and the dose was escalated to 10 mg, and then to 15 mg—based on the number of patients requesting rescue analgesia. As the doses of cannador escalated, there was a significant reduction in pain intensity at rest, and the requirement for rescue analgesia. They found that cannador at 10 and 15 mg dose was comparable to acetaminophen and ibuprofen to prevent the need for rescue analgesia. However, as the cannador doses rose, there was a significant increase in sedation and adverse events. In fact, the study was terminated due to a serious vasovagal attack in a patient at the 15 mg dose. Nevertheless, these researchers felt the evidence was compelling enough to advocate the use of 10 mg of cannador to treat postoperative pain (Holdcroft et al., 2006).

There is still much heterogeneity in study design and outcomes to make a definitive statement on the effect of cannabis in postoperative pain in clinical trials (Table 46.2).

SYNERGISM BETWEEN CANNABINOIDS AND OPIOIDS

Although there are mixed results from clinical studies investigating the analgesic effects of cannabinoids, researchers have suggested that they may be beneficial through synergistic effects, when combined with opioid analgesics. This important concept follows the principles of multimodal therapy, where cannabinoids produce greater-than-additive effect on the antinociceptive effect of opioids. Therefore, the dose of opioids could be reduced, producing less worrisome side effects. This is important, since morphine remains the mainstay of pharmacologic treatment to control severe postoperative pain, and is associated with a significant risk of respiratory depression at high doses (Hamilton & Baskett, 2000).

Animal experiments have revealed some of the mechanisms for the synergistic effects. Firstly, stimulation of cannabinoid receptors may also stimulate the release of endogenous opioids: CB1 stimulation has been shown to lead to the release of dynorphin A (Pugh, Smith, Dombrowski, & Welch, 1996), and selective CB2 stimulation by AM241 has been shown to stimulate the release of β-endorphin from surrounding keratinocytes (Ibrahim

TABLE 46.2 Clinical Trials Assessing the Role of Cannabinoids in the Postoperative Setting

Authors	Study design	Cannabinoid tested/dose	Study population	Outcomes/results	Adverse events
Jain et al. (1981)	Randomized double-blind trial comparing four doses with placebo	Levonantradol at doses of 1.5, 2, 2.5, and 3 mg compared with placebo	56 patients with moderate to severe postoperative or traumatic pain	Levonantradol was found to have significantly superior analgesic effect compared with placebo. 2.5 mg dose provided best analgesia, but no statistically significant difference among treatment groups	57% of 40 patients who received Levonantradol experienced side effects. Most commonly, sedation (47%)
Buggy et al. (2003)	Randomized double-blind placebo controlled single dose trial	Δ^9-THC/5 mg compared with placebo	40 patients after elective abdominal hysterectomy	No difference among groups in visual analog scale pain scores at movement or rest. No difference in mean sum of pain intensity differences at 6 h. No difference among groups in time to request rescue analgesia	Significantly increased awareness of surroundings in patients who received 5 mg THC (40%) compared with patients who received placebo (5%)
Naef et al. (2003)	Randomized, placebo-controlled, double-blinded, crossover study	Placebo compared with 20 mg THC, compared with 30 mg morphine, compared with a combined preparation of 30 mg morphine + 20 mg THC, all given orally and as single doses	12 healthy volunteers subjected to experimental pain tests: heat, cold, pressure, and transcutaneous electrical stimulation	THC did not reduce pain scores for pressure, heat, cold. THC increased pain scores in cold and heat testing. There was a slight additive analgesic effect with THC-morphine, in electrical stimulation tests	Increased psychotropic and somatic side effects: drowsiness, euphoria, anxiety, confusion, nausea, dizziness
Seeling et al. (2006)	Randomized double blind, placebo controlled trial	5 mg THC given three times a day starting on the evening preoperatively to day 2 post-op compared with placebo	100 patients who underwent radical prostatectomy	No significant difference in opioid (piritramide consumption) or analgesia	Increase in vasovagal reactions in THC group, and a decrease in postoperative nausea and vomiting
Beaulieu (2006)	Randomized double-blind, placebo controlled, parallel group trail	Nabilone at doses of 1 and 2 mg, compared with ketoprofen 50 mg or placebo	41 patients undergoing gynecological (46%), orthopedic (Naef et al., 2003) (44%), or other surgery	No statistically significant difference in 24 h morphine consumption with administration of nabilone. Intensity of pain at rest and on movement (assessed by pain scores) was significantly greater in the group who received 2 mg nabilone	Sedation scores were significantly higher in the group who received 2 mg nabilone. Incidence of nausea and vomiting showed no difference between groups

TABLE 46.2 Clinical Trials Assessing the Role of Cannabinoids in the Postoperative Setting (*cont.*)

Authors	Study design	Cannabinoid tested/dose	Study population	Outcomes/results	Adverse events
Holdcroft et al. (2006)	Multicentre dose-escalation study	Δ^9-THC at doses of 5, 10 or 15 mg	65 patients who underwent breast, general, gynecological, orthopedic, or plastic surgery	There was significant decrease in request for rescue analgesia at increasing dose. 5 mg dose 100% requested rescue analgesia, compared to 50% at 10 mg dose, and 25% at 15 mg dose ($p < 0.001$) There was significant improvement in pain intensity at rest with increasing dose	Significantly increased sedation and nausea with increasing dosage. One serious vasovagal adverse event at the 15 mg dose

This table summarizes the current data assessing the interaction of cannabinoids with mechanisms of acute pain.

et al., 2005). There is also evidence to suggest that Δ^9-THC has some activity at the κ-opioid and δ-opioid receptors (Smith, Cichewicz, Martin, & Welch, 1998).

Experimental animal studies supported the theory of cannabinoid-opioid synergism (Smith et al., 1998; Pugh et al., 1996; Cichewicz & McCarthy, 2003; Ibrahim et al., 2005). Smith et al. (1998) carried out experiments in mice, comparing tail flick responses to painful thermal stimuli, when treated with morphine alone or morphine/Δ^9-THC combinations. They were able to demonstrate a significant potentiation of morphine's analgesic effect, when combined with Δ^9-THC. The synergistic effect was also present when a synthetic selective cannabinoid (CP-55-940) was used in place of Δ^9-THC, and the effects could be reliably reversed when a CB1 agonist (SR141716A) was administered (Smith et al., 1998). Similar synergistic effects between Δ^9-THC and morphine, with a left shift in the morphine dose-response curve, and greater antinociceptive in mice, were demonstrated in mice by Pugh et al. (1996) and Cichewicz and McCarthy (2003). Again, there was reliable attenuation of the morphine/Δ^9-THC synergism, when κ-antagonists (norbinaltorphimine) and δ-antagonists (naltrindole) were administered to interrupt the action of Δ^9-THC (Pugh et al., 1996).

However, human studies did not support the theories of synergism, when evaluated in human trials. Naef et al. (2003) exposed 12 healthy volunteers to a variety of noxious stimuli. The volunteers were then prospectively randomized to analgesia with either placebo, 20 mg of THC, 30 mg of morphine, or combinations of 20 mg THC + 30 mg morphine. Interestingly, in response to thermal stimuli, THC resulted in hyperalgesia. Otherwise, there was a slight additive analgesic effect that did not bear statistical significance (Naef et al., 2003). Similar results were reported by Beaulieu (2006) in a study randomizing 44 patients to nabilone (a synthetic Δ^9-THC analog), ketoprofen, or placebo at 8-h dosing intervals, after undergoing a range of operative procedures. The patients receiving nabilone reported *significantly greater* pain scores at rest and during movement, when compared to ketoprofen or placebo. There was also no difference in rescue morphine consumption, quality of sleep, or side effects (Beaulieu, 2006). Similarly, in a cohort of 100 patients randomized to receive placebo or 5 mg drobanibol (Δ^9-THC analog) after radical prostatectomy, there was no analgesic effect, and no difference in the amount of morphine used as rescue analgesia by patients who received drobanibol (Seeling et al., 2006).

The use of morphine in combination with cannabinols has shown more promising results in the experimental literature but has not translated fully into human trials. Admittedly, this deserves further study to determine the effect of synergism. If there is a role for the use of cannabis as an adjunct to morphine in postoperative pain, this would allow us to reduce the dosage of morphine and the attendant risk of respiratory depression. Additionally, cannabinoid receptors are found in low concentration in the brainstem centers that modulate respiratory drive, hence they do not cause respiratory depression. Furthermore, decreased dosing of cannabinoids will produce decreased undesirable CNS effects associated with high dose cannabinoid use.

ANALGESIC REQUIREMENTS IN THE CHRONIC CANNABIS USER, POSTOPERATIVELY

The National Survey on Drug Use and Health of 2012 reported that there were about 6600 new cannabis users per day. Cumulatively, there were 2.4 million new users of cannabis, in the year of 2012 (US Department of Health and Human Services, 2012). The World Health Organization describes cannabis as the most widely used illicit drug, currently being used by 2.5% of the global population. They estimate that cannabis use is 12 times more prevalent than cocaine and heroin (World Health Organization, 2016). The exceedingly high prevalence of cannabis use has even sparked a significant political debate advocating global legalization of medicinal and recreational usage (Spurgeon, 2001; Morris et al., 2014; Martínez-Rodríguez et al., 2008; Sevigny et al., 2014). With the escalating prevalence of cannabis use, more patients who are chronic cannabis users will be encountered in surgical practice. Therefore, the effects of opioid analgesia on acute pain in the chronic cannabis user must be explored.

Gofeld, Robinson, and Faclier (2006) reported on a case in which a chronic marijuana user experienced intractable postoperative pain despite adequate multimodal therapy. They resorted to the use of nabilone, a synthetic cannabinoid, for supplemental analgesia with good effect. There was a dramatic reduction in visual pain scores, and a decrease in morphine consumption (Gofeld et al., 2006). Jefferson et al. (2013) then carried out a prospective randomized blinded trial in 73 patients undergoing orthopedic operations. They compared postoperative pain control in cannabis users and cannabis naive patients. Cannabis users were found to have a significantly greater mean pain intensity difference in the first postoperative hour, and a significantly greater demand for rescue morphine (Jefferson et al., 2013).

The available data suggest that chronic cannabis users have reduced pain thresholds. Several theories have been proposed to explain this effect, and focus predominantly on three mechanisms: neuroadaptation, cross-tolerance, and withdrawal. Long-term cannabis use in animal studies produces drug tolerance with attenuated cannabinoid-mediated effects (Sulipta, Einstein, Neely, Moise, & Hohmann, 2008; Gonzales, Cebiera, & Fernandez-Ruiz, 2005; Hoffman, Oz, Caulder, & Lupica, 2003). This is believed to be due to a combination of CB1 downregulation (Hirvonen et al., 2010; Villares, 2007), attenuation of G-protein activation (Jefferson et al., 2013), and impaired mRNA second messenger effects (Villares, 2007; Sim-Selley, 2003). The degree of tolerance can be erratic, varying with time (Manning, 1976), individuals (Jefferson et al., 2013), behavior-specific responses (Whitlow et al., 2004), and the degree of tolerance (Ramaekers, Kauert, Theunissen, Toennes, & Moeller, 2009).

In light of the evidence to support interaction between the cannabinoid and opioid receptors, some have hypothesized that cross-tolerance also occurs, leading to neuroadaptation at opioid receptors (Garzón, de la Torre-Madrid, Rodríguez-Muñoz, Vicente-Sánchez, & Sánchez-Blázquez, 2009). This would lead to the observed effect of resistance to opioids, and has also been theorized to produce similar forms of resistance to barbiturates, benzodiazepines, and phenothiazines (Ashton, 1999).

Finally, some have theorized that chronic users develop cannabis dependence. This cannabis withdrawal syndrome may play a role in the observed hyperalgesia that is seen in chronic cannabis users (Jefferson et al., 2013).

CONCLUSIONS

Further research is needed before cannabis can be used as a part of multimodal therapy for postoperative analgesia. There is more experimental evidence for its use as an adjunct to morphine for postoperative analgesia, rather than for use on its own. There are increased analgesic requirements in chronic cannabis users but further work is needed to elucidate the resistance mechanisms.

While experimental research continues to pave the way in knowledge of cannabinoids, animal models have not translated into clinical trials. Without larger, high quality human trials, the use of cannabinoids will continue to be cautious and limited. At this time, based on the current evidence, it would not be harmful to observe the pain requirement of cannabis users more closely during the postoperative period.

MINI-DICTIONARY

Analgesic A medication that attenuates the sensation of pain.
Cannabinoid Chemical compounds with the ability to bind to CB1 or CB2.
Hyperalgesia An exaggerated sensation of pain, out of keeping with that expected in response to the noxious stimulus applied.
Levonotradol A synthetic cannabinoid.
Nabilone A synthetic cannabinoid.
Nociception The processes by which sensory neurons encode and transmit the presence of a harmful stimulus, resulting in the sensation of pain.
Nociceptor Sensory receptor that receives and transmits information indicating the presence of noxious stimuli.
Psychotropic A chemical that acts on the central nervous system to alter mood, sensorium, or level of consciousness.

References

Alkaitis, M. S., Solorzano, C., Landry, R. P., Piomelli, D., DeLeo, J. A., & Romero-Sandoval, E. A. (2010). Evidence for a role of endocannabinoids, astrocytes and p38 phosphorylation in the resolution of postoperative pain. *PLoS One*, *5*(5), e10891.

Apfelbaum, J. L., Chen, C., Mehta, S. S., & Gan, T. J. (2003). Postoperative pain experience: results from a national survey suggest postoperative pain continues to be undermanaged. *Anesthesia and Analgesia*, *97*(2), 534–540.

Ashton, C. H. (1999). Adverse effects of cannabis and cannabinoids. *British Journal of Anaesthesia*, *83*(4), 637–649.

Beaulieu, P. (2006). Effects of nabilone, a synthetic cannabinoid, on postoperative pain. *Canadian Journal of Anaesthesia—Journal Canadien D'anesthésie*, *53*(8), 769–775.

Borgelt, L. M., Franson, K. L., Nussbaum, A. M., & Wang, G. S. (2013). The pharmacologic and clinical effects of medical cannabis. *Pharmacotherapy: Journal of Human Pharmacology and Drug Therapy*, *33*(2), 195–209.

Brennan, T. J. (2011). Pathophysiology of postoperative pain. *Pain*, *152*(3S), 33–40.

Buggy, D. J., Toogood, L., Maric, S., Sharpe, P., Lambert, D. G., & Rowbotham, D. J. (2003). Lack of analgesic efficacy of oral δ-9-tetrahydrocannabinol in postoperative pain. *Pain*, *106*(1–2), 169–172.

Cichewicz, D. L., & McCarthy, E. A. (2003). Antinociceptive synergy between delta(9)-tetrahydrocannabinol and opioids after oral administration. *Journal of Pharmacology and Experimental Therapeutics*, *304*(3), 1010–1015.

Costa, B., Colleoni, M., Conti, S., Parolaro, D., Franke, C., Trovato, A. E., & Giagnoni, G. (2004). Oral anti-inflammatory activity of cannabidiol, a non-psychoactive constituent of cannabis, in acute carrageenan-induced inflammation in the rat paw. *Naunyn-Schmiedebergs Archives of Pharmacology*, *369*(3), 294–299.

Dirks, J., Møiniche, S., Hilsted, K. L., & Dahl, J. B. (2002). Mechanisms of postoperative pain: clinical indications for a contribution of central neuronal sensitization. *Anesthesiology*, *97*(6), 1591–1596.

Elikkottil, J., Gupta, P., & Gupta, K. (2009). The analgesic potential of cannabinoids. *Journal of Opioid Management*, *5*(6), 341–357.

Garzón, J., de la Torre-Madrid, E., Rodríguez-Muñoz, M., Vicente-Sánchez, A., & Sánchez-Blázquez, P. (2009). Gz mediates the long-lasting desensitization of brain CB1 receptors and is essential for cross-tolerance with morphine. *Molecular Pain*, *5*, 11.

Gofeld, M., Robinson, C., & Faclier, G. (2006). Administration of nabilone for postoperative pain control in the marijuana-addicted: case study. *Acute Pain*, *8*, 29–32.

Gonzales, S., Cebeira, M., & Fernandez-Ruiz, J. (2005). Cannabinoid tolerance and dependence: a review of studies in laboratory animals. *Pharmacology Biochemistry and Behaviour*, *81*(2), 300–318.

Grape, S., & Tramèr, M. R. (2007). Do we need pre-emptive analgesia for the treatment of postoperative pain? *Best Practice and Research Clinical Anaesthesiology*, *21*(1), 51–63.

Guindon, J., & Hohmann, A. G. (2009). The endocannabinoid system and pain. *CNS and Neurological Disorders Drug Targets*, *8*(6), 403–421.

Hamilton, G. R., & Baskett, T. F. (2000). In the arms of Morpheus the development of morphine for postoperative pain relief. *Canadian Journal of Anaesthesia*, *47*(4), 367–374.

Hill, A. J., Williams, C. M., Whalley, B. J., & Stephens, G. J. (2012). Phytocannabinoids as novel therapeutic agents in CNS disorders. *Pharmacology and Therapeutics*, *133*(1), 79–97.

Hirvonen, J., Goodwin, R. S., Li, C. T., Morse, C., Zoghbi, S. S., Pike, V. W., & Innis, R. B. (2010). Chronic heavy cannabis use downregulates brain cannabinoid CB1 receptors in humans. *NeuroImage*, *52*(S1), 14.

Hoffman, A. F., Oz, M., Caulder, T., & Lupica, C. R. (2003). Functional tolerance and blockade of long-term depression at synapses in the nucleus accumbens after chronic cannabinoid exposure. *Journal of Neurosciences*, *23*(12), 4815–4820.

Holdcroft, A., Maze, M., Doré, C., Tebbs, S., & Thompson, S. (2006). A multicenter dose-escalation study of the analgesic and adverse effects of an oral cannabis extract (Cannador) for postoperative pain management. *Anesthesiology*, *104*(5), 1040–1046.

Ibrahim, M. M., Porreca, F., Lai, J., Albrecht, P. J., Rice, F. L., Khodorova, A., & Malan, T. P., Jr. (2005). CB2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(8), 3093–3098.

Jain, A. K., Ryan, J. R., McMahon, F. G., & Smith, G. (1981). Evaluation of intramuscular levonantradol and placebo in acute postoperative pain. *Journal of Clinical Pharmacology*, *21*(S89), 320S–326S.

Jefferson, D. A., Harding, H. E., Cawich, S. O., & Jackson-Gibson, A. (2013). Postoperative analgesia in the Jamaican cannabis user. *Journal of Psychoactive Drugs*, *45*(3), 227–232.

Karst, M., Wippermann, S., & Ahrens, J. (2010). Role of cannabinoids in the treatment of pain and (painful) spasticity. *Drugs*, *70*(18), 2409–2438.

Mackie, K. (2008). Cannabinoid receptors: where they are and what they do. *Journal of Neuroendocrinology*, *20*(S1), 10–14.

Manning, R. J. (1976). Chronic delta-9-tetrahydrocannabinol: transient and lasting effects on avoidance behavior. *Pharmacology Biochemistry and Behaviour*, *4*(1), 17–21.

Martínez-Rodríguez, J. E., Munteis, E., Carreño, M., Blanco, Y., Roquer, J., Abanades, S., & Saiz, A. (2008). Cannabis use in Spanish patients with multiple sclerosis: fulfilment of patients' expectations? *Journal of the Neurological Sciences*, *273*(12), 103–107.

Morris, R. G., TenEyck, M., Barnes, J. C., & Kovandzic, T. V. (2014). The effect of medical marijuana laws on crime: evidence from State Panel data: 1990–2006. *PLoS One*, *9*(3), e92816.

Myles, P. S., & Power, I. (2007). Clinical update: postoperative analgesia. *Lancet*, *369*(9564), 810–812.

Naef, M., Curatolo, M., Petersen-Felix, S., Arendt-Nielsen, L., Zbinden, A., & Brenneisen, R. (2003). The analgesic effect of oral delta-9-tetrahydrocannabinol (THC), morphine, and a THC-morphine combination in healthy subjects under experimental pain conditions. *Pain*, *105*(1–2), 79–88.

Pogatzki, E. M., Gebhart, G. F., & Brennan, T. J. (2002). Characterization of Adelta- and C-fibers innervating the plantar rat hindpaw one day after an incision. *Journal of Neurophysiology*, *87*(2), 721–731.

Pugh, G., Jr., Smith, P. B., Dombrowski, D. S., & Welch, S. P. (1996). The role of endogenous opioids in enhancing the antinociception produced by the combination of delta 9-tetrahydrocannabinol and morphine in the spinal cord. *Journal of Pharmacology and Experimental Therapeutics*, *279*(2), 608–616.

Ramaekers, J. G., Kauert, G., Theunissen, E. L., Toennes, S. W., & Moeller, M. R. (2009). Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. *Journal of Psychopharmacology*, *23*(3), 266–277.

Roykulcharoen, V., & Good, M. (2004). Systematic relaxation to relieve postoperative pain. *Journal of Advanced Nursing*, *48*(2), 140–148.

Schug, S. A., & Manopas, A. (2007). Update on the role of non-opioids for postoperative pain treatment. *Best Practice & Research Clinical Anaesthesiology*, *21*(1), 15–30.

Seeling, W., Kneer, L., Büchele, B., Gschwend, J. E., Maier, L., Nett, C., & Rockemann, M. (2006). Delta (9)-tetrahydrocannabinol and the opioid receptor agonist piritramide do not act synergistically in postoperative pain. *Der Anaesthesist*, *55*(4), 391–400.

Sevigny, E. L., Pacula, R. L., & Heaton, P. (2014). The effects of medical marijuana laws on potency. *International Journal on Drug Policy*, *25*(2), 308–319.

Sim-Selley, L. J. (2003). Regulation of cannabinoid CB1 receptors in the central nervous system by chronic cannabinoids. *Critical Review of Neurobiology*, *15*, 91–119.

Smith, F. L., Cichewicz, D., Martin, Z. L., & Welch, S. P. (1998). The enhancement of morphine antinociception in mice by delta9-tetrahydrocannabinol. *Pharmacology, Biochemistry, and Behavior*, *60*(2), 559–566.

Spurgeon, D. (2001). Canadian doctors brace themselves as cannabis becomes legal. *BMJ*, *323*(7308), 302.

Sulipta, R. L., Einstein, S. A., Neely, M. H., Moise, A. M., & Hohmann, A. G. (2008). Cross-sensitization and cross-tolerance between exogenous cannabinoid anti-nociception and endocannabinoid-mediated stress-induced analgesia. *Neurophamacology*, *54*(1), 161–171.

Taylor, A., & Stanbury, L. (2009). A review of postoperative pain management and the challenges. *Current Anaesthesia and Critical Care*, *20*(4), 188–194.

US Department of Health and Human Services. (2012). *Results from the 2012 National Survey on Drug Use and Health: Summary of National Findings*. Washington, DC: US Department of Health and Human Services.

Villares, J. (2007). Chronic use of marijuana decreases cannabinoid receptor binding and mRNA expression in the human brain. *Neuroscience*, *145*(1), 323–334.

Whitlow, C. T., Liguori, A., Livengood, L. B., Hart, S. L., Mussat-Whitlow, B. J., Lamborn, C. M., Laurienti, P. J., & Porrino, L. J. (2004). Long-term heavy marijuana users make costly decisions on a gambling task. *Drug and Alcohol Dependency*, *76*(1), 107–111.

World Health Organization. (2016). Epidemiology of cannabis use, disorders and treatment. In W. Hall, M. Renström, & V. Poznyak (Eds.), *The Health and Social Effects of Non-Medical Cannabis Use* (pp. 9–12). Geneva, Switzerland: WHO Press (Chapter 3).

Wu, C. L., & Raja, S. N. (2011). Treatment of acute postoperative pain. *Lancet*, *377*(9784), 2215–2225.

Zuardi, A. W. (2006). History of cannabis as a medicine: a review. *Revista Brasileira de Psiquiatria*, *28*(2), 153–157.

CHAPTER

e3

Aggressive Behavior and Cannabis Use

W. Liu, H. Petras***

***NORC at the University of Chicago, Bethesda, MD, United States**
****American Institutes for Research, Washington, DC, United States**

SUMMARY POINTS

- Substance use (eg, cannabis use) and aggressive and disruptive behavior (ADB) are considered to be major public health problems around the world.
- In middle school, cannabis use and ADB tend to cooccur, but unbiased information about their causal relationship is not available.
- Knowledge about whether ADB causes cannabis use or vice versa is relevant for the design and implementation of interventions targeting such behaviors.
- ADB during the elementary school years has been hypothesized as an antecedent for cannabis use, but a causal relationship has not been established yet.
- During the middle and high school years, both ADB causing cannabis use and vice versa are plausible, and a causal relationship has not been established yet.
- Uncertainty about the relationship between these behaviors is likely due to the large variety in study designs, and this ultimately hinders the identification of typical pathways, as well as documenting the variation across individuals.

KEY FACTS OF AGGRESSION

- Aggressive, disruptive behavior (ADB) is considered to be normal, and highly frequent in young children.
- Approximately 50% of social exchanges between children 12–18 months of age can be considered ADB.
- ADB tends to decrease significantly during late childhood and adolescence.
- For a small number of children, ADB does not decrease, or starts to reemerge in later childhood.
- Persistent ADB is one of the strongest risk factors for negative outcomes later in life, including substance use, juvenile delinquency, school dropout, and adult criminality.

LIST OF ABBREVIATIONS

ADB Aggressive and disruptive behavior
PBT Problem Behavior Theory

INTRODUCTION

The prevention and treatment of both substance use and aggressive, disruptive behavior (ADB) are national priorities in countries around the world. Good epidemiological evidence exists suggesting that both behaviors lead to significant proximal (eg, truancy and suspension), as well as distal (eg, school dropout and criminality), negative social and health outcomes (Petras et al., 2008; Petras, Masyn, & Ialongo, 2011).

Substance use, especially cannabis use, has been often found to have a positive link with aggressive behavior, found in cross-sectional and longitudinal studies (Goodman, 2010; Jester et al., 2008). Liu, Lynne-Landsman, Petras, Masyn, and Ialongo (2013) found that early childhood aggressive behavior (grade 1–3) doubles the odds of high-frequency marijuana use in adolescence (grade 8–12). Petras et al. (2011) found that early childhood aggressive behavior during grade 1–3 increased

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00029-6
Copyright © 2017 Elsevier Inc. All rights reserved.

the odds of conduct problems during grade 6–12 by eightfold. The common cooccurrence of cannabis and aggressive behavior in adolescence is often explained by the Problem Behavior Theory (PBT; Jessor, 1991). PBT proposes that many problem behaviors, including substance use, conduct problems, and other antisocial behaviors, are interrelated in that the personal and situational factors influencing one behavior may be the same as those influencing another.

Huizinga, Loeber, Thornberry, and Cothern (2000) assessed the level of cooccurrence of multiple problem behaviors in three large longitudinal studies, including the Denver Youth Survey (PI: David Huizinga), the Pittsburgh Youth Study (PI: Rolf Loeber), and the Rochester Youth Development Study (PI: Terence Thornberry). It was found that 34–44% of male delinquents were also drug users, and between 54% and 70% of male drug users were also delinquents. While these relationships are statistically significant, it cannot be assumed that most delinquents are also drug users, and causal inferences are not possible insofar as a causal relationship indicates that changes in one variable cause changes in another (Rosenbaum, 2002).

This raises questions about the causal basis for this association, in particular during adolescence. For example, if the association between early childhood aggression and later cannabis use is noncausal, then harm reduction and substance use prevention/intervention programs in adolescence are expected to be effective. On the other hand, evidence for causality would make cannabis use a hidden, but preventable, public health problem responsible for many psychosocial problems.

This book chapter represents an intermediate step toward the goal of assessing the causality, by conceptually summarizing the current literature focusing on the cooccurrence of these two behaviors during childhood and adolescence. The chapter is structured as follows: building upon the descriptive epidemiology of cannabis use and ADB, we will review theoretical models, as well as the extent of empirical support for the different possible hypothesized associations between the two behaviors.

Descriptive Epidemiology of Cannabis Use

Cannabis is the most widely cultivated, trafficked, and abused illicit drug worldwide. It is estimated that 147 million people, or 2.5% of the world population, consume cannabis. This annual prevalence is over 10 times higher than the prevalence for cocaine or opiates. The most rapid abuse starting in the 1960s has occurred in developed countries, including North America, Western Europe, and Australia.

Initiation and experimentation with cannabis typically begin during late adolescence (Fuller et al., 2005). The hazard of first use follows a typical age-curve characterized by an increase in risk at age 10–11, and peaking around age 18 (Wagner & Anthony, 2002). Recent data from the Monitoring the Future study (Johnston, O'Malley, Bachman, Schulenberg, & Miech, 2014) revealed that, between 2008 and 2013, past-month use increased from 5.8% to 7.0% among 8th graders, from 13.8% to 18.0% among 10th graders, and from 19.4% to 22.9% among 12th graders. The 2011 ESPAD Report (Hibell et al., 2011) reported that the prevalence among 15- and 16-year-old school students was 13% in Europe, with the highest prevalence found in France at 35%.

Descriptive Epidemiology of Aggressive/Disruptive Behavior

ADB (eg, lying and disobeying) is considered to be normal, and highly frequent in young children. Approximately 50% of social exchanges between children 12–18 months of age can be considered ADB (Holmberg, 1980). During this age period, ADB is seen as adaptive, and part of the infants' training ground to develop effective social strategies for assertiveness, ownership of objects, and resolution of social conflict. For the majority of children, ADB tends to decrease during childhood and adolescence, due to increases in self-control, hormonal regulation, and cognitive capacity (Tremblay, 2006).

However, for a small but significant number of children, ADB does not decrease, or starts to reemerge in later childhood, as discussed in two prominent theoretical models. The model by Patterson, DeBaryshe, and Ramsey (1989) features two distinct pathways toward antisocial behavior: *early starters* (ie, involving coercive parenting, school failure, and antisocial behavior starting in childhood), and *late starters* (ie, involving poor parental monitoring, oppositionality, and deviant peer involvement starting in early adolescence). The model by Moffitt (1993) also proposes two mutually exclusive subgroups of antisocial youth: *life-course persistent offenders*, who show high levels of aggressive behavior throughout development, and continue to be violent as adults; and *adolescence-limited offenders*, who engage in nonviolent forms of antisocial behavior only during the teen years.

THEORIES AND EMPIRICAL SUPPORT

Building on the descriptive epidemiology of aggressive behavior and cannabis use, we reviewed theories and empirical support of cannabis use-aggressive behavior relationship. Our review was conceptually motivated by a model depicting three developmental periods: preelementary, elementary, and middle school (Fig. e3.1).

During the preelementary school period, the focus is on delineating etiological factors of ADB, namely family factors and individual vulnerabilities. During the

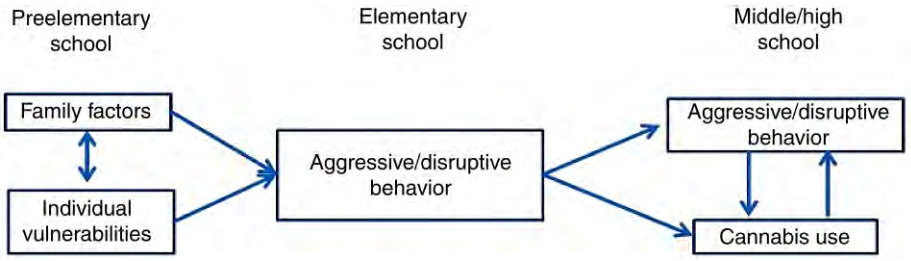


FIGURE e3.1 **Risk factors for cannabis use during three developmental periods.** Risk factors for cannabis use in three developmental periods are depicted in this figure. *Arrows* are not meant to indicate causal pathways. During the preelementary school years, family factors interacting with individual vulnerabilities are hypothesized to influence levels of aggressive behavior during the elementary school years. These levels, in turn, may influence cannabis use directly or indirectly through increasing levels of aggressive and disruptive behavior during the middle and high school years. In addition, it is hypothesized that both cannabis use and levels of aggressive and disruptive behaviors during this period may influence each other.

elementary school period, the focus is on ADB as a risk factor, for both later aggressive behavior, as well as cannabis use. Lastly, during the middle-school period, we focus on the cooccurrence between the two behaviors of interest.

In the following sections, we will discuss three likely pathways of the relationship between cannabis use and aggressive behavior, in elementary school, as well as in middle and high school, respectively: ADB in elementary school leading to later cannabis use (see section "ADB in Elementary School Leading to Later Cannabis Use"); ADB leading to cannabis use in middle and high school (see section "ADB Leading to Cannabis Use in Middle and High school"); cannabis use leading to ADB in middle and high school (see section "Cannabis Use Leading to ADB in Middle and High School").

ADB in Elementary School Leading to Later Cannabis Use

Theory

Life course–social field theory (Kellam, Branch, Agrawal, & Ensminger, 1975) and its integration with the developmental model of antisocial behavior (Patterson et al., 1989) have been used to explain the relationship between early ADB and cannabis use. Life course–social field theory posits that, over the course of development, individuals are evaluated on how they respond to different task demands in various social fields (eg, the family, the classroom, etc.). At the heart of this theory is the tenet that early success in meeting these demands promotes success in later stages of life, as individuals face new challenges (Cicchetti & Schneider-Rosen, 1984).

According to life course–social field theory (Kellam et al., 1975), when first transitioning into formal education, children are confronted with teachers' demands for following classroom rules, and participating in classroom activities. Successfully meeting these demands will promote the successful transition into early adolescence, a period characterized by a different set of demands defined by family, school, and peers. In contrast, engagement in ADB in early elementary school may indicate the failure to meet classroom demands, and eventually lead to negative behavior and consequences in adolescence, such as, engaging in antisocial behavior, and frequent use of illicit drugs.

The origins of early classroom ADB are explained by referring to Patterson's developmental model of antisocial behavior (Patterson et al., 1989). The early starter model by Patterson et al. (1989) posits that aggressive children are more likely to provoke parents' and teachers' punitive discipline, leading to their rejection, which can facilitate children's drift into deviant peer groups, where a range of antisocial behaviors, including drug use and sustained levels of ADB, are reinforced, and further opportunities to engage in these behaviors may be provided.

Peer rejection is another common correlate of chronic aggressive behavior problems (Hektner, August, & Realmuto, 2000) and, in accord with Patterson, Reid, and Dishion (1992), may serve to hasten the aggressive and disruptive child's drift into a deviant peer group in late childhood and early adolescence. In these deviant peer groups, ADB is reinforced, thereby canalizing the pathway to later antisocial behavior, for example, by providing access and opportunities to use illicit drugs, such as, cannabis.

Empirical Support

Large prospective longitudinal studies with sufficiently long follow-ups have been utilized to discover this relationship (Table e3.1). Together, these studies have found a positive relationship between childhood ADB and later cannabis use, that is, increases in aggressive behavior in childhood are longitudinally associated with early onset and/or frequency of cannabis use. Using longitudinal data collected from a community sample consisting predominantly of inner-city African-American youths, a group of scholars have examined the relationship between aggressive behavior, measured as

TABLE e3.1 Empirical Studies Supporting ADB in Elementary School Leading to Later Cannabis Use

Author (year)	Location	Sample
Liu et al. (2013)	Baltimore, MD, United States	A sample of 678 urban first-graders from 27 classrooms, in nine elementary schools, who participated in the JHU PIRC's Second-Generation Intervention Trial
Lynne-Landsman, Bradshaw, and Ialongo (2010)	Baltimore, MD, United States	A sample of 678 urban first-graders from 27 classrooms, in nine elementary schools, who participated in the JHU PIRC's Second-Generation Intervention Trial
Reboussin, Hubbard, and Ialongo (2007)	Baltimore, MD, United States	A sample of 678 urban first-graders from 27 classrooms, in nine elementary schools, who participated in the JHU PIRC's Second-Generation Intervention Trial
Kim, Catalano, Haggerty, and Abbott (2011)	Seattle, WA, United States	A longitudinal community sample of 957 young people from the Raising Healthy Children project
Schofield, Bierman, Heinrichs, and Nix (2008)	Durham, NC; Nashville, TN; Seattle, WA; rural central Pennsylvania (United States)	A longitudinal sample of 694 boys and girls from four geographical locations was utilized, with data collected from kindergarten through high school, from Fast Track project
Wu, Witkiewitz, McMahon, and Dodge (2010)	Durham, NC; Nashville, TN; Seattle, WA; Pennsylvania (United States)	A sample of 446 high-risk control group who participated in the follow-up study from the Fast Track project
Korhonen et al. (2010)	The Netherlands	Dutch adolescents (N = 1606; 854 girls and 752 boys) from the Tracking Adolescents' Individual Lives Survey (TRAILS) study
Creemers et al. (2009)	The Netherlands	Data from 81% (n = 1804) of the participants (51.9% girls) of the TRAILS study, a prospective general population study, were analyzed
Jester et al. (2008)	Michigan, IL, United States	A sample of 335 children of alcoholic and nonalcoholic fathers collected behavioral ratings by parents and teachers of aggression and inattention/hyperactivity across ages 7–16
Fergusson, Boden, and Horwood (2008)	New Zealand	A 25-year prospective longitudinal study of the health, development, and adjustment of a birth cohort of 1265 New Zealand children
Galera et al. (2010)	France	A sample of 1107 participants (aged 4–18 years at baseline) recruited from the population-based longitudinal Gazel Youth study with a follow-up assessment 8 years later
Flory, Milich, Lynam, Leukefeld, and Clayton (2003)	Metropolitan area, United States	A sample of 481 young adults with 10–12 years follow-up
Ensminger, Juon, and Fothergill (2002)	Chicago, IL, United States	An inner-city community sample from a Mid-West city followed from age 6 to 32 (N = 1242)
Pedersen, Mastekaasa, and Wichstrom (2001)	Norway	A prospective longitudinal study of a national sample of 2436 adolescents followed up over a year and a half, when the adolescents were in their early teens
Prinstein and La Greca (2004)	Metropolitan area, United States	Participants were 148 girls from diverse ethnic backgrounds, who were initially assessed in Grades 4–6 and again in Grades 10–12

early as in first grade of elementary school, on adolescent substance use. Liu et al. (2013) reported that trajectories of ADB measured at grades 1–3 are significantly associated with frequency of cannabis use in grades 8–12. A similar finding was also documented in Lynne-Landsman et al. (2010), using the same dataset. In addition, Reboussin et al. (2007) also found that aggressive behavior in first grade was associated with an increased risk of cannabis exposure opportunities, as well as cannabis use and problems in middle school.

Using data from the Raising Health Children (RHC) study, Kim et al. (2011) found that self-reported bullying behavior at grade 5 is significantly associated with increased cannabis use at age 21. Analyzing a longitudinal sample of youths followed from kindergarten to high school, Schofield et al. (2008) found that, regardless of gender and race, high rates of ADB at school entry increased risk of substance use, including cannabis use, in middle school.

Using a community sample of children at high risk for conduct disorder, Wu et al. (2010) found that higher levels of childhood conduct problems, including aggressive behavior, in kindergarten predicted higher levels of cannabis use in adolescence.

Korhonen et al. (2010) analyzed data collected from Dutch adolescents as part of the Tracking Adolescents' Individual Survey (TRAILS), and reported that childhood externalizing behavior (including aggressive behavior) measured at age 10–12 predicts the initiation and use of cannabis at age 12–18, both directly and indirectly, through the initiation of cigarettes smoking. Using the same data set, Creemers et al. (2009) found that childhood/early adolescent conduct problems at ages 10–12 predicted early onset of cannabis use in adolescence, at ages 12–14.

Similarly, Jester et al. (2008) found that aggression trajectories in childhood measured between the ages of 7 and 16 predict early onset of cannabis use. Analyzing a 25-year prospective longitudinal study of a New Zealand birth cohort, Fergusson et al. (2008) reported that childhood and early adolescent conduct problems, including aggressive behavior, were significantly associated with illicit drug abuse, including cannabis abuse and dependence, measured at ages 16–25.

Studies also found that, when coupled with other behavioral problems, aggressive behavior is a stronger predictor of cannabis use and abuse/dependence. Using Gazel Youth study, a population-based longitudinal sample, Galera et al. (2010) found that conduct disorder combined with hyperactivity-inattention in childhood predicts early initiation of cannabis use in males.

Using a community sample of youths from multiple schools, Flory et al. (2003) found that disruptive behavior at grade 6 significantly contributed to later cannabis use and dependence in young adulthood, ages 19–22, while this effect on cannabis dependence is stronger, when Hyperactivity–Impulsivity–Inattention is presented, in addition to disruptive behavior. Similarly, using an inner-city community sample from a Mid-West city, followed from age 6 to 32, Ensminger et al. (2002) examined childhood antecedents of cannabis use in adulthood. They found that males who were both shy and aggressive in first grade were more likely to use cannabis in adulthood than those who were neither.

Some studies have specifically examined gender differences in the aggressive behavior–cannabis use relationship. Taken together, these studies suggest that the effect of aggressive behavior on cannabis use may differ by gender, consistent with the general hypothesis that risk factors of cannabis use may be gender-specific (van den Bree & Pickworth, 2005). Using a prospective longitudinal study of a national sample of adolescents in their early teens in Norway, Pedersen et al. (2001) found a strong association between early conduct problems and subsequent early initiation of cannabis use, with strong effect found for girls than for boys. Liu et al. (2013) reported that ADB measured at grades 1–3 is significantly associated with frequency of cannabis use among males in grades 8–12, but not females.

Other studies found that ADB only leads to cannabis use in certain circumstances. For example, Prinstein and La Greca (2004) analyzed a sample of girls assessed in grades 4–6, and then again in grades 10–12. They reported an interaction between aggressive behavior and peer rejection, whereby aggressive behavior was significantly associated with adolescent cannabis use only among those experiencing peer rejection. When girls were accepted by peers, the association between childhood aggression and adolescent cannabis use did not hold.

ADB Leading to Cannabis Use in Middle and High School

Theory

It has been argued that, for adolescents who engage in both conduct disorder and substance use, conduct disorder typically precedes the initiation of substance use (Le Blanc & Loeber, 1998). Khantzian (1985) proposed a "self-medication hypothesis," whereby individuals with mental health problems, including aggression and other externalizing behavioral problems, tend to resort to substance use as a "solution" to their problems. The short-term effects of alcohol and illicit drugs help these individuals to cope with stressful states experienced as a result of their mental health issues. More recently, Khantzian (2003) posits that cannabis has both stimulating (ie, boosting energy and calming hyperactivity) and sedatory (ie, calming intense rage and creating the illusion of relief) effects.

TABLE e3.2 Empirical Studies Supporting ADB Leading to Cannabis Use in Middle and High School

Author (year)	Location	Sample
King, Iacono, and McGue (2004)	Minnesota, United States	A total of 699 twin girls and 665 twin boys from Minnesota Twin Family Study interviewed at age 11 and age 14
Goodman (2010)	United Kingdom	A sample of 3,607 British national cohort (age 11–16 at baseline)
Griffith-Lendering, Huijbregts, Mooijaart, Vollebergh, and Swaab (2011)	The Netherlands	Data were used from the TRAILS study, a longitudinal cohort study of (pre)adolescents (n = 1,449), with measurements at age 11.1 (T1), age 13.6 (T2) and age 16.3 (T3)
van den Bree and Pickworth (2005)	United States	Data were from Add Health study collected over two time points with a sample of middle and high school students (N = 13,718, aged 11–21 years)
Paradise and Cauce (2003)	Seattle, WA, United States	Using a sample of 200 adolescents (age 13–18) from Seattle Homeless Adolescent Research Project (SHARP)
Hayatbakhsh, Najman, Bor, O'Callaghan, and Williams (2009)	Australia	A longitudinal cohort of 2,493 young adults from Mater Hospital and University of Queensland Study of Pregnancy
Doherty, Green, and Ensminger (2008)	Chicago, IL, United States	A community sample of 1,242 African American youths followed from first grade to age 42
Skara et al. (2008)	Los Angeles and Ventura, CA, United States	Self-reported data were gathered from 2,064 high school students at pretest and 1-year post-test

Empirical Support

A large body or empirical studies have found that adolescent ADB is likely to lead to later substance use (Table e3.2). Using a longitudinal community sample of twins (at age 11 and age 14), King et al. (2004) found that externalizing behavior measured at age 11 (including aggressive behavior) predicts onset and regular use of cannabis use by age 14.

Goodman (2010) examined the longitudinal associations in both directions between externalizing behavior and cannabis use in adolescence, using a British national cohort (age 11–16 at baseline). It was found that externalizing behavior problems (including aggressive behavior) measured at baseline independently predicted cannabis use 3 years later, with a dose–response relationship. No evidence was found that baseline cannabis use predicted externalizing behavior at follow-up.

Using data from the longitudinal TRAILS study, Griffith-Lendering et al. (2011) found that externalizing behavior at age 11 and 13 predicted cannabis use at 13 and 16, respectively. van den Bree and Pickworth (2005) examined the risk factors of five stages of cannabis involvement, including "initiation of experimental use," "initiation of regular use," "progression to regular use," "failure to discontinue experimental use," and "failure to discontinue regular use" among middle and high school students, as part of the Add Health study. They reported that delinquency was one of the strong predictors of all stages, 1 year later.

Using a sample of adolescents (age 13–18) from Seattle Homeless Adolescent Research Project (SHARP), Paradise and Cauce (2003) found that delinquent behavior is associated with changes in frequency of cannabis use, measured 3 and 6 months later. Using data from Mater Hospital and the University of Queensland Study of Pregnancy, a birth cohort study in Australia, Hayatbakhsh et al. (2009) found that adolescent aggressive behavior/delinquency measured at age 14 were strongly associated with cannabis use and disorder in young adulthood. Using a community sample of African-American youths, Doherty et al. (2008) found that serious adolescent delinquency has at least some causal influence on cannabis use initiation through mid-life (age 42).

Some mixed findings were revealed when separate types of aggression were examined. Using self-reported data gathered from high school students, Skara et al. (2008) found that relational aggression at baseline was a strong predictor for cannabis use 1 year later for females, but not for males. However, physical aggression at baseline does not predict cannabis use, either for males or females.

Cannabis Use Leading to ADB in Middle and High School

Theory

Despite the abundant literature supporting the "self-medication" hypothesis, which posits that adolescent

aggressive behavior leads to cannabis use, some studies have pointed out that cannabis use may influence levels of aggressive behavior. It has been proposed that cannabis use can damage the individual's cognitive capacity to process complex stimuli in conflict and, in turn, decreases the ability to constrain oneself from inappropriate responses, and increase premature responses (Moore & Stuart, 2005).

Importantly, different mechanisms were proposed for regular versus novice users. For regular users, it is possible that cannabis intoxication may trigger maladjusted individuals, with previous aggressive behavior and violence history, to behave violently (Levinthal, 2008). It has also been argued that for those who develop cannabis dependence, cannabis withdrawal and abstinence may also increase anger and irritability (Moore & Stuart, 2005; Ostrowsky, 2011). The link between cannabis use and violent behavior for more novice users can be explained by panic reactions and paranoid feelings, in combination with a sense of losing control, which is more commonly experienced by novice users (Moore & Stuart, 2005).

Above and beyond the above short-term effects of cannabis use applicable to both adolescence and adulthood, another mechanism points to the long-term impact of cannabis use in adolescence. High levels of cannabis use in adolescence may interfere with the development of communication and social skills, and increase deviant peer associations, which may together lead to life-long persistent patterns of antisocial behavior manifesting aggressive behavior and violence.

Lastly, it has also been argued that cannabis use may decrease aggressive and violent behavior through mechanisms consistent with the "self-mediation hypothesis," which posits that individuals with mental health problems may seek to relive their problems through using cannabis. First, as cannabis has both stimulant and sedatory effects, under certain circumstances, users may experience calmness rather than increased energy, which typically does not lead to violent behavior (Pujazon-Zazik & Park, 2009). As a related mechanism, cannabis may also create a positive mood, providing the users with a sense of contentment and humor (Goode, 2008). A third reason explaining why cannabis may decrease violent behavior is that users self-reported to be calmer, more open-minded, and tolerant of others' opinions, over time, as a result of long-time leisure consumption cannabis (Hathaway, 1997; Tart, 1970). Fourth, users typically expect cannabis to relax them, and to give them a sense of pleasure and happiness, which increases the chance of them acting according to this expectation, and avoid the use of violence (Moore & Stuart, 2005).

Empirical Support

Empirical studies yield mixed results (see Table e3.3). Using a school-based sample of adolescents, Temple et al. (2013) found cannabis use in grade 10 predicted perpetration of physical dating violence 1 year later, for both males and females. Analyzing longitudinal data on Mexican and European-American adolescents, Brady et al. (2008) found that adolescents who used cannabis at age 15 were more likely to engage in violence perpetration at age 19.

A body of studies examines the relationship between adolescent cannabis use and dating violence, specifically. It was suggested that the association may differ for males and females. For example, using longitudinal data, Foshee et al. (2010) found that cannabis use predicted the onset of dating violence perpetration among girls, but not among boys. Examining a longitudinal sample of youths between grades 8 and 12, Reyes et al. (2014) also found that the effects of cannabis use on dating violence held only for violence perpetrated by girls, not boys.

There are also some mixed results in the literature. Using longitudinal self-reported survey on high school students, Ellickson and McGuigan (2000) reported that frequency of cannabis use in grade 7 was a significant predictor only for the amount of predatory violence in grade 12, not the likelihood of any predatory violence in grade 12. Using data from the Pittsburgh Youth Study, Wei et al. (2004) found the longitudinal relationship between frequent cannabis use and violence (and vice versa) in adolescence and early adulthood to be spurious, after controlling for common risk factors, such as, race, and hard drug use. They concluded that the observed cannabis–violence relationship is due to selection effects, in that both are influenced by shared risk factors.

Using data from the National Longitudinal Study of Adolescence Health, Melander et al. (2010) reported that cannabis use in adolescence was not related to perpetration of interpartner violence. A similar finding was reported by Marie et al. (2008) using a longitudinal study of a New Zealand birth cohort.

The majority of research that supports the claim that cannabis use is associated with decreases in aggressive and violent behavior have been conducted using adult samples, thus, beyond the scope of this study. We identified two studies using adolescent samples. Kaplan et al. (2001) found that cannabis use in early adolescence is inversely related to violent behavior measured 3 years later. The findings were used to support the theoretical explanation that the use of cannabis can assuage distressful feelings that initiate violence. In a four-wave study, White and Hansell (1998) found that heavy cannabis use in early to mid-adolescence predicted decreases in aggressive behavior in late adolescence and young adulthood.

CONCLUSIONS

This chapter examines the current evidence base supporting the cooccurrence hypothesis, and the two directional hypotheses about the relationship between

TABLE e3.3 Empirical Studies Supporting Cannabis Use Leading to ADB in Middle and High School

Author (year)	Location	Sample
Temple, Shorey, Fite, Stuart, and Le (2013)	Houston, TX, United States	A sample of 1042 9th and 10th grade high schools students were recruited and assessed in the spring of 2010, and 93% of the original sample completed the 1-year follow-up in the spring of 2011
Brady, Tschann, Pasch, Flores, and Ozer (2008)	CA, United States	Longitudinal data on a sample of 302 Mexican and European-American adolescents
Foshee, McNaughton Reyes, and Ennett (2010)	NC, United States	A sample of 1666 students in 8th–10th grade who reported ever dated
Reyes, Luz, Foshee, Bauer, and Ennett (2014)	NC, United States	A sample of 2455 students followed up from 6th grade
Ellickson and McGuigan (2000)	CA and OR, United States	A 5-year follow-up of 4300 high school seniors and dropouts
Wei, Loeber, and Whie (2004)	Pittsburg, PA, United States	A sample of 503 youths from the Pittsburgh Youth Study
Melander, Noel, and Tyler (2010)	United States	A sample of National Longitudinal Study of Adolescence Health
Marie, Fergusson, and Boden (2008)	New Zealand	A longitudinal study of over 1000 New Zealand birth cohort sample
Kaplan, Tolle, and Yoshida (2001)	Houston, TX, United States	Using panel data (N = 2222) from subjects tested during early adolescence and 3 years later
White and Hansell (1998)	Rutgers, NJ, United States	1380 subjects followed up at age 12, 15, and 18 from the Rutgers Health and Human Development Project

aggressive behavior and cannabis use. Any support for these hypotheses has the potential to guide public health-based preventive interventions by focusing on the underlying causes of ADB and cannabis use (ie, cooccurrence hypothesis), or by specifically targeting one of the behaviors to prevent the other one (ie, directional hypothesis). To this end, we inspected both the theories underlying these hypotheses, and the existing empirical literature supporting them.

Clear theoretical and empirical support exists to support the claim that early aggressive behavior (ie, during the elementary school years) is an antecedent not only of later ADB, but also of cannabis use. However, the claim for a causal relationship between ADB and cannabis use remains unsupported. In the case of a clear causal relationship, interventions aiming to reduce early aggressive behavior should lead to reductions in cannabis use. In the absence of a causal relationship, factors more proximal to the onset of cannabis use, such as, peer rejection, deviant peer affiliation, and neighborhood disadvantage may be more suitable intervention targets (Liu et al., 2013). A cascading model of accumulating risk during the major socialization phases may be a more appropriate model for capturing the dynamic relationship between ABD and cannabis use.

Similarly, inconsistent results were found for the interrelationship between the two behaviors during adolescence, and no clear support for the directionality could be found. In fact, there exists both theoretical and empirical evidence (albeit not causal) supporting the claim that ABD leads to cannabis use, or vice versa.

Together with Ostrowsky (2011), we can speculate that several study characteristics are plausible culprits for the inconsistent results. Due to a lack of a comprehensive but integrated substance use prevention theory, little guidance exists for researchers how to design their studies appropriately. Consequently, studies differ significantly with respect to measurement (eg, diagnosis vs self-report; minor aggressive behavior vs violence), developmental periods studied (eg, early adolescence and late adolescence), length of time between waves (eg, long-term and short-term relationship); as well as the selection of the population under study (eg, at-risk samples vs population sample).

These inconsistencies hinder the identification of typical pathways, and how they may differ by gender, race/ethnic groups, as well as in different cultures and locations, guided by different substance use policies. It is our hope that the current chapter can help stimulate better coordination of efforts in assessing the etiology and prevention of ADBs and cannabis, as well as other illicit substances.

DISCLAIMER

This study is not meant to be an exhaustive literature review, but it rather aims to describe the quite complex relationship between cannabis-aggressive behavior, and, by identifying important research gaps, this chapter may inspire future empirical research to respond to such challenges.

MINI-DICTIONARY

Aggressive, disruptive behavior A range of rule-breaking behavior during childhood and adolescence, in home and classroom settings.
Causality The relationship between cause and effect.
Conduct disorder Antisocial behavior displayed in childhood or adolescence.
Delinquency Criminal behavior committed by youth.
Prevention The action of stopping something from happening.

References

Brady, S. S., Tschann, J. M., Pasch, L. A., Flores, E., & Ozer, E. J. (2008). Violence involvement, substance use, and sexual activity among Mexican-American and European-American adolescents. *Journal of Adolescent Health*, *43*(3), 285–295.

Cicchetti, D., & Schneider-Rosen, K. (1984). Toward a transactional model of childhood depression. In D. Cicchetti, & K. Schneider-Rosen (Eds.), *Childhood depression* (pp. 5–28). San Francisco, CA: Jossey-Bass, Inc. Publishers.

Creemers, H. E., van Lier, P. A., Vollebergh, W. A., Ormel, J., Verhulst, F. C., & Huizink, A. C. (2009). Predicting onset of cannabis use in early adolescence: the interrelation between high-intensity pleasure and disruptive behavior. The TRAILS Study. *Journal of Studies on Alcohol and Drugs*, *70*(6), 850–858.

Doherty, E. E., Green, K. M., & Ensminger, M. E. (2008). Investigating the long-term influence of adolescent delinquency on drug use initiation. *Drug and Alcohol Dependence*, *93*(1–2), 72–84.

Ellickson, P. L., & McGuigan, K. A. (2000). Early predictors of adolescent violence. *American Journal of Public Health*, *90*(4), 566–572.

Ensminger, M. E., Juon, H. S., & Fothergill, K. E. (2002). Childhood and adolescent antecedents of substance use in adulthood. *Addiction*, *97*(7), 833–844.

Fergusson, D. M., Boden, J. M., & Horwood, L. J. (2008). The developmental antecedents of illicit drug use: evidence from a 25-year longitudinal study. *Drug and Alcohol Dependence*, *96*(1–2), 165–177.

Flory, K., Milich, R., Lynam, D. R., Leukefeld, C., & Clayton, R. (2003). Relation between childhood disruptive behavior disorders and substance use and dependence symptoms in young adulthood: individuals with symptoms of attention-deficit/hyperactivity disorder and conduct disorder are uniquely at risk. *Psychology of Addictive Behaviors*, *17*(2), 151–158.

Foshee, V. A., McNaughton Reyes, H. L., & Ennett, S. T. (2010). Examination of sex and race differences in longitudinal predictors of the initiation of adolescent dating violence perpetration. *Journal of Aggression Maltreatment and Trauma*, *19*(5), 492–516.

Fuller, E., Bates, B., Blenkinsop, S., Boreham, R., Hills, A., McGee, A., et al. (2005). *Smoking, drinking and drug use among young people in England in 2004*. Leeds, UK: Health and Social Care Information Centre.

Galera, C., Bouvard, M. P., Melchior, M., Chastang, J. F., Lagarde, E., Michel, G., & Fombonne, E. (2010). Disruptive symptoms in childhood and adolescence and early initiation of tobacco and cannabis use: the Gazel Youth study. *European Psychiatry*, *25*(7), 402–408.

Goode, E. (2008). *Drugs in American society* (7th ed.). Boston, MA: McGraw-Hill.

Goodman, A. (2010). Substance use and common child mental health problems: examining longitudinal associations in a British sample. *Addiction*, *105*(8), 1484–1496.

Griffith-Lendering, M. F., Huijbregts, S. C., Mooijaart, A., Vollebergh, W. A., & Swaab, H. (2011). Cannabis use and development of externalizing and internalizing behaviour problems in early adolescence: a TRAILS study. *Drug and Alcohol Dependence*, *116*(1–3), 11–17.

Hathaway, A. D. (1997). Marijuana and lifestyle: exploring tolerable deviance. *Deviant Behavior*, *18*(3), 213–232.

Hayatbakhsh, M. R., Najman, J. M., Bor, W., O'Callaghan, M. J., & Williams, G. M. (2009). Multiple risk factor model predicting cannabis use and use disorders: a longitudinal study. *American Journal of Drug and Alcohol Abuse*, *35*(6), 399–407.

Hektner, J. M., August, G. J., & Realmuto, G. M. (2000). Patterns and temporal changes in peer affiliation among aggressive and nonaggressive children participating in a summer school program. *Journal of Clinical Child Psychology*, *29*(4), 603–614.

Hibell, B., Guttormsson, U., Ahlström, S., Balakireva, O., Bjarnason, T., Kokkevi, A., & Kraus, L. (2011). *The 2011 ESPAD Report: Substance use among students in 36 European countries*. Stockholm, Sweden: Swedish Council for Information on Alcohol and other Drugs (CAN).

Holmberg, M. C. (1980). The development of social interchange patterns from 12–42 months. *Child Development*, *51*, 448–456.

Huizinga, D., Loeber, R., Thornberry, T. P., & Cothern, L. (2000). Co-occurrence of delinquency and other problem behaviors. *Juvenile Justice Bulletin, November*, 1–7.

Jessor, R. (1991). Risk behavior in adolescence: a psychosocial framework for understanding and action. *Journal of Adolescent Health*, *12*(8), 597–605.

Jester, J. M., Nigg, J. T., Buu, A., Puttler, L. I., Glass, J. M., Heitzeg, M. M., & Zucker, R. A. (2008). Trajectories of childhood aggression and inattention/hyperactivity: differential effects on substance abuse in adolescence. *Journal of the American Academy of Child and Adolescent Psychiatry*, *47*(10), 1158–1165.

Johnston, L. D., O'Malley, P. M., Bachman, J. G., Schulenberg, J. E., & Miech, R. A. (2014). *Monitoring the future national survey results on drug use, 1975–2013: Vol. I, Secondary school students*. Ann Arbor, MI: Institute for Social Research, The University of Michigan.

Kaplan, H. B., Tolle, G. C., & Yoshida, T. (2001). Substance use-induced diminution of violence: a countervailing effect longitudinal perspective. *Criminology*, *39*(1), 205–224.

Kellam, S. G., Branch, J. D., Agrawal, K. C., & Ensminger, M. E. (1975). *Mental health and going to school: The Woodlawn program of assessment, early intervention, and evaluation*. Chicago, IL: University of Chicago Press.

Khantzian, E. J. (1985). The self-medication hypothesis of addictive disorders: focus on heroin and cocaine dependence. *American Journal of Psychiatry*, *142*(11), 1259–1264.

Khantzian, E. J. (2003). The self-medication hypothesis revisited: the dually diagnosed patient. *Primary Psychiatry*, *10*, 47–48.

Kim, M. J., Catalano, R. F., Haggerty, K. P., & Abbott, R. D. (2011). Bullying at elementary school and problem behaviour in young adulthood: a study of bullying, violence and substance use from age 11 to age 21. *Criminal Behaviour and Mental Health*, *21*(2), 136–144.

King, S. M., Iacono, W. G., & McGue, M. (2004). Childhood externalizing and internalizing psychopathology in the prediction of early substance use. *Addiction*, *99*(12), 1548–1559.

Korhonen, T., van Leeuwen, A. P., Reijneveld, S. A., Ormel, J., Verhulst, F. C., & Huizink, A. C. (2010). Externalizing behavior problems and cigarette smoking as predictors of cannabis use: the TRAILS Study. *Journal of the American Academy of Child and Adolescent Psychiatry*, *49*(1), 61–69.

Le Blanc, M., & Loeber, R. (1998). Developmental criminology updated. In M. Tonry (Ed.), *Crime and justice: A review of research* (Vol. 23, pp. 115–198). Chicago, IL: University of Chicago Press.

Levinthal, C. F. (2008). *Drugs, society, and criminal justice* (2nd ed.). Boston, MA: Pearson Education.

Liu, W., Lynne-Landsman, S. D., Petras, H., Masyn, K., & Ialongo, N. (2013). The evaluation of two first-grade preventive interventions on childhood aggression and adolescent marijuana use: a latent transition longitudinal mixture model. *Prevention Science*, *14*(3), 206–217.

Lynne-Landsman, S. D., Bradshaw, C. P., & Ialongo, N. S. (2010). Testing a developmental cascade model of adolescent substance use trajectories and young adult adjustment. *Development and Psychopathology*, *22*(4), 933–948.

Marie, D., Fergusson, D. M., & Boden, J. M. (2008). Links between ethnic identification, cannabis use and dependence, and life outcomes in a New Zealand birth cohort. *Australian and New Zealand Journal of Psychiatry*, *42*(9), 780–788.

Melander, L. A., Noel, H., & Tyler, K. A. (2010). Bidirectional, unidirectional, and nonviolence: a comparison of the predictors among partnered young adults. *Violence and Victims*, *25*(5), 617–630.

Moffitt, T. E. (1993). Adolescence-limited and life-course-persistent antisocial behavior: a developmental taxonomy. *Psychology Review*, *100*(4), 674–701.

Moore, T. M., & Stuart, G. L. (2005). A review of the literature on marijuana and interpersonal violence. *Aggression and Violent Behavior*, *10*(2), 171–192.

Ostrowsky, M. K. (2011). Does marijuana use lead to aggression and violent behavior? *Journal of Drug Education*, *41*(4), 369–389.

Paradise, M. J., & Cauce, A. M. (2003). Substance use and delinquency during adolescence: a prospective look at an at-risk sample. *Substance Use and Misuse*, *38*(3–6), 701–723.

Patterson, G. R., DeBaryshe, B. D., & Ramsey, E. (1989). A developmental perspective on antisocial behavior. *American Psychologist*, *44*(2), 329–335.

Patterson, G. R., Reid, J. B., & Dishion, T. J. (1992). *A social learning approach: Vol. 4. Antisocial boys*. Eugene, OR: Castilia.

Pedersen, W., Mastekaasa, A., & Wichstrom, L. (2001). Conduct problems and early cannabis initiation: a longitudinal study of gender differences. *Addiction*, *96*(3), 415–431.

Petras, H., Kellam, S. G., Brown, C. H., Muthen, B. O., Ialongo, N. S., & Poduska, J. M. (2008). Developmental epidemiological courses leading to antisocial personality disorder and violent and criminal behavior: effects by young adulthood of a universal preventive intervention in first- and second-grade classrooms. *Drug and Alcohol Dependence*, *95*(Suppl. 1), S45–S59.

Petras, H., Masyn, K., & Ialongo, N. (2011). The developmental impact of two first grade preventive interventions on aggressive/disruptive behavior in childhood and adolescence: an application of latent transition growth mixture modeling. *Prevention Science*, *12*(3), 300–313.

Prinstein, M. J., & La Greca, A. M. (2004). Childhood peer rejection and aggression as predictors of adolescent girls' externalizing and health risk behaviors: a 6-year longitudinal study. *Journal of Consulting and Clinical Psychology*, *72*(1), 103–112.

Pujazon-Zazik, M., & Park, M. J. (2009). Marijuana: use among young males and health outcomes. *American Journal of Men's Health*, *3*(3), 265–274.

Reboussin, B. A., Hubbard, S., & Ialongo, N. S. (2007). Marijuana use patterns among African-American middle-school students: a longitudinal latent class regression analysis. *Drug and Alcohol Dependence*, *90*(1), 12–24.

Reyes, M., Luz, H., Foshee, V. A., Bauer, D. J., & Ennett, S. T. (2014). Proximal and time-varying effects of cigarette, alcohol, marijuana and other hard drug use on adolescent dating aggression. *Journal of Adolescence*, *37*(3), 281–289.

Rosenbaum, P. (2002). *Observational studies* (2nd ed.). New York, NY: Springer.

Schofield, H. L., Bierman, K. L., Heinrichs, B., & Nix, R. L. (2008). Predicting early sexual activity with behavior problems exhibited at school entry and in early adolescence. *Journal of Abnormal Child Psychology*, *36*(8), 1175–1188.

Skara, S., Pokhrel, P., Weiner, M. D., Sun, P., Dent, C. W., & Sussman, S. (2008). Physical and relational aggression as predictors of drug use: gender differences among high school students. *Addictive Behaviors*, *33*(12), 1507–1515.

Tart, C. T. (1970). Marijuana intoxication: common experiences. *Nature*, *226*(5247), 701–704.

Temple, J. R., Shorey, R. C., Fite, P., Stuart, G. L., & Le, V. D. (2013). Substance use as a longitudinal predictor of the perpetration of teen dating violence. *Journal of Youth and Adolescence*, *42*(4), 596–606.

Tremblay, R. E. (2006). Prevention of youth violence: why not start at the beginning? *Journal of Abnormal Child Psychology*, *34*(4), 481–487.

van den Bree, M. B., & Pickworth, W. B. (2005). Risk factors predicting changes in marijuana involvement in teenagers. *Archives of General Psychiatry*, *62*(3), 311–319.

Wagner, F. A., & Anthony, J. C. (2002). From first drug use to drug dependence; developmental periods of risk for dependence upon marijuana, cocaine, and alcohol. *Neuropsychopharmacology*, *26*(4), 479–488.

Wei, E. H., Loeber, R., & Whie, H. R. (2004). Teasing apart the developmental associations between alcohol and marijuana use and violence. *Journal of Contemporary Criminal Justice*, *20*(2), 166–183.

White, H. R., & Hansell, S. (1998). Acute and long-term effects of drug use on aggression from adolescence into adulthood. *Journal of Drug Issues*, *28*(4), 837–858.

Wu, J., Witkiewitz, K., McMahon, R. J., & Dodge, K. A. (2010). A parallel process growth mixture model of conduct problems and substance use with risky sexual behavior. *Drug and Alcohol Dependence*, *111*(3), 207–214.

CHAPTER

e4

COMT Genotypes, Cannabis Use, and Psychosis: Gene-Environment Interaction Evidence from Human Populations, and Its Methodological Concerns

M. Fatjó-Vilas, C. Prats, L. Fañanás

Faculty of Biology, Anthropology Unit, Department of Animal Biology, University of Barcelona, Biomedicine Institute of the University of Barcelona (IBUB), Barcelona, Spain; CIBER of Mental Health (CIBERSAM), Madrid, Spain

SUMMARY POINTS

- This chapter focuses on reviewing the evidences concerning the putative interaction between the Val188Met polymorphism of the *Catecol-O-methyltransferase* gene (*COMT*) and cannabis use on the development of psychosis.
- The Dunedin Multidisciplinary Health and Development Study reported for the first time that the relationship between adolescent cannabis use and the risk of developing psychotic disorders is modulated by *COMT* Val158Met genotype.
- The following cohort, case-control, and case-based studies have offered contradictory results, and have pointed toward the interest of studying the effect of other genetic and environmental factors operating on specific phenotypic subgroups.
- To date, few neuroimaging studies have been conducted in humans, and these have showed that brain changes associated with cannabis use may be influenced by the *COMT* genotype.
- The divergence across *COMT* and cannabis interaction studies has raised several aspects to be considered in the interpretation of these results: the pleiotropic biological and behavioral effects of *COMT* gene, the gene-gene interactions and other gene expression mechanisms, the role of other environmental factors, among others.

KEY FACTS

- Systematic reviews of prospective studies have consistently evidenced the role of cannabis use as a risk factor for the emergence of psychosis.
- Sensitivity to cannabis effects is proposed to be mediated by the genetic variability observed in human populations.
- *COMT* is one of several enzymes that degrade catecholamines such as dopamine, which is central to one of the major neurochemical theories of psychotic disorders.
- *COMT* gene contains a functional single nucleotide polymorphism (Val158Met) which is associated with

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00031-4
Copyright © 2017 Elsevier Inc. All rights reserved.

changes in enzyme activity in brain (Val allele higher activity than Met allele).
- Diverse strategies and results have led to the conclusion that psychosis liability by itself may explain part of the association between cannabis and psychosis mediated by *COMT*, suggesting that there probably exists a bidirectional relationship between the risk factor and the disorder.

LIST OF ABBREVIATIONS

CB Cannabinoid
CB1 Cannabinoid receptor type 1
COMT Catecol-O-methyltransferase
DA Dopamine
DAT Presynaptic dopamine transporter
GxE Gene–environment interaction
Met Methionine
PFC Prefrontal cortex
SNP Single nucleotide polymorphism
THC Tetrahydrocannabinol
Val Valine

THE ROLE OF CANNABIS USE AS A RISK FACTOR FOR PSYCHOSIS

The endocannabinoid system plays a modulatory role on reward dopamine (DA) neurons, and this is substantiated by the expression of type 1 cannabinoid (CB1) receptors, and the abundance of their endogenous ligands, or endocannabinoids. From a general point of view, endocannabinoids are released on demand by the postsynaptic neurons, and travel retrogradely across the synapse to bind to and activate CB1 receptors located on the presynaptic terminals (Fig. e4.1). The result of such activation is a decreased neurotransmitter release in a short- or long-term manner (Melis, Muntoni, & Pistis, 2012). In this regard, endocannabinoids serve as retrograde signaling molecules at many synapses within the central nervous system. Synapses on midbrain DA neurons (in the ventral tegmental area—VTA) make no exception to this rule. In fact, the effects of cannabinoids on DA transmission, as well as DA-related behaviors, are generally exerted through the modulation of inhibitory and excitatory afferents impinging onto DA neurons (Melis et al., 2012).

Systematic reviews have consistently evidenced the role of cannabis use as a risk factor for the emergence of psychosis, ranging from psychotic symptoms, such hallucinations and delusions, to clinically significant disorders, such as, schizophrenia (2–4). The effects of cannabinoids on increasing DA, reducing GABA, and reducing glutamatergic neurotransmission may contribute to their capacity to induce transient positive, negative, and cognitive symptoms, but the precise mechanism remains unclear (D'Souza, Sewell, & Ranganathan, 2009). However, as expected in multifactorial complex diseases, cannabis use is not sufficient or necessary to cause schizophrenia or related disorders—meaning that only a small proportion of cannabis users develop psychosis, or that not all persons with a psychotic disorder have been exposed to cannabis. Accordingly, the genetic variability observed in human populations is proposed to be the most important factor mediating the sensitivity to cannabis effects on increased risk for mental disorders. In this sense, genetically sensitive studies indicate that the risk of suffering from cannabis-related psychotic experiences is much higher in individuals with evidence of psychosis liability (measured psychometrically, or defined as having an affected first degree relative). These results have led to the view that the differential effect of cannabis may be related to individual predisposition to psychosis, which is strongly accepted to be influenced by genetic factors.

THE *COMT* GENE MOLECULAR VARIABILITY AND THE RISK FOR PSYCHOSIS

Since dopamine is hypothesized to represent a crucial common final pathway between brain biology and experiences of psychosis, the enzyme COMT has been a great focus of attention. COMT is one of several enzymes that degrade catecolamines, such as, dopamine, epinephrine, and norepinephrine. In the brain, COMT-dependent dopamine degradation is of particular importance in brain regions with low expression of the presynaptic dopamine transporter (DAT), such as, the prefrontal cortex (Matsumoto et al., 2003).

In humans, catechol-O-methyltransferase protein is encoded by the *COMT* gene, placed at 22q11.21-q11.23 chromosomal region (Fig. e4.2). The gene has several allelic variants, and the most relevant is the single nucleotide polymorphism (SNP) Val158Met (rs4680) that results in a Valine to Methionine change at protein position 158 (Lotta et al., 1995). The Val variant catabolizes dopamine at up to 4 times the rate of its Met counterpart. The lower activity associated with Met allele results in higher synaptic dopamine levels, following neurotransmitter release, ultimately increasing dopaminergic stimulation of the postsynaptic neuron. Postmortem studies (Akil et al., 2003) have also shown that the number of Val-encoding alleles predicted increased dopamine synthesis in the midbrain, and suggested that this functional polymorphism can modulate prefrontal-midbrain

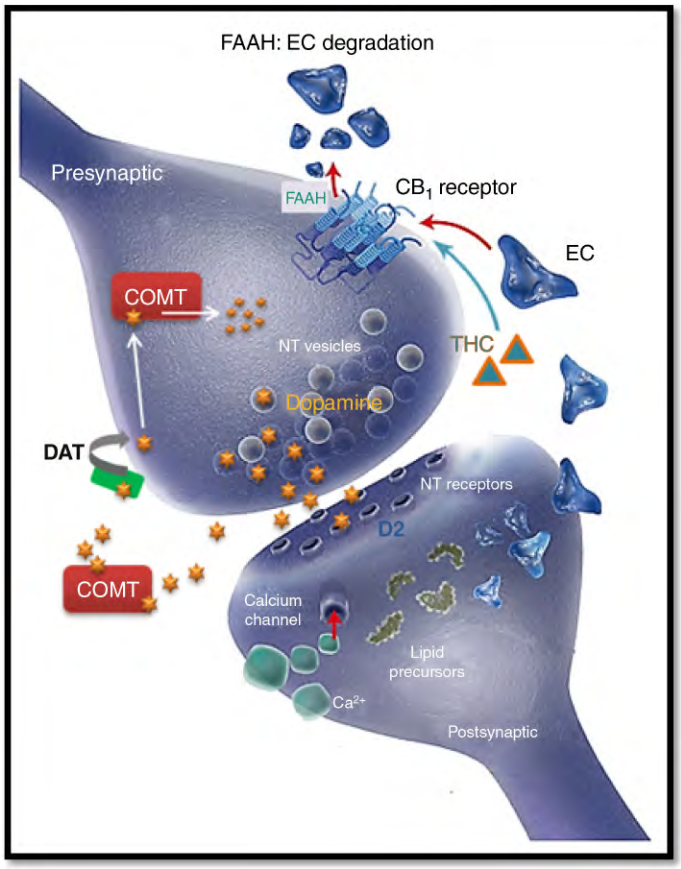


FIGURE e4.1 Schematic representation of the endocannabinoid system in pre- and postsynaptic neurons. The endocannabinoid signaling system (eCBS) is composed of cannabinoid (CB) receptors, their endogenous ligands (the endocannabinoids, EC) and the enzymes that produce and inactivate these ligands. Endogenous cannabinoids and the Δ^9-*tetrahydrocannabinol* (THC) produce effects by binding to and activating the cannabinoid receptor (CB1), the major cannabinoid receptor in the brain. Then, both components compete for access to the CB1 receptor classified as a 1-G-protein, coupled receptors that activate $G_{i/o}$-type G-proteins. The activation of these G-protein subtypes normally produces inhibition of adenylyl cyclase, inhibition of voltage-gated calcium channels, and activation of certain potassium channels. Note that the fatty acid amide hydrolase (FAAH) is distributed in the central nervous system, and degrades fatty acid amide signaling molecules, such as, endocannabinoids. In general, CBs act as a "dimmer switch" for presynaptic neurons, limiting the amount of neurotransmitter (eg, dopamine) that gets released. The *dopamine transporter* (DAT) plays a critical role in terminating the action of *dopamine* by rapid *reuptake* into the presynaptic neuron. Catecol-O-methyltransferase (COMT) is an important degradation enzyme of monoamines, and participates in both the extracellular and intracellular metabolism of dopamine. *Source: Adapted from: http://www.treccani.it/enciclopedia/endocannabinoidi_(Dizionario-di-Medicina)*

interaction. To this respect, using multimodal neuroimaging in healthy subjects, it has been shown that the interaction between prefrontal activity and midbrain dopaminergic synthesis is modulated by *COMT* Val158Met polymorphism (Meyer-Lindenberg et al., 2005).

Although different metaanalyses have not found a clear association between the Val158Met *COMT* polymorphism and vulnerability for schizophrenia (eg, Fan et al., 2005; Munafo, Bowes, Clark, & Flint, 2005), it has been suggested that it might be related to schizophrenia via its effect on cognitive function. In this regard, the study of Egan et al. (2001) reported that *COMT* genotype was associated with variations in executive cognition and with PFC physiological activity during working memory, functions for which DA levels in the PFC are known to be important.

Clinical and epidemiological studies have been interested in understanding the role that *COMT* gene could exert on the differential risk to develop psychoses, between cannabis users and nonusers (Di Forti et al., 2014). Considering that the blockage of CB1 by the THC component of the cannabis can impair dopamine release (Fig. e4.1), weak or subtle changes in COMT activity may contribute to explain individual risk differences in the development of dopaminergic related psychotic symptoms in cannabis consumers. This phenomenon is also called gene–environment interaction (GxE) and represents the genetic control of the sensitivity to the environment. In this scenario (Fig. e4.3), subjects exposed to similar environmental risk factors (eg, cannabis use at the same period of brain development, such as, age), will be at a substantial different risk to develop a particular

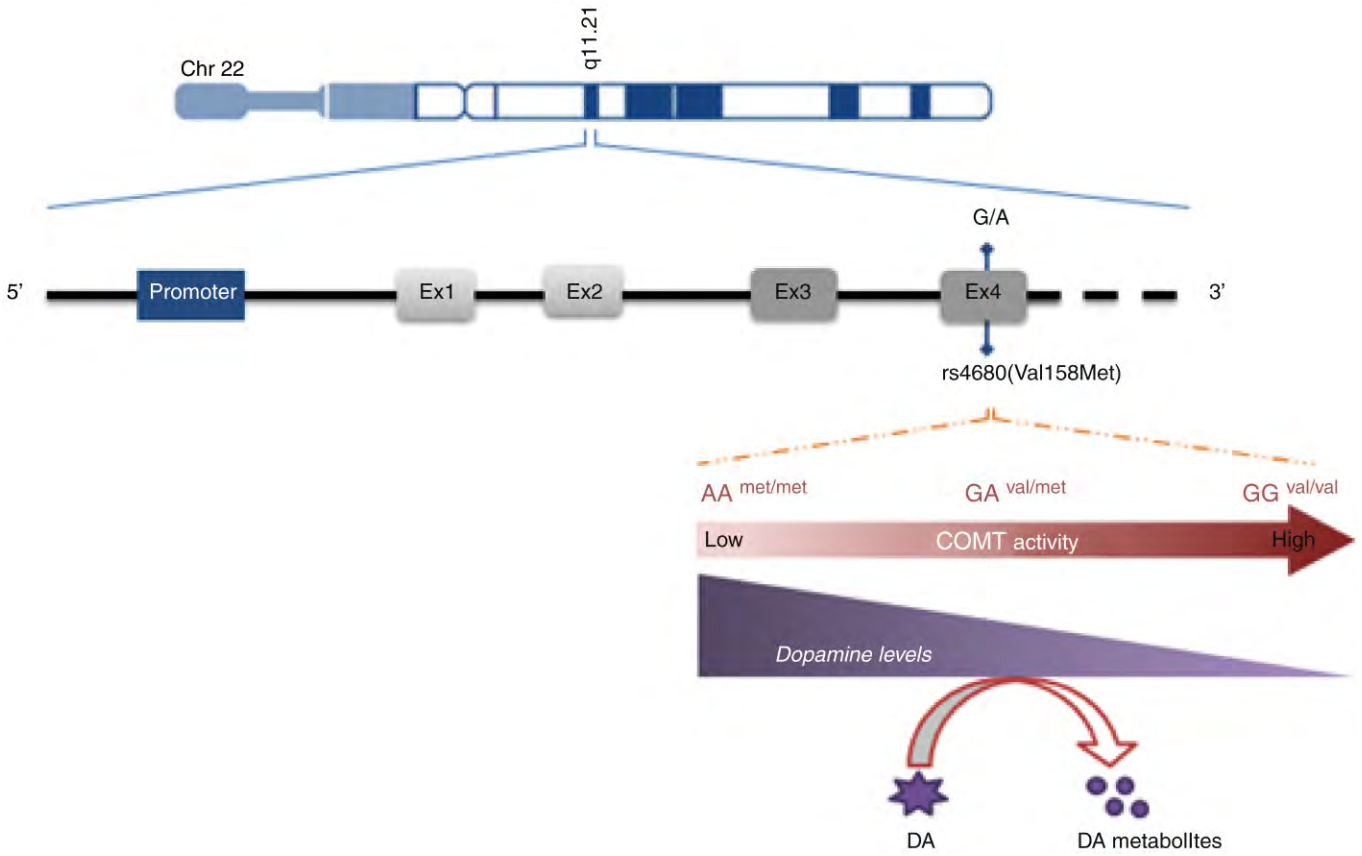


FIGURE e4.2 Diagram showing *COMT* gene and the effect of Val158Met polymorphism on COMT activity. *COMT* gene is located on chromosome 22q11.21, and it contains a functional single nucleotide polymorphism (SNP: Val158Met or rs4680) which entails a nucleotide change from Guanine (G) to Adenine (A) in the sequence of the gene. This polymorphism produces an amino acid change from Valine (Val) to Methionine (Met), at codon 158 of the membrane-bound protein form. The Val allele is associated with higher enzyme activity in the brain than the Met allele (Chen et al., 2004) and, consequently, it is presumed to result in reduced dopamine availability. Coding exons of *COMT* gene are represented in dark gray (wide bars), while noncoding exons are in light gray.

brain dysfunction (eg, psychotic symptoms), depending on their genetic background.

Taking into account evidences from different study designs based on Caucasian subjects (Table e4.1), in the following section we will review the evidences concerning the putative interaction between the Val188Met polymorphism of the *COMT* gene and cannabis use on the development of psychosis and, eventually, cognitive associated conditions.

EVIDENCES OF COMT GENE AND CANNABIS USE INTERACTIONS, AND THE EFFECT ON THE RISK FOR PSYCHOSIS

Evidences From Longitudinal Studies

The first study that provided evidence of interaction between cannabis and the Val158Met polymorphism was conducted by Caspi et al. (2005), using a longitudinal prospective design involving 803 individuals. This showed that adolescent cannabis use, but not adult-onset use, was associated with a 10-fold increase in the risk of developing schizophreniform disorder at the age of 26, among individuals homozygous for the Val allele, whereas cannabis use had no such adverse influence on individuals with two copies of the Met allele. These results, together with studies that have described cannabis effects on the brain, limited to adolescence both in human research and in experimental animal models (Bossong & Niesink, 2010; Schneider & Koch, 2003), have shed light on the relationship between cannabis use, "neurodevelopmental processes", and the emergence of psychiatric disorders, and also on the fact that sensitivity to cannabis effects may depend on the state of brain development and maturity.

Not surprisingly, these results triggered several other studies with diverse designs aiming to replicate and to further understand the interaction between cannabis and *COMT* gene, in relation to the risk for psychosis.

In this respect, another cohort study, based on 2630 individuals aged 16, detected that cumulative use of

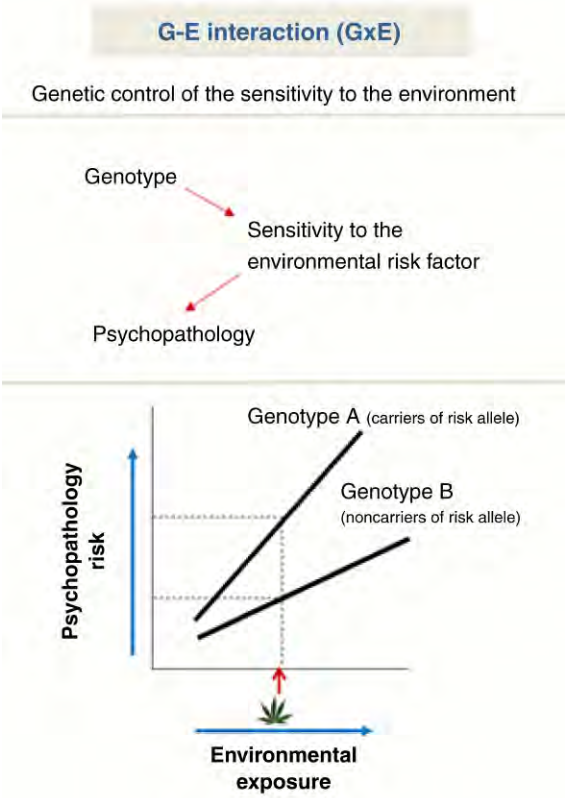


FIGURE e4.3 Example of Gene–environment interaction (GxE) on a hypothetical exposure to cannabis, in a population sample with carriers and noncarriers of a hypothetical risk allele. This theoretical model example assumes similar cannabis quality and age at onset for all the individuals. *Source: Adapted from van Os & Marcelis (1998); van Os et al. (2008).*

cannabis at age 14 increased the risk for self-reported psychotic experiences, irrespective of the underlying *COMT* Val158Met genotype or haplotypes (Zammit, Owen, Evans, Heron, & Lewis, 2011). However, when several sensitivity analyses were conducted, some contradictory interaction effects emerged: the risk for hallucinations after cannabis use was greater in Val allele homozygotes, compared with Met ones; or the risk for psychotic experiences was greater in Met allele homozygotes, but only in subjects that never had definite experiences before the age of 14. Although these findings do not support that the risk for developing psychosis following use of cannabis is dependent upon variation within *COMT*, the observed variation in the interaction in sample subgroups pointed toward the interest of studying the effect of other genetic and environmental factors operating in these specific groups that could account for these variations.

Evidences From Case-Only and Case-Control Studies

Unlike longitudinal data collection, case-only design relies on people recalling the age at first use of cannabis, and relating this in time to the date of the emergence of the first psychotic symptoms. This kind of studies can result in some recall or time sequence bias, but allow having larger genotype and cannabis use sample subgroups, fact that increases the power to detect interaction effects. Despite this, some of these studies have replicated the interaction, while others have failed or have offered controversial results. Firstly, in a sample of 493 patients with schizophrenia, no association was found between three SNPs at *COMT* and cannabis use (Zammit et al., 2007). Secondly, a study based on 169 patients with a first episode of psychosis showed a significant effect of the interaction between Val158Met genotype and cannabis use, on both age of onset and duration of untreated illness (Pelayo-Teran et al., 2010). Due to the fact that this effect was observed only in the group of cannabis nonusers, the authors propose that the use of cannabis modulated the genotype effect by suppressing the delay in the onset of the symptoms, in patients carrying the Met allele. Thirdly, a marginal interaction between Val158Met genotype and lifetime cannabis use on age at emergence of psychotic disorders was reported in a sample of 157 adolescent psychiatric patients, with Val/Val subjects presenting an earlier onset than Met carriers (Estrada et al., 2011). Fourthly, the analysis of five SNPs in *COMT* in two samples of Spanish patients with schizophrenia (n = 383 and n = 365) allowed the definition of three common haplotypes described to have different enzymatic activity (Costas et al., 2011). Association was detected between cannabis use and low activity variants; however, when considering only the Val158Met polymorphism, patients homozygous for the Met allele were observed to double the probability of lifetime prevalence of cannabis use, in comparison to Val homozygotes. Although these data are in contrast with previous studies that identified the Val allele as the one presenting the risk, they add evidence to the cannabis and *COMT* interaction effect on the risk for schizophrenia and, moreover, suggest the interest of studying the haplotype molecular variability over single-nucleotide polymorphisms in order to characterize better the enzymatic activity of each individual. In this respect, a study based on 403 patients with schizophrenia or schizoaffective diagnoses that also analyzed several SNPs in *COMT* reported a sex-specific interaction between cannabis use and age at onset of schizophrenia, but did not detect an interaction between cannabis and *COMT* genotypes (De Sousa et al., 2013).

Finally, few studies have explored the interplay between *COMT* gene and cannabis use on cognitive performance. For example, a study that analyzed the effect of cannabis use on executive functions, by comparing 86 cannabis users with 58 nondrug users controls, described that cannabis users carrying the Val allele exhibited lower performance in attention and monitoring/shifting

TABLE e4.1 Summary of Studies Examining the Effect of the Interaction Between Cannabis Use and *COMT* Gene Val158Met Polymorphism (rs4680) on the Risk for Psychosis in Caucasian Subjects

Study	Study design	Sample size and characteristics	Cannabis use measure	Val158Met *COMT* genotype	Outcome measure	Main results
Longitudinal studies						
Caspi et al. (2005)	General population *(Dunedin Multidisciplinary Health and Development Study)*	*n* = 803 Follow-up from 3 to 26 years 26% classified as adolescent-onset cannabis users	Assessed at ages 13, 15, and 18	Val/Val (25%) Val/Met (50%) Met/Met (25%)	Psychosis outcomes assessed at age 26 (Diagnostic Interview Schedule): • 3.6% met DSM-IV diagnosis criteria for schizophreniform disorder • 13% reported at least one hallucinatory experience • 21% reported at least one delusional belief	Adolescent cannabis use was associated with increased risk of: • schizophreniform disorder in adulthood among Val/Val individuals [OR (95% CI) = 10.9 (2.2–54.1)] • hallucinatory experiences in adulthood among Val/Val individuals [OR (95% CI) = 5.3 (2.2–12.37)] • delusional beliefs in adulthood among Val/Val individuals [OR (95% CI) = 3.5 (1.7–7.0)]
Zammit et al. (2011)	General population *(Avon Longitudinal Study of Parents and Children, ALSPAC)*	*n* = 2630 Annual assessments from 7.5 to 16 years	Assessed at age 14 Cumulative cannabis use: none, <5 times, 5–20, 21–60, >60. 6.5% of individuals ever used cannabis at age 14	Subjects with psychotic symptoms: Val/Val (20.9%) Val/Met (53.1%) Met/Met (26%) Subjects without psychotic symptoms: Val/Val (23%) Val/Met (49%) Met/Met (28%)	Psychosis-like symptoms assessed at age 16 (PLIKS-Q): • 8.6% responded yes to any of the 11 questions	Cannabis use by age 14 increased the risk to present psychotic experiences at age 16: OR (95% CI) = 2.5 (1.62–3.77) No evidence of statistical interaction under a multiplicative model between cannabis use and *COMT* Val15rs4680, or between cannabis use and the other *COMT* SNPs
Cross-sectional studies with retrospective evaluation of cannabis use						
Zammit et al. (2007)	Case-only study: schizophrenia patients	*n* = 493	Binary measure: users/nonusers Age at onset of cannabis use	—	DSM-IV schizophrenia diagnosis assessed with semistructured interview (SCAN)	No evidence of association between *COMT* and cannabis use Results were identical when restricting the analysis to participants who first used cannabis before age 18 and at least 1 year before prior to the illness onset
Pelayo-Teran et al. (2010)	Case-only study: first-episode psychotic patients *(Longitudinal intervention programe of first episode psychosis, PAFIP)*	*n* = 169	Binary measure: users/nonusers 46.15% of cannabis users	Val/Val = 25.4%, Val/Met = 60.4%, Met/Met = 14.2%	DSM-IV psychotic disorders diagnoses assessed with structured interview (SCID-I): • 61.54% schizophrenia Age of onset of psychosis Duration of untreated psychosis (DUP)	Cannabis use had a significant effect on earlier age of onset ($F = 16.89$, $p < 0.001$) but not on DU *COMT* by cannabis use interaction significant effect on both age of onset ($F = 3.82$, $p = 0.024$) and DUP ($F = 3.29$, $p = 0.039$) Age of onset/DUP significantly later/longer in Met homozygote group than in the Val homozygote group ($F = 2.18$, $p = 0.009$), only in cannabis nonusers group

Estrada et al. (2011)	Case-only study: psychiatric patients	n = 157	Binary measure: users/nonusers 45.5% of cannabis users	Schizophrenia-spectrum disorders: Val/Val = 23.75% Val/Met = 52.05% Met/Met = 23.75% Nonpsychotic disorders: Val/Val = 36.30% Val/Met = 45.50% Met/Met = 18.20%	DSM-IV diagnoses : 51% schizophrenia-spectrum disorders 49% nonpsychotic disorders (conduct and affective disorders) Age at disorder onset	Age at first cannabis use correlated with age at onset in both psychotic (β = 1.59, $p < 0.001$) and nonpsychotic disorders (β = 0.4, p = 0.006). *COMT* genotype was associated with age at onset of psychotic disorders only within cannabis users group (β = 01.66, p = 0.04) *COMT* by cannabis use interaction effect on age at onset within schizophrenia-spectrum disorders (β = 2.72, p = 0.04). Val/Val carriers showed an earlier age at onset than Met carriers.
Costas et al. (2011)	Case-only study: schizophrenia patients	Two samples: (1) n = 383 (2) n = 365	Binary measure: users/nonusers 16.9 and 24.6% of cannabis users	(1) Val/Val = 38.7% Val/Met = 42.5% Met/Met = 18.8% (2) Val/Val = 32.9% Val/Met = 46.6% Met/Met = 20.5% Also genotyped four other SNPs (rs4633, rs4818, rs6269, and rs737865) and conducted haplotype analyses	DSM-IV schizophrenia diagnosis	Met/Met subjects doubled the probability of lifetime prevalence of cannabis use, compared to Val homozygous [OR (95% CI) = 2.07 (1.27–3.26), in the combined sample (a + b)]. Association between cannabis use and low activity *COMT* haplotypic variants in patients with schizophrenia [OR (95% CI) = 0.56 (0.38–0.84)]
De Sousa et al. (2013)	Case-only study: schizophrenia or schizoaffective patients	n = 403	Binary measure: low users/high users 60.1% of cannabis high users	Also genotyped 3 other SNPs: rs4633, rs4818, rs6269	Age at onset of schizophrenia	High users of cannabis had an earlier onset relative to low users (p = 0.003) and high cannabis use was more prevalent in males than females [OR (95% CI) = 3.47 (2.0–-6.0)] No interaction between *COMT* genotypes and cannabis use was found

(*Continued*)

TABLE e4.1 Summary of Studies Examining the Effect of the Interaction Between Cannabis Use and *COMT* Gene Val158Met Polymorphism (rs4680) on the Risk for Psychosis in Caucasian Subjects (*cont.*)

Study	Study design	Sample size and characteristics	Cannabis use measure	Val158Met *COMT* genotype	Outcome measure	Main results
Experimental and experience sampling method based studies (ESM)						
Henquet et al. (2006)	Double-blind, placebo-controlled cross-over design Evaluation of psychotic experiences and cognitive performance after exposure to THC/placebo	30 patients 12 relatives 32 healthy subjects	Two test sessions, blinded subjects received in randomized order either 300 µg THC/kg, or 0 µg THC/kg (placebo) in tobacco cigarettes	Total sample Val/Val = 27% Val/Met = 47% Met/Met = 26%	Diagnosis made according to Research Diagnostic Criteria using OPCRIT. PANSS used to measure psychotic symptoms during the previous 2 weeks: patients: 36.7% schizophrenia, 36.7% schizoaffective, 26.6% psychosis not otherwise specified Psychometric psychosis liability determined by using CAPE Cognitive measures: different tasks on verbal and visual memory, attention	A significant three-way condition x genotype x Cape-trait interaction was observed ($X^2 = 9.00$, $df = 1$; $p = 0.003$): carriers of the Val allele were more sensitive to THC induced psychotic experiences, but this was conditional on prior evidence of psychometric psychotic liability Carriers of the Val allele were also more sensitive to THC-induced memory and attention impairments, compared to carriers of the Met allele
van Winkel et al. (2008)	Case-control: psychotic patients with comorbid cannabis misuse and healthy cannabis users	31 cases 25 controls	Dichotomic: users/nonusers	Total sample: Val/Val = 30.4% Val/Met = 55.4% Met/Met = 14.3%	Emotional and psychotic symptoms (ESM)	Significant interaction between *COMT* Val158Met genotype and ESM stress on negative affect in patients ($X^2 = 7.4$, $p = 0.02$), but not in controls ($X^2 = 3.8$, $p = 0.15$) Met/Met patients showed the largest increase in psychotic experiences, in reaction to ESM stress ($X^2 = 10.5$, $p = 0.06$).
Henquet et al. (2009)	Case-control: psychotic patients and controls Case-control: psychotic patients and controls	32 patients 29 controls	Dichotomic: users/nonusers	Total sample: Val/Val = 30% Val/Met = 55% Met/Met = 14%	Psychotic symptoms (ESM) Psychometric psychosis liability determined by using CAPE	Carriers of the Val allele showed an increase in hallucinations after cannabis exposure conditional on prior evidence of psychometric psychosis liability [$\beta = 078$ (95% CI: 0.36−1.19, $p < 0.01$]

tasks than Val carriers who were nonusers (Verdejo-Garcia et al., 2013). From a different approach, these results add to those from an experimental study in which it is described that Val allele carriers are more sensitive to THC-induced memory and attention impairments than Met allele carriers (Henquet et al., 2006). Nonetheless, it must also be taken into account that cognitive performance has also been shown to be modulated by the interaction of Val158Met polymorphisms with other illegal psychostimulants, such as, ecstasy (Cuyas et al., 2011).

Evidences From Experimental and Experience Sampling Method Based Studies

A double-blind placebo-controlled study of THC exposure in 30 patients with psychotic illness and 32 healthy controls (Henquet et al., 2006) was the first to provide understanding of the importance of the role of the genetic vulnerability background in modulation of the *COMT* and cannabis interaction. The authors observed that carriers of the Val allele were most sensitive to THC-induced psychotic experiences, but this was conditional on prior evidence of increased psychosis proneness, as measured by psychometric tests. More recently, the experience sampling method, a structured diary technique assessing current context and emotional and psychotic experiences in daily life (Myin-Germeys, van Os, Schwartz, Stone, & Delespaul, 2001), was used to show that among Val allele carriers the psychometric psychosis liability also mediates the link between cannabis exposure and the hallucinatory phenomena in the flow of daily life (Henquet et al., 2009). This sampling method has also been used to explore the effect of stress on psychotic experiences in 31 patients with a psychotic disorder and comorbid cannabis misuse and 25 healthy cannabis users (van Winkel et al., 2008). In contrast with other studies, Met/Met cannabis users patients showed the largest increase in psychotic experiences in reaction to experience sampling method stress, extending the evidence of the complex mechanisms that underlie gene–environment interactions related to the development of psychotic symptoms by identifying the importance of the daily life stress as another mediating factor of the relationship between cannabis and psychosis proneness.

Evidences From Neuroimaging Studies

Neuroimaging techniques, including functional neuroimaging, provide a powerful means of investigating the central actions of cannabis in human. On the one hand, THC administration has been associated with changes in brain structure and function (Bossong, Jansma, Bhattacharyya, & Ramsey, 2014). On the other hand, stronger evidence for a role of the Val158Met polymorphism in schizophrenia and cognition comes from functional imaging studies (eg, Egan et al., 2001; Pomarol-Clotet et al., 2010). However, to date, few neuroimaging studies have been carried out to measure the combined effects of cannabis, *COMT* genotype, and psychosis in humans. Preliminary data of several genes modulating the adverse effects of cannabis on the brain, including *COMT* polymorphism, were reported in long-term cannabis users (Solowij, Fernandez, Murray, & Yücel, 2012). *COMT* polymorphism has also been described to influence the volume of the bilateral ventral caudate nucleus in both chronic cannabis users and healthy subjects, but in an opposite direction: more copies of Val allele led to lesser volume in chronic cannabis users, and more volume in controls (Batalla et al., 2014). These findings support for the first time that brain changes associated with cannabis use may be influenced by the *COMT* genotype.

CONSIDERATIONS FOR THE INTERPRETATION OF THE STUDIES ON *COMT* AND CANNABIS USE INTERACTION

The divergence across *COMT* and cannabis interaction studies has raised several aspects that need to be considered in the interpretation of these results and in order to design future studies.

The Pleiotropic Biological and Behavioral Effects of *COMT* Gene

The association of the Val allele with diminished executive cognitive functions has been described to be associated with lower incidence of anxiety-related traits, in subjects carrying this allele, while the improved cognitive performance found for Met carriers goes along with impaired emotional processing, such as, increased sensitivity to psychosocial stress (Smolka et al., 2005). It has been proposed that this reciprocal effect on emotion and cognition represents a tradeoff between the cognitive efficiency and emotional resiliency that maintains each allele in the population because each confers an environment-specific selective advantage (Goldman, Oroszi, & Ducci, 2005). Such balance between genotypic variants and mechanisms of cognition and emotion processing may be, therefore, implicated in the gene–environment interaction variability and may be underlying the *COMT* gene and cannabis differential interaction, conditional on other genetic and environmental factors. Accordingly, it is proposed that, by influencing the phenotypic response to environmental exposures, *COMT* gene may thus operate as a plasticity gene, where a genotype can have either a protective or a susceptibility

role, depending on specific genetic background and environmental conditions (Belsky & Pluess, 2009). It is noteworthy that certain periods of brain development are specific vulnerability windows because of both the gene expression patterns associated to brain maturation, but also the sensitivity of the ongoing developmental processes to environmental factors (Sundram, 2006).

Genetic Interactions and Other Biological Mechanisms

Far from being independent units, genes usually interact in complex networks whose expression is tightly regulated and coordinated. Indeed, genetic interactions underlying human complex traits and disease are considered as one possible explanation for their persistence within populations. Then, as already suggested by the description of the interaction of *COMT* Val158Met genotype and psychometric psychosis liability to moderate cannabis effects on psychotic symptoms (Henquet et al., 2009; Henquet et al., 2006), the identification of gene–gene interactions may probably better account for the differential sensitivity to cannabis than a single gene. In this regard, interactions between genes have been described in schizophrenia research before, and genetic and statistical analyses advances are facilitating this approach. For example, consistent with the biological processes related to schizophrenia, a three-way interaction on schizophrenia risk was reported between three functional polymorphisms in *COMT*, brain-derived neurotrophic factor (*BDNF*), and v-akt murine thymoma viral oncogene homolog 1 (*AKT1*) genes (Tan et al., 2012). Interestingly, *AKT1* gene has also been described to interact with cannabis use, with CC homozygous subjects for the rs29494732 SNP having approximately two fold odds of being diagnosed with a psychotic disorder when having used cannabis (van Winkel, 2011). More recently, another study has reported an odds ratio greater than 7 for developing a psychotic disorder for daily cannabis users who are homozygous for the risk allele (CC) of the same polymorphism (Di Forti et al., 2012). In addition, this interaction has also been suggested to mediate long-term changes in cognition measures, such as, sustained attention (van Winkel, van Beveren, & Simons, 2011).

Moreover, biological mechanisms that regulate gene expression and, thus, could indirectly regulate COMT activity, should also be taken into account. As mentioned, only few studies have included not only the Val158Met genotype, but also other polymorphisms along the gene (Costas et al., 2011; De Sousa et al., 2013; Zammit et al., 2007). On the other hand, *COMT* gene promoter differences methylation density has been associated with gene expression levels, and *COMT* gene promoter methylation differences have been reported between schizophrenia patients and healthy subjects (Abdolmaleky et al., 2006). More recently, it has been described that the interaction between *COMT* genotype and *COMT* promoter methylation is associated with cannabis use (van der Knaap et al., 2014), suggesting that combining genetic and epigenetic variation may be helpful in elucidating the gene–environment mechanisms involved in psychosis proneness.

Other Environmental Factors Interaction

In addition to genetic interaction, environmental factors may also interact with each other, as well as on psychosis risk. Some evidence comes from the study of the interaction between cannabis use and childhood trauma. For example, sexual trauma has been associated to an increase of the risk to develop psychosis, but only in those individuals with an early use of cannabis (Houston, Murphy, Adamson, Stringer, & Shevlin, 2008). These results have been extended by three-way interaction studies that have shown, in general population-based samples, that *COMT* genotype may act as a modulator of the interaction between cannabis use and childhood maltreatment on the risk for developing psychotic symptoms (Alemany et al., 2014; Vinkers et al., 2013). Overall, these results suggest that *COMT* gene variability may be involved in the sensitization to several environmental factors.

Methodological Concerns and Challenges

After a decade of gene–environment research on candidate genes in psychiatry, increasing concerns about this kind of approach have appeared, mainly addressing the problem of spurious findings as a result of multiple testing problems, and the large sample sizes required to reliably detect the proposed effects (Duncan & Keller, 2011). However, other authors have indicated that, in the detection of gene–environment interactions, the most appropriate strategy is to weigh the tradeoff between sample size and measurement precision (Wong, Day, Luan, Chan, & Wareham, 2003). Then, while debate is currently open, there is agreement about the need for further well-powered GxE studies based on accurate variables to explain between-subject differences in the risk to develop psychosis, following exposure to cannabis.

Regarding the reliability of cannabis measures, retrospective evaluation can be limited by recall bias and length of time between cannabis use and the assessment of the participant. However, in contrast to other environmental exposures, cannabis use measure is considered to have a fair level of confidence. On the other hand, based on studies that relate intensity/duration of cannabis use, and the cannabis variety used (due to the differences in THC content) (Di Forti et al., 2014), a more detailed

characterization of cannabis use should be considered in future studies.

Moreover, whether the association of cannabis and *COMT* gene with psychosis could be confounded has been a largely discussed issue (Henquet, Murray, Linszen, & van Os, 2005). Studies have included several confounders (such as, age, sex, social class, ethnic group, urbanicity, use of other drugs) in order to ascertain that the cannabis and psychosis association persisted after adjustment for these factors. In parallel, as those on a psychotic spectrum are more likely to experiment with cannabis, studies have also dealt in different ways with reverse causality (or the fact that cannabis use could be a consequence of the individuals' psychosis vulnerability expression). In this respect, a recent GWAS-based study has tested whether the cumulative burden of schizophrenia risk alleles carried by an individual predict cannabis use (Power et al., 2014). Although directly predicting only a small amount of the variance, results from this study have led to the conclusion that psychosis liability by itself may explain part of the association between cannabis and psychosis, suggesting that there probably exists a bidirectional relationship between the risk factor and the disorder. Similarly, studies facing the gene–environment correlation phenomena (the genotype influence on an individual's exposure to cannabis) have shown that the liability to use cannabis is influenced by genetic factors (Kendler, Schmitt, Aggen, & Prescott, 2008). Then, evidence seems to indicate that cannabis use and psychosis share at least part of their genetic roots, and that both GxE and correlation mechanisms should be better explored.

Finally, due to the polygenic architecture of psychotic disorders, it is unlikely that few genes account for cannabis sensitivity. Thus, certainly one of the challenges of genetic epidemiology in the coming years will be the integration of genome-wide association studies (GWAS) and environmental factors. Therefore, the development of gene-environment-wide interaction studies (GEWIS) represents a conceptual and methodological challenge of much interest for their potential and possible applications.

MINI-DICTIONARY

Allele One of two or more alternative forms of a gene or DNA sequence at a specific chromosomal location. In the case of the Val158Met polymorphism at *COMT* gene, there are two possible alleles: the allele G (corresponding to the presence of a Guanine nucleotide), or the allele A (corresponding to the presence of an Adenine nucleotide).

Amino acids Organic compounds containing an amino group (NH_2), a carboxylic acid group (COOH), that combines to form proteins. There are 20 different amino in proteins. The name of the Val158Met polymorphism comes from a single nucleotide substitution that results in an amino acid change from Valine (Val) to Methionine (Met), at codon 158.

Cross-sectional study An observational study that compares different population groups at a single point in time.

Experimental study A type of evaluation in which the participants undergo some kind of intervention (eg, new drug) in order to evaluate its impact. The experimental researcher has control over the intervention, its timing, and dose or intensity.

Genotype Refers to the specific allelic combination for a given locus. In the case of the Val158Met polymorphism at *COMT* gene, the alleles G and A are combined in the following genotypes: homozygote GG, heterozygote GA, or homozygote AA.

Longitudinal study An observational research method in which data is gathered for the same subjects repeatedly, over a period of time. These studies allow researchers to study changes over time, and can be extended over years, or even decades.

Pleiotropic effects Producing or having multiple effects from a single gene. Genes that affect multiple, apparently unrelated, phenotypes are thus called pleiotropic genes.

Polymorphism A DNA sequence variation that is common in the population. To be classed as a polymorphism, the least common allele must have a frequency of 1% or more in the population. The less frequent allele (A) of the Val158Met polymorphism has a frequency of 39% in Caucasian populations.

Single nucleotide polymorphism (SNP) The most common type of polymorphism in the human genome, where a single base (or nucleotide) differs between individuals. The Val158Met polymorphism at *COMT* gene is a single nucleotide substitution of the allele G for the allele, A at the exon 3 of the gene.

References

Abdolmaleky, H. M., Cheng, K. H., Faraone, S. V., Wilcox, M., Glatt, S. J., Gao, F., et al. (2006). Hypomethylation of MB-COMT promoter is a major risk factor for schizophrenia and bipolar disorder. *Human Molecular Genetics*, *15*(21), 3132–3145.

Akil, M., Kolachana, B. S., Rothmond, D. A., Hyde, T. M., Weinberger, D. R., & Kleinman, J. E. (2003). Catechol-O-methyltransferase genotype and dopamine regulation in the human brain. *Journal of Neuroscience*, *23*(6), 2008–2013.

Alemany, S., Arias, B., Fatjó-Vilas, M., Villa, H., Moya, J., Ibanez, M. I., et al. (2014). Psychosis-inducing effects of cannabis are related to both childhood abuse and COMT genotypes. *Acta Psychiatrica Scandinavica*, *129*, 54–62.

Batalla, A., Soriano-Mas, C., Lopez-Sola, M., Torrens, M., Crippa, J. A., Bhattacharyya, S., et al. (2014). Modulation of brain structure by catechol-O-methyltransferase Val(158) Met polymorphism in chronic cannabis users. *Addiction Biology*, *19*(4), 722–732.

Belsky, J., & Pluess, M. (2009). Beyond diathesis stress: differential susceptibility to environmental influences. *Psychology Bulletin*, *135*(6), 885–908.

Bossong, M. G., & Niesink, R. J. (2010). Adolescent brain maturation, the endogenous cannabinoid system and the neurobiology of cannabis-induced schizophrenia. *Progress in Neurobiology*, *92*(3), 370–385.

Bossong, M. G., Jansma, J. M., Bhattacharyya, S., & Ramsey, N. F. (2014). Role of the endocannabinoid system in brain functions relevant for schizophrenia: an overview of human challenge studies with cannabis or 9-tetrahydrocannabinol (THC). *Progress in Neuropsychopharmacology and Biological Psychiatry*, *52*, 53–69.

Caspi, A., Moffitt, T. E., Cannon, M., McClay, J., Murray, R., Harrington, H., et al. (2005). Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biological Psychiatry*, *57*(10), 1117–1127.

Chen, J., Lipska, B. K., Halim, N., Ma, Q. D., Matsumoto, M., Melhem, S., et al. (2004). Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *American Journal of Human Genetics*, *75*(5), 807–821.

Costas, J., Sanjuan, J., Ramos-Rios, R., Paz, E., Agra, S., Tolosa, A., et al. (2011). Interaction between COMT haplotypes and cannabis in schizophrenia: a case-only study in two samples from Spain. *Schizophrenia Research*, *127*(1–3), 22–27.

Cuyas, E., Verdejo-Garcia, A., Fagundo, A. B., Khymenets, O., Rodriguez, J., Cuenca, A., et al. (2011). The influence of genetic and environmental factors among MDMA users in cognitive performance. *PLoS One*, *6*(11), e27206.

De Sousa, K. R., Tiwari, A. K., Giuffra, D. E., Mackenzie, B., Zai, C. C., & Kennedy, J. L. (2013). Age at onset of schizophrenia: cannabis, COMT gene, and their interactions. *Schizophrenia Research*, *151*(1–3), 289–290.

Di Forti, M., Iyegbe, C., Sallis, H., Kolliakou, A., Falcone, M. A., Paparelli, A., et al. (2012). Confirmation that the AKT1 (rs2494732) genotype influences the risk of psychosis in cannabis users. *Biological Psychiatry*, *72*(10), 811–816.

Di Forti, M., Sallis, H., Allegri, F., Trotta, A., Ferraro, L., Stilo, S. A., et al. (2014). Daily use, especially of high-potency cannabis, drives the earlier onset of psychosis in cannabis users. *Schizophrenia Bulletin*, *40*(6), 1509–1517.

D'Souza, D. C., Sewell, R. A., & Ranganathan, M. (2009). Cannabis and psychosis/schizophrenia: human studies. *European Archives of Psychiatry and Clinical Neurosciences*, *259*(7), 413–431.

Duncan, L. E., & Keller, M. C. (2011). A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. *American Journal Psychiatry*, *168*(10), 1041–1049.

Egan, M. F., Goldberg, T. E., Kolachana, B. S., Callicott, J. H., Mazzanti, C. M., Straub, R. E., et al. (2001). Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(12), 6917–6922.

Estrada, G., Fatjó-Vilas, M., Munoz, M. J., Pulido, G., Minano, M. J., Toledo, E., et al. (2011). Cannabis use and age at onset of psychosis: further evidence of interaction with COMT Val158Met polymorphism. *Acta Psychiatrica Scandinavica*, *123*(6), 485–492.

Fan, J. B., Zhang, C. S., Gu, N. F., Li, X. W., Sun, W. W., Wang, H. Y., et al. (2005). Catechol-O-methyltransferase gene Val/Met functional polymorphism and risk of schizophrenia: a large-scale association study plus meta-analysis. *Biological Psychiatry*, *57*(2), 139–144.

Goldman, D., Oroszi, G., & Ducci, F. (2005). The genetics of addictions: uncovering the genes. *Nature Review Genetics*, *6*(7), 521–532.

Henquet, C., Murray, R., Linszen, D., & van Os, J. (2005). The environment and schizophrenia: the role of cannabis use. *Schizophrenia Bulletin*, *31*(3), 608–612.

Henquet, C., Rosa, A., Krabbendam, L., Papiol, S., Fananas, L., Drukker, M., et al. (2006). An experimental study of catechol-o-methyltransferase Val158Met moderation of delta-9-tetrahydrocannabinol-induced effects on psychosis and cognition. *Neuropsychopharmacology*, *31*(12), 2748–2757.

Henquet, C., Rosa, A., Delespaul, P., Papiol, S., Fananas, L., van Os, J., et al. (2009). COMT ValMet moderation of cannabis-induced psychosis: a momentary assessment study of 'switching on' hallucinations in the flow of daily life. *Acta Psychiatrica Scandinavica*, *119*(2), 156–160.

Houston, J. E., Murphy, J., Adamson, G., Stringer, M., & Shevlin, M. (2008). Childhood sexual abuse, early cannabis use, and psychosis: testing an interaction model based on the National Comorbidity Survey. *Schizophrenia Bulletin*, *34*(3), 580–585.

Kendler, K. S., Schmitt, E., Aggen, S. H., & Prescott, C. A. (2008). Genetic and environmental influences on alcohol, caffeine, cannabis, and nicotine use from early adolescence to middle adulthood. *Archives of General Psychiatry*, *65*(6), 674–682.

Lotta, T., Vidgren, J., Tilgmann, C., Ulmanen, I., Melen, K., Julkunen, I., et al. (1995). Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry*, *34*(13), 4202–4210.

Matsumoto, M., Weickert, C. S., Beltaifa, S., Kolachana, B., Chen, J., Hyde, T. M., et al. (2003). Catechol O-methyltransferase (COMT) mRNA expression in the dorsolateral prefrontal cortex of patients with schizophrenia. *Neuropsychopharmacology*, *28*(8), 1521–1530.

Melis, M., Muntoni, A. L., & Pistis, M. (2012). Endocannabinoids and the processing of value-related signals. *Frontiers in Pharmacology*, *3*, 7.

Meyer-Lindenberg, A., Kohn, P. D., Kolachana, B., Kippenhan, S., McInerney-Leo, A., Nussbaum, R., et al. (2005). Midbrain dopamine and prefrontal function in humans: interaction and modulation by COMT genotype. *Nature Neuroscience*, *8*(5), 594–596.

Munafo, M. R., Bowes, L., Clark, T. G., & Flint, J. (2005). Lack of association of the COMT (Val158/108 Met) gene and schizophrenia: a meta-analysis of case-control studies. *Molecular Psychiatry*, *10*(8), 765–770.

Myin-Germeys, I., van Os, J., Schwartz, J. E., Stone, A. A., & Delespaul, P. A. (2001). Emotional reactivity to daily life stress in psychosis. *Archives of General Psychiatry*, *58*(12), 1137–1144.

Pelayo-Teran, J. M., Perez-Iglesias, R., Mata, I., Carrasco-Marin, E., Vazquez-Barquero, J. L., & Crespo-Facorro, B. (2010). Catechol-O-Methyltransferase (COMT) Val158Met variations and cannabis use in first-episode non-affective psychosis: clinical-onset implications. *Psychiatry Research*, *179*(3), 291–296.

Pomarol-Clotet, E., Fatjo-Vilas, M., McKenna, P. J., Monte, G. C., Sarro, S., Ortiz-Gil, J., et al. (2010). COMT Val158Met polymorphism in relation to activation and de-activation in the prefrontal cortex: a study in patients with schizophrenia and healthy subjects. *Neuroimage*, *53*(3), 899–907.

Power, R. A., Verweij, K. J. H., Zuhair, M., Montgomery, G. W., Henders, A. K., Heath, A. C., et al. (2014). Genetic predisposition to schizophrenia associated with increased use of cannabis. *Molecular Psychiatry*, *19*, 1201–1204.

Schneider, M., & Koch, M. (2003). Chronic pubertal, but not adult chronic cannabinoid treatment impairs sensorimotor gating, recognition memory, and the performance in a progressive ratio task in adult rats. *Neuropsychopharmacology*, *28*(10), 1760–1769.

Smolka, M. N., Schumann, G., Wrase, J., Grusser, S. M., Flor, H., Mann, K., et al. (2005). Catechol-O-methyltransferase val158met genotype affects processing of emotional stimuli in the amygdala and prefrontal cortex. *Journal of Neuroscience*, *25*(4), 836–842.

Solowij, N., Fernandez, F., Murray, R., & Yücel, M. (2012). Genetic modulation of the long-term effects of cannabis on brain structure, function and symptomatology. *Schizophrenia Research*, *136*(Suppl. 1), S134.

Sundram, S. (2006). Cannabis and neurodevelopment: implications for psychiatric disorders. *Human Psychopharmacology*, *21*(4), 245–254.

Tan, H. Y., Chen, A. G., Chen, Q., Browne, L. B., Verchinski, B., Kolachana, B., et al. (2012). Epistatic interactions of AKT1 on human medial temporal lobe biology and pharmacogenetic implications. *Molecular Psychiatry*, *17*(10), 1007–1016.

van der Knaap, L. J., Schaefer, J. M., Franken, I. H., Verhulst, F. C., van Oort, F. V., & Riese, H. (2014). Catechol-O-methyltransferase gene methylation and substance use in adolescents: the TRAILS study. *Genes, Brain and Behavior*, *13*(7), 618–625.

van Os, J., & Marcelis, M. (1998). The ecogenetics of schizophrenia: a review. *Schizophrenia Research*, *32*(2), 127–135.

van Os, J., Rutten, B. P., & Poulton, R. (2008). Gene-environment interactions in schizophrenia: review of epidemiological findings and future directions. *Schizophrenia Bulletin*, *34*(6), 1066–1082.

van Winkel, R. (2011). Family-based analysis of genetic variation underlying psychosis-inducing effects of cannabis: sibling analysis and proband follow-up. *Archives of General Psychiatry*, *68*(2), 148–157.

van Winkel, R., Henquet, C., Rosa, A., Papiol, S., Fananas, L., De Hert, M., et al. (2008). Evidence that the COMT(Val158Met) polymorphism moderates sensitivity to stress in psychosis: an experience-sampling study. *American Journal of Medical Genetics, Part B Neuropsychiatric Genetics*, *147B*(1), 10–17.

van Winkel, R., van Beveren, N. J., & Simons, C. (2011). AKT1 moderation of cannabis-induced cognitive alterations in psychotic disorder. *Neuropsychopharmacology*, *36*(12), 2529–2537.

Verdejo-Garcia, A., Fagundo, A. B., Cuenca, A., Rodriguez, J., Cuyas, E., Langohr, K., et al. (2013). COMT val158met and 5-HTTLPR genetic polymorphisms moderate executive control in cannabis users. *Neuropsychopharmacology*, *38*(8), 1598–1606.

Vinkers, C. H., Van Gastel, W. A., Schubart, C. D., Van Eijk, K. R., Luykx, J. J., van Winkel, R., et al. (2013). The effect of childhood maltreatment and cannabis use on adult psychotic symptoms is modified by the COMT Val(1)(5)(8)Met polymorphism. *Schizophrenia Research*, *150*(1), 303–311.

Wong, M. Y., Day, N. E., Luan, J. A., Chan, K. P., & Wareham, N. J. (2003). The detection of gene-environment interaction for continuous traits: should we deal with measurement error by bigger studies or better measurement? *International Journal of Epidemiology*, *32*(1), 51–57.

Zammit, S., Spurlock, G., Williams, H., Norton, N., Williams, N., O'Donovan, M. C., et al. (2007). Genotype effects of CHRNA7, CNR1 and COMT in schizophrenia: interactions with tobacco and cannabis use. *British Journal of Psychiatry*, *191*, 402–407.

Zammit, S., Owen, M. J., Evans, J., Heron, J., & Lewis, G. (2011). Cannabis, COMT and psychotic experiences. *British Journal of Psychiatry*, *199*(5), 380–385.

CHAPTER

e5

Neuroimaging and Genetics of the Acute and Chronic Effects of Cannabis

*R. Martín-Santos**, *J.A. de Souza Crippa***, *S. Bhattacharyya*†

Department of Psychiatry and Psychology, Hospital Clinic, IDIBAPS, University of Barcelona, CIBERSAM, Barcelona, Spain

****Department of Neuroscience and Behaviour, Hospital das Clinicas, University of Sao Paulo, Ribeirao Preto (Sao Paulo), Brazil**

†**Department of Psychosis Studies, King's College London, Institute of Psychiatry, Psychology & Neuroscience, London, United Kingdom**

SUMMARY POINTS

- This chapter gives an overview of neuroimaging studies of the acute and chronic effects of cannabis on the brain, and also preliminary evidence of the genetic modulation of these effects.
- Cannabinoids showed an acute increase in resting state brain activity in areas rich in CB1R, and altered neural activity during the performance of several cognitive tasks.
- Pharmacological challenge studies involving acute administration of delta-9-THC and CBD showed opposite patterns of brain activation during several cognitive tasks (response inhibition, emotional processing, sensory and salience processing) consistent with their opposite symptomatic effects.
- Chronic cannabinoid use has been associated with gray matter volume reduction in the hippocampus, amygdala, prefrontal and cerebellum, and with the amount of cannabinoid exposure; adolescents showed similar alterations to adults, with preliminary evidence indicating gender differences in sensitivity to these effects.
- Chronic cannabis use has been associated with reduced resting state activation, both globally and in areas rich in CB1R, and altered neural activity during several cognitive tasks, even in the absence of unaltered performance. However, the studies reviewed reveal a great degree of heterogeneity.
- Preliminary data suggest that both the acute and chronic effects of cannabinoids on the brain may be genetically modulated. This may be more important during the neurodevelopment period, and may explain interindividual differences in the effects of cannabis.
- Limitations of current neuroimaging research include the high heterogeneity of studies published, methodological aspects, and the small number of longitudinal studies.

KEY FACTS FOR NEUROIMAGING STUDIES OF ACUTE AND CHRONIC CANNABIS EFFECTS

- Cannabis is the world's most commonly used illicit drug, mainly in young adulthood.
- There is a growing interest in its relationship with mental health.
- Neuroimaging tools offer an opportunity to study in vivo the acute effects of two main compounds of cannabis, delta-9-tetrahydrocannabinol (delta-9-THC) and cannabidiol (CBD), in both healthy volunteers and occasional cannabis users.
- Imaging research into the chronic effects of cannabis show both structural and functional brain impairment.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00040-5
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

AKT1	V-akt murine thymoma viral oncogene homolog 1 gene
BOLD	Blood oxygen level-dependent signal
CB1R	Cannabinoid receptor-1
CB2R	Cannabinoid receptor-2
CBD	Cannabidiol
CBF	Cerebral blood flow
COMT	Catechol-O-methyltransferase gene
DAT1	Dopamine transporter gene
Delta-9-THC	Δ^9-Tetrahydrocannabinol
DTI	Diffusion tensor imaging
eCB	Endocannabinoid
FDG	Fludeoxyglucose
fMRI	Functional magnetic resonance imaging
GABA	Gamma-aminobutyric acid
PET	Positron emission tomography
PRISMA	Preferred reporting items for systematic reviews and metaanalyses
rCBF	Regional cerebral blood flow
ROI	Region of interest
SNP	Single nucleotide polymorphism
SPECT	Single photon emission tomography
VBM	Voxel-based morphometry

INTRODUCTION

Cannabis is the world's most widely available and most commonly used illicit drug. In Europe, approximately 20 million adults—around 6% of the population aged 15–64 years—are reported to have used the drug in the previous year (EMCDDA, 2013).

Even though many individuals discontinue cannabis use after initial experimentation, the percentage of long-term dependence is around 9% (EMCDDA, 2013). There is a consistent association and a dose-response relationship between the frequency of adolescent cannabis use and adverse health outcomes in young adults (Horwood et al., 2014).

Cannabis use may be associated acutely with effects such as anxiety and panic attacks (especially in first-time users), perception abnormalities, psychotic symptoms at higher doses (or in vulnerable individuals), learning and memory impairment, and road crashes if driving in an intoxicated state (Ramaekers et al., 2004; Hall & Degenhardt, 2009). It has also been associated with neurocognitive alterations, and deficits such as memory impairments, in some (though not all) studies, as well as increased risk of depression and psychotic symptoms and disorders (Bolla et al., 2002; Moore et al., 2007; Schreiner & Dunn, 2012).

However, the neural mechanisms underlying these effects have not been clearly identified. The extract of the *Cannabis sativa* plant has over 60 different cannabinoids (Mechoulam & Gaoni, 1967). Δ^9-Tetrahydrocannabinol (delta-9-THC) is its main psychoactive constituent, and is responsible for most of the drug's psychotropic effects, and cannabidiol (CBD) has been found to induce anxiolytic (Crippa et al., 2004), antipsychotic (Zuardi et al., 2012), and neuroprotective effects (Perez et al., 2013). In the last decade, a tendency for strains with high proportions of delta-9-THC and low proportions of CBD has been noted, a finding that may have clinical and neuroanatomical consequences.

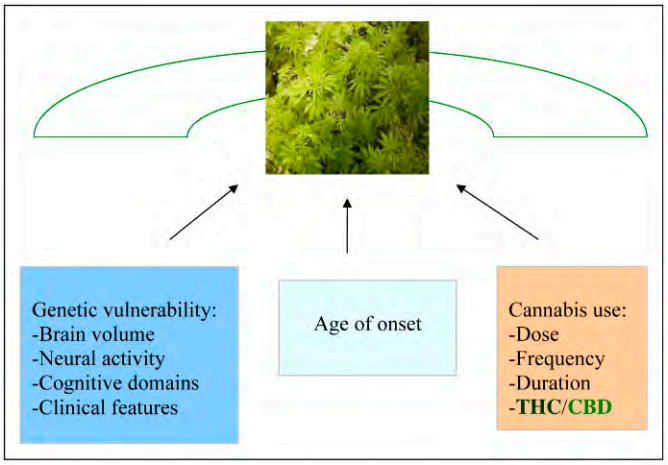


FIGURE e5.1 **Neurodevelopment influences on brain cannabis effects.**

Cannabinoids exert their effects through the endocannabinoid system (eCB), which can modulate the neuronal activity of other neurotransmitter systems such as dopamine, glutamate, or GABA through its action on the most abundant cannabinoid receptor in the brain, CB1. High densities of CB1R are found in the prefrontal cortex, basal ganglia, medial temporal areas and cerebellum (Mechoulam & Parker, 2013). The effect of CB1R activation on neurotransmitter release in these areas is critical for various cognitive processes and for reward, motor function, and psychiatric symptoms. CB2R are found mainly in hematopoietic and immune cells, but are also present in the brain (Onaive, 2006). The eCB system plays an important role in neuromaturation and synaptic pruning, and controls proliferation and neurogenesis in the hippocampus (Fogaça, Galve-Roperh, Guimaraes, & Campos, 2013). During adolescence, the brain may be more vulnerable to disturbances from exogenous cannabinoids which may alter normal brain functioning, as studies with animal models have shown (Maldonado et al., 2011) (Fig. e5.1).

Lastly, cannabis-induced impairment may depend on factors such as gender, age of onset, quantity, frequency and duration of use, comorbidity, as well as genetic aspects (Batalla et al., 2014a).

In this chapter, we review the main results of neuroimaging studies of the effects on the brain of acute and chronic cannabis use, and assess the preliminary evidence of genetic modulation of these effects. We discuss the methodological limitations of the existing evidence, and suggest some directions for future research in the field.

ACUTE EFFECTS OF CANNABINOIDS ON BRAIN FUNCTION

Systematic reviews of the acute effects of cannabinoids on brain functions (Martín-Santos et al., 2010; Batalla et al., 2014b; reviews) in cannabinoid-naïve individuals and infrequent users have provided consistent findings.

During Resting State

After Acute Administration of Delta-9-THC or CBD

Acute administration of cannabinoids has been shown to increase cerebral blood flow in CB1R-rich brain areas during baseline brain perfusion. These areas are involved in the reward pathway, and several other cognitive processes (O'Leary et al., 2003). Measuring baseline perfusion and resting state BOLD signal (fMRI) brain activity in the same group, van Hell et al. (2010) indicated that brain activity was similar in delta-9-THC and placebo in areas with different cerebral blood flows (CBF).

These changes in CBF have been associated with acute symptoms of delta-9-THC use, such as increased anxiety (Mathew & Wilson, 1993), depersonalization and time perception (Mathew et al., 1999), intoxication levels, and feeling "high" effects (van Hell et al., 2010).

The acute effect of CBD on CBF in the resting state has also been investigated in naïve cannabis users. CBD was found to modulate resting brain activity in limbic and paralimbic cortical areas (Crippa et al., 2004), a finding consistent with their anxiolytic clinical effect (Fig. e5.2).

During Cognitive Tasks

In the last decade, researchers have combined pharmacological challenges with cannabinoids and neuroimaging to investigate the neural mechanisms underlying their acute effects on cognition and emotional processing, but also to shed light on psychiatric symptomatology and disorders. Using fMRI or SPECT neuroimaging techniques, the acute experimental administration of cannabis to naïve or occasional cannabis users, during the performance of cognitive tasks such as emotion processing or the inhibitory response, has shown alterations in neural activity (Table e5.1).

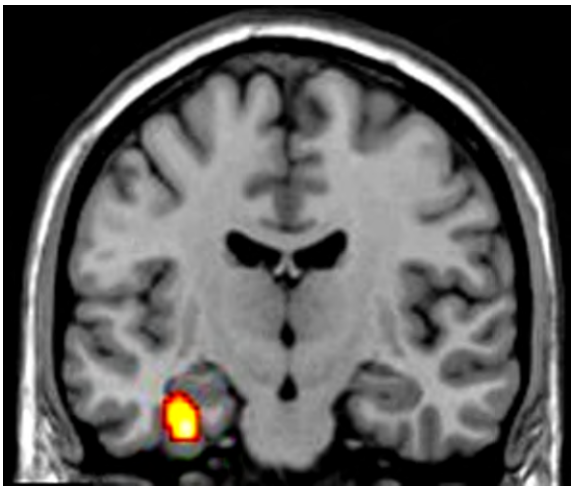


FIGURE e5.2 The brain region where there was significantly increased regional cerebral blood flow (rCBF) located in the left parahippocampal gyrus in healthy volunteers (n = 10) during 400 mg oral cannabidiol (CBD) administration versus placebo has been overlaid on coronal sections (−13, y-axis) *(Crippa et al., 2004)*.

Acute Administration of Delta-9-THC/CBD/Placebo Condition or Delta-9-THC/Placebo Condition

MEMORY

One of the first fMRI studies to apply a learning task involving the repeated presentation of verbal stimuli demonstrated that the impairment in learning and memory induced by cannabis may be mediated by its effects on medial temporal and prefrontal function (Bhattacharyya et al., 2009). In that study, which used a randomized, double-blind, placebo-controlled, crossover design, 10 mg of delta-9-THC was administered in a single oral dose versus 600 mg of CBD. The study also showed that the acute induction of psychotic symptomatology by delta-9-THC was related to its effects on the striatal function.

A second fMRI experimental study (Bossong et al., 2012a), using a randomized, placebo-controlled crossover design but with 9 mg of delta-9-THC administered through the inhalation route, investigated the effects of cannabis on associative memory using a task with pictorial stimuli. These authors reported attenuated activity of the insula, right inferior frontal gyrus, and the left side of the middle occipital gyrus during the encoding condition, and an increase in the activity of the cuneus and precuneus under the effect of delta-9-THC. Task performance was not modified by the administration of delta-9-THC, compared to placebo, in either study. However, using a working memory task, the same authors (Bossong et al., 2012b) found a decline in cognitive performance under the experimental administration of delta-9-THC, with increased activity in brain areas related to working memory, such as the dorsolateral prefrontal, inferior temporal and parietal cortices.

TABLE e5.1 Neuroimaging Studies of Acute Administration of THC and CBD in Naïve Cannabis Users

Author (year)	Type study	Design	Results
RESTING STATE			
Crippa et al. (2004)	90mTC-ECD-SPECT	CBD 400 mg versus placebo	*CBD versus placebo* Decrease rCBF L amygdala, hipoccapus, hypothalamus; posterior cingulate gyrus Increase rCBF L parahypocampus and fusiforms gyrus
fMRI			
Borgwardt et al. (2008)	fMRI 1.5 T Response inhibition task	THC 10 mg versus CBD 600 mg versus placebo	*THC versus placebo* Decrease BOLD R inferior frontal gyrus; L/R anterior cinglated cortex, and precuneus Increase BOLD R hippocampus, transverse temporal and fusiform gyrus, parahippocampus and caudate; L precuneus and prefrontal cortex *CBD versus placebo* Decrease BOLD L insula and superior and transverse temporal gyrus
Fusar-Poli et al. (2009)	fMRI 1.5 T Affective stimuli	THC 10 mg versus CBD 600 mg versus placebo	*THC versus placebo* Neutral face: increase BOLD posterior temporal and L inferior parietal lobule Mildly fearful face: increase BOLD R inferior parietal lobule and decrease BOLD inferior frontal gyrus Intensely fearful face: decrease BOLD L/R frontal gyrus, Pcgyrus; increase BOLD L precuneus, L/R primary sensorimotor cortex *CBD versus placebo* Neutral face: no brain activation differences Mildly fearful face: decrease BOLD L7R posterior lobe cerebellum Intensely fearful face: decrease BOLD amygdala, anterior parahippocampus, anterior and posterior cyngulate gyrus, L occipital gyrus, R posterior lobe cerebellum
Bhattacharyya et al. (2009)	fMRI 1.5 T Verbal paired associated task	THC 10 mg versus CBD 600 mg versus placebo	*THC versus placebo* Encoding block 1: decrease BOLD parahippocampus, 2 & 3: increase BOLD parahippocampus Recall block 1 & 2: no brain activation differences *CBD versus placebo* No brain activation differences
Winton-Brown et al. (2011)	fMRI 1.5 T Auditory and visual stimuli task	THC 10 mg versus CBD 600 mg versus placebo	*THC versus placebo* Auditory: decrease BOLD L/R anterior and posterior superios and middle temporal gyrus, insula, supramarginal gyrus, R inferior frontal gyrus and L cerebellum Visual: decrease BOLD L/R extrastriate visual cortex, increase BOLD R lingual and occipital gyrus, L lingual and fusiform gyrus *CBD versus placebo* Auditory: increase BOLD L/R temporal cortex, insula, parahipocampus and hippocampus, decrease BOLD L superior temporal gyrus, insula, posterior temporal, and supramarginal gyrus Visual: increase BOLD R middle and inferior occipital gyrus, lingual gyrus, and cuneus
Bhattacharyya et al. (2012b)	fMRI 1.5 T Salience stimuli task	THC 10 mg versus CBD 600 mg versus placebo	*THC versus placebo* Increase BOLD R prefrontal cortex, orbitofrontal cortex, and frontal lobe; decrease BOLD R head of caudate, putamen, insula and thalamus *CBD versus placebo* Decrease BOLD R prefrontal cortex; increase BOLD L caudate, parahippocampus, insula, precentral gyrus, and thalamus *THC versus CBD* Increase BOLD R orbitofrontal cortex and decrease BOLD L parahippocampus, caudate, putamen, thalamus, and lingual gyrus
Bhattacharyya et al. (2015)	fMRI 1.5 T Go-No-Go	THC 10 mg versus placebo	*THC versus placebo* During the response inhibition condition (No-Go minus Oddball trials) THC was associated to a decrease in BOLD L inferior frontal gyrus, insula and L precuneus, and increase BOLD hippocampus and caudate nucleus

THC, delta-9-tetrahydrocannabinol; *CBD*, cannabidiol; *BOLD*, blood oxygenation-level dependence; *D*, right hemisphere; *fMRI*, functional magnetic resonance imaging; *L*, left hemisphere; *rCBF*, regional cerebral blood flow; *90mTC-ECD-SPECT*, etyl-cesteinate-dimer labeled with technetium-99m, single-photon emission computed tomography.

These investigations provided evidence that the impairments in learning and memory induced by THC are mediated by its effect on medial temporal and prefrontal functioning.

Emotional Faces Processing Task

Acute use of cannabis has been associated with acute affective symptomatology (Martín-Santos et al., 2012; Crippa et al., 2009).

An initial fMRI study with a randomized, placebo-controlled, crossover design, in which 7.5 mg of delta-9-THC was administered orally to occasional cannabis users, found an attenuation of amygdala activation in response to threatening stimuli, under the effect of delta-9-THC (Phan et al., 2008). The authors suggested that these results were related to the anxiolytic effect of delta-9-THC.

Later, another study using a randomized, placebo-controlled, crossover design and oral route of drug administration investigated the neural effects of 10 mg of delta-9-THC versus 600 mg of CBD, during an emotional processing task (fearful faces) (Fusar-Poli et al., 2009). These authors reported an increase in engagement of the right inferior parietal lobule, and attenuation of engagement of the left medial frontal gyrus while viewing mildly fearful faces. When the naïve cannabis users were exposed to intensely fearful faces, delta-9-THC was found to increase the engagement of the left precuneus and primary sensorimotor cortex bilaterally, and to decrease the engagement of the middle frontal gyrus bilaterally, and in the posterior cingulate gyrus. These results were associated with increases in anxiety symptoms and in skin conductance response. However, no relationship was found with amygdala brain activity (Fusar-Poli et al., 2009).

Taken together, these results suggest the presence of a dose-response relationship between anxiety and delta-9-THC (Crippa et al., 2009). In contrast, CBD showed attenuated activation in the amygdala, and in the anterior and posterior cingulate cortices (Fusar-Poli et al., 2009), as well as in the skin conductance response, thus providing evidence of its anxiolytic role.

These effects of delta-9-THC and CBD while performing emotional processing tasks suggest a role of the eCB system in the modulation of emotion, and corroborate earlier findings in this field (Fogaça et al., 2013).

Response Inhibition Task

Cannabis use has been associated with impulsivity and impaired psychomotor control, resulting in risk behavior such as dangerous driving (Ramaekers et al., 2004). Few studies have examined the brain effects of acute cannabis use, or its main psychoactive component (delta-9-THC) on psychomotor control function under experimental conditions, using different paradigms. Those carried out to date have produced inconsistent results (Ramaekers et al., 2009; Borgwardt et al., 2008). CBD deactivated the left temporal cortex and insula, brain areas which are not involved in this cognitive process (Borgwardt et al., 2008).

Another adverse effect of cannabis use is the acute induction of psychotic symptoms, and the increased risk of schizophrenia following regular use. Impairment of inhibitory control and processing are known to occur under the influence of cannabis and schizophrenia. A recent fMRI investigation studied whether cannabis-induced impairment in inhibitory processing is related to the acute induction of psychotic symptoms (Bhattacharyya et al., 2015). Under experimental conditions, these authors administered delta-9-THC to 36 naïve cannabis users, in a study with a placebo-controlled, double blind, within-subjects design, and tracked their performance on a response inhibition (Go-No-Go) task using fMRI. Interestingly, they observed that, compared to placebo, delta-9-THC caused transient psychotic symptoms, anxiety, intoxication, and sedation, inhibition errors, and impaired inhibition efficiency. The severity of the psychotic symptoms was directly correlated with inhibition error frequency and, inversely, with inhibition efficiency under the influence of delta-9-THC. Moreover, the administration of delta-9-THC attenuated left inferior frontal activation, which was inversely correlated with the frequency of inhibition errors, and the severity of psychotic symptoms, and possibly with inhibition efficiency.

The results suggest that the impairment in the inhibitory control of thoughts, behaviors, and emotions, and the altered inferior frontal function observed under the influence of cannabis may have a role in the transient psychotic symptoms associated with its use.

Sensory Processing Task

As mentioned earlier, acute use of cannabis can lead to sensory–perceptual alterations similar to those experienced during psychotic episodes. This effect of cannabis has also been studied with fMRI (Winton-Brown et al., 2011), using an auditory–visual processing task in naïve cannabis users.

Compared with placebo, delta-9-THC attenuated activation bilaterally in the anterior and posterior superior temporal gyri and middle temporal gyrus, the insulae and in the supramarginal gyri, and in the right inferior frontal gyrus and left cerebellum during auditory processing. Attenuated temporal cortical activation was correlated with psychotic symptomatology. CBD showed the opposite effect to delta-9-THC, for temporal cortical activation. Under the visual processing condition, compared with placebo, delta-9-THC attenuated activation in the extrastriate visual cortex and enhanced activation in lingual and middle occipital gyri (primary visual cortex), on the right side and parts of the lingual and fusiform gyri, extending anteriorly on the left side. Increased

activation in the primary visual cortex was correlated with the severity of psychotic symptomatology.

Attentional Salience Processing

Attentional salience processing has been implicated in schizophrenia, in which aberrant salience attribution has been associated with the presence of psychotic symptoms, such as delusions. Due to this, and to the association between chronic cannabis use and the increased risk of schizophrenia and worse outcome (Moore et al., 2007), the acute effects of 10 mg oral delta-9-THC versus CBD were investigated in a sample of naïve cannabis users using a visual oddball detection task (Bhattacharyya et al., 2012b). Compared with placebo, delta-9-THC attenuated activation in the right caudate, but increased activation in the right prefrontal cortex, including the inferior frontal gyrus during the processing of salient, relative to nonsalient, oddball stimuli. Delta-9-THC also reduced the response latency of salient and nonsalient stimuli, though the effect was greater on the nonsalient stimuli. This may suggest that, under the influence of delta-9-THC, insignificant stimuli became significant. Moreover, the effect of delta-9-THC on striatal activation was inversely correlated to the severity of the psychotic symptomatology it induced. Striatal and lateral prefrontal function are altered during salience processing in psychosis (Juckel et al., 2006), and in drug-induced psychotic states (Roiser, 2010). The study provided evidence that the effect of cannabis use on the risk of psychosis might be mediated by its effect on attentional salience processing.

CBD showed an opposite pattern of activation to placebo. It attenuated activation in the prefrontal cortex on the right side, but increased activation in the left caudate and putamen, and in the left parahippocampal gyrus, lingual gyrus, and thalamus.

Genetic Vulnerability to the Acute Effects of Cannabis

Several studies have shown the genetic modulation of brain activation under cognitive tasks such as response inhibition (Bhattacharyya et al., 2014), and verbal learning (Bhattacharyya et al., 2012a), after a single 10 mg oral dose of delta-9-THC.

Verbal Learning Task

While cannabis use may induce acute psychotic symptoms, increase the long-term risk of schizophrenia, and worsen outcome, as we stated previously, there is marked interindividual variability in the susceptibility to these effects. This variability probably has a genetic basis (Fig. e5.1).

Induction of psychotic symptoms by delta-9-THC is related to its effect on the striatum, a brain region rich in dopaminergic terminals. The marked interindividual variation in the psychotic effects of cannabis may therefore reflect differences in genes that influence the transmission or metabolism of brain dopamine. A preliminary study (Bhattacharyya et al., 2012a) tested the effect of variation in two candidate genes altering central dopaminergic neurotransmission on the symptomatic and neurophysiological response to delta-9-THC: the DAT1 gene, which codes for the dopamine transporter that removes dopamine from central synapses (Dreher et al., 2009), and the protein kinase B AKT1, an integral component of the dopamine signaling cascade (Beaulieu, Gainetdinov, & Caron, 2007).

In an fMRI study with a double-blind, repeated measure, placebo-controlled, within-subject design, the authors examined the ability of the DAT 1 3UTR VNTR and AKI 1 rs1130233 polymorphisms to modulate the acute effects of delta-9-THC on psychotic symptoms and regional brain activation, in 35 healthy volunteers with minimal experience of cannabis use (<25 times/life) (Bhattacharyya et al., 2012a). The authors found that individuals carrying the risk variants of either the DAT1 30 UTR VNTR or the AKI1 rs1130233 polymorphism have an increased sensitivity to the psychotomimetic effects of delta-9-THC, and that the sensitivity was especially high in subjects who carried both these variants. Moreover, the more delta-9-THC attenuated the striatal and midbrain activation, the greater the severity of the psychotic symptoms.

The results showed that the acute psychotogenic effects of cannabis were moderated by two genes that code for proteins which influence dopamine neurotransmission, and modulate the effects of delta-9-THC on midbrain and striatal function.

Response Inhibition Task (Go, No-Go Task) and AKT1 Polymorphism

Another recent fMRI study using a double-blind, repeated-measure design investigated the genetic and neural basis of variable sensitivity to cannabis-induced behavioral dyscontrol in healthy occasional cannabis users during a response inhibition task (Bhattacharyya et al., 2014). Results showed that carriers of the A allele at rs1130233 of AKT1, a protein kinase B (AKT1) which influences central dopaminergic neurotransmission, have a significantly greater risk of impairment in the control of prepotent response under the influence of delta-9-THC. In carriers of the A allele, associated with reduced AKT1 expression (Blasi et al., 2011), and impaired prefrontal executive cognition (Tan et al., 2008), administration of delta-9-THC was associated with a more than twofold increase in the frequency of impaired inhibitory control, and concomitant attenuation of the engagement of the inferior frontal gyrus, the principal neural

substrate for inhibitory control. Furthermore, there was a direct relationship between the effects of delta-9-THC on psychomotor control behavior, and the physiological response of its neural substrate, in that the more delta-9-THC attenuated inferior frontal engagement, the greater the risk of errors of inhibition.

The results of this preliminary study suggest that variation in a gene coding for a protein which influences central dopaminergic neurotransmission may moderate the sensitivity to the acute impairments in psychomotor control induced by use of delta-9-THC.

As we noted previously, cannabis is one of the psychoactive substances most frequently associated with road-traffic accidents (Ramaekers et al., 2004). Although the act of driving involves a complex cognitive process, the results of the study suggest that this effect has a genetic basis related to dopaminergic signaling in the brain. This may help to explain why certain persons are more sensitive to the effects of cannabis on psychomotor control.

Acute Effects on Dopamine Release

Some publications have used functional neurochemical imaging to measure dopamine in cannabis users after acute administration of delta-9-THC with ^{11}C raclopride PET (Bossong et al., 2009; Stokes et al., 2009), and with 123 IBZM SPECT (Barkus et al., 2011). The first study found a decrease in ^{11}C raclopride binding in the ventral striatum and precommisural dorsal putamen, after delta-9-THC inhalation, reflecting an increase in dopamine in these regions. The second study with a large sample of subjects did not find significant differences in ^{11}C raclopride striatal binding with oral delta-9-THC, compared with placebo. Lastly, using ^{123}IBZM SPECT, differences in radioligand binding in caudate or putamen were found under delta-9-THC, compared with placebo. Future studies should explore this area further.

CHRONIC EFFECTS OF CANNABIS USE ON BRAIN STRUCTURE AND FUNCTION

Structural Effects

Specific regional brain analyses demonstrated evidence of structural abnormalities in adult chronic cannabis users, compared with healthy controls. The abstinence period for subjects before undergoing structural MRI was between 12 and 24 h in most studies (Martín-Santos et al., 2010; Batalla et al., 2013 reviews). The most consistently reported brain alteration was reduced hippocampal volume (Lorenzetti et al., 2015). This alteration may persist after several months of abstinence, and may be related to the amount of cannabis used. Morphological alterations were also found in the amygdala (Ashtari et al., 2011), the cerebellum (Cousijn et al., 2012), and the frontal cortex (Matochik et al., 2005). Moreover, some diffusion tensor imaging (DTI) studies have found differences in the mean diffusivity or fractional anisotropy in the corpus callosum and the frontal white matter fiber tract (Arnone et al., 2008; Gruber, Silveri, Dahlgren, & Yurgelun-Todd, 2011). It may be that cannabis also alters white matter structural integrity. More studies are needed in this area (Table e5.2).

On the other hand, the few studies done in adolescents (Ashtari et al., 2011; Medina et al., 2009; Medina, Nagel, & Tapert, 2010; McQueeny et al., 2011) suggest that the effect of chronic cannabis use may appear soon after initiation, may persist after a month of abstinence, and may be moderated by gender. It has been suggested that adolescent females may be at a higher risk of morphological effects than males (McQueeny et al., 2011). These results need to be replicated in further studies.

These effects of chronic cannabis use on brain structure are consistent with those found in studies showing similar alterations in schizophrenia which reported volume reductions of up to 6% in the hippocampus and the amygdala, in schizophrenic patients, particularly during the first years of the illness (Andreasen et al., 2011). Moreover, cannabis misuse in schizophrenia has been associated with a more pronounced loss of gray matter volume than in nonusers (Rais et al., 2010), suggesting that individuals with schizophrenia may be particularly vulnerable to cannabis exposure.

Functional Neuroimaging Studies

Resting State

Using various imaging methods ($H2^{15}O$-PET, ^{133}Xe-SPECT, ^{18}F-FDG-PET, ^{11}C-raclopride PET and ^{18}F FMPEPd2), several studies have evaluated rCBF in resting state in abstinent adult chronic cannabis users (Martín-Santos et al., 2010; Batalla et al., 2013; reviews). The abstinence period before the imaging test was between 12 h and 542 days, depending on the study. These studies showed that, in the global resting state, areas with high concentrations of CB1R (the prefrontal cortex and cerebellar and striatal regions) showed a lower blood flow, compared to healthy controls. These results may indicate a downregulation of CB1R in chronic cannabis users, which are area-specific and reversible in the neocortex and limbic areas (Hirvonen et al., 2012). Furthermore, the resting state findings are consistent with the impairments in attention and working memory (Solowij & Battisti, 2008), decision-making (Bechara et al., 2001), and psychomotor control (Bolla et al., 2002) described in chronic cannabis users. Additional studies are needed to investigate the causes of these results.

TABLE e5.2 Structural Neuroimaging Studies in Chronic Adult Cannabis Users Versus Nonusers Controls[a]

Author (year)	Type study	Results
MRI		
Block et al. (2000)	MRI 1.5 T	*VBM*: no differences between chronic cannabis users and controls
Tzilos et al. (2005)	MRI 1.5 T	*ROI analysis*: negative results
Matochik et al. (2005)	MRI 1.5 T	*VBM*: lower GM density in the R parahippocampus and in the precentral gyrus and R thalamus in cannabis users, compared to controls *ROI analysis*: decreased in hippocampus L/R
Jager et al. (2007)[b]	MRI 1.5 T	*VBM*: no differences between chronic cannabis users and controls
Yücel et al. (2008)	MRI 1.5 T	*ROI analysis*: decrease in hippocampus L/R and amygdala R in chronic cannabis users versus controls
Ashtari et al. (2011)[c]	MRI 1.5 T	*ROI analysis*: decrease in hippocampus L/R in chronic cannabis users, compared to controls
Cousijn et al. (2012)	MRI 3 T	*VBM*: increased cerebellum in chronic cannabis users, compared to controls
Batalla et al. (2014a)	MRI 1.5T	*VBM*: increased in postcentral gyrus L in chronic cannabis users compared to controls
Lorenzetti et al. (2015)	MRI 3T	*ROI analysis*: smaller hippocampus and amygdala volume in chronic heavy cannabis users, compared to controls
DTI		
Gruber et al. (2005)	DTI 3 T	*VBM and ROI analysis*: negative results
DeLisi et al. (2006)	DTI 1.5 T	*VBM and ROI analysis*: negative results
Arnone et al. (2008)	DTI 1.5 T	*ROI analysis*: increased mean diffusivity in the corpus callosum in chronic cannabis users
Gruber et al. (2011)	DTI 3 T	*ROI analysis*: increased mean diffusivity in the R genus, and reduction in L frontal fractional anisotropy in chronic cannabis users

[a] *All chronic cannabis users studies were done after 12–24 h abstinence, except*
[b] *Jager et al. (2007) done after 7 days abstinence, and*
[c] *Study by Ashtari et al. (2011) that was done after 6.7 months abstinence*
D, Right hemisphere; *L*, left hemisphere; *VBM*, voxel-based morphometry; *ROI*, region of interest analysis; *DTI*, diffusion tensor.

Under Cognitive Tasks

Functional neuroimaging studies in chronic cannabis users comparing brain activation with healthy controls under different cognitive tasks (attention memory, inhibition and impulsivity, decision making, motor performance, and affective processing) indicated that chronic cannabis users showed an altered pattern of brain activity in the areas engaged in these cognitive processes (Block et al., 2002; Bolla, Eldreth, Matochik, & Cadet, 2005; Gruber et al., 2011; Jager et al., 2007; Wesley, Hanlon, & Porrino, 2011).

The fact that in most of the studies the level of performance on cognitive tasks was similar in chronic cannabis users and healthy controls indicated that these tasks engage similar brain areas. It has been suggested that, in chronic cannabis users, there is a process of neuroadaptation, possibly activating additional areas as a compensatory mechanism, in order to maintain normal cognitive performance. The few fMRI studies carried out in adolescents did not find differences with respect to adult in studies carried out during the performance of memory (Schweinsburg et al., 2010, 2011), inhibition, and impulsivity tasks (Tapert et al., 2007).

Genetic Vulnerability to Chronic Cannabis Use and its Effect on Brain Structure

In recent years, some studies have shown that genetic variation may play an important role in determining brain morphology. The *catechol-O-methyl-transferase* (*COMT*) gene has a functional polymorphism at codon 156 causing a valine (Val) to methionine (Met) substitution, resulting in three genotypes: Val/Val, Val/Met, and Met/Met. The enzymatic activity of Met/Met is 40% lower, indicating low levels of extrasynaptic dopamine. COMT has an important role in the prefrontal cortex, in subcortical regions such as the basal ganglia and medial temporal lobe, and in the cerebellum and spinal cord. It has been associated with modulation of brain adverse effects in chronic cannabis users (Solowij et al., 2012); the Val allele carrier seems to be more sensitive to the chronic and acute effects of delta-9-THC, especially in subjects at risk of psychosis (Estrada et al., 2011).

Recently, the modulation of the brain structure by the COMT polymorphism has been explored in a sample of early onset chronic cannabis users (Batalla et al., 2014a). The study provided evidence that the COMT genotype influenced the bilateral ventral caudate nucleus in

opposite directions, with more copies of the Val allele being associated with smaller volumes, in chronic cannabis users, and greater volumes in controls. The opposite pattern was found in the left amygdala. The results corroborate previous reports that neuroanatomical changes are associated with chronic cannabis use, and show, for the first time, that these changes may be modulated by the COMT genotype.

METHODOLOGICAL LIMITATIONS OF NEUROIMAGING STUDIES

Several systematic reviews have highlighted the major methodological differences between the studies included (Martín-Santos et al., 2010; Batalla et al., 2013, 2014b; Rochetti et al., 2013), a situation which has often limited the generalization of the results in the area, and hampered comparisons between them. The use of strict definitions (eg, naïve, or recreational/occasional), and predetermined selection criteria, as well as the application of PRISMA guidelines, is essential in order to reduce the heterogeneity. Some of the studies use different designs (for instance within-subject or between-group) and apply different neuroimaging techniques, and few studies apply a multimodal, complementary approach to the same sample; even fewer replicate previous methodologies and objectives, thus making comparisons particularly difficult.

The studies reviewed present several sociodemographic differences, for example, investigating only men in order to obtain a homogeneous sample, but losing information on what happens in the female brain (Crane, Schuster, Fusar-Poli, & Gonzalez, 2013). Patterns of cannabis use (eg, frequency of use, lifetime use) vary widely, as do administration routes (eg, smoking, oral, inhalation, intravenous), which have different pharmacokinetics (Martín-Santos et al., 2012). In nonexperimental studies, the use of diverse units to measure cannabis exposure (eg, joints, grams) may help to explain some discrepancies in the results.

Furthermore, comorbidity with other substance use disorders, and with other axis I/II mental disorders, may be very high in cannabis users. The use of specific structured clinical interviews for screening and diagnosis is also an important issue, and will help to study the cannabis user phenotype (Torrens et al., 2004).

Since the introduction of functional neuroimaging techniques, the question has arisen of whether the drug effect reflects an influence on cerebral blood flow rather than neural activity. In this regard, the results of a group of pharmacological challenges with delta-9-THC and CBD have shown that the same participant in different cognitive and emotional paradigms during fMRI underwent opposite effects of the same drug, in same brain areas, during the same scanning session, both with delta-9-THC and CBD (Bhattacharyya et al., 2012c). Moreover, some of the drug effects observed were similar to the results obtained by electrophysiological methods not related to the CBF (Morrison et al., 2011).

Ethical aspects are also important when studying the acute effects of a psychoactive substance which may induce dependence, and has been linked to psychiatric disorders. Careful participant selection, appropriate choice of screening and settings, adequate counseling, and provision of information and the scheduling of follow-up assessments will minimize the risk.

SOME POINTERS FOR FUTURE STUDIES

Future studies should consider the need to standardize methodology, to replicate known facts with greater methodological rigor, and to combine experimental designs and prospective large samples involving subjects of both genders, from adolescence to adulthood. Collaborative initiatives will allow the joint use of neuroimaging, genetic, psychiatry, and cognitive data from multiple sites around the world.

Finally, the eCB system is a promising target in the treatment of symptoms of psychiatric disorders such as schizophrenia, depression, and anxiety. More experimental studies using different cannabinoid compounds such as CBD should be carried out in the near future.

MINI-DICTIONARY

Functional Magnetic Resonance Imaging (fMRI) A neuroimaging technique for measuring brain activity. It detects changes in blood oxygenation and flow in response to neural activity.

Positron Emission Tomography (PET) A neuroimaging technique that uses trace amounts of short-lived radioactive material to map functional processes in the brain. Areas of high radioactivity are associated with brain activity.

Single Photon Emission Computed Tomography (SPECT) A functional neuroimaging technique that evaluates regional cerebral perfusion through the administration of a gamma-emitting radioisotope injection into the bloodstream. Cerebral blood flow is closely linked to neuronal activity.

Acknowledgments

This chapter has been funded in part by Spanish grants from the Plan Nacional sobre Drogas, Ministerio de Sanidad y Consumo: PNSD/2011/050(R.Martín-Santos); and the support of AGAUR, Catalonia Government SGR2014/1114(R. Martín-Santos), and INCT Translational Medicine (INCT-TM, CNPq), Brazil (J.A. Crippa); S. Bhattacharyya is the recipient of a Clinician Scientist award from the NIHR, United Kingdom; and J.A. Crippa is the recipient of a CNPq-Brazil productivity award. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health, UK.

The authors have no conflicts of interest to report with regard to the contents of this chapter.

References

Andreasen, N. C., Nopoulos, P., Magnotta, V., et al. (2011). Progressive brain change in schizophrenia: a prospective longitudinal study of first-episode schizophrenia. *Biological Psychiatry*, *70*, 672–679.

Arnone, D., Barrick, T. R., Chengappa, S., et al. (2008). Corpus callosum damage in heavy marijuana use: preliminary evidence from diffusion tensor tractography and tract-based spatial statistics. *Neuroimage*, *41*, 1067–1074.

Ashtari, M., Avants, B., Cyckowski, L., et al. (2011). Medial temporal structures and memory functions in adolescents with heavy cannabis use. *Journal of Psychiatry Research*, *45*, 1055–1066.

Barkus, E., Morrinson, P. D., Vulectic, D., et al. (2011). Does intravenous delta-9-tetrahydrocannabinol increase dopamine release? A SPECT study. *Journal of Psychopharmacology*, *25*, 1462–1468.

Batalla, A., Bhattacharyya, S., Yucel, M., et al. (2013). Structural and functional imaging studies in chronic cannabis users: a systematic review of adolescent and adult findings. *PLoS One*, *8*, e55821.

Batalla, A., Bhattacharyya, S., Yucel, M., et al. (2014a). Modulation of brain structure by catechol-O-methyltransferase Val159Met polymorphism in chronic cannabis users. *Addiction Biology*, *19*, 722–732.

Batalla, A., Crippa, J. A., Busatto, G. F., et al. (2014b). Neuroimaging studies of acute effects of THC and CBD in humans and animals: a systematic review. *Current Pharmaceutical Design*, *20*, 2168–2185.

Beaulieu, J. M., Gainetdinov, R. R., & Caron, M. G. (2007). The AKT-GSK-3 signalling cascade in the actions of dopamine. *Trends in Pharmacology Science*, *28*, 166–172.

Bechara, A., Dolan, S., Denburg, N., et al. (2001). Decision-making deficits, linked to a dysfunctional ventromedial prefrontal cortex, revealed in alcohol and stimulant abusers. *Neuropsychologia*, *39*, 376–389.

Bhattacharyya, S., Atakan, Z., Martín-Santos, R., et al. (2012a). Preliminary report of biological basis of sensitivity to the effects of cannabis on psychosis: AKT1 and DAT1 genotype modulates the effects of D-9-tetrahydrocannabinol on midbrain and striatal function. *Molecular Psychiatry*, *17*, 1152–1155.

Bhattacharyya, S., Atakan, Z., Martin-Santos, R., Crippa, J. A., & McGuire, P. K. (2012c). Neural mechanisms for the cannabinoid modulation of cognition and affect in man. A critical review of neuroimaging studies. *Current Pharmacology Design*, *18*, 5045–5054.

Bhattacharyya, S., Atakan, Z., Martín-Santos, R., et al. (2015). Impairment of inhibitory control processing related to acute psychotomimetic effects of cannabis. *European Neuropsychopharmacology*, *25*, 26–37.

Bhattacharyya, S., Crippa, J. A., Allen, P., et al. (2012b). Induction of psychosis by D-9-tetrahydrocannabinol reflects modulation of prefrontal and striatal function during attentional salience processing. *Archives of General Psychiatry*, *69*, 27–36.

Bhattacharyya, S., Fusar-Poli, P., Borgwardt, S., et al. (2009). Modulation of mediotemporal and ventrostriatal function in humans by Delta9-tetrahydrocannabinol: a neural basis for the effects of Cannabis sativa on learning and psychosis. *Archives of General Psychiatry*, *66*, 442–451.

Bhattacharyya, S., Iyegbe, C., Atakan, Z., et al. (2014). Protein kinase B (AKT1) genotype mediates sensitivity to cannabis-induced impairments in psychomotor control. *Psychological Medicine*, *44*, 3315–3328.

Blasi, G., Napolitano, F., Ursini, G., et al. (2011). DRD2/AKT1 interaction on D2 cmAMP independent signalling attentional processing and response to olanzapine treatment in schizophrenia. *Proceeding of the National Academy of Sciences of the United States of America*, *108*, 15158–15163.

Block, R. I., O'Leary, D. S., Ehrhardt, J. C., et al. (2000). Effects of frequent marijuana use on brain tissue volume and composition. *Neuroreport*, *11*, 491–496.

Block, R. I., O'Leary, D. S., Hichwa, R. D., et al. (2002). Effects of frequent marijuana use on memory-related regional cerebral blood flow. *Pharmacology Biochemistry and Behaviour*, *72*, 237–250.

Bolla, K. I., Brown, K., Eldreth, D., et al. (2002). Dose-related neurocognitive effects of marijuana use. *Neurology*, *59*, 1337–1343.

Bolla, K. I., Eldreth, D. A., Matochik, J. A., & Cadet, J. L. (2005). Neural substrates of faulty decision-making in abstinent marijuana users. *Neuroimage*, *26*, 480–492.

Borgwardt, S. J., Allen, P., Bhattacharyya, S., et al. (2008). Neural basis of Delta-9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. *Biological Psychiatry*, *64*, 966–973.

Bossong, M. G., Jager, G., van Hell, H. H., et al. (2012a). Effects of delta9-tetrahydrocannabinol administration on human encoding and recall memory function: a pharmacological fMRI study. *Journal of Cognitive Neuroscience*, *24*, 588–599.

Bossong, M. G., Jansma, J. M., van Hell, H. H., et al. (2012b). Effects of delta-9-tetrahydrocannabinol on human working memory function. *Biological Psychiatry*, *71*, 693–699.

Bossong, M. G., van Berckel, B. N., Boellard, R., et al. (2009). Delta-9-tetrahydrocanabinol induces dopamine release in the human striatum. *Neuropsychopharmacology*, *34*, 759–766.

Cousijn, J., Wiers, R. W., Ridderinkhof, K. R., et al. (2012). Grey matter alterations associated with cannabis use: results of a VBM study in heavy cannabis users and healthy controls. *Neuroimage*, *59*, 3845–3851.

Crane, N. A., Schuster, R. M., Fusar-Poli, P., & Gonzalez, R. (2013). Effects of cannabis on neurocognitive functioning: recent advances, neurodevelopmental influences, and sex differences. *Neuropsychological Review*, *23*, 117–137.

Crippa, J. A., Zuardi, A. W., Garrido, G. E., et al. (2004). Effects of cannabidiol (CBD) on regional cerebral blood flow. *Neuropsychopharmacology*, *29*, 417–426.

Crippa, J. A., Zuardi, A. W., Martín-Santos, R., et al. (2009). Cannabis and anxiety: a critical review of the evidence. *Human Psychopharmacology*, *24*, 515–523.

Delisi, L. E., Bertisch, H. C., Szulc, K. U., et al. (2006). A preliminary DTI study showing no brain structural change associated with adolescent cannabis use. *Harm Reduction Journal*, *3*, 17–22.

Dreher, J. C., Kohn, P., Kolachana, B., et al. (2009). Variation in dopamine genes influence responsivity of the human reward system. *Proceeding of the National Academy of Sciences of the United States of America*, *106*, 617–622.

EMCDDA. (2013). Perspectives on drugs. Characteristics of frequent and high-risk cannabis users. Lisbon, Portugal: EMCDDA. Available from www.emcdda.europa.eu/topics/pods/frequent-cannabis.users

Estrada, G., Fatjo-Vilas, M., Munoz, M. J., et al. (2011). Cannabis use and age at onset of psychosis: further evidence of interaction with COMT Val158Met polymorphism. *Acta Psychiatrica Scandinavica*, *123*, 485–492.

Fogaça, M. V., Galve-Roperh, I., Guimaraes, F. S., & Campos, A. C. (2013). Cannabinoids, neurogenesis and antidepressant drugs: is there a link? *Current Neuropharmacology*, *11*, 263–275.

Fusar-Poli, P., Crippa, J. A., Bhattacharyya, S., et al. (2009). Distinct effects of {delta}9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Archives of General Psychiatry*, *66*, 95–105.

Gruber, S. A., Silveri, M. M., Dahlgren, M. K., & Yurgelun-Todd, D. (2011). Why so impulsive? White matter alterations are associated with impulsivity in chronic marijuana smokers. *Experimental and Clinical Psychopharmacology*, *19*, 231–242.

Gruber, S. A., & Yurgelun-Todd, D. A. (2005). Neuroimaging of marijuana smokers during inhibitory processing: a pilot investigacion. *Brain Research and Cognitive*, *23*, 107–118.

Hall, W., & Degenhardt, L. (2009). Adverse health effects of non-medical cannabis use. *Lancet*, *374*, 1383–1391.

Hirvonen, J., Goodwin, R. S., Li, C. T., et al. (2012). Reversible and regionally selective downregulation of brain cannabinoid CB(1) receptors in chronic daily cannabis smokers. *Molecular Psychiatry*, *17*, 642–649.

Horwood, E. J., Patton, G. C., Fergusson, D. M., et al. (2014). Young adult sequelae of adolescent cannabis use: an integrative analysis. *Lancet Psychiatry*, *1*, 286–293.

Jager, G., van Hell, H. H., De Win, M. M., et al. (2007). Effects of frequent cannabis use on hippocampal activity during an associative memory task. *European Neuropsychopharmacology*, *17*, 289–297.

Juckel, G., Schlagenhauf, F., Koslowski, M., et al. (2006). Dysfunction of ventral striatal reward prediction in schizophrenic patients treated with typical, not atypical, neuroleptics. *Psychopharmacology*, *187*, 222–228.

Lorenzetti, V., Solowji, N., Whittle, S., et al. (2015). Gross morphological brain changes with chronic, heavy cannabis use. *British Journal of Psychiatry*, *206*, 77–78.

Maldonado, R., Berrendero, F., Ozaita, A., et al. (2011). Neurochemical basis of cannabis addiction. *Neuroscience*, *181*, 1–17.

Martín-Santos, R., Crippa, J. A., Seal, M., et al. (2012). Acute effects of a single, oral dose of d9-tetrahydrocannabinol (THC) and Cannabidiol (CBD) administration in healthy volunteers. *Current Pharmaceutical Design*, *18*, 4966–4979.

Martín-Santos, R., Fagundo, A. B., Crippa, J. A., et al. (2010). Neuroimaging in cannabis use: a systematic review of the literature. *Psychological Medicine*, *40*, 383–398.

Mathew, R. J., & Wilson, W. H. (1993). Acute changes in cerebral blood flow after smoking marijuana. *Life Science*, *52*, 757–767.

Mathew, R. J., Wilson, W. H., Chiu, N. Y., et al. (1999). Regional cerebral blood flow and depersonalization after tetrahydrocannabinol administration. *Acta Psychiatrica Scandinavica*, *100*, 67–75.

Matochik, J. A., Eldreth, D. A., Cadet, J. L., & Bolla, K. I. (2005). Altered brain tissue composition in heavy marijuana users. *Drug and Alcohol Dependence*, *77*, 23–30.

McQueeny, T., Padula, C. B., Price, J., et al. (2011). Gender effects on amygdala morphometry in adolescent marijuana users. *Behavior and Brain Research*, *224*, 128–134.

Mechoulam, R., & Gaoni, Y. (1967). Recent advances in the chemistry of hashish. *Fortschritte der Chemie Organischer Naturstoffe*, *25*, 175–213.

Mechoulam, R., & Parker, L. A. (2013). The endocannabinoid system and the brain. *Annual Review of Psychology*, *64*, 21–47.

Medina, K. L., McQueeny, T., Nagel, B. J., et al. (2009). Prefrontal cortex morphometry in abstinent adolescent marijuana users: subtle gender effects. *Addiction Biology*, *14*, 457–468.

Medina, K. L., Nagel, B. J., & Tapert, S. F. (2010). Abnormal cerebellar morphometry in abstinent adolescent marijuana users. *Psychiatry Research*, *182*, 152–159.

Moore, T. H., Zammit, S., Lingford-Hughes, A., et al. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet*, *370*, 319–328.

Morrison, P. D., Nottage, J., Stone, J. M., et al. (2011). Disruption of frontal theta coherence by delta-9-tetrahydrocannabinol is associated with positive psychotic symptoms. *Neuropsychopharmacology*, *36*, 827–836.

O'Leary, D. S., Block, R. I., Turner, B. M., et al. (2003). Marijuana alters the human cerebellar clock. *Neuroreport*, *14*, 1145–1151.

Onaive, E. S. (2006). Neuropsychobiological evidence for the functional presence and expresión of cannabinoid CB2 receptors in the brain. *Neuropsychobiology*, *54*, 231–246.

Perez, M., Benitez, S. J., Cartarozzi, L. P., et al. (2013). Neuroprotection and reduction of glial reaction by cannabidiol treatment after sciatic nerve transection in neonatal rats. *European Journal of Neuroscience*, *38*, 3424–3434.

Phan, K. L., Angstadt, M., Golden, J., et al. (2008). Cannabinoid modulation of amygdala reactivity to social signals of threat in humans. *Journal of Neuroscience*, *28*, 2313–2319.

Rais, M., van Haren, N. E., Cahn, W., et al. (2010). Cannabis use and progressive cortical thickness loss in areas rich in CB1 receptors during the first five years of schizophrenia. *European Neuropsychopharmacology*, *20*, 855–865.

Ramaekers, J. G., Berghaus, G., van Laar, M., et al. (2004). Dose related risk of motor vehicle crashes after cannabis use. *Drug and Alcohol Dependence*, *73*, 109–119.

Ramaekers, J. G., Kauert, G., Theunissen, E. L., et al. (2009). Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. *Journal of Psychopharmacology*, *23*, 266–277.

Rochetti, M., Crescini, A., Borgward, S., et al. (2013). Is cannabis neurotoxic for the health brain. A meta-analystic review of structural brain alterations in non-psychotic users. *Psychiatry and Clinical Neuroscience*, *67*, 483–492.

Roiser, J. (2010). Aberrant salience in subjects at high risk of psychosis related to altered dorsolateral prefrontal function. *Schizophrenia Research*, *117*, 168.

Schreiner, A. M., & Dunn, M. E. (2012). Residual effects of cannabis use on neurocognitive performance alter prolonged abstinence: a metaanalysis. *Experimental and Clinical Psychopharmacology*, *20*, 420–429.

Schweinsburg, A. D., Schweinsburg, B. C., Medina, K. L., et al. (2010). The influence of recency of use on fMRI response during spatial working memory in adolescent marijuana users. *Journal of Psychoactive Drugs*, *42*, 401–412.

Schweinsburg, A. D., Schweinsburg, B. C., Nagel, B. J., et al. (2011). Neural correlates of verbal learning in adolescent alcohol and marijuana users. *Addiction*, *106*, 564–573.

Solowij, N., & Battisti, R. (2008). The chronic effects of cannabis on memory in humans: a review. *Current Drug Abuse Review*, *1*, 81–98.

Solowij, N., Fernandez, F., Murray, R., et al. (2012). Genetic modulation of the long-term effects of cannabis on brain structure, function and symptomatology. *Schizophrenia Research*, *136*(Suppl. 1), S134.

Stokes, P. R., Metha, M. A., Curran, H. V., et al. (2009). Can recreations doses of THC produce significant dopamine release in human striatum? *Neuroimage*, *48*, 186–190.

Tan, H. Y., Nicodemus, K. K., Chen, Q., et al. (2008). Genetic variation in AKT1 is linked to dopamine associated prefrontal cortical structure and function in humans. *Journal of Clinical Investigation*, *118*, 2200–2208.

Tapert, S. F., Schweinsburg, A. D., Drummond, S. P., et al. (2007). Functional MRI of inhibitory processing in abstinent adolescent marijuana users. *Psychopharmacology*, *194*, 173–183.

Torrens, M., Serrano, D., Astals, M., et al. (2004). Diagnosing comorbid psychiatric disorders in substance abusers: validity of the Spanish versions of the Psychiatric Research Interview for Substance and Mental Disorders and the Structured Clinical Interview for DSM-IV. *American Journal of Psychiatry*, *161*, 1231–1237.

Tzilos, G. K., Cintron, C. B., Wood, J. B., et al. (2005). Lack of hippocampal volume change in long-term heavy cannabis users. *American Journal of Addiction*, *14*, 64–72.

van Hell, H. H., Vink, M., Ossewaarde, L., et al. (2010). Chronic effects of cannabis use on the human reward system: an fMRI study. *European Neuropsychopharmacology*, *20*, 153–163.

Wesley, M. J., Hanlon, C. A., & Porrino, L. J. (2011). Poor decision-making by chronic marijuana users is associated with decreased functional responsiveness to negative consequences. *Psychiatry Research*, *191*, 51–59.

Winton-Brown, T. T., Allen, P., Bhattacharyya, S., et al. (2011). Modulation of auditory and visual processing by delta-9-tetrahydrocannabinol and cannabidiol: an FMRI study. *Neuropsychopharmacology*, *36*, 1340–1348.

Yücel, M., Solowij, N., Respondek, C., et al. (2008). Regional brain abnormalities associated with long-term heavy cannabis use. *Archives of General Psychiatry*, *65*(6), 694–701.

Zuardi, A. W., Crippa, J. A., Hallak, J. E., et al. (2012). A critical review of the antipsychotic effects of cannabidiol: 30 years of a translational investigation. *Current Pharmaceutical Design*, *18*, 5131–5140.

CHAPTER

e6

Gray Matter, Lateral Ventricle Volumes, and Executive Functioning in Cannabis Users with First-Episode Psychosis

P.J. Cunha[*,**,†], *P.G.P. Rosa*[*,**], *F.L.S. Duran*[*,**], *L.C. Santos*[*,**], *J.A.S. Crippa*[*,‡], *G.F. Busatto*[*,**], *M.S. Schaufelberger*[*,**,‡]

*Department of Psychiatry, Faculty of Medicine, Laboratory of Psychiatric Neuroimaging (LIM-21), University of São Paulo, São Paulo, Brazil

**Center for Interdisciplinary Research on Applied Neurosciences (NAPNA), University of São Paulo, São Paulo, Brazil

†Department of Psychiatry, Faculty of Medicine, University of São Paulo, São Paulo, Brazil

‡Department of Neuroscience and Behavior, Faculty of Medicine, Ribeirão Preto, University of São Paulo, Ribeirao Preto, Brazil

SUMMARY POINTS

- Cannabis is a risk factor for psychosis.
- Cannabis users who develop psychosis initially have less brain alterations because they commonly have a better premorbid functioning.
- Executive deficits and brain structural abnormalities are common in patients with first-episode psychosis, after a period of cannabis use.
- Patients only with psychosis present a higher degree of neurodevelopmental involvement, and greater brain abnormalities.
- Individuals with psychosis and cannabis use usually present in their clinical history more "environmental" risk factors in their causal pathway.
- It is important to develop strategies aiming to prevent cannabis use among adolescents, whose brain is still developing, especially those with prodromal psychotic symptoms, and a family history of psychosis.
- Early interventions for patients with FEP could help them prevent relapse on cannabis use for a better prognosis.

KEY FACTS OF NEUROIMAGING

- Neuroimaging has advanced our understanding on the nervous system by investigating both brain structure and functioning.
- Magnetic Resonance Imaging (MRI) is the most used neuroimaging technique in order to study alterations in gray matter (GM) and ventricles' volumes.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00043-0
Copyright © 2017 Elsevier Inc. All rights reserved.

KEY FACTS OF GRAY MATTER

- Gray matter (GM) is distributed at the surface of the brain, and it is also known as the "cerebral cortex."
- GM is mainly composed by neuron cell bodies, dendrites, capillaries, and glial cells, thus being differentiated of white matter (WM)—composed by myelinated axons—the difference in colors being basically caused by the whiteness of myelin, predominantly composed by lipid tissue.
- GM is predominantly associated with cognitive processing, while WM has been related with communication among different brain cells and regions.

LIST OF ABBREVIATIONS

ACC	Anterior cingulate cortex
ARMS	At-risk mental state
BD	Backward Digits
CBD	Cannabidiol
CB1	Type-1 cannabinoid receptors
CPT	Continuous Performance Test
DARTEL	Diffeomorphic registration algorithm
FD	Forward Digits
FEP	First-episode psychosis
FES	First-episode schizophrenia
GM	Gray matter
LVs	Lateral ventricles
MRI	Magnetic resonance imaging
PCC	Posterior cingulate cortex
PFC	Prefrontal cortex
ROIs	Regions of interest
ROP	Recent-onset psychosis
ROS	Recent-onset schizophrenia
THC	Δ^9-Tetrahydrocannabinol
THs	Temporal horns
VBM	Voxel-based morphometry
VBRs	Ventricle-to-brain ratios
WCST	Wisconsin Card Sorting Test
WMS-III	Wechsler Memory Scale

INTRODUCTION

Cannabis is still one of the most widely used illegal substance worldwide (UNODC, 2014). Community and scientists are still debating the implications of cannabis legalization, and the consequences are unclear, except for an expected tendency toward an increased prevalence of cannabis use among people (UNODC, 2014; Volkow, Baler, Compton, & Weiss, 2014), leading to an increased healthcare utilization related to cannabis use (Monte, Zane, & Heard, 2015). More importantly, considering that cannabis use is associated with a significant increased risk for developing psychosis (Bangalore et al., 2008; Koskinen, Löhönen, Koponen, Isohanni, & Miettunen, 2010; Moore et al., 2007; Murray, Morrison, Henquet, & Di Forti, 2007), it is inevitable to hypothesize that the number of persons who will develop psychosis will increase substantially (Monte et al., 2015). Actually, the prevalence of cannabis use may reach 43% (Bersani, Orlandi, Kotzalidis, & Pancheri, 2002) or even 60% in patients with psychosis (Duke, Pantelis, McPhillips, & Barnes, 2001). The aim of this chapter is to review the brain structural consequences of a chronic exposure to cannabis in the presence of psychosis, more specifically focusing on gray matter (GM) and ventricular alterations.

CANNABIS EFFECTS ON THE BRAIN AND IMPACT ON COGNITION

There is a frequent finding linking cannabis use with a negative impact on brain structure, especially the prefrontal cortex (PFC), amygdala, and hippocampus (Bolla, Brown, Eldreth, Tate, & Cadet, 2002; Batalla et al., 2013; Battistella et al., 2014; Lorenzetti et al., 2015; Rapp, Bugra, Riecher-Rössler, & Borgwardt, 2012; Yücel et al., 2008), but structural abnormalities remain inconclusive due to methodological differences across studies (Filbey et al., 2014; Malchow et al., 2012; Martín-Santos et al., 2010; Weiland et al., 2015). Functional neuroimaging studies have shown more consistent findings. During acute exposure, cannabis users present greater activation in brain areas related to the reward pathway, such as the ventral tegmental area, nucleus accumbens, thalamus, anterior cingulate, orbitofrontal cortex, insula, and amygdala (Filbey, Schacht, Myers, Chavez, & Hutchison, 2009; Martín-Santos et al., 2010). Subjective levels of intoxication and mental confusion have been correlated with such changes (Martín-Santos et al., 2010; Mathew & Wilson, 1993). However, after a long-term period of use, studies suggest that resting global, prefrontal, and anterior cingulate cortex (ACC) blood flow are lower in cannabis users than in controls (Martín-Santos et al., 2010).

Neuropsychological studies have supported such findings, Bolla et al. (2002) have demonstrated that chronic cannabis users have persistent dose-related cognitive deficits on memory, psychomotor speed, manual dexterity, and executive global functioning, even after 28 days of abstinence. Moreover, earlier cannabis use has a greater impact on cognitive functioning than later use. Fontes et al. (2011b) showed that chronic cannabis users who started to use before 15 years old (early-onset) are cognitively more impaired, as compared to late-onset users and controls, suggesting that early exposure to cannabis is associated with more adverse effects on

brain development. Considering that the endocannabinoid system plays an important role in brain development, it is plausible to hypothesize that prolonged use during adolescence results in a disruption in the normative maturational processes that occur during this period (Lubman, Cheetham, & Yücel, 2014). Also, perseverative responding measured by a traditional neuropsychological task named Wisconsin Card Sorting Test (WCST) indicates that early exposure to cannabis is associated with poorer mental flexibility, helping to explain why it is so hard for a cannabis chronic user to stop their compulsive behavior (Fontes et al., 2011a).

CANNABIS, PSYCHOSIS, AND THE BRAIN: COMPLEX MECHANISMS AND PREMORBID FUNCTIONING

Patients with first-episode psychosis (FEP) show significant regional GM alterations in frontal and temporal lobe structures, midline brain abnormalities, and poorer cognitive functioning (Ayres et al., 2007, 2010; Minatogawa-Chang et al., 2009; Rosa et al., 2010; Schaufelberger et al., 2007; Trzesniak et al., 2012). Abnormalities of brain structures have been suggested to underlie cognitive and emotional disturbances commonly seen in schizophrenia (Wobrock, Sittinger, Behrendt, D'Amelio, & Falkai, 2009). Apparently, a patient with FEP who is also cannabis user would presumably have greater alterations on brain structure, and impaired cognitive functioning (Rapp et al., 2012; Wobrock et al., 2009). In fact, Szeszko et al. (2007) used a method in which the frontal regions of interest (ROIs) were outlined manually, and reported that patients with first-episode schizophrenia (FES) who had a history of cannabis use had less anterior cingulate GM, compared with similar patients who did not use cannabis, and with healthy volunteers (see Table e6.1). In the same line, Bangalore et al. (2008) used voxel-based morphometry (VBM) and found decreased GM density in the posterior cingulate cortex (PCC) and in the left hippocampus in FEP cannabis users, when compared with FEP nonusers (see Fig. e6.1), suggesting that cannabis use is associated with altered brain structure, in particular regions rich in type-1 cannabinoid receptors (CB1) (Bangalore et al., 2008).

Also using VBM, Jarvis et al. (2008) found that bipolar adolescents with cannabis use had greater structural abnormalities than adolescents with bipolar disorder alone, in frontotemporal brain regions, as well as in certain subcortical areas associated with emotion, executive functioning, and motivational regulation.

More recently, Rapp et al. (2013) conducted a study with 23 patients with FEP, and 37 subjects in at-risk mental state (ARMS), and they found a significant negative effect of cannabis use on the GM volume of the posterior cingulum, and the left anterior cingulum, which were independent of the diagnostic group. The authors argue that both ARMS and FEP subjects may be particularly sensitive to activation of CB1 receptors that are very concentrated in the cingulate cortex (Rapp et al., 2013).

On the other hand, Cahn et al. (2004) examined brain volumes in cannabis-exposed patients with recent-onset schizophrenia (ROS), but the authors did not find any statistically significant difference between groups (cannabis users × nonusers), except for ventricular volumes (see next section). Wobrock et al. (2009) also found no differences in the brain morphology between the two subgroups of recent-onset psychosis (ROP) with and without substance use, primarily cannabis use. In a prospective study, Haller et al. (2013) did not found any significant differences among 50 patients with FEP, divided in 33 FEP with cannabis use and 17 FEP without cannabis use. However, the authors argue that they cannot exclude subtle morphometric changes associated with cannabis consumption, given the limitations of the study (Haller et al., 2013).

In general, literature involving neuroimaging in FEP with history of cannabis is relatively recent, comprehending different methodologies and distinct results (Malchow et al., 2012). Given the relevance and the high prevalence of cannabis use among patients with FEP, we consider that this issue deserves more attention. Although cannabis use has been associated with cognitive impairments and brain dysfunction in nonpsychotic users (Cunha, Nicastri, de Andrade, & Bolla, 2010; Fontes et al., 2011a,b; Hester, Nestor, & Garavan, 2009; Solowij et al., 2012), there is also evidence that a history of cannabis use could be associated with less abnormalities in brain structure and cognitive functioning, in people with schizophrenia (Rodríguez-Sánchez et al., 2010; Yücel et al., 2012) and bipolar disorder (Braga, Burdick, Derosse, & Malhotra, 2012). Thus, research studies using neuroimaging methods and neuropsychological testing have demonstrated heterogeneous results, due to different neuroimaging methods, cannabis use definitions (ie, lifetime versus recent use, age of onset, and others variables), and distinct cognitive tasks. For example, in two recent studies, individuals who develop psychosis in the context of cannabis use had less GM and lateral ventricles abnormalities than those patients who had no history of substance use (Cunha et al., 2013; Schnell, Kleiman, Gouzoulis-Mayfran, Daumann, & Becker, 2012). Schnell et al. (2012) evaluated 30 FES with cannabis use and 24 FES without cannabis use, using VBM and a diffeomorphic registration algorithm (DARTEL). They investigated specific ROIs such as insula, medial temporal gyrus, and middle frontal gyrus. They found less severe cognitive impairments (verbal and working memory), and less GM deficits in the PFC in patients with schizophrenia and cannabis use, when compared with patients without

TABLE e6.1 Structural Magnetic Resonance Studies in Patients With First Episode Psychosis and Cannabis Use

Authors (year)	Diagnosis	Comparative analysis (N)	Neuroimaging methods	Controlled variables	Cognitive assessment	Structural brain results
Cahn et al. (2004)	ROS	27 patients with ROS and cannabis use, 20 patients with ROS, and no healthy controls (HC)	Quantitative measurements were obtained manually. ROIs: total brain, cerebellum, caudate, lateral and third ventricle volumes	The two groups were matched for sex, height, weight, handedness, and parental education/controlled for age (different among groups)	No cognitive assessment	No differences between ROS with and without cannabis use, except for a decreased asymmetry of the lateral ventricles in the cannabis-exposed group
Szeszko et al. (2007)	FES	20 FES with cannabis use or dependence, 31 FES with no cannabis use and 56 healthy controls	All regions were outlined manually. ROIS: SFG, ACG, and OFC	Patients were volunteers in clinical trials comparing the efficacy of atypical antipsychotic drugs. HC were more educated/controlled by age and gender	No cognitive assessment	Reduced GM in ACG in FES patients with cannabis use compared with both patients with FES and HC
Bangalore et al. (2008)	FES patients	15 FES with cannabis use, 24 FES without cannabis use, and 42 healthy controls	VBM/ROIs consisting of the CB1 receptor rich brain regions that included DLPFC, hippocampus, posterior cingulate, and cerebellum	Age and gender/drug naive	No cognitive assessment	Decreased GM density in the right PCC and left hippocampus in FES patients with cannabis use, when compared with FES patients without cannabis use
Jarvis et al. (2008)	Bipolar disorder (inpatients)	7 patients with bipolar disorder and cannabis use, 7 patients with bipolar disorder without cannabis use, and no healthy controls (HC)	VBM	Adolescents (12–18 years old)	No cognitive assessment	Bipolar patients with cannabis use demonstrated decreased GM in the left fusiform gyrus and increased GM in the right caudate and precentral gyrus, as well as increased GM density in the right middle occipital and fusiform gyri and cerebellar vermis
Rais et al. (2008)	ROS	19 patients with ROS and cannabis use, 32 patients with ROS without cannabis use, and 31 HC	Prospective study examining total brain, GM, and lateral and third ventricle volumes. MRIs were obtained at inclusion (T0) and at 5-year follow-up (T5)	No differences among patients and controls in gender, handedness, age, parental education, and scan interval	No cognitive assessment	No significant differences at baseline/patients with FES and cannabis use demonstrated larger GM loss and larger increase in lateral and third ventricle volumes
Wobrock, Sittinger, Behrendt, D'Amelio, & Falkai (2009)	ROS	20 patients with ROS with comorbid SUD, and 21 patients with ROS without SUD, and no HC	Volumetric measurement of ROIs including STG, amygdala-hippocampus, and cingulum	ROS with SUD were significantly younger, predominantly male, and had a lower socioeconomic status	Premorbid intelligence was estimated/patients with SUD demonstrated elevated anxiety, depression, and less attentional impairments	No differences between ROS with and without substance/cannabis use

Rais et al. (2010)	ROS	19 patients with ROS and cannabis use, 32 patients with ROS without cannabis use, and 31 HC	Prospective study examining differences in cortical thickness/MRIs were obtained at inclusion in the study and at a 5-year follow-up	Patients and HC matched for confounding variables	No cognitive assessment	After 5 years, patients who used cannabis showed additional thinning in the left DLPFC, left ACC, and left occipital lobe, as compared to those patients that did not use cannabis
Schnell, Kleiman, Gouzoulis-Mayfrank, Daumann, & Becker (2012)	FES	30 FES with cannabis use, 24 FES without cannabis use, and no HC	DARTEL and VBM. ROIs investigated: insula, medial temporal gyrus, and middle frontal gyrus	Groups were similar in terms of age, gender, education, psychopathology, medication, alcohol or nicotine use	Patients with FES and cannabis use presenting less severe cognitive impairments	Patients with FES and cannabis use had less severe middle frontal GM deficits/GM density was positively associated with cognitive functioning
Cunha et al. (2013)	FEP	28 FEP with cannabis use, 78 FEP without cannabis use, and 80 HC	VBM/LVs were measured by the manual tracing ROI	Controlled by age and gender/medicated/cognitive results	Patients with FEP and cannabis use had less attentional and executive impairments	Patients with FEP and a history of cannabis use had less brain abnormalities, characterized by GM and ventricle volumes preservation
Rapp et al. (2013)	ARMS subjects with FEP	23 patients with FEP, 37 subjects with ARMS, and no HC	Manually traced cingulum	Corrected by age, gender, alcohol use, brain volume, and psychiatric medication	No cognitive assessment	Decreased GM volume in the posterior and left anterior cingulum associated with cannabis use
Haller et al. (2013)	FEP	33 FEP subjects with cannabis use and 17 FEP patients without cannabis use	Prospective study examining GM by VBM using FSL	Controlled by age and gender	No significant differences in attention and memory performance	No significant differences in GM between user and nonuser FEP groups

Abbreviations: *VBM*, voxel-based morphometry; *GM*, gray matter; *ROI*, region of interest; *LV*, lateral ventricles; *ROS*, recent-onset schizophrenia; *FES*, first-episode schizophrenia; *FEP*, first-episode psychosis; *HC*, healthy controls; *DARTEL*, diffeomorphic registration algorithm; *SUD*, substance use disorder; *DLPFC*, dorsolateral prefrontal cortex; *ARMS*, subjects at-risk mental state; *CB1*, cannabinoid type-1 receptors; *MRI*, magnetic resonance imaging; *FSL*, FMRIB Software Library.

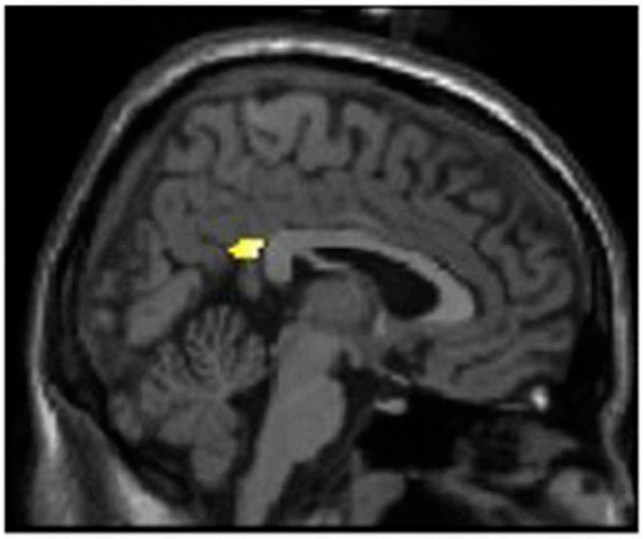


FIGURE e6.1 Gray Matter (GM) Reduction in the Posterior Cingulate Cortex (PCC) of Patients with First-Episode Schizophrenia (FES) and Cannabis Use. *Source: Bangalore et al. (2008) (reproduced with permission).*

cannabis use (Schnell et al., 2012). Also, GM density was positively associated with cognitive performance in the Continuous Performance Test (CPT), which is highly dependent on prefrontal cortex integrity. The authors argue that deficits in these regions might represent preexisting risk factors particularly linked to the biological vulnerability for schizophrenia. In a more recent controlled VBM study, including a healthy control group, investigating GM and cognitive performance in patients with psychosis and a history of cannabis use, compared with psychotic patients without any history of cannabis use, we confirmed that patients with a history of cannabis use had less structural brain abnormalities and less cognitive alterations than patients without a history of cannabis use. Patients were selected from a sample of people with FEP identified for an epidemiological study about the incidence of psychotic disorders in Brazil (Menezes et al., 2007). We included 106 patients aged between 18 and 50 and with the diagnosis of psychosis (affective or nonaffective), who were categorized among those with a lifetime history of cannabis use (with a frequency of at least 3 times/month for at least 1 year, mean duration of cannabis use = 6.5 years, $N = 28$) and those patients who had no history of cannabis use ($N = 78$). Both groups of patients were compared with a control group of 80 nonpsychotic individuals, with no history of cannabis use, living in the same geographical area. As a result, we found GM deficits in medial temporal structures (hippocampus and parahippocampal gyrus), and in the PFC of patients only with FEP, but not in patients with FEP and cannabis use (see Fig. e6.2).

In fact, those abnormalities were found in patients without cannabis use, when directly compared to patients with a history of cannabis use. At first sight, one could precipitately interpret this finding as a "positive" result of cannabis use on brain structure. For example, one of the natural compounds in cannabis is cannabidiol (CBD), known to have opposite effects on the functional connectivity between the dorsal striatum, prefrontal cortex, and hippocampus, when compared with Δ^9-tetrahydrocannabinol (THC), the main psychoactive compound of cannabis, associated with abnormalities in brain functions (Bhattacharyya et al., 2014). Patients submitted to CBD for a period of time show better functional connectivity in brain areas associated with important cognitive functions (idem). For example, Δ^9-THC reduced fronto-striatal connectivity, related to its effect on task performance, whereas this connection was enhanced by CBD (Bhattacharyya et al., 2014). Also, experimental studies confirm that Δ^9-THC may be neurotoxic to the prefrontal cortical dopamine metabolism (Verrico, Jentsch, & Roth, 2003) that, in turn, has an important role in the neurobiology of schizophrenia (Szeszko et al., 2007). Unfortunately, in our study we did not have detailed information about the quality and estimated THC/CBD ratios in the cannabis the patients smoked. Also, patients

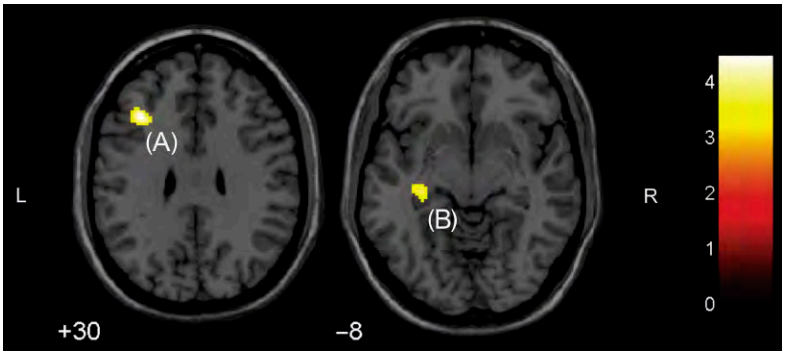


FIGURE e6.2 Brain Structural Analysis Displaying Clusters of Significant Decreased Grey Matter (GM) in Psychotic Patients Without History of Cannabis Use. Note: data indicates GM reduction in the left PFC (A) and in the left hippocampus (B) of patients with FEP, when compared with patients with FEP and a history of cannabis use. *Source: Cunha et al. (2013) (reproduced with permission).*

frequently use cannabis because of its psychoactive effects, so a significant concentration of Δ^9-THC may be part of the cannabis they smoke, so it is expected that at least some degree of impairments would be present after a long period of cannabis use.

In order to investigate this hypothesis, we also divided the cannabis users with FEP into two groups, the first with patients with an abstinence period of at least 6 months (N = 10), and the second comprising patients with less than 6 months of abstinence (N = 18, including "current" users). Results indicated that cannabis users with FEP with a longer period of abstinence had less GM abnormalities than recent users, in the left and right hippocampus/parahippocampal gyrus, thus indicating that some negative effects of cannabis on the brain may depend on the period of abstinence. We conclude that some other factors (other than cannabis use) would explain the lesser GM reduction of psychotic patients with a history of cannabis use. For them, contrary to those who developed psychosis without exposure to such environmental risk factor, previous exposure to cannabis may have had an effect on the development of psychosis in a structurally "preserved" brain, but, after the illness onset, a persistent use of cannabis could lead to GM deficits (Cunha et al., 2013). In other words, cannabis use would affect the late neurodevelopmental processes, involving synaptic plasticity, leading to the development of psychosis, and the continued cannabis use could lead to cognitive deficits.

It is interesting to note that participants in our study also underwent cognitive testing composed by a brief neuropsychological battery, including Forward Digits (FD) and Backward Digits (BD), from the Wechsler Memory Scale (WMS-III) (Wechsler, 1997), measures of attention and working memory (executive functioning), respectively. In this same line, FEP patients with history of cannabis use had a more preserved pattern of executive functioning, when compared with those without history of cannabis use, more specifically with regard to verbal working memory (BD). In other words, patients with history of cannabis use present a more preserved ability to retain and manipulate "*on line*" verbal information while performing a task. Indeed, executive abilities are necessary to find, acquire, and sustain illegal cannabis use (Joyal, Hallé, Lapierre, & Hodgins, 2003). Another finding in this study linked the ability of working memory with GM morphology in the left superior frontal gyrus. Also, it is plausible to hypothesize that patients with more preserved cognitive functioning are precisely those who may be more interactive, less socially withdrawn, and tend to have better social skills, thus having greater propensity to contact with cannabis by peers-influence or not.

Last, but not least, it is very relevant to note that a subsample of our patients with history of cannabis use had significant greater mean IQ scores than those without history of drug use, thus indicating that a supposedly better premorbid IQ could influence the brain structure and neuropsychological measures (Bolla et al., 2002). This is consistent with the recent findings of Ferraro et al. (2013).

Taken together, both studies (Cunha et al., 2013; Schnell et al., 2012) are congruent with the idea that the group of cannabis users with FEP presented less GM reduction in some brain areas, and less cognitive deficits, than the usual patients with FEP, without cannabis use. For these patients, it is plausible to suggest that a psychotic disorder emerged in a more "structurally preserved" brain, probably associated with a better pattern of premorbid functioning, and a higher cognitive potential (Cunha et al., 2013; Meijer et al., 2012; Wobrock et al., 2013).

VENTRICULAR ALTERATIONS IN CANNABIS USERS WITH FIRST-EPISODE PSYCHOSIS

Studies involving volumetric measurements obtained by magnetic resonance imaging (MRI) have demonstrated ventricular enlargement in psychosis (Rosa et al., 2010). In consonance with GM abnormalities, however, studies investigating ventricular alterations in cannabis users with psychosis have shown heterogeneous findings.

Cahn et al. (2004) investigated 27 patients with ROS and cannabis use, and compared them with 20 patients with ROS without cannabis use, and they found a decreased asymmetry of the lateral ventricles in the cannabis-exposed patients.

In our study, we also investigated lateral ventricles (LVs), measured by manual tracing ROI approach (Rosa et al., 2010).

We found significant group differences in VBRs among groups (Table e6.2). Patients with FEP without cannabis use had significant larger left LV and right TH, when compared with the control subjects. At the same time, they present an enlarged left LV, when directly compared with those from cannabis users with psychosis. There were no correlations between VBRs and neuropsychological measures. The absence of alterations in ventricular volumes in patients with FEP and a history of cannabis use would confirm a more preserved pattern of brain structure in these patients, when compared with those with FEP without history of cannabis use, which is compatible with our data on GM described before. In a prospective study, Rais et al. (2008) also observed a more preserved pattern of ventricles in patients with FES at baseline. However, after the period of 5 years, they found a more pronounced lateral ventricle enlargement,

TABLE e6.2 Ventricular Measures in Patients with FEP With History of Cannabis Use, FEP Without Cannabis Use, and Healthy Controls

VBR	FEP with cannabis use (FEP C+)	FEP without cannabis use (FEP C–)	Healthy controls (HC)	*Statistics* (*p* value)	
				FEP C– vs HC	*FEP C– vs FEP C+*
Right LV	0.0041 (0.0015)	0.0047 (0.0021)	0.0041 (0.0026)	0.082	0.727
Left LV	0.0042 (0.0017)	0.0054 (0.0026)	0.0045 (0.0026)	0.016	0.06
Right TH	0.00032 (0.00008)	0.00039 (0.00017)	0.00032 (0.0001)	0.015	0.126
Left TH	0.0003 (0.0001)	0.00036 (0.0002)	0.0003 (0.0001)	0.145	0.549

Note: VBR (ventricle-to-brain ratio) is expressed as a percentage (%) of the brain volume that is occupied by the region of interest; numbers are expressed by means (s.d.); *LV*, lateral ventricle; *TH*, temporal horn.
Source: Cunha et al. (2013) (reproduced with permission)

and third ventricle increase in cannabis-using patients. Also, altered ventricles were associated with more problems in daily life functioning (Rais et al., 2008).

BRAIN CHANGES AND COGNITIVE FUNCTIONING IN PSYCHOTIC CANNABIS USERS OVER TIME

Despite the fact that cannabis users with psychosis present less brain abnormalities and less cognitive deficits, it does not mean that these patients will not develop some degree of deficits. In a prospective study, cannabis-using psychotic patients presented similar cortical thickness, when compared with noncannabis users at baseline, but, after a period of 5 years, they showed progressive alterations in the dorsolateral PFC, and in the ACC (Rais et al., 2010), as well as decreased GM, and larger lateral and third ventricle volumes (Rais et al., 2008). Patients with FEP who continue to use cannabis after illness onset are particularly vulnerable to brain alterations in areas rich in CB1 receptors, related to negative symptoms and to poorer cognitive functioning (Rais et al., 2010). Problems in the ACC could have a role in poor decision-making, and in choosing more risky outcomes in patients with FEP and a history of cannabis use, which could contribute to substance-taking behavior (Szeszko et al., 2007).

It is possible that the development of psychosis should involve genetic factors that are associated with differential cerebral cortical sensitivity to developmental environmental exposures such as cannabis use, and exposure to emotional trauma (Habets, Marcelis, Gronenschild, Drukker, & van Os, 2011). In this line, cannabis may amplify the preexistent brain vulnerability associated with psychosis, but the mechanism by which cannabis may cause neuronal damage in patients with FEP remains unclear (Rais et al., 2008). Future and prospective studies are needed to better characterize the effects of cannabis use on brain structure and cognitive functioning in patients with psychosis.

CONCLUSIONS

While psychosis is often associated with neurodevelopmental deficits, including brain structural and executive abnormalities, cannabis users who develop psychosis are more likely to present a relatively "preserved" brain, and less deficits in executive functioning, at least at the beginning of the disorder, depending on their premorbid functioning. As cannabis use progresses, it is possible that the patient will have more executive deficits and brain structure abnormalities, such as GM reductions and lateral ventricle enlargements. Neuroimaging data presented here suggest that individuals with psychosis would be represented by those patients with a higher degree of neurodevelopmental involvement and greater brain abnormalities, whereas individuals with psychosis and cannabis use would be represented by patients with exposure to other environmental risk factors, such as cannabis, in their causal pathway. In clinical practice, it would be relevant to develop strategies aiming to prevent cannabis use among adolescent individuals and young people, especially those with prodromal symptoms, and a family history of psychosis. Also, early implementation of rehabilitation strategies for patients with FEP could help them prevent relapse on cannabis use for a better prognosis.

MINI-DICTIONARY

Amygdala A subcortical brain region mostly associated with emotional processing.
Cognitive functioning A set of mental functions, including attention, memory, language, executive functioning, and intelligence.
Executive functioning Complex cognitive functions that involve motivation, planning/abstracting, executing, and evaluating the result of a complex task.
First-episode psychosis (FEP) First occurrence of a psychotic episode that usually includes major psychiatric symptoms such as delusion, hallucination and/or motor agitation. Psychosis is basically characterized by the loss of contact with reality.

Frontotemporal brain regions Areas integrating the frontal and temporal cortex of the brain.
Hippocampus Brain area located in the temporal cortex that is predominantly associated with memory and learning new information.
Negative symptoms Common symptoms in patients with psychosis characterized by lack of drive, motivation and energy (ie, apathy), as well as social withdrawal and diminished affective/speech/motor responsiveness.
Prefrontal cortex (PFC) The anterior part of the cortex.
Prodromal symptoms or "at risk mental state" (ARMS) A set of symptoms and clinical signs that might indicate the beginning of a disease such as psychosis. They may include unusual thinking (ie, suspiciousness, thoughts of death, among others), mental confusion, anxiety, less intense perceptual alterations, persistent lack of motivation, and decline in cognitive and social functioning.
Subcortical areas Brain areas under the "cortex;" subcortical areas are basically composed by white matter (WM).
Ventricles They are basically "cavities" inside the brain, filled of cerebrospinal fluid (CSF).

ACKNOWLEDGMENTS

The authors would like to thank FAPESP, CNPq and CAPES. Dr José A. Crippa and Geraldo F. Busatto are partly funded by the CNPq-Brazil. Paulo Cunha and Geraldo Busatto are partly funded by FAPESP (Grant Numbers: 2010/15786-1 to Dr Geraldo Busatto e 2010/15604-0 to Dr Paulo Cunha).

Note: J.A. Crippa is one of the owners of the patent: Fluorinated CBD compounds, compositions and uses thereof. Pub. No.: WO/2014/108899. International Application No.: PCT/IL2014/050023 (Raphael Mechoulam, Antonio Waldo Zuardi, Flavio Pereira Kapczinski, Jaime Eduardo Cecilio Hallak, Francisco Silveira Ghimarães, Jose Alexandre de Souza Crippa, Aviva Breuer).

References

Ayres, A. M., Busatto, G. F., Menezes, P. R., Schaufelberger, M. S., Coutinho, L., Murray, R. M., McGuire, P. K., Rushe, T., & Scazufca, M. (2007). Cognitive deficits in first-episode psychosis: a population-based study in São Paulo, Brazil. *Schizophrenia Research*, *90*, 338–343.

Ayres, A. M., Scazufca, M., Menezes, P. R., Nakano, E. Y., Regina, A. C., Schaufelberger, M. S., Murray, R. M., McGuire, P. K., Rushe, T., & Busatto, G. F. (2010). Cognitive functioning in subjects with recent-onset psychosis from a low-middle-income environment: multiple-domain deficits and longitudinal evaluation. *Psychiatry Research*, *179*, 157–164.

Bangalore, S. S., Prasad, K. M., Montrose, D. M., Goradia, D. D., Diwadkar, V. A., & Keshavan, M. S. (2008). Cannabis use and brain structural alterations in first episode schizophrenia—a region of interest, voxel based morphometric study. *Schizophrenia Research*, *99*, 1–6.

Batalla, A., Bhattacharyya, S., Yücel, M., Fusar-Poli, P., Crippa, J. A., Nogué, S., Torrens, M., Pujol, J., Farré, M., & Martin-Santos, R. (2013). Structural and functional imaging studies in chronic cannabis users: a systematic review of adolescent and adult findings. *PLoS One*, *8*(2), e55821.

Battistella, G., Fornari, E., Annoni, J. M., Chtioui, H., Dao, K., Fabritius, M., Favrat, B., Mall, J. F., Maeder, P., & Giroud, C. (2014). Long-term effects of cannabis on brain structure. *Neuropsychopharmacology*, *39*(9), 2041–2048.

Bersani, G., Orlandi, V., Kotzalidis, G. D., & Pancheri, P. (2002). Cannabis and schizophrenia: impact on onset, course, psychopathology and outcomes. *European Archives of Psychiatry and Clinical Neuroscience*, *252*, 86–92.

Bhattacharyya, S., Falkenberg, I., Martin-Santos, R., Atakan, Z., Crippa, J. A., Giampietro, V., Brammer, M., & McGuire, P. (2014). Cannabinoid modulation of functional connectivity within regions processing attentional salience. *Neuropsychopharmacology*, *40*(6), 1343–1352.

Bolla, K. I., Brown, K., Eldreth, D., Tate, K., & Cadet, J. L. (2002). Dose-related neurocognitive effects of marijuana use. *Neurology*, *59*, 1337–1343.

Braga, R. J., Burdick, K. E., Derosse, P., & Malhotra, A. K. (2012). Cognitive and clinical outcomes associated with cannabis use in patients with bipolar I disorder. *Psychiatry Research*, *200*, 242–245.

Cahn, W., Hulshoff Pol, H. E., Caspers, E., van Haren, N. E., Schnack, H. G., & Kahn, R. S. (2004). Cannabis and brain morphology in recent-onset schizophrenia. *Schizophrenia Research*, *67*, 305–307.

Cunha, P. J., Nicastri, S., de Andrade, A. G., & Bolla, K. I. (2010). The frontal assessment battery (FAB) reveals neurocognitive dysfunction in substance-dependent individuals in distinct executive domains: abstract reasoning, motor programming, and cognitive flexibility. *Addiction Behavior*, *35*, 875–881.

Cunha, P. J., Rosa, P. G., Ayres, A. M., Duran, F. L., Santos, L. C., Scazufca, M., Menezes, P. R., dos Santos, B., Murray, R. M., Crippa, J. A., Busatto, G. F., & Schaufelberger, M. S. (2013). Cannabis use, cognition and brain structure in first-episode psychosis. *Schizophrenia Research*, *147*(2–3), 209–215.

D'Souza, D. C., Abi-Saab, W. M., Madonick, S., Forselius-Bielen, K., Doersch, A., Braley, G., Gueorguieva, R., Cooper, T. B., & Krystal, J. H. (2005). Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biological Psychiatry*, *57*(6), 594–608.

Duke, P. J., Pantelis, C., McPhillips, M. A., & Barnes, T. R. (2001). Comorbid non-alcohol substance misuse among people with schizophrenia: epidemiological study in central London. *British Journal of Psychiatry*, *179*, 509–513.

Ferraro, L., Russo, M., O'Connor, J., Wiffen, B. D., Falcone, M. A., Sideli, L., Gardner-Sood, P., Stilo, S., Trotta, A., Dazzan, P., Mondelli, V., Taylor, H., Friedman, B., Sallis, H., La Cascia, C., La Barbera, D., David, A. S., Reichenberg, A., Murray, R. M., & Di Forti, M. (2013). Cannabis users have higher premorbid IQ than other patients with first onset psychosis. *Schizophrenia Research*, *150*(1), 129–135.

Filbey, F. M., Schacht, J. P., Myers, U. S., Chavez, R. S., & Hutchison, K. E. (2009). Marijuana craving in the brain. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(31), 13016–13021.

Filbey, F. M., Aslan, S., Calhoun, V. D., Spence, J. S., Damaraju, E., Caprihan, A., & Segall, J. (2014). Long-term effects of marijuana use on the brain. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(47), 16913–16918.

Fontes, M. A., Bolla, K. I., Cunha, P. J., Almeida, P. P., Jungerman, F., Laranjeira, R. R., Bressan, R. A., & Lacerda, A. L. (2011a). Frontal Assessment Battery (FAB) is a simple tool for detecting executive deficits in chronic cannabis users. *Journal of Clinical and Experimental Neuropsychology*, *33*, 523–531.

Fontes, M. A., Bolla, K. I., Cunha, P. J., Almeida, P. P., Jungerman, F., Laranjeira, R. R., Bressan, R. A., & Lacerda, A. L. (2011b). Cannabis use before age 15 and subsequent executive functioning. *British Journal of Psychiatry*, *198*, 442–447.

Habets, P., Marcelis, M., Gronenschild, E., Drukker, M., & van Os, J. Genetic Risk and Outcome of Psychosis (GROUP) Investigators. (2011). Reduced cortical thickness as an outcome of differential sensitivity to environmental risks in schizophrenia. *Biological Psychiatry*, *69*(5), 487–494.

Haller, S., Curtis, L., Badan, M., Bessero, S., Albom, M., Chantraine, F., Alimenti, A., Lovblad, K. O., Giannakopoulos, P., & Merlo, M.

(2013). Combined grey matter VBM and white matter TBSS analysis in young first episode psychosis patients with and without cannabis consumption. *Brain Topography*, *26*(4), 641–647.

Hester, R., Nestor, L., & Garavan, H. (2009). Impaired error awareness and anterior cingulate cortex hypoactivity in chronic cannabis users. *Neuropsychopharmacology*, *34*, 2450–2458.

Jarvis, K., DelBello, M. P., Mills, N., Elman, I., Strakowski, S. M., & Adler, C. M. (2008). Neuroanatomic comparison of bipolar adolescents with and without cannabis use disorders. *Journal of Child and Adolescent Psychopharmacology*, *18*(6), 557–563.

Joyal, C. C., Hallé, P., Lapierre, D., & Hodgins, S. (2003). Drug abuse and/or dependence and better neuropsychological performance in patients with schizophrenia. *Schizophrenia Research*, *63*, 297–299.

Koskinen, J., Löhönen, J., Koponen, H., Isohanni, M., & Miettunen, J. (2010). Rate of cannabis use disorders in clinical samples of patients with schizophrenia: a meta-analysis. *Schizophrenia Bulletin*, *36*, 1115–1130.

Lorenzetti, V., Solowij, N., Whittle, S., Fornito, A., Lubman, D. I., Pantelis, C., & Yücel, M. (2015). Gross morphological brain changes with chronic, heavy cannabis use. *British Journal of Psychiatry*, *206*(1), 77–78.

Lubman, D. I., Cheetham, A., & Yücel, M. (2014). Cannabis and adolescent brain development. *Pharmacology and Therapeutics*, *148*, 1–16.

Malchow, B., Hasan, A., Fusar-Poli, P., Schmitt, A., Falkai, P., & Wobrock, T. (2014). Cannabis abuse and brain morphology in schizophrenia: a review of the available evidence. *European Archives of Psychiatry and Clinical Neuroscience*, *263*(1), 3–13.

Martín-Santos, R., Fagundo, A. B., Crippa, J. A., Atakan, Z., Bhattacharyya, S., Allen, P., Fusar-Poli, P., Borgwardt, S., Seal, M., Busatto, G. F., & McGuire, P. (2010). Neuroimaging in cannabis use: a systematic review of the literature. *Psychological Medicine*, *40*(3), 383–398.

Mathew, R. J., & Wilson, W. H. (1993). Acute changes in cerebral blood flow after smoking marijuana. *Life Sciences*, *52*, 757–767.

Meijer, J. H., Dekker, N., Koeter, M. W., Quee, P. J., van Beveren, N. J., & Meijer, C. J. Genetic Risk and Outcome of Psychosis (GROUP) Investigators. (2012). Cannabis and cognitive performance in psychosis: a cross-sectional study in patients with non-affective psychotic illness and their unaffected siblings. *Psychological Medicine*, *42*(4), 705–716.

Menezes, P. R., Scazufca, M., Busatto, G., Coutinho, L. M., McGuire, P. K., & Murray, R. M. (2007). Incidence of first-contact psychosis in São Paulo, Brazil. *British Journal of Psychiatry*, *191*, 102–106.

Minatogawa-Chang, T. M., Schaufelberger, M. S., Ayres, A. M., Duran, F. L., Gutt, E. K., Murray, R. M., Rushe, T. M., McGuire, P. K., Menezes, P. R., Scazufca, M., & Busatto, G. F. (2009). Cognitive performance is related to cortical grey matter volumes in early stages of schizophrenia: a population-based study of first-episode psychosis. *Schizophrenia Research*, *113*, 200–209.

Monte, A. A., Zane, R. D., & Heard, K. J. (2015). The implications of marijuana legalization in Colorado. *JAMA*, *313*(3), 241–242.

Moore, T. H., Zammit, S., Lingford-Hughes, A., Barnes, T. R., Jones, P. B., Burke, M., & Lewis, G. (2007). Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. *Lancet*, *370*, 319–328.

Murray, R. M., Morrison, P. D., Henquet, C., & Di Forti, M. (2007). Cannabis, the mind and society: the hash realities. *Nature Reviews Neuroscience*, *8*, 885–895.

Rais, M., Cahn, W., Van Haren, N., Schnack, H., Caspers, E., Hulshoff Pol, H., & Kahn, R. (2008). Excessive brain volume loss over time in cannabis-using first-episode schizophrenia patients. *American Journal of Psychiatry*, *165*, 490–496.

Rais, M., van Haren, N. E., Cahn, W., Schnack, H. G., Lepage, C., Collins, L., Evans, A. C., Hulshoff Pol., H. E., & Kahn, R. S. (2010). Cannabis use and progressive cortical thickness loss in areas rich in CB1 receptors during the first five years of schizophrenia. *European Neuropsychopharmacology*, *20*, 855–865.

Rapp, C., Bugra, H., Riecher-Rössler, A., & Borgwardt, S. (2012). Effects of cannabis use on human brain structure in psychosis: a systematic review combining in vivo structural neuroimaging and post-mortem studies. *Current Pharmaceutical Design*, *18*, 5070–5080.

Rapp, C., Walter, A., Studerus, E., Bugra, H., Tamagni, C., Röthlisberger, M., Borgwardt, S., Aston, J., & Riecher-Rössler, A. (2013). Cannabis use and brain structural alterations of the cingulate cortex in early psychosis. *Psychiatry Research*, *214*(2), 102–108.

Rodríguez-Sánchez, J. M., Ayesa-Arriola, R., Mata, I., Moreno-Calle, T., Perez-Iglesias, R., González-Blanch, C., Periañez, J. A., Vazquez-Barquero, J. L., & Crespo-Facorro, B. (2010). Cannabis use and cognitive functioning in first-episode schizophrenia patients. *Schizophrenia Research*, *124*, 142–151.

Rosa, P. G., Schaufelberger, M. S., Uchida, R. R., Duran, F. L., Lappin, J. M., Menezes, P. R., Scazufca, M., McGuire, P. K., Murray, R. M., & Busatto, G. F. (2010). Lateral ventricle differences between first-episode schizophrenia and first-episode psychotic bipolar disorder: a population-based morphometric MRI study. *World Journal of Biological Psychiatry*, *11*, 873–887.

Schaufelberger, M. S., Duran, F. L., Lappin, J. M., Scazufca, M., Amaro, E., Jr., Leite, C. C., de Castro, C. C., Murray, R. M., McGuire, P. K., Menezes, P. R., & Busatto, G. F. (2007). Grey matter abnormalities in Brazilians with first-episode psychosis. *British Journal of Psychiatry*, *51*, 117–122.

Schnell, T., Kleiman, A., Gouzoulis-Mayfrank, E., Daumann, J., & Becker, B. (2012). Increased gray matter density in patients with schizophrenia and cannabis use: a voxel-based morphometric study using DARTEL. *Schizophrenia Research*, *138*, 183–187.

Solowij, N., Jones, K. A., Rozman, M. E., Davis, S. M., Ciarrochi, J., Heaven, P. C., Pesa, N., Lubman, D. I., & Yücel, M. (2012). Reflection impulsivity in adolescent cannabis users: a comparison with alcohol-using and non-substance-using adolescents. *Psychopharmacology (Berlin)*, *219*, 575–586.

Szeszko, P. R., Robinson, D. G., Sevy, S., Kumra, S., Rupp, C. I., Betensky, J. D., Lencz, T., Ashtari, M., Kane, J. M., Malhotra, A. K., Gunduz-Bruce, H., Napolitano, B., & Bilder, R. M. (2007). Anterior cingulate grey-matter deficits and cannabis use in first-episode schizophrenia. *British Journal of Psychiatry*, *190*, 230–236.

Trzesniak, C., Schaufelberger, M. S., Duran, F. L., Santos, L. C., Rosa, P. G., McGuire, P. K., Murray, R. M., Scazufca, M., Menezes, P. R., Hallak, J. E., Crippa, J. A., & Busatto, G. F. (2012). Longitudinal follow-up of cavum septum pellucidum and adhesio interthalamica alterations in first-episode psychosis: a population-based MRI study. *Psychological Medicine*, *42*, 2523–2534.

UNODC. (2014). *World Drug Report 2014 [online]*. New York, NY: United Nations Office on Drugs and Crime. http://www.unodc.org

Verrico, C. D., Jentsch, J. D., & Roth, R. H. (2003). Persistent and anatomically selective reduction in prefrontal cortical dopamine metabolism after repeated, intermittent cannabinoid administration to rats. *Synapse*, *49*, 61–66.

Volkow, N. D., Baler, R. D., Compton, W. M., & Weiss, S. R. (2014). Adverse health effects of marijuana use. *New England Journal of Medicine*, *370*(23), 2219–2227.

Wechsler, D. (1997). *Wechsler Memory Scalem* (3rd ed.). San Antonio, TX: The Psychological Corporation–Harcourt Brace (WMS-III).

Weiland, B. J., Thayer, R. E., Depue, B. E., Sabbineni, A., Bryan, A. D., & Hutchison, K. E. (2015). Daily marijuana use is not associated with brain morphometric measures in adolescents or adults. *Journal of Neuroscience*, *35*(4), 1505–1512.

Wobrock, T., Sittinger, H., Behrendt, B., D'Amelio, R., & Falkai, P. (2009). Comorbid substance abuse and brain morphology in recent-onset psychosis. *European Archives of Psychiatry and Clinical Neuroscience*, *259*, 28–36.

Wobrock, T., Falkai, P., Schneider-Axmann, T., Hasan, A., Galderisi, S., Davidson, M., Kahn, R. S., Derks, E. M., Boter, H., Rybakowski, J. K., Libiger, J., Dollfus, S., López-Ibor, J. J., Peuskens, J., Hranov, L. G., Gaebel, W., & Fleischhacker, W. W. EUFEST study group. (2013). Comorbid substance abuse in first-episode schizophrenia: effects on cognition and psychopathology in the EUFEST study. *Schizophrenia Research*, *147*(1), 132–139.

Yücel, M., Solowij, N., Respondek, C., Whittle, S., Fornito, A., Pantelis, C., & Lubman, D. I. (2008). Regional brain abnormalities associated with long-term heavy cannabis use. *Archives of General Psychiatry*, *65*, 694–701.

Yücel, M., Bora, E., Lubman, D. I., Solowij, N., Brewer, W. J., Cotton, S. M., Conus, P., Takagi, M. J., Fornito, A., Wood, S. J., McGorry, P. D., & Pantelis, C. (2012). The impact of cannabis use on cognitive functioning in patients with schizophrenia: a meta-analysis of existing findings and new data in a first-episode sample. *Schizophrenia Bulletin*, *38*, 316–330.

CHAPTER

e7

Cannabis Use and Attention-Deficit/Hyperactivity Disorder: Potential Moderators

K.E. Maple, N.E. Wright, K.M. Lisdahl

Department of Psychology, University of Wisconsin-Milwaukee, Milwaukee, WI, United States

SUMMARY POINTS

- The literature remains unclear regarding whether ADHD is a risk factor for, or a consequence of, cannabis use.
- In some samples, childhood ADHD no longer predicts subsequent cannabis use, after controlling for ODD and CD symptoms.
- Some evidence suggests that deviant peer relationships and cigarette use may mediate the relationship between childhood ADHD and later cannabis use.
- Only one known study has investigated neurocognitive functioning in cannabis users with comorbid ADHD. Neuroimaging and longitudinal studies are needed to further investigate this population.
- Greater understanding of the impact of THC and CBD content on neurocognition may provide insight into the relationship between cannabis use and psychiatric problems.

KEY FACTS OF THC:CBD RATIOS IN THE IMPACT OF CANNABIS USE

- CBD has been shown to reduce anxiety in some studies.
- One study suggests that THC increases heart rate, anxiety, and psychosis-like symptoms, compared to either CBD or placebo.
- Decreased preference for cannabis has been associated with higher CBD:THC ratios.
- Using cannabis with increased THC:CBD ratios has been associated with earlier onset of psychosis, while higher CBD content is associated with experiencing fewer psychotic symptoms.
- Cannabis with higher CBD content may be associated with diminished memory impairment, after acute intoxication.
- THC content in many cannabis samples is drastically increasing, while CBD is decreasing.
- More longitudinal studies are needed in adolescent samples to assess the long-term effects of chronic cannabis use, with varying THC:CBD ratios.

LIST OF ABBREVIATIONS

Brain Imaging Terms

fMRI Functional magnetic resonance imaging
MRI Magnetic resonance imaging

Cannabis Components

CBD Cannabidiol
THC Δ^9-Tetrahydrocannabinol

Psychiatric Terms

ADHD Attention-deficit/hyperactivity disorder
CD Conduct disorder
CUD Cannabis use disorder
FEP First-episode psychosis
ODD Oppositional defiant disorder

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00053-3
Copyright © 2017 Elsevier Inc. All rights reserved.

INTRODUCTION

Cannabis use is often comorbid with other psychiatric disorders, including attention-deficit/hyperactivity disorder (ADHD), mood disorders, and schizophrenia (Tims et al., 2002; Agosti, Nunes, & Levin, 2002). This chapter will explore the association between cannabis use and ADHD, supplementing other chapters in this volume, which focus on the cooccurrence of cannabis use with mood disorders and psychotic disorders. Secondly, an emerging field of research suggests that cannabis content may influence the relationship between psychiatric disorders and cannabis use. Two cannabis components, Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), may have differential effects on cognition and mood. Thus, we will also review studies of THC and CBD levels, which might partially explain differential findings in the cannabis use comorbidity literature.

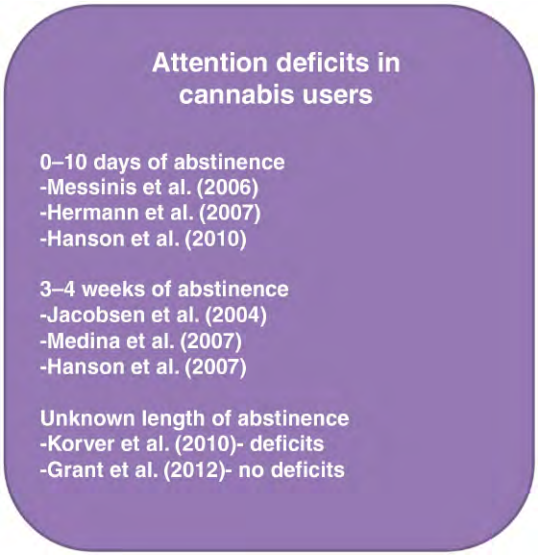


FIGURE e7.1 Attention deficits in cannabis users. Overview of studies examining attention deficits in cannabis users at various levels of abstinence.

ATTENTION-DEFICIT/HYPERACTIVITY DISORDER AND CANNABIS USE COMORBIDITY

Several studies support an association between chronic cannabis use and poor attention and executive functioning, in adolescents and adults without comorbid psychiatric disorders (for reviews, see Crane, Schuster, Fusar-Poli, & Gonzalez, 2013; Lisdahl, Wright, Medina-Kirchner, Maple, & Shollenbarger, 2014). After 3 days or fewer of abstinence, sustained and executive attention deficits have been found in adolescent and adult cannabis users (Hermann et al., 2007; Hanson et al., 2010). Messinis, Kyprianidou, Malefaki, and Papathanasopoulos (2006) found decreased psychomotor speed and sustained attention in adults who were abstinent 1–10 days prior to testing. Deficits in complex and sustained attention persist 3–4 weeks after abstinence, in samples that exclude for childhood ADHD diagnosis (Jacobsen, Mencl, Westerveld, & Pugh, 2004; Medina et al., 2007; Hanson et al., 2010). After an unknown period of abstinence, some studies have found cannabis-related attention differences (Korver et al., 2010), while others have found none (Grant, Chamberlain, Schreiber, & Odlaug, 2012). Age of cannabis use onset may also be related to attention problems. Early onset (before age 16) was associated with impaired reaction times on a visual scanning task, within a group of cannabis using adolescents and young adults, but not other measures of attention, such as alertness, divided attention, and flexibility (Ehrenreich et al., 1999) (Fig. e7.1).

It is also possible that prenatal cannabis exposure or regular cannabis use in youth may predict childhood ADHD symptoms. Increased cannabis use during pregnancy has been associated with sustained attention deficits at the age of 4 (Noland et al., 2005), as well as increased hyperactivity, impulsivity, and inattention problems at the age of 10 (Goldschmidt, Day, & Richardson, 2000). Taken together, the literature indicates that chronic cannabis use is often associated with long-term sustained and divided attention problems, particularly in youth. According to the *Diagnostic and Statistical Manual for Mental Disorders—Fifth Edition* (DSM-V), ADHD is diagnosed in individuals surpassing a threshold level of either inattentive, hyperactive, or combined inattentive/hyperactive problems (American Psychiatric Association, 2013).

Cross-sectional research has indicated that increased ADHD symptoms are associated with greater cannabis use, and cannabis use disorders (CUD), in adolescence and adulthood (De Alwis et al., 2014a; Tims et al., 2002; Walther, Morgenstern, & Hanewinkel, 2012). For example, Loflin, Earleywine, De Leo, and Hobkirk (2014) reported that daily cannabis users are more likely than moderate (5–30 days/month) or casual ($\leq$5 days/month) users to meet ADHD criteria in adults aged 18–74. In addition, more daily users were of the ADHD combined or hyperactive/impulsive subtypes than of the inattentive subtype, suggesting that cannabis use may be more prevalent in ADHD individuals with hyperactive/impulsive symptoms (Loflin et al., 2014). However, as Loflin et al. (2014) note, they did not account for additional substance use or comorbid psychiatric disorders; therefore, confounding factors may be driving the relationship between ADHD and cannabis use. Further, this study did not measure childhood ADHD diagnosis, but rather used a current symptom questionnaire; therefore, it is

not possible to disentangle whether the ADHD symptoms were independent from cannabis use exposure.

Approximately half of those with childhood ADHD will demonstrate persistent ADHD into adulthood (Halperin, Trampush, Miller, Marks, & Newcorn, 2008; Lara et al., 2009), driven primarily by inattention symptoms (Faraone, Biederman, & Mick, 2006). Regular cannabis use in individuals with ADHD may exacerbate symptoms of inattention and executive functioning, placing teens at increased risk for having persistent clinical symptoms into adulthood. Indeed, one longitudinal study found that cannabis use between the ages of 15–25 predicted persistence of ADHD symptoms at the age of 25 (Fergusson & Boden, 2008). Among a sample of adult treatment-seeking primary cocaine abusers, those with adult ADHD had a lower age of cannabis use initiation, compared to those with no history of ADHD (Perez de los Cobos et al., 2011). None of those in the ADHD sample had been previously diagnosed with childhood onset ADHD, suggesting their adult ADHD diagnosis may be dependent upon their adolescent substance use. Thus, preliminary data suggest that early cannabis exposure may be a risk factor for developing ADHD symptoms, or increasing one's risk for having persistent ADHD symptoms into adulthood.

Several studies have attempted to determine the direction of causality between cannabis use and ADHD (for a metaanalytic review, see Charach, Yeung, Climans, & Lillie, 2011). Adolescent boys with high familial risk of substance use score higher on measures of hyperactivity, compared to controls, suggesting a shared genetic factor for hyperactivity and substance use, in general (Aytaclar, Tarter, Kirisci, & Lu, 1999). Elkins et al. (2007) concluded that hyperactivity and inattentive symptoms in early adolescence (M_{age} = 11 years) predicted increased substance use, including cannabis abuse and dependence, at two follow-up points (M_{age} = 14 years, and M_{age} = 18 years). Similarly, Hechtman et al. (1984) conducted a longitudinal study following both children diagnosed with hyperactivity between ages 6–12 and healthy controls. Ten years later, significantly more hyperactive children than controls had tried cannabis or hashish, although there was no difference in past 3 month use levels.

Retrospective studies have also provided evidence for ADHD symptoms predicting later cannabis use (Fig. e7.2). A study of adolescent males determined that those with a history of childhood ADHD had an earlier onset, and more severe history of cannabis use, compared to those without an ADHD history (Kousha et al., 2012). Three studies of young adults determined that both increased hyperactive and inattentive symptoms before the age of 18 predicted lifetime cannabis use, and incidence of CUD (Murphy et al., 2002; Ameringer & Leventhal, 2013; De Alwis et al., 2014b). Alternatively, Lambert and Hartsough (1998) found no differences between adults diagnosed with childhood ADHD and

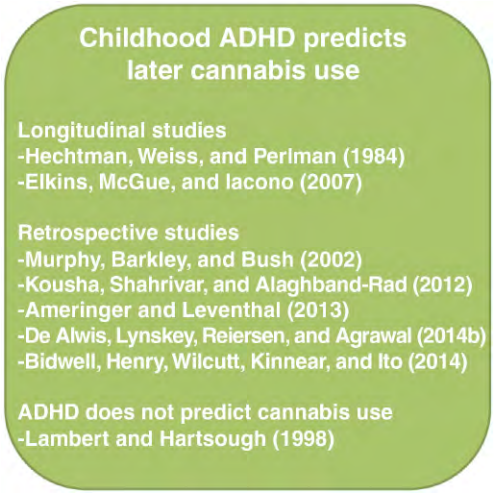


FIGURE e7.2 Associations between ADHD and cannabis use. Overview of studies examining the relationship between ADHD and subsequent cannabis use.

controls on measures of cannabis use. Other evidence indicates that inattention and hyperactivity may differentially predict later cannabis use. Bidwell et al. (2014) found that while inattentive symptoms in childhood were related to more cannabis use and craving in young adulthood, childhood hyperactivity symptoms were related to earlier cannabis use onset. Therefore, it is possible that cannabis use outcomes may depend on specific ADHD symptoms. Therefore, childhood ADHD diagnosis places one at increased risk for cannabis use, earlier age of cannabis use onset, and developing CUD.

Other externalizing disorders, including oppositional defiant disorder (ODD) and conduct disorder (CD), are often comorbid with ADHD, and may also be associated with cannabis use outcomes (Angold, Costello, & Erkanli, 1999) (Fig. e7.3). ADHD, CD, and ODD at the age of 11 have been shown to independently predict cannabis use

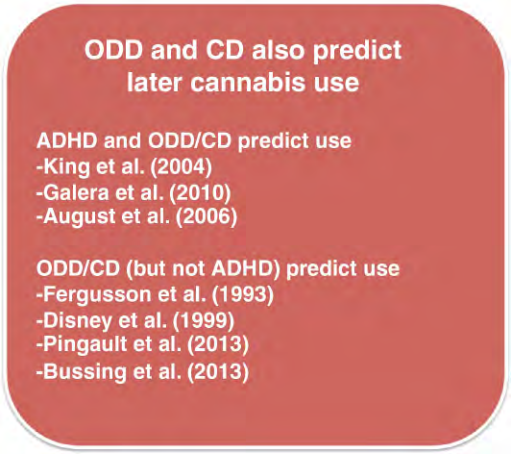


FIGURE e7.3 Associations between ADHD, ODD/CD, and cannabis use. Overview of studies examining the relationship between ADHD and ODD/CD and subsequent cannabis use.

initiation, and monthly use, by the age of 14 (King, Iacono, & McGue, 2004). In males, increased hyperactive-inattentive and CD symptoms in childhood (ages 4–18) have been associated with higher levels of cannabis use onset 8 years later (Galéra et al., 2010). August et al. (2006) found that those diagnosed at aged 11–16 with childhood ADHD, combined with either CD or ODD, reported greater lifetime cannabis use at ages 17–19, compared to both controls and those with only ADHD. In fact, some evidence suggests that the association between ADHD and cannabis use disappears, after controlling for CD and ODD. In one study, conduct problems in middle childhood predicted cannabis use by the age of 15, and early ADHD did not predict cannabis use after controlling for the association between ADHD and CD (Fergusson, Lynskey, & Horwood, 1993). Furthermore, Pingault et al. (2013) assessed behavioral problems in children aged 6–12, and then measured cannabis use at the age of 21. Opposition was the only predictor of adult cannabis use; inattention, hyperactivity/impulsivity, anxiety, and depression were not associated with cannabis outcomes. Similarly, Bussing, Mason, Bell, Porter, and Garvan (2010) observed that ODD, but not ADHD, at the ages of 5–11 increased risk of cannabis use 8 years later. In yet another study, CD, but not ADHD, was associated with cannabis use in 17-year-olds (Disney, Elkins, McGue, & Iacono, 1999). On the other hand, within a sample of first-episode psychosis (FEP) patients, greater childhood inattention problems, but not hyperactivity, ODD, or CD, predicted lifetime cannabis use and difficulty maintaining cannabis use abstinence during FEP treatment (Cassidy, Joober, King, & Malla, 2011). It is possible that comorbidity with psychosis might moderate the relationship between cannabis use and externalizing problems, although more research is needed. Thus, whether ADHD predicts cannabis use independently of CD and ODD in otherwise healthy samples remains unclear. It has been proposed that ADHD, CD, and ODD share underlying socioemotional dispositions, including prosociality, negative emotionality, and daring (Taylor, Allan, Mikolajewski, & Hart, 2013). Therefore, it is possible that individuals with ADHD possessing more aspects of the shared dispositions are at increased risk for cannabis use.

Mediating factors may also help explain the link between ADHD and cannabis use. Affiliation with deviant peers may drive the relationship between childhood ADHD and past 6-month adolescent cannabis use (Marshal, Molina, & Pelham, 2003). Moreover, Korhonen et al. (2010) demonstrated that cigarette use at the ages of 12–15 mediated the relationship between parent reports of externalizing behaviors (conduct, oppositional, and attention-deficit/hyperactivity problems) at the ages of 10–12, and cannabis use at age 15–18. Therefore, further research should investigate the specific mechanisms driving the relationship between externalizing disorders and cannabis use.

Considering that ADHD is twice as prevalent in males as in females (American Psychiatric Association, 2013), gender may moderate the relationship between cannabis use and ADHD symptoms. For example, hyperactivity and inattention symptoms in childhood may be related to increased cannabis use 8 years later in males, but not females (Galéra, Bouvard, Messiah, & Fombonne, 2008). In contrast, in a study of adolescents aged 12–16, childhood ADHD was marginally associated with cannabis use in females, but not in males (Ostojic, Charach, Henderson, McAuley, & Crosbie, 2014). More research is needed to examine the influence of gender on cannabis use outcomes, in those with ADHD.

Only one known study to date has investigated neurocognitive functioning in those with comorbid ADHD and cannabis use. Executive functioning was assessed in regular cannabis users and nonusers, with and without a childhood diagnosis of ADHD (Tamm et al., 2013). Importantly, individuals were excluded for weekly binge drinking, and excessive other substance use. Tamm et al. (2013) found that nonusers without ADHD performed significantly better than those with ADHD on tests of verbal memory, processing speed, cognitive interference, decision-making, working memory, and response inhibition. However, no differences were found between cannabis users and nonusers on any executive functioning tasks. In addition, there were no interactions between ADHD and cannabis use. Although no effects for cannabis use were determined, those who started using cannabis regularly before the age of 16 performed significantly worse on tests of decision-making, working memory, and response inhibition than those who initiated use at a later age. Further, it is notable that only recent (past year) cannabis use was closely measured, so those in the "nonuser" group could have previous years of heavy cannabis exposure. Therefore, this study suggests that recent cannabis use may not worsen executive functioning abilities in emerging adults with childhood ADHD. However, early onset of regular cannabis use was associated with significantly worse executive functioning in those with ADHD. Given the high comorbidity and these initial findings, additional studies are needed to explore the relationship between cannabis use, ADHD, and neurocognition. Neuroimaging studies, in particular, are needed to characterize the independent and interactive effects of cannabis use and ADHD on brain functioning.

IMPACT OF THC AND CBD CONTENT

Cannabis samples differ in levels of THC and CBD, two major psychoactive components of the drug. Due to the unique effects of each, varying levels of THC and CBD may contribute to neurocognitive differences observed in both comorbid and healthy cannabis users.

As THC is the major psychoactive component of cannabis, it has received the largest proportion of attention from the scientific community. However, CBD has emerged as a potentially neuroprotective, antipsychotic, and antianxiety agent (for full review, see Niesink & van Laar, 2013). A single dose of CBD can reduce anxiety in individuals with social anxiety disorder, during a speech (Bergamaschi et al., 2011). In comparison to placebo, THC alone, and THC + CBD, oral inhalation doses of CBD have been shown to increase emotion recognition (Hindocha et al., 2014). CBD administered in an inhaler reduced cigarette smoking in treatment-seeking tobacco smokers (Morgan, Das, Joye, Curran, & Kamboj, 2013). CBD has also been suggested as a possible treatment for cannabis dependence, as using cannabis with a higher CBD:THC ratio has been associated with decreased preference for cannabis (Morgan, Freeman, Schafer, & Curran, 2010). In a study comparing almost pure THC, CBD, and placebo in healthy volunteers, THC was found to increase heart rate, anxiety, and psychosis-like symptoms significantly more than either CBD or placebo (Martin-Santos et al., 2012). Notably, in this study there were very few differences between CBD and placebo, suggesting that, in this sample, CBD did not have as strong an anxiolytic effect as has been previously found (Fig. e7.4).

Other psychological and physical symptoms have been analyzed (Fig. e7.5). Examining psychosis symptoms, using higher THC potency, "skunk-type," cannabis has been associated with earlier onset of psychosis, compared to using cannabis with a higher THC:CBD ratio (Di Forti et al., 2009, 2014). Higher CBD content has also been associated with experiencing fewer psychotic symptoms (Schubart et al., 2011). Therefore, THC:CBD content could provide one explanation for the complicated literature on cannabis use and psychosis. In a naturalistic study, Morgan et al. (2010) found that individuals who smoked cannabis high in CBD content, regardless of THC content, did not have memory impairments, following acute intoxication. Using a similar sample, Morgan et al. (2010) also found high CBD:THC content to predict significantly fewer drug and food attentional biases. In rhesus monkeys, acute effects of THC + CBD reduced some cognitive deficits (Wright, Vandewater, & Taffe, 2013). Yet other studies have found CBD to be less influential, or even found CBD to be harmful. For example, in male adolescent rats, CBD has been shown to potentiate the effects of equal doses of THC, when given repeatedly over a 21 day period, resulting in significantly greater THC levels in their blood content in CBD + THC rats than in THC rats alone, as well as an increase in anxiety levels (Klein et al., 2011). Male rats have also been shown to have either no difference in THC-induced hypothermia or hypolocomotion, when treated or pretreated with CBD and, at times, those who had been exposed to CBD had exaggerated symptoms of hypothermia (Taffe, Creehan, & Vandewater, 2014). More research is needed to clarify the mechanisms and effects of CBD on symptomologies such as psychosis and anxiety.

Magnetic Resonance Imaging (MRI) studies have also indicated differences due to THC:CBD content and exposure. Individuals using cannabis with high THC or low CBD content have been found to have smaller right anterior hippocampal gray matter volume (Demirakca et al., 2011). CBD has also been found to reduce anxiety response in a functional MRI (fMRI) emotion processing task (Fusar-Poli et al., 2009). In an attention task of occasional cannabis users, acute doses of THC or CBD had opposite effects in various attention regions, such that if one resulted in increased connectivity, the other attenuated similar regions (Bhattacharyya et al., 2014). Specifically, THC was associated with reduced frontostriatal connectivity, while CBD increased connectivity in these regions. On the other hand, CBD diminished

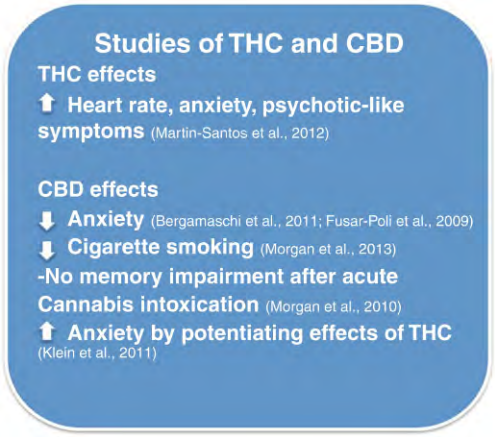


FIGURE e7.4 The effects of THC and CBD. Overview of studies examining the effects of THC and CBD.

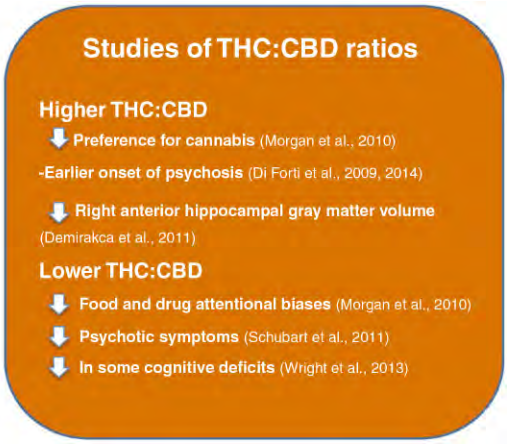


FIGURE e7.5 The effects of differential THC:CBD ratios. Overview of studies examining the effects of THC:CBD ratios.

mediotemporal-prefrontal connectivity, while THC strengthened it (Bhattacharyya et al., 2014). Together, this preliminary evidence suggests there may be neuroanatomical and neuronal functioning differences as a result of THC and CBD concentration.

As THC content is increasing (more than doubling), while CBD content is decreasing and consistently low, in marijuana samples in places like California (Burgdorf, Kilmer, & Pacula, 2011), greater understanding of the neurocognitive influences of CBD is essential for making more informed decisions regarding potential policy implications. Further, as most studies have thus far only looked at the acute effects of THC:CBD when intoxicated (eg, Fusar-Poli et al., 2009; Wright et al., 2013), more longitudinal studies examining chronic exposure are needed.

CONCLUSIONS AND FUTURE DIRECTIONS

Investigation into the effects of cannabis use in comorbid populations, including those with ADHD, is a relatively new field. While many studies report attention problems in cannabis users, the literature is still mixed regarding whether these deficits are premorbid, or if they emerge as a result of cannabis use. Some studies suggest that other factors, such as delinquent peer relationships, comorbid ODD, CD, or cigarette use may be responsible for increased cannabis use in ADHD individuals. Additionally, males and females with ADHD may have different patterns of cannabis use. Therefore, more research is needed in order to determine the specific relationships between cannabis use, ADHD, and other influences as gender and comorbidities. Notably, there is a significant dearth of research on the interactive effects of cannabis use and ADHD on neurocognition, and whether cannabis use during adolescence places individuals with childhood ADHD at increased risk for having persistent adult ADHD symptoms. Longitudinal and neuroimaging studies, in particular, may clarify neurocognitive vulnerabilities specific to ADHD patients who use cannabis. Furthermore, it is possible that inconsistent findings in the cannabis comorbidity literature are due to THC potency in cannabis (Di Forti et al., 2009, 2014). Longitudinal studies may help characterize the effects of chronic exposure to differing THC and CBD levels. Overall, further investigation into the relationships between cannabis use and both ADHD and THC:CBD content is needed.

MINI-DICTIONARY

Attention-deficit/hyperactivity disorder A psychiatric disorder involving either significant inattentive symptoms, significant hyperactive symptoms, or both, that lead to impaired functioning.

Cannabidiol Another psychoactive cannabinoid found in cannabis that is thought to be neuroprotective against the negative effects of THC.

Cannabis use disorder Problematic cannabis use, leading to impairment. Possible symptoms include (but are not limited to) an increase in tolerance and withdrawal symptoms, unintended increase in cannabis use, strong craving or urge to use cannabis, or using cannabis in hazardous situations.

Cognitive interference This measures one's ability to disregard extraneous information in order to suppress an incorrect response to a stimulus.

Conduct disorder A psychiatric disorder involving a pattern of aggression toward people and animals, destruction of property, theft, or serious violation of rules.

Executive functioning A function of the prefrontal cortex, executive functioning is involved in regulating goal-directed behavior. Working memory is a component of executive functioning.

Oppositional defiant disorder A psychiatric disorder characterized by repeated irritability, argumentative behavior, or vindictiveness, lasting at least 6 months.

Processing speed The ability to quickly and accurately react to information in one's environment.

Psychosis A psychiatric condition defined by delusions, hallucinations, disorganized speech, or catatonic behavior that leads to impairment.

Response inhibition The ability to stop oneself from responding inappropriately.

Δ^9-Tetrahydrocannabinol The main psychoactive cannabinoid in the cannabis plant that is thought to be responsible for many of the drug's effects on the brain.

Working memory The ability to briefly retain and manipulate material in one's mind in order to produce a result.

References

Agosti, V., Nunes, E., & Levin, F. (2002). Rates of psychiatric comorbidity among U.S. residents with lifetime cannabis dependence. *The American Journal of Drug and Alcohol Abuse*, *28*(4), 643–652.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Arlington, VA: American Psychiatric Publishing.

Ameringer, K. J., & Leventhal, A. M. (2013). Associations between attention deficit hyperactivity disorder symptom domains and DSM-IV lifetime substance dependence. *American Journal of Addiction*, *22*(1), 1–16.

Angold, A., Costello, E. J., & Erkanli, A. (1999). Comorbidity. *Journal of Child Psychology and Psychiatry*, *40*(1), 57–87.

August, G. J., Winters, K. C., Realmuto, G. M., Fahnhorst, T., Botzet, A., & Lee, S. (2006). *Journal of the American Academy of Child and Adolescent Psychiatry*, *45*(7), 824–832.

Aytaclar, A., Tarter, R. E., Kirisci, L., & Lu, S. (1999). Association between hyperactivity and executive cognitive functioning in childhood and substance use in early adolescence. *Journal of the American Academy of Child and Adolescent Psychiatry*, *38*(2), 172–178.

Bergamaschi, M. M., Queiroz, R. H. C., Chagas, M. H. N., de Oliveira, D. C. G., de Martinis, B. S., Kapczinski, F., Quevedo, J., Roesler, R., Schroder, N., Nardi, A. E., Martin-Santos, R., Hallak, J. E. C., Zuardi, A. W., & Crippa, J. A. S. (2011). Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naive social phobia patients. *Neuropsychopharmacology*, *36*, 1219–1226.

Bhattacharyya, S., Falkenberg, I., Martin-Santos, R., Atakan, Z., Crippa, J. A., Giampietro, V., Brammer, M., & McGuire, P. (2014). Cannabinoid modulation of functional connectivity within regions processing attentional salience. *Neuropsychopharmacology*, *40*, 1343–1352.

Bidwell, L. C., Henry, E. A., Wilcutt, E. G., Kinnear, M. K., & Ito, T. A. (2014). Childhood and current ADHD symptom dimensions are associated with more severe cannabis outcomes in college students. *Drug and Alcohol Dependence*, *135*, 88–94.

Burgdorf, J. R., Kilmer, B., & Pacula, R. L. (2011). Heterogeneity in the composition of marijuana seized in California. *Drug and Alcohol Dependence*, *117*(1), 59–61.

Bussing, R., Mason, D. M., Bell, L., Porter, P., & Garvan, C. (2010). Adolescent outcomes of childhood attention-deficit/hyperactivity disorder in a diverse community sample. *Journal of the American Academy of Child and Adolescent Psychiatry*, *49*(6), 595–605.

Cassidy, C. M., Joober, R., King, S., & Malla, A. K. (2011). Childhood symptoms of inattention-hyperactivity predict cannabis use in first episode psychosis. *Schizophrenia Research*, *132*, 171–176.

Charach, A., Yeung, E., Climans, T., & Lillie, E. (2011). Childhood attention-deficit/hyperactivity disorder and future substance use disorders: comparative meta-analyses. *Journal of the American Academy of Child and Adolescent Psychiatry*, *50*(1), 9–21.

Crane, N. A., Schuster, R. A., Fusar-Poli, P., & Gonzalez, R. (2013). Effects of cannabis on neurocognitive functioning: recent advances, neurodevelopmental influences, and sex differences. *Neuropsychology Review*, *23*(2), 117–137.

De Alwis, D., Agrawal, A., Reiersen, A. M., Constantino, J. N., Henders, A., Martin, N. G., & Lynskey, M. T. (2014a). ADHD symptoms, autistic traits, and substance use and misuse in adult Australian twins. *Journal of Studies on Alcohol and Drugs*, *75*(2), 211–221.

De Alwis, D., Lynskey, M. T., Reiersen, A. M., & Agrawal, A. (2014b). Attention-deficit/hyperactivity disorder subtypes and substance use and use disorders in NESARC. *Addictive Behaviors*, *39*(8), 1278–1285.

Demirakca, T., Sartorius, A., Ende, G., Meyer, N., Welzel, H., Skopp, G., Hermann, D., et al. (2011). Diminished gray matter in the hippocampus of cannabis users: possible protective effects of cannabidiol. *Drug and Alcohol Dependence*, *114*(2/3), 242–245.

Di Forti, M., Morgan, C., Dazzan, P., Pariante, C., Mondelli, V., Marques, T. R., Handley, R., Luzi, S., Russo, M., Paparelli, A., Butt, A., Stilo, S. A., Wiffen, B., Powell, J., & Murray, R. M. (2009). High-potency cannabis and the risk of psychosis. *The British Journal of Psychiatry*, *195*, 488–491.

Di Forti, M., Sallis, H., Allegri, F., Trotta, A., Ferraro, L., Stilo, S. A., Marconi, A., La Cascia, C., Marques, T. R., Pariante, C., Dazzan, P., Mondelli, V., Paparelli, A., Kolliakou, A., Prata, D., Gaughran, F., David, A. S., Morgan, C., Stahl, D., Khondoker, M., MacCabe, J. H., & Murray, R. M. (2014). Daily use, especially of high-potency cannabis, drives the earlier onset of psychosis in cannabis users. *Schizophrenia Bulletin*, *40*(6), 1509–1517.

Disney, E. R., Elkins, I. J., McGue, M., & Iacono, W. G. (1999). Effects of ADHD, conduct disorder, and gender on substance use and abuse in adolescence. *American Journal of Psychiatry*, *156*(10), 1515–1521.

Ehrenreich, H., Rinn, T., Kunert, H. J., Moeller, M. R., Poser, W., Schilling, L., Gigerenzer, G., & Hoehe, M. R. (1999). Specific attentional dysfunction in adults following early start of cannabis use. *Psychopharmacology*, *142*, 295–301.

Elkins, I. J., McGue, M., & Iacono, W. G. (2007). Prospective effects of attention-deficit/hyperactivity disorder, conduct disorder, and sex on adolescent substance use and abuse. *Archives of General Psychiatry*, *64*(10), 1145–1152.

Faraone, S. V., Biederman, J., & Mick, E. (2006). The age-dependent decline of attention deficit hyperactivity disorder: a meta-analysis of follow-up studies. *Psychological Medicine*, *36*(2), 159–165.

Fergusson, D. M., & Boden, J. M. (2008). Cannabis use and adult ADHD symptoms. *Drug and Alcohol Dependence*, *95*, 90–96.

Fergusson, D. M., Lynskey, M. T., & Horwood, J. L. (1993). Conduct problems and attention deficit behavior in middle childhood and cannabis use by age 15. *The Australian and New Zealand Journal of Psychiatry*, *27*(4), 673–682.

Fusar-Poli, P., Crippa, J. A., Bhattacharyya, S., Borgwardt, S. J., Allen, P., Martin-Santos, R., Seal, M., Surguladze, S. A., O'Carrol, C., Atakan, Z., Zuardi, A. W., & McGuire, P. K. (2009). Distinct effects of d9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Archives of General Psychiatry*, *66*(1), 95–105.

Galéra, C., Bouvard, M. P., Melchoir, M., Chastang, J. F., Lagarde, E., Michel, G., Encrenaz, G., Messiah, A., & Fombonne, E. (2010). Disruptive symptoms in childhood and adolescence and early initiation of tobacco and cannabis use: the Gazel Youth study. *European Psychiatry*, *25*, 402–408.

Galéra, C., Bouvard, M. P., Messiah, A., & Fombonne, E. (2008). Hyperactivity-inattention symptoms in childhood and substance use in adolescence: the youth Gazel cohort. *Drug and Alcohol Dependence*, *94*, 30–37.

Goldschmidt, L., Day, N. L., & Richardson, G. A. (2000). Effects of prenatal marijuana exposure on child behavior problems at age 10. *Neurotoxicology and Teratology*, *22*(3), 325–336.

Grant, J. E., Chamberlain, S. R., Schreiber, L., & Odlaug, B. L. (2012). Neuropsychological deficits associated with cannabis use in young adults. *Drug and Alcohol Dependence*, *121*(1–2), 159–162.

Halperin, J. M., Trampush, J. W., Miller, C. J., Marks, D. J., & Newcorn, J. H. (2008). Neuropsychological outcome in adolescents/young adults with childhood ADHD: profiles of persisters, remitters, and controls. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *49*(9), 958–966.

Hanson, K. L., Winward, J. L., Schweinsburg, A. D., Medina, K. L., Brown, S. A., & Tapert, S. F. (2010). Longitudinal study of cognition among adolescent marijuana users over three weeks of abstinence. *Addictive Behaviors*, *35*(11), 970–976.

Hechtman, L., Weiss, G., & Perlman, T. (1984). Hyperactives as young adults: past and current substance use and antisocial behavior. *American Journal of Orthopsychiatry*, *54*(3), 415–425.

Hermann, D., Santorius, A., Welzel, H., Walter, S., Skopp, G., Ende, G., & Mann, K. (2007). Dorsolateral prefrontal cortex *N*-acetylaspartate/total creatine (NAA/tCr) loss in male recreational cannabis users. *Biological Psychiatry*, *61*(11), 1281–1289.

Hindocha, C., Freeman, T. P., Schafer, G., Gardener, C., Das, R. K., Morgan, C. J. A., & Curran, H. V. (2014). Acute effects of delta-9-tetrahydrocannabinol, cannabidiol and their combination on facial emotion recognition: a randomized, double-blind, placebo-controlled study in cannabis users. *European Neuropsychopharmacology*, *25*, 325–334.

Jacobsen, L. K., Mencl, W. E., Westerveld, M., & Pugh, K. (2004). Impact of cannabis use on brain function in adolescents. *Annals of the New York Academy of Sciences*, *1021*, 384–390.

King, S. M., Iacono, W. G., & McGue, M. (2004). Childhood externalizing and internalizing psychopathology in the prediction of early substance use. *Addiction*, *99*, 1548–1559.

Klein, C., Karanges, E., Spiro, A., Wong, A., Spencer, J., Huynh, T., Gunasekaran, N., Karl, T., Long, L. E., Huang, X. F., Liu, K., Arnold, J. C., & McGregor, I. S. (2011). Cannabidiol potentiates d9-tetrahydrocannabinol (THC) behavioural effects and alters THC pharmacokinetics during acute and chronic treatment in adolescent rats. *Psychopharmacology*, *218*, 443–457.

Korhonen, T., van Leeuwen, A. P., Reijneveld, S. A., Ormel, J., Verhulst, F. C., & Huizink, A. C. (2010). Externalizing behavior problems and cigarette smoking as predictors of cannabis use: the TRAILS study. *Journal of the American Academy of Child and Adolescent Psychiatry*, *49*(1), 61–69.

Korver, N., Nieman, D. H., Becker, H. E., Reinaud van de Fliert, J., Dingemans, P. H., de Haan, L., Spiering, M., Schmitz, N., & Linszen, D. H. (2010). Symptomatology and neuropsychological functioning in cannabis using subjects at ultra-high risk for developing psychosis and healthy controls. *Australian and New Zealand Journal of Psychiatry*, *44*, 230–236.

Kousha, M., Shahrivar, A., & Alaghband-Rad, J. (2012). Substance use disorder and ADHD: is ADHD a particularly "specific" risk factor? *Journal of Attention Disorders*, *16*(4), 325–332.

Lambert, N. M., & Hartsough, C. S. (1998). Prospective study of tobacco smoking and substance dependencies among samples of ADHD non-ADHD participants. *Journal of Learning Disabilities*, *31*(6), 533–544.

Lara, C., Fayyad, J., de Graaf, R., Kessler, R. C., Aguilar-Gaxiola, S., Angermeyer, M., Demytteneare, K., de Girolamo, G., Haro, J. M., Jin, R., Karam, E. G., Lepine, J. P., Mora, M. E., Ormel, J., Posada-Villa, J., & Sampson, N. (2009). Childhood predictors of adult attention-deficit/hyperactivity disorder: results from the World Health Organization World Mental Health Survey Initiative. *Biological Psychiatry*, *65*(1), 46–54.

Lisdahl, K. M., Wright, N. E., Medina-Kirchner, C., Maple, K. E., & Shollenbarger, S. (2014). Considering cannabis: the effects of regular cannabis use on neurocognition in adolescents and young adults. *Current Addiction Reports*, *1*, 144–156.

Loflin, M., Earleywine, M., De Leo, J., & Hobkirk, A. (2014). Subtypes of attention deficit-hyperactivity disorder (ADHD) and cannabis use. *Substance Use and Misuse*, *49*, 427–434.

Marshal, M. P., Molina, B. S., & Pelham, W. E., Jr. (2003). Childhood ADHD and adolescent substance use: an examination of deviant peer group affiliation as a risk factor. *Psychology of Addictive Behaviors*, *17*(4), 293–302.

Martin-Santos, R., Crippa, J. A., Batalla, A., Bhattacharyya, S., Atakan, Z., Borgwardt, S., Allen, P., Seal, M., Langhor, K., Farre, M., Zuardi, A. W., & McGuire, P. K. (2012). Acute effects of a single, oral dose of d9-tetrahydrocannabinol (THC) and cannabidiol (CBD) administration in healthy volunteers. *Current Pharmaceutical Design*, *18*, 4966–4979.

Medina, K. L., Hanson, K. L., Schweinsburg, A. D., Cohen-Zion, M., Nagel, B. J., & Tapert, S. F. (2007). Neuropsychological functioning in adolescent marijuana users: subtle deficits detectable after a month of abstinence. *Journal of the International Neuropsychological Society*, *13*(5), 807–820.

Messinis, L., Kyprianidou, A., Malefaki, S., & Papathanasopoulos, P. (2006). Neuropsychological deficits in long-term frequent cannabis users. *Neurology*, *66*, 737–739.

Morgan, C. J. A., Das, R. K., Joye, A., Curran, H. V., & Kamboj, S. K. (2013). Cannabidiol reduces cigarette consumption in tobacco smokers: preliminary findings. *Addictive Behaviors*, *38*, 2433–2436.

Morgan, C. J. A., Freeman, T. P., Schafer, G. L., & Curran, H. V. (2010). Cannabidiol attenuates the appetitive effects of d9-tetrahydrocannabinol in humans smoking their chosen cannabis. *Neuropsychopharmacology*, *35*, 1879–1885.

Murphy, K. R., Barkley, R. A., & Bush, T. (2002). Young adults with attention deficit hyperactivity disorder: subtype differences in comorbidity, educational, and clinical history. *The Journal of Nervous and Mental Disease*, *190*(3), 147–157.

Niesink, R. J. M., & van Laar, M. W. (2013). Does cannabidiol protect against adverse psychological effects of THC? *Frontiers in Psychiatry*, *4*, 130.

Noland, J. S., Singer, L. T., Short, E. J., Minnes, S., Arendt, R. E., Kirchner, H. L., & Bearer, C. (2005). Prenatal drug exposure and selective attention in preschoolers. *Neurotoxicology and Teratology*, *27*, 429–438.

Ostojic, D., Charach, A., Henderson, J., McAuley, T., & Crosbie, J. (2014). Childhood ADHD and addictive behaviors in adolescence: a Canadian sample. *Journal of the Canadian Academy of Child and Adolescent Psychiatry*, *23*(2), 128–135.

Perez de los Cobos, J., Sinol, N., Puerta, C., Cantillano, V., Lopez Zurita, C., & Trujols, J. (2011). Features and prevalence of patients with probable adult attention deficit hyperactivity disorder who request treatment for cocaine use disorders. *Psychiatry Research*, *185*(1–2), 205–210.

Pingault, J., Côté, S. M., Galéra, C., Genolini, C., Falissard, B., Vitaro, F., & Tremblay, R. E. (2013). Childhood trajectories of inattention, hyperactivity and oppositional behaviors and prediction of substance abuse/dependence: a 15-year longitudinal population-based study. *Molecular Psychiatry*, *18*(7), 806–812.

Schubart, C. D., Sommer, I. E. C., van Gastel, W. A., Goetgebuer, R. L., Kahn, R. S., & Boks, M. P. M. (2011). Cannabis with high cannabidiol content is associated with fewer psychotic experiences. *Schizophrenia Research*, *130*, 216–221.

Taffe, M. A., Creehan, K. M., & Vandewater, S. A. (2014). Cannabidiol fails to reverse hypothermia or locomotor suppression induced by delta9-tetrahydrocannabinol in Sprague-Dawley rats. *British Journal of Pharmacology*, *172*, 1783–1791.

Tamm, L., Epstein, J. N., Lisdahl, K. M., Molina, B., Tapert, S., Hinshaw, S. P., Arnold, L. E., Velanova, K., Abikoff, H., Swanson, J. M., et al. (2013). Impact of ADHD and cannabis use on executive functioning in young adults. *Drug and Alcohol Dependence*, *133*(2), 607–614.

Taylor, J., Allan, N., Mikolajewski, A. J., & Hart, S. A. (2013). Common genetic and nonshared environmental factors contribute to the association between socioemotional dispositions and the externalizing factor in children. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *54*(1), 67–76.

Tims, F. M., Dennis, M. L., Hamilton, N., Buchanan, B. J., Diamond, G., Funk, R., & Brantley, L. B. (2002). Characteristics and problems of 600 adolescent cannabis abusers in outpatient treatment. *Addiction*, *97*(Suppl. 1), 46–57.

Walther, B., Morgenstern, M., & Hanewinkel, R. (2012). Co-occurrence of addictive behaviors: personality factors related to substance use, gambling and computer gaming. *European Addiction Research*, *18*, 167–174.

Wright, M. J., Vandewater, S. A., & Taffe, M. A. (2013). Cannabidiol attenuates deficits of visuospatial associative memory induced by d9-tetrahydrocannabinol. *British Journal of Pharmacology*, *170*, 1365–1373.

PART IV

CANNABIS, ORGANS, TISSUES AND NON-CNS ASPECTS

47 *Chronic cannabis abuse and thyroid function* 461
48 *Cannabis hyperemesis syndrome* 466
49 *Cannabis and cannabinoids and the effects on gastrointestinal function: An overview* 471
50 *Cardiovascular effects of cannabis usage* 481
51 *Cannabis and stroke* 486
52 *Cannabis smoking and the lung* 494
53 *Cannabis and hepatic injury* 505
54 *Cannabis allergy: More than a bad trip* 517
55 *Marijuana and breastfeeding* 527
56 *Hypocretins/orexins and addiction: Role in* cannabis *dependence* 533
57 *Regulatory role of cannabinoids for skin barrier functions and cutaneous inflammation* 543

Page left intentionally blank

CHAPTER

47

Chronic Cannabis Abuse and Thyroid Function

U. Bonnet

Department of Psychiatry, Psychotherapy and Psychosomatics, Evangelisches Krankenhaus Castrop-Rauxel, Academic Teaching Hospital of the University of Duisburg-Essen, Castrop-Rauxel, Germany

SUMMARY POINTS

- Cannabinoid 1 (CB1) receptors are organized compactly along the central and peripheral thyroid hormone axis.
- Chronic cannabis smoking has only negligible effects on human thyroid function.
- TSH- and free T4-levels remained stable in the serum of regular, even heavier cannabis smokers.
- Thyroid function tests did not correlate significantly with serum THC-levels of chronic cannabis smokers.
- The results of the human studies argue against a relevant influence of regular or chronic cannabis consumption on thyroid function.

KEY FACTS OF THYROID HORMONES

- Trijodthyronine (T3) and thyroxin (T4) are produced by the thyroid gland, and are primarily responsible for regulation of metabolism. Iodine is necessary for the production of T3 and T4. Thyroxin (T4) is the major thyroid hormone in the blood. It has a longer half-life than T3. The ratio of T4 to T3 released into the blood is nearly 20 to 1. T4 is converted to the active T3 within thyroid follicular cells, via deiodinase. T3 is 3–4 times more potent than T4.
- Thyroxin-binding globulin (TBG) is one of three proteins (together with transthyretin and albumin) that bind T3 and T4 in blood. Of these three proteins, TBG has the highest affinity for thyroid hormones, and carries the majority of T4 in circulation.
- TRH is produced in the paraventricular nucleus of the hypothalamus, and spreads via blood stream to the anterior pituitary gland to stimulate TSH-synthesis, and release there. TSH can be also stimulated by cold temperature and hypoglycemia.
- TSH binds to a G-protein coupled TSH-receptor on the thyroid follicular cell, which stimulates the synthesis and release of thyroid hormones.
- Small changes in serum T4 are amplified by changes in serum TSH.
- Thyroid hormones increase basal metabolic rate, stimulate lipolysis/lipogenesis, increase in adaptive thermogenesis and stimulate β-oxidation of fatty acids (Mullur, Liu, & Brent, 2014).
- A deficiency of iodine leads to decreased production of T3 and T4, which enlarges the thyroid tissue via an increase of TSH, and will cause goiter.

KEY FACTS OF CANNABINOID RECEPTORS

- CB1-receptor is expressed in the hypothalamus and the pituitary gland, and CB1-receptor stimulation is known to modulate all the endocrine hypothalamic-peripheral endocrine axes (Pagotto, Marsicano, Cota, Lutz, & Pasquali, 2006).

LIST OF ABBREVIATIONS

CB1-receptor	Cannabinoid 1-receptor
HPT-axis	Hypothalamic-pituitary-thyroid axis
T3	Triiodthyronine
T4	Thyroxin

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00054-5
Copyright © 2017 Elsevier Inc. All rights reserved.

TGB	Thyroxin-binding-globulin
THC	Δ^9-Tetrahydrocannabinol
THC-COOH	11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid
THC-OH	11-hydroxy-delta-9-tetrahydrocannabinol
TRH	Thyrotropin releasing hormone
TSH	Thyroid stimulating hormone (thyrotropin)

INTRODUCTION

Cannabinoid 1 (CB1) receptors are organized compactly along the central and peripheral thyroid hormone axis (Fig. 47.1, Brown & Dobs, 2002; Porcella et al., 2002; Pagotto et al., 2006). Former reports have shown that acute administration of Δ^9-tetrahydrocannabinol (THC), the major psychoactive cannabinoid ingredient of cannabis, transiently reduced levels of thyroxine (T4) and thyroid-stimulating hormone (TSH) to a great extent, in rats (Nazar, Kairys, Fowler, & Harclerode, 1981). Furthermore, cannabis decreased the release of radioactive iodine from rats' thyroid (Lomax, 1970). Both depressing effects were reversed by exogenously applied TSH, which pointed to a hypothalamic site of action (Lomax, 1970; Nazar et al., 1981). Data about the effects of cannabis on thyroid function in humans are increasing. The author is aware of four studies, which addressed this subject (Table 47.1). These cohort-studies analyzed the thyroid function in patients who abused cannabis regularly, and did not consume any other illegal drug or alcohol (Rubin & Comitas, 1972; Parshad, Kumar, & Melville, 1983; Herning, Better, & Cadet, 2008; Bonnet, 2013). All investigated subjects were current nicotine smokers, or used tobacco to build cannabis-containing joints.

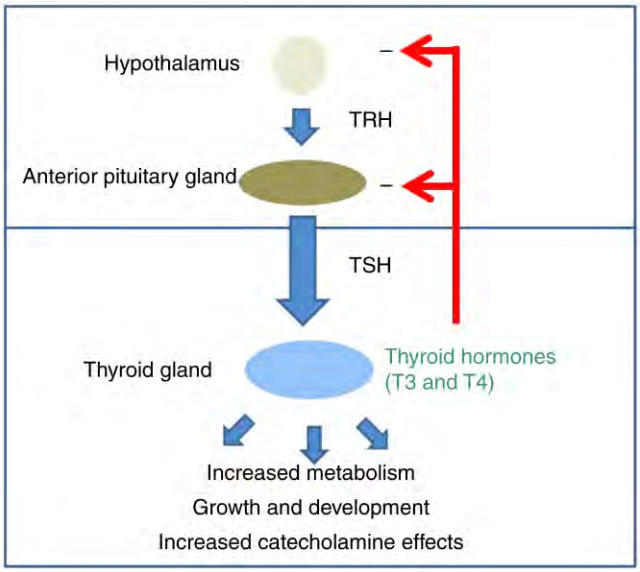


FIGURE 47.1 The hypothalamic-pituitary-thyroid axis (HPT-axis). Short overview on central (top) and peripheral (below) compounds of the HPT-axis. Hormones are transported via bloodstream. Negative feedback in *red*; for more details see Mullur et al. (2014).

TABLE 47.1 Studies on the Thyroid-Function of Chronic Cannabis Consumers

Study	Study type and *N*	Population	Results	Comments
Rubin and Comitas (1972)	Epidemiologic study, *N* = ?	Jamaican chronic cannabis consumers (predominantly African-Americans)	No relevant functional impairment in the thyroid glands (reported in Parshad et al., 1983)	Study is no longer available, according to a request of the author to NIHM
Parshad et al. (1983)	Case-control study: 26 marijuana smokers versus 30 nonsmokers	Jamaican chronic cannabis consumers (predominantly African-Americans)	Serum total T4, TSH and T3-uptake were found to be not significantly different between both groups and also were all within normal limits	
Herning et al. (2008)	Case control study: 108 cannabis consumers versus 33 control subjects "cannabis group" was subdivided into two groups, one consuming marijuana less than 8 years ("MJ-short," *N* = 56) and the other consuming marijuana longer than 8 years ("MJ-long," *N* = 19)	Chronic cannabis consumers from US (75% African Americans)	"MJ-long"-group showed significantly lower total serum T4 levels and higher T3 uptake levels in comparison to the "MJ-short"-group and the control group. TSH remained stable in the whole active sample	Study primarily focused on EEG-abnormalities
Bonnet (2013)	Cohort study: 39 cannabis consumers seeking inpatient detoxification treatment	German chronic cannabis consumers (all Caucasian)	None of the patients had serum TSH, T3, and free T4 tests outside normal limits at admission	Serum levels of thyroid hormones did not correlate significantly with serum levels of THC, THC-OH or THC-COOH at admission

EFFECT OF CHRONIC CANNABIS USE ON HUMAN THYROID FUNCTION

The first two studies were performed on Jamaican chronic cannabis smokers, and found no relevant functional impairment in the thyroid glands in this population (Rubin & Comitas, 1972; Parshad et al., 1983). However, the study of Parshad et al. (1983), which compared 26 marijuana smokers with 30 nonsmokers, described significantly lower serum total T3-levels in smokers (Parshad et al., 1983). Serum total T4, TSH, and T3-uptake were found to be not significantly different between both groups, and also were all within normal limits (Parshad et al., 1983). In the case of the study of Parshad et al. (1983), the validity was weakened by vague description of the amount of the recent cannabis intake, and strengthened by a comparison with a control group .

The study of Herning et al. (2008), which primarily focused on EEG-abnormalities, comprised 108 individuals (22.8 ± 5.3 years old, 53% women, 75% African American). Among them, there were 75 marijuana-smoking individuals, and 33 control subjects (Herning et al., 2008), all of whom were not seeking treatment, and were selected from a larger National Institute on Drug Abuse (NIDA) sample. All subjects of the active "cannabis group" smoked marijuana between 15 and 30 days before study entry, a fact that ensured an actual burden with cannabis. Furthermore, this "cannabis group" was subdivided into two further groups, one consuming marijuana for less than 8 years ("MJ-short," $N = 56$), and the other consuming marijuana for longer than 8 years ("MJ-long," $N = 19$) (Herning et al., 2008). The following thyroid function tests were investigated in the serum of all groups: TSH, total T4, and T3-uptake, which also corresponds well with the concentration of thyroxin-binding-globulin (TGB) that carries free T4 and free T3 in blood.

There were no statistical significant differences between the control group and "MJ-short"-group, with respect to these thyroid function tests. However, the "MJ-long"-group showed significantly lower total T4 levels, and higher T3 uptake levels, in comparison to the "MJ-short"-group, and the control group. In the whole active sample, TSH remained stable, and there were only two subjects with abnormal thyroid function tests. Both subjects were in the "MJ-long"-group, and had lower total T4-levels and higher T3-uptake levels than normal (Herning et al., 2008). The latter reflected a reduced T4-level, or increased activity of hormone binding proteins. The validity of this study is strengthened by the control group, and weakened by the vague definition of the amount of the recent cannabis consumption in active groups.

The most recent study was performed by the author, with 39 cannabis-dependents, (28.6 ± 8 years old, 20.5% women, all Caucasian), according to ICD-10 (Bonnet, 2013). The patients were seeking treatment, and were voluntarily admitted for inpatient detoxification because of serious problems with cannabis. On average, this cohort reported to have smoked 2.5 ± 1.2 g cannabis nearly daily, for a period of 54.2 ± 62.5 months, and to be 9.9 ± 6.7 years dependent on cannabis. The patients were included in the study if they (1) had consumed cannabis by inhalation daily or almost daily during the 6 months before admission, (2) had consumed cannabis within 24 h before admission, (3) had used no other psychotropic substances (except tobacco) during 4 weeks before admission, and (4) had no active comorbid psychiatric or somatic disorder that could noticeably affect the course of cannabis detoxification treatment.

Thyroid function tests (TSH, total T3, free T4) were determined directly at admission. Simultaneously, the serum levels of THC and its two major metabolites, water-soluble THC-OH and lipid-soluble THC-COOH (Fig. 47.2; Grotenhermen, 2003) were measured. None of the patients had THC-levels below the detection limit (1.0 ng/mL). On average, levels of THC, THC-OH, and THC-COOH were 13.1 ± 23.8 ng/mL ($N = 30$), 4.3 ± 8.6 ng/mL ($N = 16$), and 146.4 ± 149.9 ng/mL ($N = 30$), respectively. These values underlined a substantial and sustained ("chronic") cannabis intake (Musshoff & Madea, 2006), and reflect the self-reported nearly daily abuse in the last weeks (Bonnet, 2013).

None of the included 39 patients had thyroid function tests outside normal limits. Furthermore, the serum levels of the thyroid hormones did not correlate significantly with the serum levels of THC or its metabolites, which suggested no relevant interplay between thyroid-hormones and the measured cannabinoids after chronic cannabis abuse. The validity of this study is

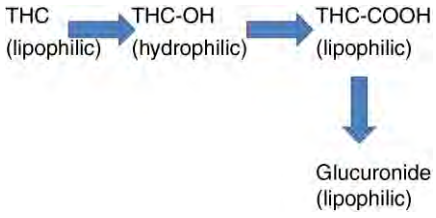


FIGURE 47.2 Main metabolic route for THC. THC is oxidized in humans mainly to 11-hydroxy-delta-9-tetrahydrocannabinol (THC-OH). This metabolite is also psychoactive, and degraded to non-psychoactive 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH). More than 100 different THC-metabolites have been identified, nearly all of which are not psychoactive. The metabolism takes place in the liver and substantially via the cytochrome P450 enzymes 2C9, 2C19, and 3A4. Due to their lipophilic properties, residual THC and metabolites are then stored in adipose tissue, from which they are removed only very slowly. More than 65% of the initial THC is excreted as metabolites in the feces, and approximately 25% in urine, a small part is broken down in the body itself. The main metabolites in urine are free THC-COOH and THC-COOH esterified with glucuronic acid, whereas THC-OH dominates in the feces (Musshoff & Madea, 2006).

strengthened by the clear definition of the amount of the recent cannabis consumption, and weakened by the missing control group.

DISCUSSION

Serum TSH values were within normal limits in the cannabis consuming cohorts of all studies (Rubin & Comitas, 1972; Parshad et al., 1983; Herning et al., 2008; Bonnet, 2013). A somewhat closer look is allowed by the studies of Parshad et al. (1983) and Herning et al. (2008), which found no significant differences between the TSH levels of regular cannabis smokers and normal controls. Even sustained heavier cannabis use was unable to put TSH-levels out of norm (Bonnet, 2013). In support, THC-levels from this sample were not significantly correlated with the thyroid function tests. Thus, regular cannabis intake seems to be not associated with appreciable changes in the thyroid function in human beings, as found in various ethnic cohorts, two with Jamaicans of African origin (Rubin & Comitas, 1972; Parshad et al., 1983), another dominated by African Americans (Herning et al., 2008), and a further with Caucasians (Bonnet, 2013).

In one study, the total T4-levels were found to be reduced, however, not before 8 months of cannabis consumption, while the T3-uptake increased (Herning et al., 2008). Because this study did not determine the free, nor the protein bounded hormone levels, the results might be influenced by an effect of cannabis on hormone-binding proteins, such as TGB.

The most recent study had the opportunity to determine the free T4 levels, which were all within the normal limits in patients with long-lasting and pronounced cannabis abuse (Bonnet, 2013).

Of note, all these studies on chronic cannabis abusers (Rubin & Comitas, 1972; Parshad et al., 1983; Herning et al., 2008; Bonnet, 2013) did not find any evidence for central hyperthyroidism, which has been described in two females suffering from cannabis hyperemesis syndrome (cyclic emesis that improved with hot bathing), after chronic cannabis abuse (Bonnet, Chang, & Scherbaum, 2012). Subclinical elevated TSH, free T4, and total T3 levels returned to normal within a few days of abstinence (Bonnet et al., 2012). In the light of the studies mentioned earlier, these abnormalities in the females' thyroid function tests could be based most likely on their emesis and loss of appetite, than on their chronic cannabis intake. Furthermore, there are experimental data that the activity of thermosensitive neurons is coupled to the activity of thyrotropin releasing hormone (TRH) in the hypothalamus (Salzman & Beckman, 1981) which would provide an explanation for a relationship between central hyperthyroidism and hot bathing, which is a typical feature of the cannabis hyperemesis syndrome (Allen, de Moore, Heddle, & Twartz, 2004; Wallace, Andrews, & Garmany, 2011).

Current nicotine smoking is associated with a slight dose-dependent fall of serum TSH, likely secondary to an increase of serum free T4 and free T3, induced by activation of the sympathetic nervous system (Wiersinga, 2013). This effect was not found in the studies mentioned earlier, which might be due to the inhibitory effect of repeated cannabis exposure on sympathetic activity (Jones, 2002).

Altogether, the results of the human studies argue against a relevant influence of regular or chronic cannabis consumption on thyroid function. An acute effect of cannabis on human thyroid function is not sufficiently investigated hitherto (Brown & Dobs, 2002). With respect to the results from animal experiments (Nazar et al., 1981; Brown & Dobs, 2002), one might suppose an acute depressant effect of cannabis also on human T4-release. Nonetheless, an acute pronounced fall in T4-levels could have been weakened by the development of tolerance in the regularly cannabis smoking cohorts, just as it was found in rats (Nazar et al., 1981).

Future detoxification studies with chronic cannabis addicts, which also analyze several thyroid function tests over time should give more insights in the finer effects of cannabis on human thyroid function. There exists no ultrasonography study of the thyroids of chronic cannabis addicts. Occurrence of goiter was not described in the studies presented previously.

MINI-DICTIONARY

Autonomic nervous system Part of the peripheral nervous system; acts largely below the level of consciousness to control visceral functions, such as digestion, heart rate, perspiration, breathing, salivation, swallowing, pupillary dilatation, and sexual arousal.
Cannabinoid receptors There are at least two known subtypes of cannabinoid receptors (CB1 and CB2); activation of CB1-receptor by THC mediates psychotropic (psychoactive) effects.
Cannabis hyperemesis syndrome Cyclic vomiting and emesis that improved with hot bathing. It emerges after chronic cannabis abuse, and shares many features with the cyclic vomiting syndrome.
Hyperthyroidism Overactive thyroid, develops when the thyroid gland produces and secretes excessive amounts of the free (not protein bound) thyroid hormones. Serum TSH is low, and free T4 is elevated. Graves' disease is the most common cause of hyperthyroidism.
Hypothalamus Located below the thalamus, and forms the ventral part of the diencephalon. It links the nervous system to the endocrine system, and is responsible for activities of the autonomic nervous system, including thermoregulation, sleep, and circadian rhythms.
Hypothyroidism Underactive thyroid, develops when the thyroid gland fails to synthesize or secrete as much thyroid hormones as the body needs, one of the most common diseases. Serum TSH is elevated, and serum free T4 is low.
Δ^9-Tetrahydrocannabinol (THC) Principal psychotropic constituent of cannabis; stimulates CB1-receptors.

Thyroid hormone Triiodthyronine (T3) and thyroxin (T4) are produced by the thyroid gland, and primarily responsible for regulation of metabolism. Iodine is necessary for the production of T3 and T4.

Thyroxin-binding globulin (TBG) One of three proteins (together with transthyretin and albumin) that bind T3 and T4 in blood. Of these three proteins, TBG has the highest affinity for thyroid hormones, and carries the majority of T4 in circulation.

CONFLICT OF INTEREST

The author received fees for lectures and the organization of training courses by the following pharmaceutical companies: Actelion, Boehringer-Ingelheim, Bristol-Myers Squibb, esparma, Glaxo-SmithKline, Janssen, Lilly, Lundbeck, Merz, and Servier.

References

Allen, J. H., de Moore, G. M., Heddle, R., & Twartz, J. C. (2004). Cannabinoid hyperemesis: cyclical hyperemesis in association with chronic cannabis abuse. *Gut*, *53*, 1566–1570.

Bonnet, U. (2013). Chronic cannabis abuse, delta-9-tetrahydrocannabinol and thyroid function. *Pharmacopsychiatry*, *46*, 35–36.

Bonnet, U., Chang, D. -I., & Scherbaum, N. (2012). Cannabis-Hyperemesis-syndrome. *Fortschritte Neurologie Psychiatrie*, *80*, 98–101 (in German).

Brown, T. T., & Dobs, A. S. (2002). Endocrine effects of marijuana. *Journal of Clinical Pharmacology*, *42*, 90S–96S.

Grotenhermen, F. (2003). Pharmacokinetics and pharmacodynamics of cannabinoids. *Clinical Pharmacokinetics*, *42*, 327–360.

Herning, R. I., Better, W., & Cadet, J. L. (2008). EEG of chronic marijuana users during abstinence: relationship to years of marijuana use, cerebral blood flow and thyroid function. *Clinical Neurophysiology*, *119*, 321–331.

Jones, R. T. (2002). Cardiovascular system effects of marijuana. *Journal of Clinical Pharmacology*, *42*(11 Suppl.), 58S–63S.

Lomax, P. (1970). The effect of marijuana on pituitary thyroid activity in the rat. *Agents and Actions*, *1*, 252–257.

Mullur, R., Liu, Y. -Y., & Brent, G. A. (2014). Thyroid hormone regulation of metabolism. *Physiological Reviews*, *94*, 355–382.

Musshoff, F., & Madea, B. (2006). Review of biologic matrices (urine, blood, hair) as indicators of recent or ongoing cannabis use. *Therapeutic Drug Monitoring*, *28*, 155–163.

Nazar, B., Kairys, D. J., Fowler, R., & Harclerode, J. (1981). Effects of delta-9-tetrahydrocannabinol on serum thyroxine concentrations in the rat. *Journal of Pharmacy and Pharmacology*, *21*, 208–214.

Pagotto, U., Marsicano, G., Cota, D., Lutz, B., & Pasquali, R. (2006). The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocrinological Reviews*, *27*, 73–100.

Parshad, O., Kumar, M., & Melville, G. N. (1983). Thyroid-gonad relationship in marijuana smokers. A field study in Jamaica. *West Indian Medical Journal*, *32*, 101–105.

Porcella, A., Marchese, G., Casu, M. A., Rocchitta, A., Lai, L. M., Gessa, G. L., & Pani, L. (2002). Evidence for functional CB1 cannabinoid receptor expressed in the rat thyroid. *European Journal of Endocrinology*, *147*, 255–261.

Rubin, V. D., & Comitas, L. (1972). *Effects of chronic smoking of cannabis in Jamaica: a report by the Research Institute for the Study of Man to the Center for Studies of Narcotic and Drug Abuse*. NIMH, NIMH contract No HSM-42-70-97. Bethesda, USA.

Salzman, S. K., & Beckman, A. L. (1981). Effects of thyrotropin releasing hormone on hypothalamic thermo-sensitive neurons of the rat. *Brain Research Bulletin*, *7*, 325–332.

Wallace, E. A., Andrews, S. E., & Garmany, C. L. (2011). Cannabinoid hyperemesis syndrome: literature review and proposed diagnosis and treatment algorithm. *Southern Medical Journal*, *104*, 659–664.

Wiersinga, W. M. (2013). Smoking and thyroid. *Clinical Endocrinology (Oxford)*, *79*, 145–151.

CHAPTER

48

Cannabis Hyperemesis Syndrome

U. Bonnet

Department of Psychiatry, Psychotherapy and Psychosomatics, Evangelisches Krankenhaus Castrop-Rauxel, Academic Teaching Hospital of the University of Duisburg-Essen, Castrop-Rauxel, Germany

SUMMARY POINTS

- Since its first description in 2004, the reports on CHS are mounting.
- In its core, CHS consists of cyclic periods with vomiting and, often, epigastric pain.
- Both symptoms improve with hot bathing or showering.
- CHS is often resistant to usual antiemetics.
- Haloperidol or lorazepam may be beneficial to suppress vomiting.
- Fluid and electrolyte replacements are essential basics in the treatment of CHS.
- Typically, CHS is remitted within 2–3 days after cessation of cannabis.
- In mysterious nausea or vomiting, a drug screen should be made.

KEY FACTS OF CHANGES IN CANNABIS POTENCY

- While cannabis users in the 1970s mainly smoked the leaves, cannabis users today prefer to smoke the more potent flowering tops, or buds of the plant (http://ncpic.org.au/ncpic/publications/factsheets/pdf/cannabis-potency).
- In 2009, the reported mean THC-content of hashish and marijuana (including sinsemilla—the form of herbal cannabis with the highest potency) ranged from 3% to 17%, and from 1% to 15% in Europe, respectively (http://www.emcdda.europa.eu/online/annual-report/2011/cannabis/2).
- Recent analysis of the cannabinoid-content of cannabis plants suggests a shift toward use of high potency plant material with high levels of THC and low levels of cannabidiol, in Australia (Swift, Wong, Li, Arnold, & McGregor, 2013).

KEY FACTS OF CYCLIC VOMITING SYNDROME

- Patients with cyclic vomiting syndrome (CVS) share many features with patients with CHS, such as compulsive hot water bathing to alleviate episodic nausea and vomiting.
- CVS can deteriorate over time to persistent symptoms lasting for weeks or even months (coalescent CVS) (Venkatesan et al., 2014).

LIST OF ABBREVIATIONS

CB1-receptor	Cannabinoid 1-receptor
CHS	Cannabis hyperemesis syndrome
CVS	Cyclic vomiting syndrome
THC	Δ^9-Tetrahydrocannabinol

INTRODUCTION

Since its first description, in Australia (Allen, de Moore, Heddle, & Twartz, 2004; de Moore, 2005), a mass of reports on cannabis hyperemesis syndrome (CHS) have been published worldwide (Patterson et al., 2010; Galli, Sawaya, & Friedenberg, 2011; Wallace, Andrews, & Garmany, 2011; Bonnet, Chang, & Scherbaum, 2012; Nicolson, Denysenko, Mulcare, Vito, & Chabron, 2012; Simonetto, Oxentenko, Herman, & Szostek, 2012; Gupta,

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00055-7
Copyright © 2017 Elsevier Inc. All rights reserved.

TABLE 48.1 Features of the CHS

Cyclical vomiting and emesis (core symptom)
Improvement by compulsive hot showering or bathing
Probable resistance to usual antiemetics
Complete resolution after cessation of cannabis (usually within 2–3 days)

Ojo, & Muruthettuwegama, 2013; Mohammed, Panchoo, Bartholemew, & Maharaj, 2013). CHS occurs after regular and, as a rule, longer lasting cannabis abuse. Its features are shown in Table 48.1.

Prodromal nausea prior to the onset of vomiting occurs in one-third of the cases (Nicolson et al., 2012). "Vomiting crises" itself are regularly accompanied by pronounced epigastric pain and, sometimes, by polydipsia and diaphoresis (Wallace et al., 2011; Nicolson et al., 2012; Simonetto et al., 2012). Hyperemesis can last over several days, and up to 6 months (Nicolson et al., 2012), before self-limiting, and it can reemerge with shorter symptom-free intervals, between the cycles. The patients would have usually gone several times through regional emergency and medical health service systems, often with extensive medical workup, before the physicians or the patients themselves attributed the symptoms to cannabis (Galli et al., 2011; Nicolson et al., 2012). CHS was found to occur in one out of a hundred detoxification treatments from cannabis (Bonnet et al., 2012). This certainly underestimates the true prevalence in real life, because most cases did not seek an addiction treatment (Wallace et al., 2011; Nicolson et al., 2012; Simonetto et al., 2012). CHS is not restricted to adults. In 2010, the first two pediatric cases were published (Miller et al., 2010). Most recently, the first case of "familial" CHS was described in father and daughter (Gupta et al., 2013).

PATHOGENESIS

The pathogenesis of CHS is not understood. As medical marijuana and its main psychoactive ingredient, Δ^9-tetrahydrocannabinol (THC), are antiemetic (Machado Rocha, Stéfano, De Cássia Haiek, Rosa Oliveira, & Da Silveira, 2008), the CHS can be assumed to be a paradoxical reaction to cannabis or THC (Patterson et al., 2010; Galli et al., 2011; Wallace et al., 2011). In contrast to the rather prompt antiemetic effect of medical marijuana, for example, in chemotherapy-induced vomiting (Machado Rocha et al., 2008), CHS occurred after regular, and often heavier cannabis consumption. This possibly points to a sensitization process (Bonnet et al., 2012). According to a large case series ($N = 98$), cannabis consumption prior to the onset of CHS lasted ≤1 year, 2–5 years, and ≥6 years in 32, 44, and 24% of the cases, respectively (Simonetto et al., 2012). The frequency of the cannabis consumption was daily in 59%, and 1–3 times per week in 25% of the cases (Simonetto et al., 2012).

Most hypotheses of the pathogenesis of CHS considered effects of chronic THC: (1) on the autonomic nervous system (inhibition of peristalsis, gastroparesis, and splanchnic vasodilatation). and (2) on the central nervous system (modulation of thermoregulatory and rhythm-forming relays in the hypothalamus, as well as emetic- and compulsion-controlling networks) (Patterson et al., 2010; Galli et al., 2011; Wallace et al., 2011; Bonnet et al., 2012; Nicolson et al., 2012; Simonetto et al., 2012). Chronic cannabis consumption may sensitize and stimulate the cannabinoid network in the gut to override the central antiemetic effect of THC, thereby inducing hyperemesis in susceptible individuals (Galli et al., 2011; Nicolson et al., 2012; Simonetto et al., 2012). Most recently, it was hypothesized that CHS is a subtype of cyclic vomiting syndrome (CVS), speculated to result from abnormal central regulation of predominantly gastrointestinal autonomic function. Exposure to adverse early life events, chronic stress, sleep deprivation, mood disorders, and chronic cannabis and/or opiate use, might lead to neuroplastic changes in central neural structures promoting chronically increased sympathetic responsivity that characterizes CVS-attacks (Levinthal & Bielefeldt, 2014). Moreover, cannabinoids other than THC, in crude cannabis, or the contamination of cannabis with, for example, lead, solvents, or pesticides, are speculated to be involved in the genesis of CHS (Galli et al., 2011; Nicolson et al., 2012).

The worldwide increase of cannabis consumption in combination with the simultaneously rising THC—and falling cannabidiol—contents in marijuana, due to selective breeding of the *Cannabis sativa* plant (Licata, Verri, & Beduschi, 2005; Knight et al., 2012; UNDOC, 2013; Swift et al., 2013) may have unmasked the CHS in cannabis addicts, in the last 10 years (Patterson et al., 2010; Galli et al., 2011; Wallace et al., 2011; Bonnet et al., 2012; Nicolson et al., 2012; Simonetto et al., 2012). In support, intravenous injection of THC or inhalation of herbal blends with admixed synthetic cannabinoids with stronger affinity to CB-1 receptors than THC, such as JWH 122 or JWH 210, were found to induce emesis (Vaziri et al., 1981; Hermanns-Clausen, Kneisel, Szabo, & Auwärter, 2013).

There are some ideas with respect to the improvement of the vomiting during hot baths. An increase of body temperature should (1) influence endocannabinoid-modulated thermoregulatory networks in the hypothalamus, which might readjust the sensitized emesis threshold (Chang & Windish, 2009; Bonnet et al., 2012), or (2) divert blood from the gut to the periphery, thereby

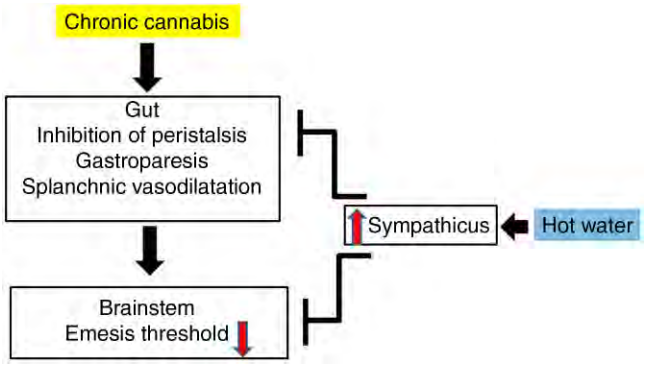


FIGURE 48.1 **Hypothetical mechanism of CHS.** Long-term repetitive (chronic) cannabis consumption repeatedly stimulates parasympathetic afferences, which are associated with a sensitization of the emesis threshold in the human brainstem. Hot water activates the sympathetic nervous system, which raises and readjusts emesis threshold.

limiting the effect of THC-induced splanchnic vasodilatation (cutaneous steel phenomenon) (Patterson et al., 2010), or (3) activate the sympathetic nervous system, which might increase the emesis threshold (Fig. 48.1).

TABLE 48.2 Differential Diagnoses of CHS

Psychogenic vomiting
Cyclic vomiting syndrome (CVS)
Intoxication or withdrawal symptoms
Hyperemesis gravidarum
Drug-seeking behavior
Eating disorders
Migraine
Elevated intracranial pressure
Gastrointestinal motility disorders, such as in Parkinson disease or diabetes mellitus
Pancreatitis
Gallbladder inflammation
Porphyria
Heart diseases
Renal diseases
Addison disease

DIAGNOSIS, COMPLICATIONS, AND TREATMENT

The physical and neurological examinations of patients suffering from CHS are strikingly unremarkable, so that a pure psychosomatic disease, such as psychogenic vomiting or cyclic vomiting, might be assumed (Allen et al., 2004; Wallace et al., 2011). A mild esophagitis or *Helicobacter pylori*-negative gastritis was frequently described (Galli et al., 2011; Nicolson et al., 2012), and suggested to be secondary to the often-pronounced vomiting. CHS did not improve with proton pump inhibitors (Galli et al., 2011). However, the differential diagnoses in Table 48.2 should be considered (Wallace et al., 2011; Nicolson et al., 2012; Bonnet et al., 2012).

Mental status may be influenced by the sequels of long-term cannabis consumption, such as cognitive and motivational impairments, psychosis, affective symptoms, or reduced drive (Hall & Degenhardt, 2009; Bonnet & Scherbaum, 2012). Chronic cannabis consumption is also associated with schizophrenia, bipolar disorder, and addiction to other illegal drugs or alcohol (Hall & Degenhardt, 2009; Bonnet & Scherbaum, 2012). It is assumed that alcohol may play a greater role in the vomiting of cannabis-addicted patients than they concede to their physicians (Nicolson et al., 2012).

Pronounced vomiting could be complicated with massive dehydrations, causing acute prerenal failure, and life-threatening electrolyte imbalances (Bramstedt & Dissmann, 2011). Therefore, fluid and electrolyte replacements are essential basics in the treatment of CHS (Bramstedt & Dissmann, 2011; Wallace et al., 2011). As the patients realized that their "vomiting crises" could be mitigated by hot bathing or showering, some of them showed self-inflicted skin scalds by hot tap water (Allen et al., 2004; Bonnet et al., 2012), or produced enormous water-bills (Fleig & Brunkhorst, 2011). One patient has possibly spent 300 of 365 days in the bathtub (Wallace, Martin, & Park, 2007).

Usual antiemetics, such as metoclopramide, domperidone, ondansetron, or promethazine, probably fail to relieve the vomiting (Wallace et al., 2011). There are some positive experiences with haloperidol (Hickey, Witsil, & Mycyk, 2013) and lorazepam (Cox, Chhabra, Adler, Justin Simmons, & Randlett, 2012) in emergency settings (Table 48.3).

The symptoms of the CHS could be a diagnostic conundrum, when the patients hide their cannabis consumption, and refuse to undergo regular urine or serum

TABLE 48.3 Treatment of CHS Attacks

Fluid and electrolyte replacements
Lorazepam
Haloperidol
Detoxification of cannabis, and treatment of cannabis addiction

drug-screenings. Characteristically, CHS disappeared with ongoing cessation of cannabis, and did not reoccur under this abstinence condition (Bonnet et al., 2012; Nicolson et al., 2012; Simonetto et al., 2012). Taken altogether, CHS is a somatic sequel of chronic cannabis abuse. Its prognosis clearly depends on the treatment of the cannabis addiction.

MINI-DICTIONARY

Autonomic nervous system Part of the peripheral nervous system; acts largely below the level of consciousness to control visceral functions, such as digestion, heart rate, perspiration, breathing, salivation, swallowing, pupillary dilatation, and sexual arousal.
Cannabidiol Nonpsychotropic phytocannabinoid in cannabis. It acts in some experimental models as an antiinflammatory, anticonvulsant, antioxidant, antiemetic, anxiolytic, and antipsychotic agent.
Cannabinoid receptors At least two known subtypes of cannabinoid receptors (CB1 and CB2); activation of CB1-receptor by THC mediates psychotropic (psychoactive) effects.
Endocannabinoids Specific neuromodulatory lipids endogenously produced in the brain, involved in a variety of physiological processes, including regulation of appetite, affect, pain sensation, and memory; ligands to cannabinoid receptors.
Helicobacter pylori Bacterium found in the stomach, associated with chronic gastritis and gastric ulcers, and also linked to the development of stomach and duodenal cancer. There is some evidence that it also has a protective effect against gastroesophageal reflux disease and its complications such as Barrett's esophagus, and esophageal adenocarcinoma.
Hypothalamus Located below the thalamus; forms the ventral part of the diencephalon. It links the nervous system to the endocrine system, and is responsible for activities of the autonomic nervous system, including thermoregulation, sleep, and circadian rhythms.
Medical cannabis Use of cannabis as medical therapy to treat disease or alleviate symptoms, such as nausea and vomiting in chemotherapy, or anorexia associated with AIDS, or pain and muscle spasticity in multiple sclerosis.
Prevalence The proportion of a population found to have a condition.
Sensitization Nonassociative learning process in which repeated administrations of a stimulus results in the progressive amplification of a response.
Synthetic cannabinoids Research chemicals to investigate the endocannabinoid system, for example, JWH 122 and JWH 210 bind 60- and 88-times stronger to CB1-receptor than THC, respectively.
Δ^9-Tetrahydrocannabinol (THC) Principal psychotropic constituent of cannabis; stimulates CB1-receptors.

CONFLICT OF INTEREST

The author received fees for lectures, and the organization of training courses by the following pharmaceutical companies: Actelion, Boehringer-Ingelheim, Bristol-Myers Squibb, esparma, Glaxo-SmithKline, Janssen, Lilly, Lundbeck, Merz, and Servier.

References

Allen, J. H., de Moore, G. M., Heddle, R., & Twartz, J. C. (2004). Cannabinoid hyperemesis: cyclical hyperemesis in association with chronic cannabis abuse. *Gut*, *53*, 1566–1570.

Bonnet, U., Chang, D. -I., & Scherbaum, N. (2012). Cannabis-Hyperemesis-syndrome. *Fortschritte Neurologie Psychiatrie*, *80*, 98–101 (in German).

Bonnet, U., & Scherbaum, N. (2012). Cannabis use disorders. Part II: psychiatric and somatic sequelae and comorbidities. *Fortschritte Neurologie Psychiatrie*, *78*, 360–367 (in German).

Bramstedt, J., & Dissmann, R (2011). Cannabinoid hyperemesis syndrome inducing acute prerenal failure and electrolyte disturbance. *Deutsche Medizinische Wochenschrift*, *136*, 1720–1722 (in German).

Chang, Y. H., & Windish, D. M. (2009). Cannabinoid hyperemesis relieved by compulsive bathing. *Mayo Clinic Proceedings*, *84*, 76–78.

Cox, B., Chhabra, A., Adler, M., Justin Simmons, J., & Randlett, D. (2012). Cannabinoid hyperemesis syndrome: case report of a paradoxical reaction with heavy marijuana use. *Case Reports in Medicine*, *2012*, 757696.

de Moore, G. M. (2005). Value of the single case report. Marijuana, vomiting and compulsive bathing: a new clinical syndrome? *Australian and New Zealand Journal of Psychiatry*, *39*, 1046.

Fleig, S., & Brunkhorst, R. (2011). Hyperemesis and a high water bill. *Zeitschrift für Gastroenterologie*, *49*, 1479–1481.

Galli, J. A., Sawaya, R. A., & Friedenberg, F. K. (2011). Cannabinoid hyperemesis syndrome. *Current Drug Abuse Reviews*, *4*, 241–249.

Gupta, N., Ojo, O., & Muruthettuwegama, K. (2013). Cannabis hyperemesis syndrome: an enigma. *Indian Journal of Psychological Medicine*, *35*, 405–406.

Hall, W., & Degenhardt, L. (2009). Adverse health effects of non-medical cannabis use. *Lancet*, *374*, 1283–1291.

Hermanns-Clausen, M., Kneisel, S., Szabo, B., & Auwärter, V. (2013). Acute toxicity due to the confirmed consumption of synthetic cannabinoids: clinical and laboratory findings. *Addiction*, *108*, 534–544.

Hickey, J. L., Witsil, J. C., & Mycyk, M. B. (2013). Haloperidol for treatment of cannabinoid hyperemesis syndrome. *American Journal of Emergency Medicine*, *31*, 1003e5–1003e6.

Knight, G., Hansen, S., Connor, M., Poulsen, H., McGovern, C., & Stacey, J. (2012). The results of an experimental indoor hydroponic Cannabis growing study, using the 'Screen of Green' (ScrOG) method—Yield, tetrahydrocannabinol (THC) and DNA analysis. *Forensic Science International*, *202*, 36–44.

Levinthal, D. J., & Bielefeldt, K. (2014). Adult cyclical vomiting syndrome: a disorder of allostatic regulation? *Experimental Brain Research*, *232*(8), 2541–2547.

Licata, M., Verri, P., & Beduschi, G. (2005). δ-9 THC content in illicit cannabis products over the period 1997–2004 (first four months). *Annali dell'Istituto Superiore di Sanità*, *41*, 483–485.

Machado Rocha, F. C., Stéfano, S. C., De Cássia Haiek, R., Rosa Oliveira, L. M., & Da Silveira, D. X. (2008). Therapeutic use of Cannabis sativa on chemotherapy-induced nausea and vomiting among cancer patients: systematic review and meta-analysis. *European Journal of Cancer Care*, *17*, 431–443.

Miller, J. B., Walsh, M., Patel, P. A., Rogan, M., Arnold, C., Maloney, M., & Donnino, M. (2010). Pedriatric cannabinoid hyperemesis: two cases. *Pediatric Emergency Care*, *26*, 919–920.

Mohammed, F., Panchoo, K., Bartholemew, M., & Maharaj, D. (2013). Compulsive showering and marijuana use—the cannabis hyperemisis syndrome. *American Journal of Case Reports*, *14*, 326–328.

Nicolson, S. E., Denysenko, L., Mulcare, L., Vito, J. P., & Chabron, B. (2012). Cannabinoid hyperemesis syndrome: a case series and review of previous reports. *Psychosomatics*, *53*, 212–219.

Patterson, D. A., Smith, E., Monahan, M., Medvecz, A., Hagerty, B., Krijger, L., Chauhan, A., & Walsh, M. (2010). Cannabinoid hyperemesis and compulsive bathing: a case series and paradoxical pathophysi-

ological explanation. *Journal of the American Board of Family Medicine*, *23*, 790–793.

Simonetto, D. A., Oxentenko, A. S., Herman, M. L., & Szostek, H. (2012). Cannabinoid hyperemesis: a case series of 98 patients. *Mayo Clinic Proceedings*, *87*, 114–119.

Swift, W., Wong, A., Li, K. M., Arnold, J. C., & McGregor, I. S. (2013). Analysis of cannabis seizures in NSW, Australia: cannabis potency and cannabinoid profile. *PLoS One*, *8*, e70052.

UNDOC (United Nations Office on Drug and Crime). (2013). *World Drug Report 2013*. New York, NY: United Nations publications, www.undoc.org.

Vaziri, N. D., Thomas, R., Sterling, M., Seiff, K., Pahl, M. V., Davila, J., & Wilson, A. (1981). Toxicity with intraveneous injection of crude marijuana extract. *Clinical Toxicology*, *18*, 353–366.

Venkatesan, T., Sengupta, J., Lodhi, A., Schroeder, A., Adams, K., Hogan, W. J., Wang, Y., Andrews, C., & Storr, M. (2014). An Internet survey of marijuana and hot shower use in adults with cyclic vomiting syndrome (CVS). *Experimental Brain Research*, *232*(8), 2563–2570.

Wallace, D., Martin, A. L., & Park, B. (2007). Cannabinoid hyperemesis: marijuana puts patients in hot water. *Australasian Psychiatry*, *15*, 156–158.

Wallace, E. A., Andrews, S. E., & Garmany, C. L. (2011). Cannabinoid hyperemesis syndrome: literature review and proposed diagnosis and treatment algorithm. *Southern Medical Journal*, *104*, 659–664.

CHAPTER

49

Cannabis and Cannabinoids and the Effects on Gastrointestinal Function: An Overview

M. Sałaga*, R. Abalo**,†, J. Fichna*

*Department of Biochemistry, Faculty of Medicine, Medical University of Lodz, Lodz, Poland
**Area of Pharmacology and Nutrition, Faculty of Health Sciences, University Rey Juan Carlos, Alcorcón, Madrid, Spain
†Associated Unit I+D+i of the Institute of Medicinal Chemistry (IQM) and of the Institute of Research in Food Sciences (CIAL), Spanish National Research Council (CSIC), Madrid, Spain

SUMMARY POINTS

- This chapter focuses on the role of both exo- and endogenous cannabinoids in gut homeostasis.
- It also refers to the localization of the endocannabinoid system (ECS) in the gut and its organization at the molecular level, and to the details of endocannabinoid degradation pathways involving enzymes, such as fatty acid amide hydrolase and monoacyl glycerol lipase.
- Various components of ECS, such as cannabinoid receptors, endogenous ligands, and degrading enzymes are located throughout the gastrointestinal tract.
- Not only "classical" components of the ECS, but also other receptors (such as GPR55) or ligands (such as *N*-palmitoylethanolamide) play important functions in the gastrointestinal tract.
- ECS modulates physiological functions of the gastrointestinal tract, such as food uptake, motility, and visceral sensation.
- Stimulation of cannabinoid receptors may alleviate pathological states in the gastrointestinal tract, such as inflammation and tumorigenesis.
- ECS is strongly involved in pain signaling, and cannabinoid receptors agonists may be used to alleviate abdominal pain.

KEY FACTS OF INFLAMMATORY BOWEL DISEASES

- Diagnosed in 1–2% of population.
- The prevalence of inflammatory bowel diseases (IBD) is higher in developed countries.
- Extraintestinal manifestations of IBD occur in 30% of patients.
- Crohn's disease (CD) may occur in all parts of the gastrointenstinal (GI) tract, while ulcerative colitis (UC) is located specifically in the colon.
- There are several disease susceptibility genes found on chromosomes 3, 7, 12, and 16.
- Smoking is an important environmental factor in IBD with different effects in UC and CD. Never smoking and formerly smoking increase the risk of UC, whereas smoking exacerbates the course of CD.

KEY FACTS OF IRRITABLE BOWEL SYNDROME

- The concept of an "irritable bowel" first time appeared in the Rocky Mountain Medical Journal, in 1950.
- Irritable bowel syndrome (IBS) symptoms may be reduced following antibiotic therapy for small intestinal bacterial overgrowth.
- Alternative methods, such as yoga and acupuncture, may alleviate the symptoms of IBS.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00056-9
Copyright © 2017 Elsevier Inc. All rights reserved.

- IBS is diagnosed more frequently in patients with mental disorders, such as depression, anxiety, or schizophrenia.
- Women are approximately 2–3 times more likely to be diagnosed with IBS than men. These differences likely reflect a combination of both biological and social factors.

LIST OF ABBREVIATIONS

Δ^9-THC	Δ^9-Tetrahydrocannabinol
2-AG	2-Arachidonoylglycerol
2-OG	2-Oleolylglycerol
AA-5-HT	Arachidonic acid–serotonin
AEA	*N*-arachidonoylethanolamine, anandamide
CB	Cannabinoid
CBD	Cannabidiol
CD	Crohn's disease
CNS	Central nervous system
CRC	Colorectal cancer
DNBS	Dinitrobenzene sulfonic acid
DSS	Dextran sodium sulphate
ECS	Endogenous cannabinoid system
EMT	Endocannabinoid membrane transporter
ENS	Enteric nervous system
FAAH	Fatty acid amide hydrolase
GI	Gastrointestinal
GLP-1	Glucagon-like peptide 1
GPCR	G-protein coupled receptors
IBD	Inflammatory bowel diseases
IBS	Irritable bowel syndrome
LPS	Lipopolysaccharide
MAGL	Monoacyl glycerol lipase
MAPK	Mitogen-activated protein kinase
OEA	*N*-oleoylethanolamide
PEA	*N*-palmitoylethanolamine
PGE2	Prostaglandin E2
PI3K-AKT	Phosphoinositide 3-kinase-protein kinase B
PPAR	Peroxisome proliferator-activated receptor
TLR4	Toll like receptor 4
TNBS	Trinitrobenzene sulfonic acid
TRPV1	Transient receptor potential vanilloid type 1
UC	Ulcerative colitis

INTRODUCTION

For centuries, it has been observed that the symptoms of several gastrointestinal (GI) diseases may be alleviated by plant-based remedies, such as those derived from the marijuana plant *Cannabis* sp., which can be used to treat a wide range of GI disorders, from enteric infections and inflammatory conditions, to functional and motility disorders, as well as emesis, and abdominal pain (Izzo & Sharkey, 2010).

Since Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive compound of *Cannabis* was identified and isolated, molecular and mechanistic studies that focused on its effects emerged, and lead to the identification of its site and mechanism of action in the GI tract, long before the specific receptor was cloned in 1990 (for review see Izzo & Coutts, 2005; Izzo & Sharkey, 2010). Studies by Gill, Paton, and Pertwee (1970), and afterward by Roth (1978), showed that Δ^9-THC is able to inhibit electrically evoked cholinergic transmission in the myenteric plexus of the guinea pig ileum. They also demonstrated that the site of action of Δ^9-THC is located presynaptically or prejunctionally. These early observations, together with later in vitro and in vivo studies evidenced that cannabinoids (CBs) inhibit GI motility throughout the gut, which—at least partially—explained some of the therapeutic features of *Cannabis* (Izzo & Sharkey, 2010; Abalo, Vera, López-Pérez, Martínez-Villaluenga, & Martín-Fontelles, 2012).

Thereafter, the endogenous cannabinoid system (ECS), including endogenous CB agonists (AEA, 2-AG), endocannabinoid-like compounds (PEA, OEA, which are products of endocannabinoid turnover), and biosynthesis and degradation enzymes (phospholipase D, FAAH, and MAGL), were demonstrated in the gut. Exogenous as well as endogenous CBs are pleiotropic molecules, and activation of other receptors (TRPV1, PPAR, and GPCR-like GPR119), may contribute to their actions. In addition, endocannabinoid levels may be altered with the use of pharmacological tools, in particular inhibitors of enzymes responsible for their degradation, leading to potentially significant functional consequences in both physiological (eg, regulation of appetite) and patophysiological (eg, reduction of inflammation) conditions.

Herein, we will overview the role of the ECS in gut homeostasis, pharmacological actions of CBs, and the potential application of ECS-targeting drugs in the treatment of GI tract diseases.

LOCALIZATION OF ECS COMPONENTS IN THE GUT

Cannabinoid receptors type 1 and 2 (CB1 and CB2) are present in the enteric nervous system (ENS), as shown using immunohistochemical stainings. CB1 receptors are most abundant in myenteric and submucosal plexuses of the ENS (Izzo & Sharkey, 2010). Moreover, double-staining of CB1 receptors with markers of specific types of neurons (eg, calretinin, substance P) confirmed their presence on excitatory motor neurons, interneurons, and intrinsic primary afferent neurons, but not on inhibitory neurons expressing nitric oxide synthase (Coutts, Irving,

Mackie, Pertwee, & Anavi-Goffer, 2002; Izzo & Sharkey, 2010; Kulkarni-Narla & Brown, 2000). Importantly, both CB receptors are located on cholinergic neurons, as well as on epithelial cells of the colon crypts and lamina propria mononuclear cells of the colon (Salaga, Sobczak, & Fichna, 2014b; Di Sabatino et al., 2011).

Besides the "classical" CB receptors, there are other targets for CBs in the gut, including TRPV1 and PPARα, as well as GPR55 and GPR119 receptors. AEA and—to a lesser extent—OEA bind to TRPV1 receptors, with affinity lower than that for CB1 receptors. TRPV1 receptors are involved in visceral hypersensitivity signaling, and were found on the terminals of extrinsic primary afferent fibers, within the innervation of muscle layers, in cells adjacent to blood vessels and, at the highest density, in the myenteric plexus. Although most of the TRPV1 nerves are of spinal afferent origin, some of them originate from the vagal nerve. In certain conditions (eg, inflammation), activation of TRPV1 receptors by AEA may result in functional changes opposite to those observed upon activation of "classical" CB receptors, such as increased intestinal secretion and contractility (Izzo & Camilleri, 2009).

PPARα receptors, which share features of both "classical" ligand-activated receptors and ligand-activated transcriptional factors, are expressed throughout the whole GI tract. PPARα receptors may be found in enterocytes of the small intestine, and in enteric neurons of the myenteric and submucosal plexuses (Izzo & Sharkey, 2010). PPARα receptors are also expressed on enteric glial cells, where they may be indirectly activated by PEA through TLR4-dependent pathway; such activation results in improvement of colitis (Esposito et al., 2013).

GPR55 and GPR119 are, to date, the newest among all discovered targets for ECS-related compounds. GPR55 were found in several peripheral tissues, including the GI tract (jejunum, ileum, colon, pancreas), both in GI epithelial cells and enteric neurons (Godlewski, Offertaler, Wagner, & Kunos, 2009). GPR119 display a relatively narrow expression pattern, and were found predominantly on pancreatic β cells and intestinal tissues, within the villi in a close proximity to GLP-1, indicating the association between these receptors and enteric L-cells (Godlewski et al., 2009). In fact, OEA (the main biolipid activating GPR119) may stimulate the release of GLP-1 by these cells (Godlewski et al., 2009; Izzo & Sharkey, 2010).

The enzymatic machinery involved in the synthesis and degradation of endocannabinoids in the GI tract is probably not different from that in other tissues. However, there is limited data about topography of endocannabinoid biosynthetic and degradation enzymes in the gut, therefore the cellular sources of endocannabinoids are yet to be fully elucidated. FAAH, which breaks AEA and 2-AG intracellularly, is a membrane-bound hydrolase, and it is localized on cell bodies in the myenteric plexus, both in stomach and intestine (Izzo & Sharkey, 2010; Salaga et al., 2014b). MAGL breaks down 2-AG, and is present in the nerve cells and nerve fibers, throughout the muscle and mucosal layers of the duodenum, ileum, and colon (Izzo & Sharkey, 2010). Interestingly, the activity of MAGL varies throughout the GI tract: the highest is observed in the upper GI tract (duodenum), and decreases, reaching the lowest level in the distal colon (Izzo & Sharkey, 2010).

CBs are transported by specific proteins, endocannabinoid membrane transporters (EMT), whose cellular localization and distribution in the organism is yet to be elucidated. Inhibition of EMT results in an increased level of extracellular AEA (up to 200%) and 2-AG (up to 110%) (Chicca, Marazzi, Nicolussi, & Gertsch, 2012). Moreover, inhibition of EMT may produce antiinflammatory effects in the GI tract, similar to that observed after blockade of FAAH (Salaga et al., 2014b).

CANNABINOIDS IN PHYSIOLOGICAL STATES

Food Intake and Obesity

CBs regulate energy balance and food intake, acting both in brain and GI tract. Δ^9-THC increases food uptake via CB1 receptor activation. Starvation increases both AEA levels and CB1 receptor expression in the vagus nerve; on the contrary, satiety reduces CB1 expression and signaling. These observations indicate that CB1-expressing neurons located on the vagus nerve are involved in transmission of CB-dependent signals modulating appetite (Fig. 49.1). Thus, AEA seems to be an important mediator of hunger in the organism (Izzo & Sharkey, 2010). Interestingly, whereas AEA enhances food intake and energy storage, OEA reduces food intake and promote lipolysis (Izzo & Camilleri, 2009; Tourino, Oveisi, Lockney, Piomelli, & Maldonado, 2010).

Since CBs are strong modulators of appetite, it was postulated that ECS may be targeted in the treatment and prevention of obesity. To date, several in vivo studies showed that CB1 receptors signaling was upregulated in animals prone to obesity, and fed with high fat diet (Paulino et al., 2009). Moreover, it was shown that AEA is upregulated in the small intestine and duodenum of obese rats (Izzo & Sharkey, 2010). Extrinsic sensory neurons that relay information from the gut to the brain exhibit increased sensitivity to orexigenic compounds, including cannabinoids in diet-induced obese rodent models. These data demonstrate the potential of endocannabinoids for strong appetite enhancement through peripheral receptors, which may result in an increased food intake and, in longer time periods, obesity. CB1 receptor antagonists were thus imagined as effective agents

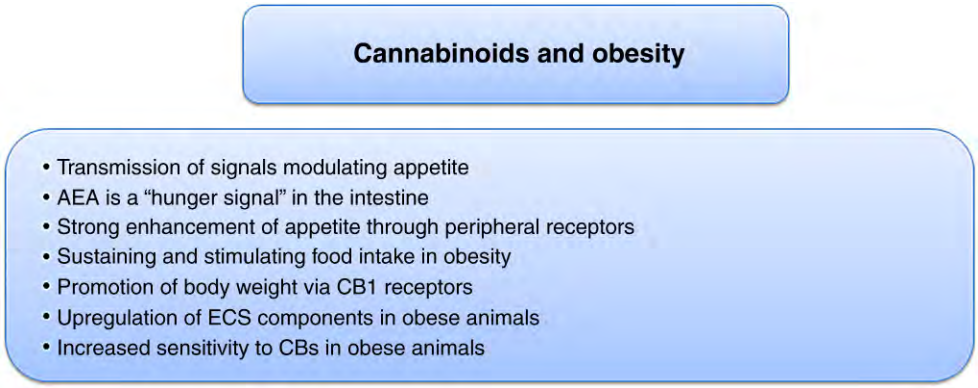


FIGURE 49.1 **Actions of the endogenous cannabinoid system in the course of obesity.**

in limiting food intake, and reducing body weight. Indeed, it was shown that rimonabant, a selective CB1 antagonist/reverse agonist diminishes food intake, and its actions were blocked after chemical vagus nerve deafferentation (Gomez et al., 2002). Another CB1 receptor antagonist, URB447, was shown to reduce food intake and body weight in genetically obese ob/ob mice (Izzo & Sharkey, 2010). However, despite the success in preclinical and clinical trials, rimonabant, the first member of this class of drugs, was withdrawn from the European market in 2008 because of an increased risk of depression due to centrally-mediated actions. The second generation of selective CB1 antagonists, unable to cross the blood–brain barrier, as well as other ECS-focused strategies, are now in the pipeline. In the next few years, we will hopefully know whether ECS can be successfully targeted without significantly affecting other vital processes related to mood and sense of wellbeing (Romero-Zerbo & Bermudez-Silva, 2014; Izzo & Sharkey, 2010).

GI Motility

Cannabinoid effects on GI motility are described in more depth in another Chapter 98 (*Cannabinoids and effects on the gastrointestinal tract: a focus on motility*) within this book.

CANNABINOIDS IN PATHOPHYSIOLOGICAL STATES

Functional Disorders of the Lower GI Tract

Irritable bowel syndrome (IBS) is a heterogeneous disorder, characterized by altered stool habits and abdominal discomfort or pain that lasts for at least 3 days per month, in 3 months prior to diagnosis, in absence of another organic disease. The prevalence of IBS is 10–20% worldwide (Sobczak, Salaga, Storr, & Fichna, 2013). IBS may involve a dysregulation of interactions between central and peripheral nervous system, so called brain-gut axis (Fichna & Storr, 2012; Sobczak et al., 2013). Patients with IBS suffer from altered bowel habits, ranging from diarrhea (IBS-D), constipation (IBS-C), or alternating diarrhea and constipation (IBS-A). The treatment of IBS is still symptomatic, without a well-defined first-line therapy.

ECS may be involved in the pathophysiology of functional diseases like IBS, with CB receptors implicated in the regulation of impaired water and electrolyte secretion, intestinal motility, and pain sensation (Fig. 49.2). CB1 receptors are involved in the reduction of net electrogenic ion transport in the bowel and, thus, may reduce intestinal secretion induced by activation of secretomotor submucosal neurons (Tyler, Hillard, & Greenwood-Van, 2000). In vivo studies indicated the potential role that ECS plays in the pathophysiology of functional diseases. Nonselective CB receptor agonists inhibit cholera toxin-induced fluid accumulation in the bowel, and this effect is blocked by selective CB1, but not CB2 antagonists (Izzo et al., 2003). Interestingly, CB1 receptors are overexpressed in the model of croton oil-induced ileitis, and the CB1 agonists were more effective in reducing transit, compared to control animals (Izzo et al., 2001). In line, Fichna, Sibaev, Salaga, Sobczak, and Storr (2013a) demonstrated that the inverse CB1 agonist, taranabant, increases motility and reduces pain in a mouse model of IBS-C . Also, indirect elevation of endogenous CBs, achieved by blockade of FAAH, may be used to inhibit gut motility in physiologic conditions, and during hypermotility states (Fichna et al., 2014). A potent and irreversible FAAH inhibitor, PF-3845 inhibited mouse colonic motility through CB1-mediated mechanisms, while this effect was not observed in FAAH-knockout mice (Fichna et al., 2013b; Nasser, Bashashati, & Andrews, 2014).

Abdominal pain, together with altered motility and bowel habits, is one of the main symptoms of IBS. ECS may be targeted in order to reduce visceral sensation and abdominal pain induced by various stimuli

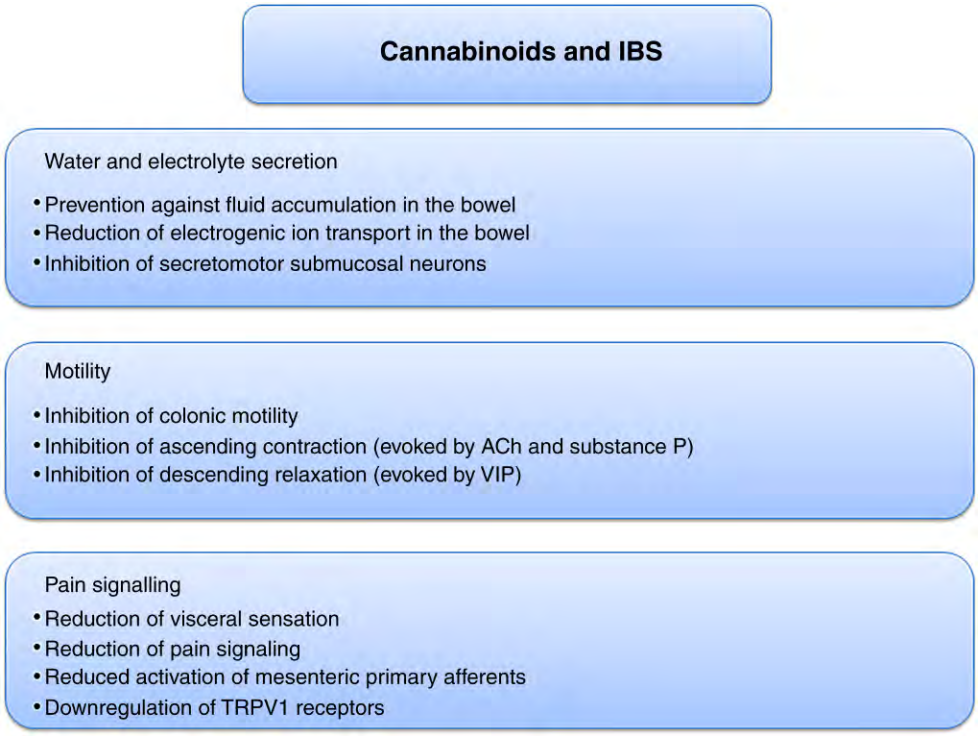


FIGURE 49.2 **Cannabinoid-mediated effects in the course of IBS.** *ACh*, Acetylcholine; *VIP*, vasoactive intestinal peptide; *TRPV1*, transient receptor potential vanilloid type 1.

(Alhouayek & Muccioli, 2012; Izzo & Coutts, 2005; Izzo & Sharkey, 2010; Salaga et al., 2014b). Both direct and indirect CB1 receptor activation, as well as direct CB2 receptor activation, inhibits visceral sensitivity and pain, in rodents. Moreover, antinociceptive effects may be also achieved by blockade of FAAH. Recently, Fichna et al. (2014) showed that PF-3845 produced a potent antinociceptive effect in the writhing test, and a mouse model of abdominal pain induced by mustard oil . Interestingly, these effects were observed after peripheral and central administration. Based on these studies, it seems that ECS is an attractive target for drugs that could treat abdominal pain, either through direct interaction with cannabinoid receptors or inhibition of endogenous cannabinoid degradation. Further translational studies are needed to validate this thesis in humans. Of note, some efforts have been already made in this direction. Fichna et al. (2013b) compared the plasma levels of endocannabinoids and colonic FAAH mRNA expression in IBS patients and healthy controls. The study showed that IBS-D patients had elevated levels of plasma 2-AG. Moreover, FAAH mRNA level was lower in patients with IBS-C, compared to healthy controls (Fichna et al., 2013b). These results suggest that ECS may become a target for future anti-IBS drugs which modulate the activity of endocannabinoid-degrading enzymes.

To date, only some compounds targeting the ECS have been tested on humans, and the results are only partially satisfactory. In a randomized double-blind placebo-controlled study, dronabinol (a synthetic nonselective CB receptor agonist) decreased fasting colonic motility, and increased compliance to colorectal distension, in healthy patients (Esfandyari et al., 2007). However, patients who received dronabinol also reported increased pain during colorectal distension, possibly due to CNS-related effects, such as modulation of perception (Esfandyari et al., 2007). In another study, dronabinol decreased fasting proximal and distal left colonic motility index, compared with placebo, and increased colonic compliance (Wong et al., 2011). The effects of dronabinol were most significant in patients with IBS-D or IBS-A. Moreover, the effects of dronabinol were influenced by genetic polymorphisms of FAAH and CB1. These data indicate that ECS plays an important role in the pathophysiology of functional GI disorders, and further translational studies are urgently needed to close the gap between animal and clinical trials on the novel ECS-targeting drugs for IBS.

Inflammatory Bowel Diseases

Inflammatory bowel diseases (IBD) are idiopathic, relapsing, and chronic disorders, which affect the GI system. Within this group of diseases, the most common are Crohn's disease (CD) and ulcerative colitis (UC), which are diagnosed in 1–2% of population (Schirbel & Fiocchi, 2010). The IBD incidence is highest between 15 and

30, and then 50–70 years of age. The major symptoms of IBD include abdominal pain, fecal bleeding, diarrhea, and weight loss. Moreover, there are extraintestinal manifestations occurring in 30% of IBD patients. The etiology of IBD remains unknown; however, several genetic and environmental factors, as well as nonbalanced immunological response, are believed to trigger its development (Salaga, Sobczak, & Fichna, 2013; Sobczak et al., 2013; Scharl & Rogler, 2012). The major therapeutic goals in IBD include control of the intestinal inflammation, and treatment of the most common symptoms, mainly abdominal pain and diarrhea.

To date, many in vitro and in vivo studies have been performed to elucidate the role of ECS in the course of IBD; here, we will summarize only the most relevant (Fig. 49.3; for a detailed review, please see Izzo & Camilleri, 2009). ECS signaling is enhanced during intestinal inflammation, as revealed by an increased expression of CB1 and CB2 receptors, altered endocannabinoid levels, and decreased expression of endocannabinoid degrading enzymes. Patients with untreated CD and UC had 2-times higher level of AEA in mucosal biopsies than healthy controls. Moreover, AEA levels significantly correlated with clinical activity of the disease (D'Argenio et al., 2006; Izzo & Camilleri, 2009). Immunostaining for CB receptors in healthy and inflamed colonic tissues revealed that CB2 receptor immunoreactivity, faintly detected in normal tissues, was significant in colonic mucosal samples from acute-phase IBD patients. Moreover, CB2 expression in ulcerated tissues was enhanced, compared to noninflamed samples from CD patients (Wright et al., 2005). Based on animal models, it was also reported that the level of FAAH mRNA is altered during the course of colitis. At the beginning of the inflammation (1 day after TNBS administration), the expression of FAAH mRNA was reduced, and when the damage was maximal (3 days after TNBS), FAAH mRNA levels increased (Storr et al., 2008).

Direct activation of CB1 and/or CB2 receptors produces a protective effect in animal models of experimental colitis, induced by mustard oil, DNBS, TNBS, DSS, and LPS (D'Argenio et al., 2006; Izzo & Camilleri, 2009; Kimball, Schneider, Wallace, & Hornby, 2006; Mathison, Ho, Pittman, Davison, & Sharkey, 2004; Storr et al., 2008). Moreover, CB1-deficient mice are less resistant to colonic inflammation than wild type (Massa et al., 2004). Indirect activation of CB receptors through inhibition of FAAH or EMT also provides protection against inflammatory stimuli (Salaga et al., 2014b). In addition, the EMT inhibitor VDM-11 not only increased the level of AEA, but also abolished inflammation (D'Argenio et al., 2006). The blockade of FAAH and EMT (with URB597 and VDM-11, respectively) protected against TNBS-induced colitis in wild type, but not in CB1- and CB2-deficient mice (Storr et al., 2008). Furthermore, the blockade of FAAH may alter the levels of non-"classical" CB receptor ligands, such as 2-OG, PGE2, and glycerol-derived biolipids (Salaga et al., 2014a). These findings highlight the protective role of all components of the ECS in inflammatory GI disorders.

Colorectal Cancer

One of the most threatening risks to which IBD patients are exposed is that of development of colorectal cancer (CRC) as a result of the chronic inflammatory

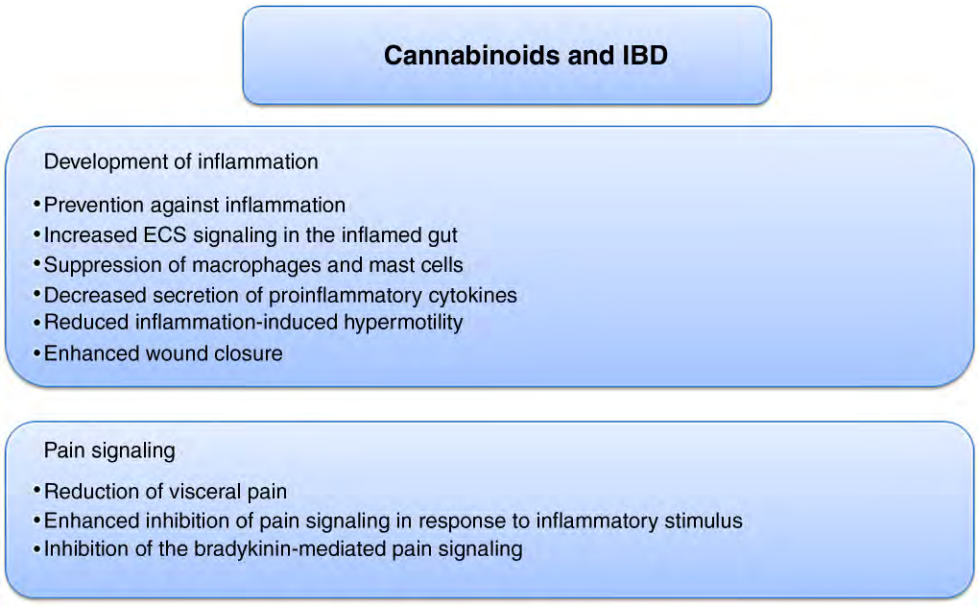


FIGURE 49.3 **Cannabinoid-mediated effects in the course of IBD.**

state in the lower GI tract. Chronic inflammation, which underlies colitis-associated colorectal cancer, may contribute to the development of up to 25% of all diagnosed colorectal tumors (Itzkowitz, Greenwald, & Meltzer, 1995). Currently, the most effective methods for colitis-associated colorectal cancer treatment are cytotoxic drugs, radiotherapy, or resection of tumor area. Low selectivity between cancer and healthy cells, and a major risk of side effects, often combined with poor efficacy and the risk of relapse, are the main reasons for which new therapies against CRC are urgently needed (Kumar et al., 2012).

Both natural and endogenous cannabinoids exert anticancer activities, as indicated in in vitro and in vivo studies (Fig. 49.4). CBs reduce proliferation, and promote apoptosis in colorectal carcinoma epithelial cells, and inhibit development of tumors in animal models of CRC (for review, see Izzo & Camilleri, 2009; Izzo & Sharkey, 2010). Studies on human intestinal biopsies revealed that ECS undergoes adaptive changes in the course of CRC. In adenomas and colorectal cancer, AEA and 2-AG levels are three- and two-fold higher, respectively, than in normal mucosa (Izzo & Camilleri, 2009). Moreover, the expression of CB1 receptors in human cancer specimens is downregulated, due to the methylation of its promoter (Wang et al., 2008). Downregulated CB1-dependent signaling in cancer cells may stimulate their growth into a tumor. On the contrary, recognizable pattern of CB2 mRNA expression can be found in tumor tissues (Izzo & Sharkey, 2010).

Activation of CB1 and, to a lesser extent, CB2 receptors exert antiproliferative and proapoptotic activity in various cancer cell lines (Izzo & Camilleri, 2009). The exact mechanism of such effects is not completely elucidated, but experimental data suggest the involvement of caspase-3, which is associated with activation of CB receptors. Moreover, experimental data demonstrate that Δ^9-THC promote apoptosis by inhibition of MAPK and PI3K-AKT (Izzo & Camilleri, 2009). Another hypothesis considers the involvement of ceramide, a well-known proapoptotic biolipid, and also a second messenger of ECS signaling. Activation of both CB receptors stimulates the synthesis of ceramide in the carcinoma cell lines. In line, it was shown in HT-29 CRC cells that the activation of CB receptors was associated with increased ceramide levels, and pharmacological blockade of ceramide-synthesizing enzymes reversed the proapoptotic changes, which occurred after CB receptor activation (Cianchi et al., 2008). There is also some evidence that antitumor actions of CBs may be mediated by non-CB mediated mechanism, such as prostamide production (Izzo & Camilleri, 2009).

Natural CBs, such as Δ^9-THC and CBD, also exhibit antiproliferative actions in human CRC (Caco-2) cells with IC50 value in low micromolar range (Izzo & Sharkey, 2010). The quinone derivative of CBD, named HU-331, has been shown to be highly effective against tumor xenografts in nude mice. Additionally, HU-331 was found to be much less cardiotoxic than doxorubicin. In HU-331 treated group, the tumor weight was 30% smaller than in doxorubicin-treated animals (Kogan et al., 2007).

CBs may also prevent metastasis. Nonselective CB receptor agonists, as well as selective CB1, but not CB2 agonists, inhibited norepinephrine-induced migration of human colon carcinoma cells SW480 (Joseph, Niggemann, Zaenker, & Entschladen, 2004).

Treatment with the FAAH inhibitor AA-5-HT reduced the formation of aberrant crypt foci in the mouse colon, via caspase-3 activation (Izzo & Camilleri, 2009). Moreover, AA-5-HT also increased AEA and 2-AG levels, indirectly activating both CB receptors.

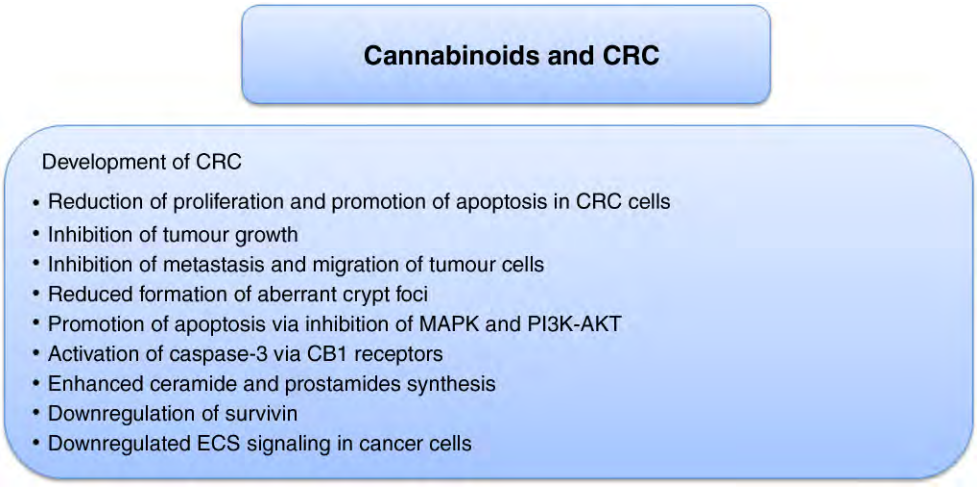


FIGURE 49.4 Cannabinoid-mediated effects in the course of CRC. *MAPK*, Mitogen-activated protein kinase; *PI3K-AKT*, phosphoinositide 3-kinase-protein kinase B.

Several studies were also performed on Apc mice, which possess a germ-like mutation in the APC gene, which is a tumor suppressor gene. These mutants spontaneously develop multiple polyps in the intestine. It has been shown that, in the CB1-deficient Apc mice, the growth rate of intestinal adenoma is significantly higher than in control animals. Moreover, the activation of CB1 receptors attenuated intestinal tumor growth through downregulation of survivin, a protein involved in prevention against apoptosis by inhibition of caspase activation (Wang et al., 2008).

CANNABIS HERBAL LEAF AND RESIN USAGE IN THE GASTROINTESTINAL TRACT

People used various *Cannabis*-derived preparations a long time before its chemical composition has been elucidated. The most popular were those made of *Cannabis* leaves and the resin, which can be obtained from flowers, stem, and leaves. Nowadays, these preparations are popular mostly among recreational users, and are forbidden in many countries due to their addictive properties.

There is limited scientific data on the effect of *Cannabis* leaves and resin on the GI tract, mostly originating from occasional reports of recreational users rather than actual scientific investigations. Bhatia, Manhas, Kumar, and Magotra (2014) points out that the whole plant of *Cannabis sativa* L. (either fresh or dry) may cause nausea and vomiting. These adverse effects of *Cannabis* significantly hamper its clinical use, even though it may be helpful in the treatment of several conditions, such as pain and muscle spasms (Borgelt, Franson, Nussbaum, & Wang, 2013). On top of the obvious legal issues and adverse events, the use of medical *Cannabis* faces a number of challenges. First, the route of administration (smoked, oral, inhaled) and individual condition of the patient (functional lung capacity, GI absorption) may both cause significant variability in the observed effects. Another serious problem is the weak quality control of *Cannabis* preparations (ie, contaminated products, nonstandarized doses). Therefore, emphasis should be put on the isolation of novel and safe therapeutic compounds from *Cannabis*, rather than the search for a safe way of using leaf or resin preparations. To date, many scientific reports showed beneficial properties of *Cannabis*-derived compounds on GI diseases and cancer (see above). Modification and improvement of these compounds is the preferred strategy in this field.

CONCLUDING REMARKS

ECS is one of the most influential endogenous systems in the GI tract, and has a tremendous impact on gut homeostasis, being involved in the regulation of motility, food intake, and intestinal secretion (Fig. 49.5). Moreover, various pathophysiological processes, such as inflammation, pain, and even colorectal cancer, are modulated by ECS-dependent pathways. Compounds targeting ECS have a great potential to become pharmacological tools to study the pathophysiology of GI diseases, as well as being used in treatment of GI diseases in the future. However, given the disappointing experience with the clinical studies on CBs, it will be essential for future drug development programs to screen for the psychotropic or depressant potential of this class of medications. Nevertheless, novel treatment strategies for GI diseases, based on the activation of CB receptors, and the regulation of endogenous cannabinoid levels in the GI tract, are currently under investigation. As more attention is paid currently to the ECS, the discovery and validation of effective therapies against GI diseases in the near future is warranted.

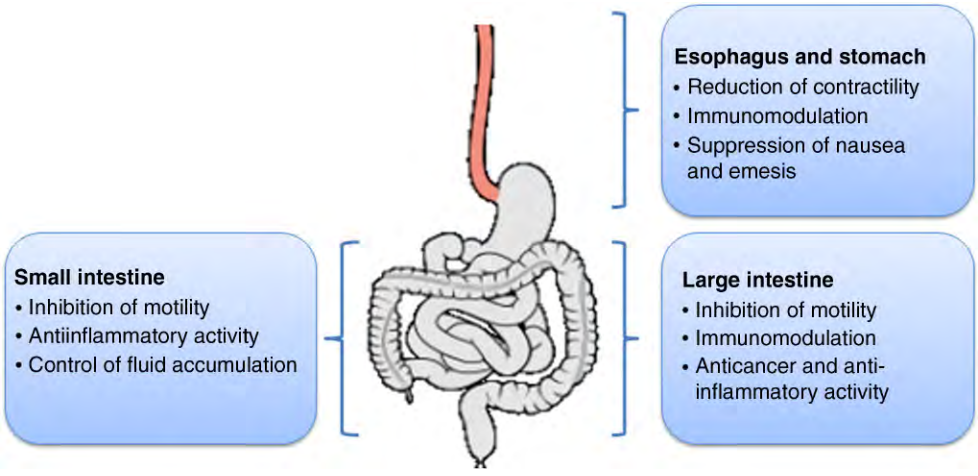


FIGURE 49.5 **A summary of cannabinoid-related actions in the gastrointestinal tract.**

MINI-DICTIONARY

Apc mice Genetically modified mice characterized by spontaneous development of polyps in the intestine.
Colitis-associated colorectal cancer A colon cancer caused by a chronic, sustained inflammatory state.
EMT A putative endocannabinoid membrane transporter, a protein that carries endocannabinoids through cell membranes.
ENS The part of the peripheral nervous system that resides in, and innervates, the gut.
GPR55 and GPR119 Non-"classical" GPCRs that may be activated by endocannabinoids.
Inflammatory bowel diseases Chronic inflammatory disorders affecting the intestine. Encompass Crohn's disease and ulcerative colitis.
Irritable bowel syndrome A functional disorder affecting the GI tract, manifested mainly by altered motility and abdominal pain.
L-cells Enteroendocrine cells located in the intestine that produce peptide hormones, such as incretins.
TNBS Trinitrobenzenesulfonic acid. Sometimes called a hapten. It is one of the chemical agents used to induce experimental colitis.
Vagus nerve The longest of the cranial nerves, it is a mixed nerve, leading to sensory and motor parasympathetic fibers. Belongs to the autonomic nervous system.

References

Abalo, R., Vera, G., López-Pérez, A. E., Martínez-Villaluenga, M., & Martín-Fontelles, M. I. (2012). The gastrointestinal pharmacology of cannabinoids: focus on motillity. *Pharmacology*, *90*(1–2), 1–10.

Alhouayek, M., & Muccioli, G. G. (2012). The endocannabinoid system in inflammatory bowel diseases: from pathophysiology to therapeutic opportunity. *Trends in Molecular Medicine*, *18*, 615–625.

Bhatia, H., Manhas, R. K., Kumar, K., & Magotra, R. (2014). Traditional knowledge on poisonous plants of Udhampur district of Jammu and Kashmir. *Indian Journal of Ethnopharmacology*, *152*, 207–216.

Borgelt, L. M., Franson, K. L., Nussbaum, A. M., & Wang, G. S. (2013). The pharmacologic and clinical effects of medical cannabis. *Pharmacotherapy*, *33*, 195–209.

Chicca, A., Marazzi, J., Nicolussi, S., & Gertsch, J. (2012). Evidence for bidirectional endocannabinoid transport across cell membranes. *Journal of Biological Chemistry*, *287*, 34660–34682.

Cianchi, F., Papucci, L., Schiavone, N., Lulli, M., Magnelli, L., Vinci, M. C., Messerini, L., Manera, C., Ronconi, E., Romagnani, P., Donnini, M., Perigli, G., Trallori, G., Tanganelli, E., Capaccioli, S., & Masini, E. (2008). Cannabinoid receptor activation induces apoptosis through tumor necrosis factor alpha-mediated ceramide de novo synthesis in colon cancer cells. *Clinical Cancer Research*, *14*, 7691–7700.

Coutts, A. A., Irving, A. J., Mackie, K., Pertwee, R. G., & Anavi-Goffer, S. (2002). Localisation of cannabinoid CB(1) receptor immunoreactivity in the guinea pig and rat myenteric plexus. *Journal of Comparative Neurology*, *448*, 410–422.

D'Argenio, G., Valenti, M., Scaglione, G., Cosenza, V., Sorrentini, I., & Di Marzo, V. (2006). Up-regulation of anandamide levels as an endogenous mechanism and a pharmacological strategy to limit colon inflammation. *FASEB Journal*, *20*, 568–570.

Di Sabatino, A., Battista, N., Biancheri, P., Rapino, C., Rovedatti, L., Astarita, G., Vanoli, A., Dainese, E., Guerci, M., Piomelli, D., Pender, S. L., Macdonald, T. T., Maccarrone, M., & Corazza, G. R. (2011). The endogenous cannabinoid system in the gut of patients with inflammatory bowel disease. *Mucosal Immunology*, *4*, 574–583.

Esfandyari, T., Camilleri, M., Busciglio, I., Burton, D., Baxter, K., & Zinsmeister, A. R. (2007). Effects of a cannabinoid receptor agonist on colonic motor and sensory functions in humans: a randomized, placebo-controlled study. *American Journal of Physiology: Gastrointestinal and Liver Physiology*, *293*, G137–G145.

Esposito, G., Capoccia, E., Turco, F., Palumbo, I., Lu, J., Steardo, A., Cuomo, R., Sarnelli, G., & Steardo, L. (2013). Palmitoylethanolamide improves colon inflammation through an enteric glia/toll like receptor 4-dependent PPAR-alpha activation. *Gut*, *63*, 1300–1312.

Fichna, J., & Storr, M. A. (2012). Brain-gut interactions in IBS. *Frontiers in Pharmacology*, *3*, 127.

Fichna, J., Sibaev, A., Salaga, M., Sobczak, M., & Storr, M. (2013a). The cannabinoid-1 receptor inverse agonist taranabant reduces abdominal pain and increases intestinal transit in mice. *Neurogastroenterology and Motility*, *25*, e550–e559.

Fichna, J., Wood, J. T., Papanastasiou, M., Vadivel, S. K., Oprocha, P., Salaga, M., Sobczak, M., Mokrowiecka, A., Cygankiewicz, A. I., Zakrzewski, P. K., Malecka-Panas, E., Krajewska, W. M., Koscielniak, P., Makriyannis, A., & Storr, M. A. (2013b). Endocannabinoid and cannabinoid-like fatty acid amide levels correlate with pain-related symptoms in patients with IBS-D and IBS-C: a pilot study. *PLoS One*, *8*, e85073.

Fichna, J., Salaga, M., Stuart, J., Saur, D., Sobczak, M., Zatorski, H., Timmermans, J. P., Bradshaw, H. B., Ahn, K., & Storr, M. A. (2014). Selective inhibition of FAAH produces antidiarrheal and antinociceptive effect mediated by endocannabinoids and cannabinoid-like fatty acid amides. *Neurogastroenterology and Motility*, *26*, 470–481.

Gill, E. W., Paton, W. D., & Pertwee, R. G. (1970). Preliminary experiments on the chemistry and pharmacology of cannabis. *Nature*, *228*, 134–136.

Godlewski, G., Offertaler, L., Wagner, J. A., & Kunos, G. (2009). Receptors for acylethanolamides-GPR55 and GPR119. *Prostaglandins and Other Lipid Mediators*, *89*, 105–111.

Gomez, R., Navarro, M., Ferrer, B., Trigo, J. M., Bilbao, A., Del Arco, I., Cippitelli, A., Nava, F., Piomelli, D., & Rodriguez de Fonseca, F. (2002). A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. *Journal of Neuroscience*, *22*, 9612–9617.

Itzkowitz, S. H., Greenwald, B., & Meltzer, S. J. (1995). Colon carcinogenesis in inflammatory bowel disease. *Inflammatory Bowel Disease*, *1*, 142–158.

Izzo, A. A., Fezza, F., Capasso, R., Bisogno, T., Pinto, L., Iuvone, T., Esposito, G., Mascolo, N., Di Marzo, V., & Capasso, F. (2001). Cannabinoid CB1-receptor mediated regulation of gastrointestinal motility in mice in a model of intestinal inflammation. *British Journal of Pharmacology*, *134*, 563–570.

Izzo, A. A., Capasso, F., Costagliola, A., Bisogno, T., Marsicano, G., Ligresti, A., Matias, I., Capasso, R., Pinto, L., Borrelli, F., Cecio, A., Lutz, B., Mascolo, N., & Di Marzo, V. (2003). An endogenous cannabinoid tone attenuates cholera toxin-induced fluid accumulation in mice. *Gastroenterology*, *125*, 765–774.

Izzo, A. A., & Coutts, A. A. (2005). Cannabinoids and the digestive tract. *Handbook of Experimental Pharmacology*. Heidelberg: Springer-Verlag, 573-598.

Izzo, A. A., & Camilleri, M. (2009). Cannabinoids in intestinal inflammation and cancer. *Pharmacology Research*, *60*, 117–125.

Izzo, A. A., & Sharkey, K. A. (2010). Cannabinoids and the gut: new developments and emerging concepts. *Pharmacology and Therapeutics*, *126*, 21–38.

Joseph, J., Niggemann, B., Zaenker, K. S., & Entschladen, F. (2004). Anandamide is an endogenous inhibitor for the migration of tumor cells and T lymphocytes. *Cancer Immunology, Immunotherapy*, *53*, 723–728.

Kimball, E. S., Schneider, C. R., Wallace, N. H., & Hornby, P. J. (2006). Agonists of cannabinoid receptor 1 and 2 inhibit experimental colitis induced by oil of mustard and by dextran sulfate sodium. *American Journal of Physiology: Gastrointestinal Liver Physiology*, *291*, G364–G371.

Kogan, N. M., Schlesinger, M., Peters, M., Marincheva, G., Beeri, R., & Mechoulam, R. (2007). A cannabinoid anticancer quinone, HU-331, is more potent and less cardiotoxic than doxorubicin: a comparative in vivo study. *Journal of Pharmacology and Experimental Therapeutics*, *322*, 646–653.

Kulkarni-Narla, A., & Brown, D. R. (2000). Localization of CB1-cannabinoid receptor immunoreactivity in the porcine enteric nervous system. *Cell and Tissue Research*, *302*, 73–80.

Kumar, M., Nagpal, R., Hemalatha, R., Verma, V., Kumar, A., Singh, S., Marotta, F., Jain, S., & Yadav, H. (2012). Targeted cancer therapies: the future of cancer treatment. *Acta Biomedica*, *83*, 220–233.

Massa, F., Marsicano, G., Hermann, H., Cannich, A., Monory, K., Cravatt, B. F., Ferri, G. L., Sibaev, A., Storr, M., & Lutz, B. (2004). The endogenous cannabinoid system protects against colonic inflammation. *Journal of Clinical Investigation*, *113*, 1202–1209.

Mathison, R., Ho, W., Pittman, Q. J., Davison, J. S., & Sharkey, K. A. (2004). Effects of cannabinoid receptor-2 activation on accelerated gastrointestinal transit in lipopolysaccharide-treated rats. *British Journal of Pharmacology*, *142*, 1247–1254.

Nasser, Y., Bashashati, M., & Andrews, C. N. (2014). Toward modulation of the endocannabinoid system for treatment of gastrointestinal disease: FAAHster but not "higher". *Neurogastroenterology and Motility*, *26*, 447–454.

Paulino, G., Barbier, d. l. S., Knotts, T. A., Oort, P. J., Newman, J. W., Adams, S. H., & Raybould, H. E. (2009). Increased expression of receptors for orexigenic factors in nodose ganglion of diet-induced obese rats. *American Journal of Physiology. Endocrinology and Metabolism*, *296*, E898–E903.

Romero-Zerbo, S. Y., & Bermudez-Silva, F. J. (2014). Cannabinoids, eating behaviour, and energy homeostasis. *Drug Testing and Analysis*, *6*, 52–58.

Roth, S. H. (1978). Stereospecific presynaptic inhibitory effect of delta9-tetrahydrocannabinol on cholinergic transmission in the myenteric plexus of the guinea pig. *Canadian Journal of Physiology and Pharmacology*, *56*, 968–975.

Salaga, M., Sobczak, M., & Fichna, J. (2013). Inhibition of proteases as a novel therapeutic strategy in the treatment of metabolic, inflammatory and functional diseases of the gastrointestinal tract. *Drug Discovery Today*, *18*, 708–715.

Salaga, M., Mokrowiecka, A., Zakrzewski, P. K., Cygankiewicz, A., Leishman, E., Sobczak, M., Zatorski, H., Malecka-Panas, E., Kordek, R., Storr, M., Krajewska, W. M., Bradshaw, H. B., & Fichna, J. (2014a). Experimental colitis in mice is attenuated by changes in the levels of endocannabinoid metabolites induced by selective inhibition of fatty acid amide hydrolase (FAAH). *Journal of Crohn's and Colitis*, *8*, 998–1009.

Salaga, M., Sobczak, M., & Fichna, J. (2014b). Inhibition of fatty acid amide hydrolase (FAAH) as a novel therapeutic strategy in the treatment of pain and inflammatory diseases in the gastrointestinal tract. *European Journal of Pharmaceutical Sciences*, *52*, 173–179.

Scharl, M., & Rogler, G. (2012). Inflammatory bowel disease pathogenesis: what is new? *Current Opinion in Gastroenterology*, *28*, 301–309.

Schirbel, A., & Fiocchi, C. (2010). Inflammatory bowel disease: established and evolving considerations on its etiopathogenesis and therapy. *Journal of Digestive Diseases*, *11*, 266–276.

Sobczak, M., Salaga, M., Storr, M. A., & Fichna, J. (2013). Physiology, signaling, and pharmacology of opioid receptors and their ligands in the gastrointestinal tract: current concepts and future perspectives. *Journal of Gastroenterology*, *49*, 24–45.

Storr, M. A., Keenan, C. M., Emmerdinger, D., Zhang, H., Yuce, B., Sibaev, A., Massa, F., Buckley, N. E., Lutz, B., Goke, B., Brand, S., Patel, K. D., & Sharkey, K. A. (2008). Targeting endocannabinoid degradation protects against experimental colitis in mice: involvement of CB1 and CB2 receptors. *Journal of Molecular Medicine (Berlin)*, *86*, 925–936.

Tourino, C., Oveisi, F., Lockney, J., Piomelli, D., & Maldonado, R. (2010). FAAH deficiency promotes energy storage and enhances the motivation for food. *International Journal of Obesity (London)*, *34*, 557–568.

Tyler, K., Hillard, C. J., & Greenwood-Van, M. B. (2000). Inhibition of small intestinal secretion by cannabinoids is CB1 receptor-mediated in rats. *European Journal of Pharmacology*, *409*, 207–211.

Wang, D., Wang, H., Ning, W., Backlund, M. G., Dey, S. K., & DuBois, R. N. (2008). Loss of cannabinoid receptor 1 accelerates intestinal tumor growth. *Cancer Research*, *68*, 6468–6476.

Wong, B. S., Camilleri, M., Busciglio, I., Carlson, P., Szarka, L. A., Burton, D., & Zinsmeister, A. R. (2011). Pharmacogenetic trial of a cannabinoid agonist shows reduced fasting colonic motility in patients with nonconstipated irritable bowel syndrome. *Gastroenterology*, *141*, 1638–1647.

Wright, K., Rooney, N., Feeney, M., Tate, J., Robertson, D., Welham, M., & Ward, S. (2005). Differential expression of cannabinoid receptors in the human colon: cannabinoids promote epithelial wound healing. *Gastroenterology*, *129*, 437–453.

CHAPTER

50

Cardiovascular Effects of Cannabis Usage

S. Menahem

Paediatric Cardiology Unit, Monash Health, Melbourne, VIC, Australia; Faculty of Medicine, Nursing and Health Sciences, Monash University, Clayton, VIC, Australia

SUMMARY POINTS

- The major active cannabinoid in marijuana is Δ^9-tetrahydrocannabinol (THC). Its physiological effects are mediated through the cannabinoid receptors CB1 and CB2.
- A low dose leads to tachycardia and blood pressure rise, while a high dose to bradycardia and hypotension.
- There is clear documentation of an increased incidence in both the morbidity and mortality of marijuana users, especially if its usage is chronic or heavy. That may result from an increased incidence of myocardial ischemia and/or infarction, the development of a cardiomyopathy, and the risk of sudden death through a probable malignant arrhythmia and/or reduced coronary blood flow.
- There is also an increased incidence of transient ischemic episodes and stroke.
- There is an increased incidence of intermittent claudication arising from peripheral vascular disease.
- All the above should suggest *caution* against *chronic* and/or *heavy* usage.

KEY FACTS OF CARDIOVASCULAR EFFECTS OF CANNABIS USAGE

- Cannabis usage is frequent and widespread.
- Excessive and/or chronic usage results in significant cardiovascular morbidity and/or mortality.
- That includes involvement of the heart, brain, and limbs.
- That may result in:
  - Angina, myocardial infarction, and/or sudden death.
  - Transient ischemic episodes and/or strokes.
  - Intermittent claudication and/or peripheral gangrene.
- All the above highlight the ill effects of excessive and/or chronic usage of cannabis and caution against its abuse.

LIST OF ABBREVIATIONS

CB1	Cannabinoid receptor 1
CB2	Cannabinoid receptor 2
THC	Δ^9-Tetrahydrocannabinol

INTRODUCTION

Marijuana use is common and widespread, especially in the developed countries (Substance Abuse & Mental Health Services Administration, 1999; Bachs & Morland, 2001; Thomas, Kloner, & Rezkalla, 2014). While often touted as being safe or the least toxic of the so-called recreational drugs, there is a substantial literature which documents potential ill effects and increasing morbidity, and often mortality—especially in its chronic users. This chapter will focus on the described physiological and pathological cardiovascular effects of marijuana, especially if taken in high dosage over a prolonged period of time.

At the outset, it is difficult to separate out the potential effects of cannabis usage, whether by inhalation or ingestion, when it is often accompanied by nicotine inhalation, alcohol, and other chemically active drugs such as cocaine, opiates, ecstasy, etc., and further compounded by at times unknown contaminants (Aryana

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00058-2
Copyright © 2017 Elsevier Inc. All rights reserved.

& Williams, 2007; Suttout & Nicole, 2009). Accurate estimation of the level of cannabis and its metabolites is often lacking, and can only be inferred by reviewing the history, where there is a tendency to underestimate the quantity taken, and the frequency of the inhalations or ingestions (Menahem, 2013).

PHYSIOLOGICAL EFFECTS

The major active cannabinoid in marijuana is Δ^9-tetrahydrocannibol (THC) (Sydney, 2002). Jones (2002) described in detail the physiological cardiovascular effects of marijuana. THC can lead to a rapid and substantial dose-dependent increase in heart rate up to 100%, and a modest increase in blood pressure probably mediated by sympathetic simulation, and reduced parasympathetic activity peaking within 15 min, and lasting for up to 3 h (Aryana & Williams, 2007; Sydney, 2002; Jones, 2002; Hollister, 1986). The cardiac output is increased by 30% or more (Sydney, 2002; Benowitz, Rozenberg, Rogers, Bachman, & Jones, 1979). THC is also described as facilitating atrioventricular node conduction, reducing left ventricular ejection time, and decreasing the peripheral vascular resistance, especially in skeletal muscle (Aryana & Williams, 2007; Sydney, 2002; Jones, 2002; Benowitz et al., 1979), which may result in orthostatic hypotension which may lead to dizziness, near syncope, or syncope (Aryana & Williams, 2007; Sydney, 2002). Kunos et al. (2000) give a more detailed account of the biological basis of action of marijuana, emphasizing that THC is the primary psychoactive ingredient of the marijuana plant. The high affinity binding of radiolabeled synthetic cannabinoids to stereoselective sites in the brain suggests the existence of specific cellular receptors for cannabinoids (Devane et al., 1992). Two cannabinoid receptors have been identified by molecular cloning—CB1, primarily in the brain, but also in some peripheral tissues, and CB2 receptors in immune cells (Metsuda, 1997). Intravenous injection of endocannabinoid anandomide into rats produced a triphasic response similar to THC. Initially, there was a brief vagally mediated drop in heart rate and blood pressure, followed by a brief pressor response, probably from vasoconstriction in certain vascular beds, followed by more prolonged hypotension and bradycardia, where CB1 appears to be involved. However, there is the possibility that there may be a decrease in sympathetic tone, or direct vasodilator action resulting in cannabinoid induced hypotension (Kunos et al., 2000). The biphasic response to cannabinoids may be dose-dependent, a low to moderate dose causing tachycardia and blood pressure rise, whereas a higher dose may lead to bradycardia and hypotension (Suttout & Nicole, 2009).

CARDIOVASCULAR CONSEQUENCES OF MARIJUANA USAGE

There are well recognized complications associated with marijuana usage, including important adverse cardiovascular disorders well described by Bachs and Morland (2001), Thomas et al. (2014), and Jones (2002). It behoves all clinicians, especially cardiologists and primary care physicians, to be aware of the potential serious consequences of prolonged and heavy use of marijuana. These adverse cardiovascular effects are summarized under the following headings.

Myocardial Ischemia/Infarction

Myocardial ischemia may lead to myocardial infarction (Arora, Goyal, Aggarwal, & Kukar, 2012; Saffan, Markham, & Jayasinghe, 2012) despite apparently normal coronary arteries on angiography. The mechanism may result from an increase in oxygen demand secondary to an increased heart rate and a decreased oxygen supply, because of a rise in the carboxyhemoglobin levels post smoking (Suttout & Nicole, 2009), or reduced coronary blood flow secondary to coronary artery vasospasm (Rezkalla, Sharma, & Kloner, 2003; Karabulut & Cakmak, 2010). Again, it is difficult to separate out the direct action of marijuana versus other drugs or contaminants involved, such as nicotine, alcohol, cocaine, etc. (Thomas et al., 2014).

Myocardial infarction is well described in autopsy series (Tormey, 2012; Caldicott, Holmes, Roberts-Thompson, & Maher, 2005), even in young subjects with apparently normal coronary arteries (Arora et al., 2012; Saffan et al., 2012). It also appears to occur more frequently in those patients with coronary artery disease who use marijuana (Aryana & Williams, 2007; Rezkalla et al., 2003; Gaziano, 2008). There is also statistical evidence that, within an hour of marijuana usage, there is a 4.8 times increase in the risk of myocardial infarction (Mittleman, Lewis, Maclure, Sherwood, & Muller, 2001). To that may be added a probable increase in mortality after myocardial infarction, in recognized users (Mukamal, Maclure, Muller, & Mittleman, 2008; Frost, Mostofsky, Rosenbloom, Mukamal, & Mittleman, 2013). There is little evidence, however, to suggest that there is either the development or acceleration of atherosclerosis, even in chronic users (Thomas et al., 2014).

Cardiomyopathy and Sudden Death

Case reports have described a possible direct action of marijuana on myocardial function (Ting, 2007; Kaushik, Alla, Madan, Arouni, & Mohiuddin, 2011). Those subjects who die suddenly with apparently normal coronary arteries and myocardium raise the possibility of a malignant cardiac arrhythmia (Suttout

& Nicole, 2009; Menahem, 2013; Lindsay, Foeli, Warren, & Henry, 2005) or possible other causes (Montisci, Thiene, Ferrara, & Basso, 2008; Daisley, Jones-Le Cointe, Hutchinson, & Simmons, 1998). Tachyarrhythmias have been frequently reported following cannabis usage (Thomas et al., 2014; Aryana & Williams, 2007; Lindsay et al., 2005; Singh, 2000). This may be related to the initial component of the biphasic response to cannabinoids producing a tachycardia (Suttout & Nicole, 2009); a higher dose may produce a sudden increase in parasympathetic tone, and parasympathetic activity which may lead to a bradycardia and/or an asystolic arrest (Suttout & Nicole, 2009; Menahem, 2013). That may be the cause of sudden death in those patients where the autopsy findings are negative (Bachs & Morland, 2001). Suttout and Nicole (2009) reported an asystolic arrest documented within 3 min of the collapse of a healthy 15 year old who had consumed alcohol and cannabis. Following cardiac pulmonary resuscitation, he developed ventricular fibrillation which was successfully cardioverted. Menahem (2013) documented, in a 25 year old heavy marijuana user, prolonged and repeated episodes of cardiac asystole, the longest lasting 5.6 s, and coinciding with the inhalation of marijuana (Fig. 50.1). His 24-h Holter returned to normal once he stopped using marijuana.

Cerebrovascular Effects of Marijuana Usage

While the psychotropic effects of marijuana use are well described elsewhere within this handbook, there does appear to be strong evidence that suggests that cerebral vascular effects can occur with chronic marijuana usage. A possible vasoconstriction effect or, alternatively, a brief hypertensive episode resulting in recurrent ischemic episodes, have been described during use of marijuana (Mouzak, Agathos, Kerezoudi, Mantas, & Vourdeli-Yiannakoura, 2000; Wolff et al., 2011). In addition, multiple reports have highlighted the development of strokes, especially in heavy users of marijuana, during marijuana inhalation (Wolff et al., 2011; Zachariah, 1991; Geller, Loftus, & Brink, 2004). In the series of Singh, Pan, Muengtaweeponsa, Geller and Cruz-Flores (2012), reexposure to marijuana resulted in recurrent stroke, as well as being described as being an important risk factor for ischemic strokes (Thomas et al., 2014; Phillips et al., 2011).

Peripheral Vascular Disease Following Marijuana Usage

Peripheral atherosclerotic disease, so-called cannabis arteritis, has been well described following marijuana usage (Grotenhermen, 2010; Noel, Ruf, & Panizzon, 2008). While it appears to be similar to thromboangiitis obliterans (Thomas et al., 2014), the latter tends to occur earlier in those individuals using marijuana (Martin-Blondel, Koskas, Cacoub, & Sene, 2011). Patients with cannabis arteritis tend to develop the usual symptoms of peripheral vascular disease, namely intermittent claudication, Reynaud phenomena, ischemic ulcers, and digital necrosis resulting from atherosclerotic changes readily demonstrated angiographically (Noel et al., 2008). Symptomatic improvement has been described, following the cessation of marijuana usage.

CONCLUSIONS

While, as stated earlier, there is little evidence to suggest that marijuana usage increases the risk of atherosclerosis, there is considerable evidence, however, to suggest that acute and/or chronic heavy usage has significant cardiac and peripheral vascular effects that may result in sudden death, a higher incidence of myocardial dysfunction, cardiac arrhythmias, acute myocardial infarction with a probable higher mortality, cerebral and peripheral vascular disease with resultant ischemic episodes, and/or stroke, or intermittent claudication and peripheral ulceration, respectively. Greater dissemination of this information describing the increased morbidity and the significant mortality from both acute and chronic marijuana usage may lead to a more cautious approach to its widespread reported usage.

MINI-DICTIONARY

Arrhythmia Describes the (electrical) activity of the heart which controls the heart rate. The rhythm may become irregular, become abnormally fast or slow, which may result in the more serious cases, a loss of cardiac output with a drop in blood pressure, or even cessation of the heart beat (contraction), resulting in a cardiac arrest leading to the (sudden) death of the person if he/she is not able to be resuscitated.

Cardiomyopathy Describes any condition that affects the heart muscle—often of unknown cause, but may be related to chronic myocardial ischemia (reduced myocardial blood supply), infarction, toxic agents or poisons, genetic disorders affecting the heart muscle, etc. That may result in reduced myocardial (heart) function, obstruction to the outflow of one or both ventricles (pumps), or the development of significant or life-threatening arrhythmias and sudden death.

Myocardial infarction Commonly known as a "heart attack," where there is irreversible necrosis or death of myocardium (heart muscle) from partial or complete occlusion or blockage of one or more coronary arteries which supply blood to the heart itself.

Myocardial ischemia Blood flow to the myocardium (heart muscle) is decreased by partial or complete blockage of the coronary arteries which supply the myocardium. Results in chest pain at rest or exercise (angina), but potentially reversible.

Peripheral vascular disease Sometimes referred to as peripheral arteritis, it results from narrowing of the peripheral (that is, outside the heart and brain) blood vessels. That may lead to intermittent claudication, that is, pain in the calf muscles on walking or running, resulting from ischemia or the reduced blood supply to the muscles of the lower limbs. Occasionally, if there is severe narrowing of the peripheral blood vessels, the skin and underlying tissue may ulcerate and/or develop gangrene (death of tissue) with loss of toes, feet, and even part of a limb.

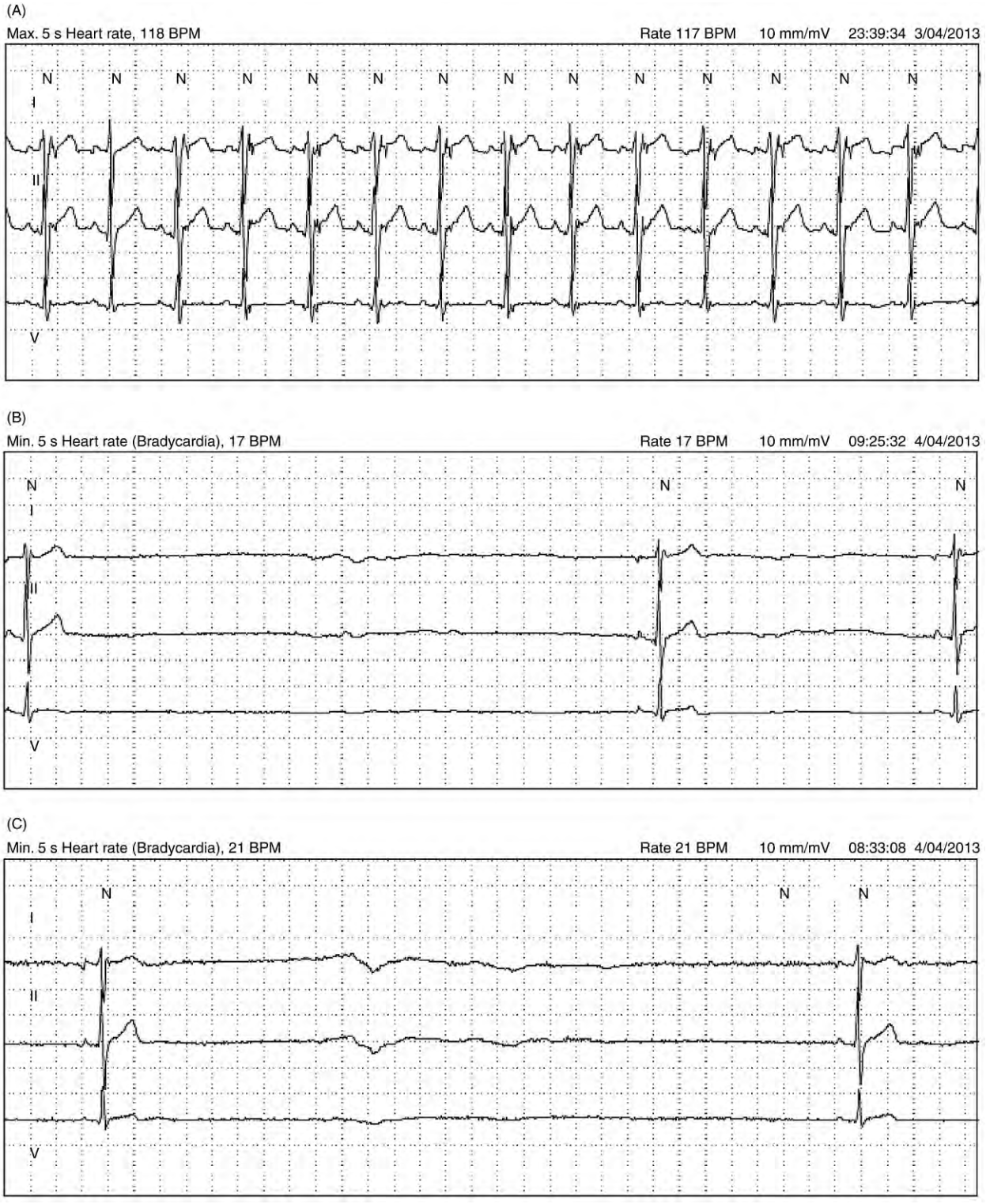


FIGURE 50.1 **Electrographic trace from a 25-year-old heavy marijuana user.** Strips from a 24-h Holter showing (A) sinus rhythm/tachycardia, (B) repeated and prolonged pauses related to cardiac asystole, associated in timing with marijuana usage; (C) longest pause: 5.8 s. *Source: From Menahem, S. (2013). Cardiac asystole following cannabis (marijuana) usage—additional mechanism for sudden death.* Forensic Science International, 233, *1–3. With permission from Elsevier.*

Stroke Sometimes called a cerebrovascular accident, occurs as a result of the loss of brain function, due to disturbance in the blood supply to the brain. That may result in ischemia (lack of blood flow) to the brain, and may occur from a thrombus or clot developing in a blood vessel supplying the brain, or from an embolism or clot dislodged from elsewhere in the body, and ending up in such a vessel. Occasionally, an affected cerebral (brain) blood vessel may rupture or leak, resulting in a hemorrhage which may also affect the brain function, leading to the loss of the use of a limb, speech, swallowing ability, comprehension, etc. Generally, recovery is often incomplete if at all, as there may be infarction or death of the affected brain tissue.

Sudden death Usually implies sudden unexpected cardiac death, often resulting from undiagnosed heart disease, possibly related to myocardial ischemia/infarction, a cardiomyopathy, or a malignant arrhythmia.

Transient ischemic attack Sometimes called a "mini-stroke," where the blood supply to the brain is interrupted for a short period of time, with generally recovery of full function.

References

Arora, S., Goyal, H., Aggarwal, P., & Kukar, A. (2012). ST segment elevation myocardial infarction in a 37 year old man with normal coronaries—it is not always cocaine. *American Journal of Emergency Medicine*, *30e3*, 2091.e3–5.

Aryana, A., & Williams, M. A. (2007). Marijuana as a trigger of cardiovascular events: speculation or scientific certainty? *International Journal of Cardiology*, *118*, 141–144.

Bachs, L., & Morland, H. (2001). Acute cardiovascular fatalities following cannabis use. *Forensic Science International*, *124*, 200–203.

Benowitz, N. L., Rosenberg, J., Rogers, W., Bachman, J., & Jones, R. T. (1979). Cardiovascular effects of intravenous Δ^9-tetrahydrocannabinol: autonomic nervous mechanisms. *Clinical Pharmacology and Therapeutics*, *25*, 440–446.

Caldicott, D. G., Holmes, J., Roberts-Thompson, K. C., & Maher, L. (2005). Keep off the grass: marijuana use and acute cardiovascular events. *European Journal of Emergency Medicine*, *12*, 236–244.

Daisley, H., Jones-Le Cointe, A., Hutchinson, G., & Simmons, V. (1998). Fatal cardiac toxicity temporally related to poly-drug abuse. *Veterinary and Human Toxicology*, *40*, 21–22.

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., Mechoulam, R., et al. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*, *228*, 1946–1949.

Frost, L., Mostofsky, E., Rosenbloom, J. I., Mukamal, K. J., & Mittleman, M. A. (2013). Marijuana use and long-term mortality among survivors of acute myocardial infarction. *American Heart Journal*, *165*, 170–175.

Gaziano, J. M. (2008). Marijuana use among those at risk for cardiovascular events. *American Heart Journal*, *155*, 395–396.

Geller, T., Loftus, L., & Brink, D. S. (2004). Cerebellar infarction in adolescent males associated with acute marijuana use. *Pediatrics*, *113*, e365–e370.

Grotenhermen, F. (2010). Cannabis-associated arteritis. *Vasa*, *39*, 43–53.

Hollister, L. E. (1986). Health aspects of cannabis. *Pharmacological Reviews*, *38*, 1–20.

Jones, R. T. (2002). Cardiovascular system effects of marijuana. *Journal of Clinical Pharmacology*, *42*(11 Suppl), 58S–63S.

Karabulut, A., & Cakmak, M. (2010). ST segment elevation myocardial infarction due to slow coronary flow occurring after cannabis consumption. *Kardiologia Polska*, *68*, 1266–1268.

Kaushik, M., Alla, V. M., Madan, R., Arouni, A. J., & Mohiuddin, S. M. (2011). Recurrent stress cardiomyopathy with variable regional involvement: insights into etiopathogenetic mechanisms. *Circulation*, *124*, e556–e557.

Kunos, G., Jarai, Z., Batkai, S., Goparaju, S. K., Ishac, E. J. N., Liu, J., Wang, L., & Wagner, J. A. (2000). Endocannabinoids as cardiovascular modulators. *Chemistry, Physics, Lipids*, *108*, 159–168.

Lindsay, A. C., Foeli, R. A., Warren, O., & Henry, J. A. (2005). Cannabis as a precipitant of cardiovascular emergencies. *International Journal of Cardiology*, *104*, 230–232.

Martin-Blondel, G., Koskas, F., Cacoub, P., & Sene, D. (2011). Is thromboangitis obliterans presentation influenced by cannabis addiction? *Annals of Vascular Surgery*, *25*, 469–473.

Menahem, S. (2013). Cardiac asystole following cannabis (marijuana) usage—additional mechanism for sudden death. *Forensic Science International*, *233*, 1–3.

Metsuda, L. A. (1997). Molecular aspects of cannabinoid receptors. *Reviews in Neurobiology*, *11*, 143–166.

Mittleman, M. A., Lewis, R. A., Maclure, M., Sherwood, J. B., & Muller, J. E. (2001). Triggering myocardial infarction by marijuana. *Circulation*, *103*, 2805–2809.

Montisci, M., Thiene, G., Ferrara, S. D., & Basso, C. (2008). Cannabis and cocaine: a lethal cocktail triggering coronary sudden death. *Cardiovascular Pathology*, *17*, 344–346.

Mouzak, A., Agathos, P., Kerezoudi, E., Mantas, A., & Vourdeli-Yiannakoura, E. (2000). Transient ischemic attack in heavy cannabis smokers—how "safe" is it? *European Neurology*, *44*, 42–44.

Mukamal, K. J., Maclure, M., Muller, J. E., & Mittleman, M. A. (2008). An exploratory prospective study of marijuana use and mortality following acute myocardial infarction. *American Heart Journal*, *155*, 465–470.

Noel, B., Ruf, I., & Panizzon, R. G. (2008). Cannabis arteritis. *Journal of the American Academy of Dermatology*, *58*, s65–s67.

Phillips, M. C., Leyden, J. M., Chong, W. K., Kleinig, T., Czepran, P., Lee, A., Kobler, S. A., & Jannes, J. (2011). Ischaemic stroke among young people ages 15 to 50 years in Adelaide South Australia. *Medical Journal of Australia*, *195*, 610–614.

Rezkalla, S. H., Sharma, P., & Kloner, R. A. (2003). Coronary no-flow and ventricular tachycardia associated with habitual marijuana use. *Annals of Emergency Medicine*, *42*, 365–369.

Saffan, A. M., Markham, R., & Jayasinghe, R. (2012). Marijuana induced recurrent acute coronary syndrome with normal coronary angiogram. *Drug and Alcohol Reviews*, *31*, 91–94.

Singh, G. K. (2000). Atrial fibrillation associated with marijuana usage. *Pediatric Cardiology*, *21*, 284.

Singh, N. N., Pan, Y., Muengtaweeponsa, S., Geller, T. J., & Cruz-Flores, S. (2012). Cannabis-related stroke: case series in review of the literature. *Journal of Stroke and Cerebrovascular Diseases*, *21*, 555–560.

Substance Abuse and Mental Health Services Administration. (1999). *National Household Survey and Drug Use* (998). Rockville, MD: SAMHSA, Office of Applied Sciences, p99–3328. DHHS No. (1999) (SMA).

Suttout, A. H., & Nicole, M. F. (2009). Cardiac arrest following cannabis usage: a case report. *Case Journal*, *2*, 208.

Sydney, S. (2002). Cardiovascular consequences of marijuana usage. *Journal of Clinical Pharmacology*, *42*(11 Suppl.), 64S–70S.

Thomas, G., Kloner, R. A., & Rezkalla, S. (2014). Adverse cardiovascular, cerebrovascular, and peripheral vascular effects of marijuana inhalation: what cardiologists need to know. *American Journal of Cardiology*, *113*, 117–190.

Ting, J. Y. (2007). Reversible cardiomyopathy associated with acute inhaled marijuana use in a young adult. *Clinical Toxicology (Philadelphia)*, *45*, 432–434.

Tormey, W. P. (2012). Royal College of Pathologists' autopsy guidelines on sudden cardiac death: role for cannabis, cotinine, NSAIDs and psychology. *Journal of Clinical Pathology*, *65*, 187–188.

Wolff, V., Lauer, V., Rouyer, O., Sellal, F., Meyer, N., Raul, J. S., Marescaux, C., et al. (2011). Cannabis use, ischaemic stroke, and multifocal intracranial vasoconstriction: a prospective study in 48 consecutive young patients. *Stroke*, *42*, 1778–1780.

Zachariah, S. B. (1991). Stroke after heavy marijuana smoking. *Stroke*, *22*, 406–409.

CHAPTER

51

Cannabis and Stroke

P.A. Barber

Department of Medicine, Centre for Brain Research, University of Auckland, Auckland, New Zealand

SUMMARY POINTS

- There have been multiple case reports, case series, and population-based studies that have shown an association between cannabis use and ischemic stroke and transient ischemic attack (TIA).
- People with stroke who have used cannabis:
  - are more likely to be younger men,
  - have recently increased cannabis intake,
  - have stroke symptoms that usually come on during or soon after cannabis use,
  - have strokes that are more likely to be in the posterior circulation.
- A causal association between cannabis and ischemic stroke and TIA is plausible for a number of reasons.
  - Cannabis is associated with myocardial infarction, atrial fibrillation and accelerated atherosclerosis, all major risk factors for ischemic stroke.
  - Cannabis may lead to a reversible cerebral arteriopathy, with segmental and multifocal narrowing of arteries, which may, in turn, lead to cerebral ischemia and infarction.
- It is important that future research clarifies whether there is a causal association, as the numbers of people presenting with ischemic stroke and TIA, after cannabis use, is increasing, and will likely continue to do so.
- Physicians should consider screening younger stroke and TIA patients for cannabis, particularly when there are no other risk factors, so that they are able to make informed decisions on future use.

KEY FACTS

- Cannabis is used by 11–27% of younger ischemic stroke and transient ischemic attack (TIA) patients.
- There is unlikely to be an association between intracerebral hemorrhage and cannabis.
- No study has shown a causal association between cannabis use and stroke, independent of tobacco use.
- The numbers of people presenting with stroke who have used cannabis is increasing, and will likely continue to do so.

LIST OF ABBREVIATIONS

ICH	Intracerebral hemorrhage
MIS	Multifocal intracranial stenosis
OR	Odds ratio
RCVS	Reversible cerebral vasoconstriction syndrome
SSRI	Selective serotonin reuptake inhibitors
TIA	Transient ischemic attack

THE ASSOCIATION BETWEEN CANNABIS USE AND STROKE

It is likely that there is an association between cannabis and ischemic stroke and transient ischemic attack (TIA). There are multiple reports of mainly younger men, with no vascular risk factors other than tobacco and alcohol use, with symptom onset during or soon after cannabis use. This often follows a marked increase in cannabis intake. The strokes are mainly in the posterior circulation, possibly reflecting a greater susceptibility to cannabis-induced autonomic changes. Population-based

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00059-4
Copyright © 2017 Elsevier Inc. All rights reserved.

studies and retrospective audits have also found an association between ischemic stroke and TIA and cannabis. The numbers of patients using cannabis and presenting with stroke has been increasing in recent years, and moves toward legalization for medical and recreational reasons will further increase these numbers.

However, a causal association between cannabis and ischemic stroke has not been proven. One of the major confounding factors is tobacco, which is often smoked with cannabis. A causal link between cannabis and stroke is plausible, and a number of lines of evidence point to such a link. These include the temporal association between cannabis use and stroke, and recurrent stroke with cannabis reexposure. Cannabis is associated with myocardial infarction and atrial fibrillation. Myocardial infarction and atrial fibrillation both increase the risk of cardiac embolism, which in turn accounts for 20–25% of ischemic strokes. There is also a reversible cerebral cannabis arteriopathy with vasospasm, seen with reversible vasoconstriction syndrome (RCVS), and (possibly) a more prolonged stenosis of cerebral arteries that has been called multifocal arterial stenosis (MIS). In this chapter, the evidence linking stroke and cannabis, and the lines of evidence pointing to a causal link, will be summarized (Table 51.1).

Cannabis Ischemic Stroke and Transient Ischemic Attack

The first possible report of ischemic stroke and TIA associated with cannabis use appeared in 1964. This was the case of an 18-year-old male who developed "giddiness" and conjugate gaze deviation to the right, after ingesting cannabis (Mohan & Sood, 1964). The patient recovered over the next 6 weeks. There were no imaging studies available, and so a diagnosis of stroke wasn't confirmed. There have been approximately 100 patients reported as single cases, or in small case series since that time (Garrett, Braithwaite, & Teale, 1977; Zachariah, 1991; Cooles & Michaud, 1987; Barnes, Palace, & O'Brien, 1992; Lawson & Rees, 1996; McCarron & Thomas, 1997; Mouzak, Agathos, Kerezoudi, Mantas, & Vourdeli-Yiannakoura, 2000; White, Martin, Geller, & Pittman, 2000; Mittleman, Lewis, Maclure, Sherwood, & Muller, 2001; Mesec, Rot, & Grad, 2001; Marinella, 2001; Mateo, Pinedo, Gomez-Beldarrain, Basterretxea, & Garcia-Monco, 2005; Finsterer, Christian, & Wolfgang, 2004; Bal, Khurana, Lal, & Prabhakar, 2009; Singh, Pan, Muengtaweeponsa, Geller, & Cruz-Flores, 2012; Wolff et al., 2011; Barber et al., 2013)

There have been three larger case series. In a retrospective study, 17 of 831 patients with ischemic stroke were reported with positive urine screens for cannabis (Singh et al., 2012). It is not clear how patients were selected for cannabis screening. Thirteen of 17 (76%) were men, and the mean age was 41 (range 15–63) years; 10 (58%) of the patients had stroke in the posterior circulation. Five had a history of alcohol use, but no alcohol was detected in blood samples, four had a history of hypertension, three had prior stroke, three used tobacco, and two used cocaine. In a prospective study, 13 of 48 (27%) consecutive ischemic stroke patients aged younger than 45 years had positive urine cannabis screens (Wolff et al., 2011). All of the patients smoked tobacco. Seven had strokes in the posterior circulation, and 10 (77%) had multifocal intracranial stenosis, and all of whom had "binged" on cannabis in the days prior to the stroke.

In a prospective case-control study, 25 out of 160 (16%) consecutive ischemic stroke (150) and TIA (10) patients aged 18–55 years had positive cannabis urine screens (Barber et al., 2013). These patients were more likely to be male, and tobacco smokers. Stroke patients were compared with 160 age, sex, and ethnicity matched control participants admitted to hospital with noncardiovascular diagnoses. Positive urine cannabis tests were obtained in 25 (16%) of the patients, and 13 (8%) control participants. In a logistic regression analysis adjusted for age, sex, and ethnicity, cannabis use was associated with increased risk of ischemic stroke and TIA (OR 2.30, 95% CI 1.08–5.08).

An association between cannabis and stroke has also been reported in population-based studies. A state-wide study of stroke hospital admissions in 18–44 year olds in Texas found an increased risk of ischemic stroke in those with a history of cannabis abuse or dependence (OR 1.76, 95% CI 1.15–2.71) (Westover, Mcbride, & Haley, 2007). An Australian study reported cannabis use in 36 out of 326 (11%) first ever ischemic stroke patients aged 15–50 years (Phillips et al., 2011). A retrospective

TABLE 51.1 Cannabis and Stroke: Summary

What we know
Multiple studies have found an association between cannabis and ischemic stroke
11–27% of younger ischemic stroke patients have recently used cannabis
Cannabis is not associated with intracerebral hemorrhage
What we don't know
Whether cannabis causes stroke, or if the association is a chance or bystander effect?
Why we need to know this
Significant numbers of younger ischemic stroke patients have recently used cannabis
These numbers are increasing, and will continue to do so
We need to know what to advise stroke patients who use cannabis

TABLE 51.2 Characteristics of Ischemic Stroke and TIA Patients Who Use Cannabis

Patients are more likely
Younger (mean age in the mid-30s)
Male (63–86%)
To have posterior circulation strokes (50–58%)
To have no vascular risk factors, apart from tobacco, alcohol and/or illicit drug use
Cannabis use more likely to be
Heavy (often more than once per week)
Increased recently
Used with tobacco, alcohol, and occasionally other illicit drugs

population-based study in the Greater Cincinnati and Northern Kentucky Study region reported cannabis use in 11% of 501 stroke patients aged 18–54 years (de Los Rios et al., 2012). This study also found increasing numbers of younger stroke patients using cannabis, in recent years, with cannabis use increasing from 0.6% in 1993, to 6% in 1999, and to 11% in 2005.

There are a number of similarities between people presenting with ischemic stroke and TIA, and who use cannabis (Table 51.2). They tend to be younger, with a mean age in the mid-30s, although some of the studies limited screening to younger patients only (Desbois & Cacoub, 2013). Most (63–86%) patients are male, possibly reflecting greater cannabis use in men. Ethnic differences have also been found (Barber et al., 2013). Patients are usually heavy users, with up to 86% reporting cannabis use more than once per week (Desbois & Cacoub, 2013). Stroke often occurred after a sudden increase in cannabis intake (Mouzak et al., 2000; Mateo et al., 2005; Renard, Taieb, Gras-Combe, & Labauge, 2012). Most patients also used tobacco and alcohol at the same time, with smaller numbers of patients using other illicit drugs. Cerebral infarction involved the vertebrobasilar arterial territories in 50–58% of patients, possibly due to less robust autoregulatory mechanisms in the posterior circulation (Desbois & Cacoub, 2013; Wolff et al., 2011, 2013). In a case-control study, no differences in age, stroke mechanism, or most vascular risk factors (apart from sex, tobacco use, and ethnicity) could be found between those with and without positive cannabis tests (Barber et al., 2013).

Cannabis and Intracerebral Hemorrhage

There is unlikely to be an association between cannabis and intracerebral hemorrhage (ICH). In a Texas-wide study of hospital admissions of 18–44-year-olds, no association was found between cannabis abuse or dependence and ICH (OR 1.36, 95% CI 0.90–2.06) (Westover et al., 2007). There is only a single case report in the literature linking cannabis use with ICH (Wolff et al., 2011). In the author's unit, only one of 25 (4%) consecutive ICH patients had a positive urine cannabis screen, compared with 13 out of 160 (8%) control patients presenting at the same time to the Internal Medicine service with nonvascular diagnoses. We have subsequently admitted a 48-year-old man with reversible cerebral vasoconstriction syndrome (RCVS) and ICH, who had smoked cannabis laced with amphetamine.

Not All Studies Have Found an Association Between Cannabis and Stroke

In a large population-based study in the Baltimore and Washington area of the United States, 783 younger adults with stroke, and 794 controls, were interviewed about cannabis use (Dutta, Ryan, Cheng, Kittner, & Cole, 2014). There were no differences between the cases and controls, after adjusting for age, sex, and tobacco use, at any time point. However, there was a nonsignificant trend to an association with stroke among frequent, but not infrequent, cannabis users (defined as cannabis use more or less than 1.88 times per month). The authors concluded that additional studies are warranted, examining the effect of heavy or frequent cannabis use. The methodology of this study is not clear, as it has only been reported in abstract form. But there is a potential for bias in studies that rely on self-report, as patients may not volunteer using what is still, in most jurisdictions, an illegal substance.

No study has found an association between stroke and cannabis that is independent of tobacco (Barber et al., 2013; Dutta et al., 2014). In a case-control study, all but three stroke patients with positive cannabis screens used tobacco (which is often mixed and smoked with cannabis), and an association independent of tobacco was unable to be confirmed (OR 1.59, 95% CI 0.71–3.70). However, cannabis does have cardiovascular effects over and above those of tobacco and nicotine, so that an association independent of tobacco is plausible. In people with established coronary artery disease, the time to angina with exercise was reduced by 50% after smoking cannabis, compared with an 8% reduction after smoking a placebo cannabis cigarette (Aronow & Cassidy, 1974), and 23% after smoking tobacco (Aronow, Kaplan, & Jacob, 1968).

One of the arguments against an association is the relative paucity of reports of patients with stroke who use cannabis, despite the widespread use of drug around the world (Thanvi & Treadwell, 2009). Surely the numbers of patients reported would be greater if a causal association existed? This is a reasonable argument, but it is not clear if clinicians routinely ask stroke patients about

cannabis use. Asking patients about, or even screening for, cannabis would likely identify more cannabis associated strokes (Barber, Roberts, Spriggs, & Anderson, 2014). Stroke patients weren't asked about cannabis use in the author's unit until the late 2000s. Once this became part of the routine assessment of patients in whom no clear cause of stroke could be identified, it became clear that one in six had recently used cannabis (Barber et al., 2013). Patients with positive screens are now routinely counselled about the potential risk of cannabis reexposure, so that they can make an informed choice about further use.

A CAUSAL LINK BETWEEN CANNABIS AND STROKE IS PLAUSIBLE

There are a number of lines of evidence that, while not proof, favor a causal association between cannabis and stroke (Table 51.3). This evidence includes the strong temporal association between cannabis and stroke onset, and recurrent stroke with cannabis reexposure, following periods of abstinence. There is an association between cannabis and heart disease, which is one of the major risk factors for stroke. A reversible cerebral cannabis arteriopathy, with RCVS and multifocal intracranial stenosis (MIS) may lead to hypoperfusion and brain ischemia.

TABLE 51.3 Evidence Suggesting a Causal Link Between Cannabis and Ischemic Stroke/TIA

Case-control study evidence
Double the rate of positive cannabis screens in stroke patients compared with controls
Temporal link between cannabis use and stroke or TIA onset
Stroke/TIA onset during or shortly after cannabis use
Stroke/TIA onset following recent increase in cannabis use
Cannabis reexposure leading to recurrent stroke/TIA
Increased risk of cardiac embolism and stroke: cannabis leads to
Shorter time to angina
Increased risk of myocardial infarction
Increased risk of atrial fibrillation
Arterial disease and vasospasm: cannabis leads to
Accelerated atherosclerosis
Reversible cerebral vasoconstriction syndrome
Multifocal intracranial stenosis
Cerebral vasculitis?[a]

[a] *To date, there is no pathological evidence that cannabis leads to cerebral vasculitis.*

Temporal Association Between Stroke Onset and Cannabis Use

A causal relationship between cannabis and stroke is strongly suggested by the close temporal link with stroke onset (Finsterer et al., 2004; Singh et al., 2012; Wolff et al., 2011). Stroke occurred while smoking cannabis in 13 of 17 (76%) patients, and during or within 30 min of last exposure in 16 (94%) patients (Singh et al., 2012). There are also multiple reports of recurrent strokes and TIAs following reexposure to cannabis. A 22-year-old man presenting with ischemic stroke had symptoms consistent with TIA while smoking cannabis 2 months, and again 4 days, prior to presenting with an ischemic stroke (Lawson & Rees, 1996). A 36-year-old man with sporadic cannabis use and no other vascular risk factors developed bilateral cerebral infarction after unusually heavy cannabis use (Mateo et al., 2005). He presented 1 year later with bilateral cerebral infarction immediately after using cannabis following a period of abstinence. He presented for a third time with an ischemic stroke, following heavy cannabis use after a further 1 ½ years of abstinence. In another study, 5 of 17 (29%) patients experienced recurrent stroke during reexposure to cannabis (Singh et al., 2012). In the study by Wolff et al. (2011), one of 10 patients with stroke and MIS did not stop using cannabis, and had a recurrent ischemic stroke.

Cannabis, Heart Disease, and Stroke

Cardiac embolism causes 20–25% of ischemic strokes. Thrombi develop in the heart as a complication of myocardial infarction or atrial fibrillation, and then embolize to the brain. Anything that increases the risk of myocardial infarction or atrial fibrillation can therefore lead to ischemic stroke and TIA.

In a case-crossover design study, the risk of myocardial infarction was elevated almost 5 times over baseline in the 60 min after cannabis use, and then rapidly tailed off with time (Mittleman et al., 2001). Bilateral cerebral infarction was reported in a 33-year-old man who had a myocardial infarction following a significant increase in his longstanding cannabis use (Renard et al., 2012).

Cannabis has also been associated with paroxysmal atrial fibrillation (Singh, 2000; Kosior, Filipiak, Stolarz, & Opolski, 2001; Fisher, Ghuran, Vadamalai, & Antonios, 2005; Charbonney, Sztajzel, Poletti, & Rutschmann, 2005; Korantzopoulos, Liu, Papaioannides, Li, & Goudevenos, 2008). The patients are younger, and the atrial fibrillation tends to be of short duration, and comes on during or shortly after cannabis use. In one review, none of six atrial fibrillation patients who had used cannabis had structural heart disease

(Korantzopoulos et al., 2008). In one of the more interesting cases, a 32-year-old medical doctor performed a provocative test by smoking cannabis during Holter monitoring, and developed paroxysmal tachycardia and atrial fibrillation lasting for 2 min (Kosior et al., 2001). He remained in sinus rhythm with cannabis abstinence over the next 12 months.

A detailed discussion of how cannabis may lead to heart disease is beyond the scope of this chapter. However, in brief, cannabis has a number of cardiovascular effects, including a dose-dependent increase in heart rate, supine hypertension, postural hypotension, and an increase in cardiac output (Jones, 2002). This is mediated through changes in autonomic tone with increases in sympathetic, and decreases in parasympathetic, activity. This increase in cardiac work, coupled with increased carboxyhemoglobin concentration and reduced blood oxygen carrying capacity, may lead to an imbalance between myocardial oxygen demand and supply (Aronow & Cassidy, 1974).

Chronic cannabis use is associated with increased cerebrovascular resistance through changes mediated through blood vessels or the brain parenchyma (Herning, Better, Tate, & Cadet, 2005). Cannabis increases apolipoprotein C levels and triglycerides, and may lead to atherosclerosis by recruiting monocytes to the vascular endothelium. This has led one group to suggest that the vasculature of 18–30-year-old cannabis users is "comparable to that of 60 year olds" (Herning, Better, Tate, & Cadet, 2001).

REVERSIBLE CEREBRAL CANNABIS ARTERIOPATHY

Vasospasm induced by cannabis may lead to ischemic stroke (Zachariah, 1991; Mesec et al., 2001; Ducros et al., 2007). In 1991, Zachariah reported two men in their 30s who developed cerebral infarction during or within 30 min of smoking cannabis (Zachariah, 1991). They had no vascular risk factors apart from tobacco use, but both patients had elevated blood pressure at presentation. The author postulated that a marked, cannabis-induced increase in blood pressure resulted in cerebral vasospasm and stroke. This couldn't be confirmed, as both patients declined angiography. In a later study of 62 RCVS patients, 20 (30%) had used cannabis, and many had also used other vasoactive substances such as selective serotonin reuptake inhibitors (SSRIs), and nasal decongestants (Ducros et al., 2007).

Patients with imaging-proven RCVS and stroke following cannabis use have been reported. A 46-year-old man was reported with recurrent thunderclap headaches and multifocal cerebral infarction, after cannabis use (Calabrese, Dodick, Schwedt, & Singhal, 2007). Vascular imaging showed multifocal segmental stenoses of the middle cerebral, posterior cerebral, basilar, and superior cerebellar arteries that resolved on follow-up imaging. One of 25 (4%) cannabis positive stroke patients in another study had clear clinical and radiological evidence of RCVS (Barber et al., 2013). This was a 54-year-old woman admitted with recurrent thunderclap headaches, and right sided weakness (Fig. 51.1). She used cannabis daily, and was treated for depression with the SSRI fluoxetine. She had left anterior circulation territory infarction and vasoconstriction in the branches of both middle cerebral arteries. She continued to use cannabis, and presented again with further thunderclap headache, and new left-sided sensory symptoms. Repeat imaging showed bilateral cerebral infarction, and more extensive cerebral vasoconstriction. At this point, it was realized that she was going outside the hospital in the evenings to smoke cannabis. The SSRI and cannabis were stopped, the headaches resolved, and repeat vascular imaging showed resolution of the cerebral vasoconstriction. Patients such as these confirm the temporal relationship between cannabis use, cerebral vasoconstriction, and stroke, with a cessation of clinical syndrome and resolution of vasospasm with abstinence (Calabrese et al., 2007). However, only a minority of cannabis-associated stroke patients have the full spectrum of clinical and imaging features of RCVS.

MIS has been reported in 10 out of 13 (77%) patients presenting with ischemic stroke and with positive cannabis screens, compared to only one of 35 (3%) patients with negative cannabis screens (Wolff et al., 2011). The stenoses preferentially involved arteries in the posterior cerebral circulation. Six patients stopped cannabis, with no evidence of relapse over the next 3–6 months, and full or partial reversal of the MIS on repeat vascular imaging. Three patients continued to smoke cannabis, and the MIS persisted in all three, and one had a recurrent ischemic stroke. The authors suggested that the persistence of arterial stenoses with ongoing cannabis exposure argued against vasospasm, although they still thought MIS could be considered a variant of RCVS (Wolff et al., 2013). This work needs to be replicated. In the meantime, the term reversible cerebral cannabis arteriopathy may be a better term to use until the exact nature of the cannabis-associated vascular changes have been clarified.

Cerebral Vasculitis and Cannabis-Associated Stroke

Cerebral vasculitis has also been postulated to cause stroke, following cannabis use (Thanvi & Treadwell, 2009). Cannabis can lead to a severe peripheral arteritis similar to thromboangiitis obliterans, in young male cannabis users (Disdier et al., 2001; Peyrot et al., 2007). A toxic or

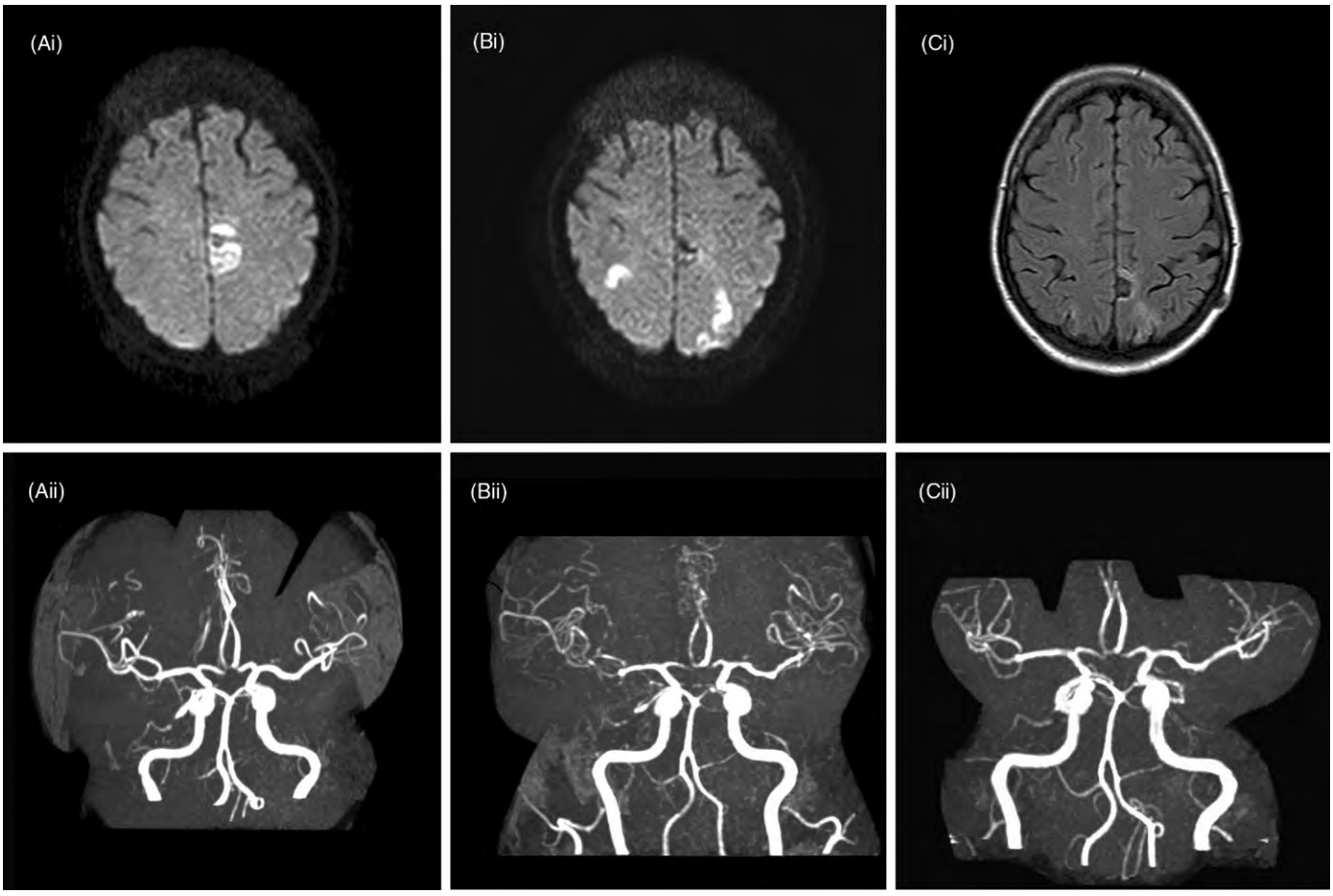


FIGURE 51.1 MRI diffusion-weighted imaging (DWI) (Ai and Bi), and FLAIR imaging (Ci) and angiography (MRA) (Aii, Bii, and Cii), in a 54-year-old woman presenting with recurrent thunderclap headaches and right-sided weakness, and a clinical and imaging course consistent with reversible cerebral vasoconstriction syndrome. The patient used cannabis daily, and was treated for depression with the SSRI antidepressant medication, fluoxetine.

- At presentation (A), the DWI shows left anterior circulation territory infarction and vasoconstriction in the branches of right middle cerebral artery (MCA).
- Ten days after admission (B), she had a further thunderclap headache, and developed new left-sided sensory symptoms. Repeat imaging showed bilateral cerebral infarction on the DWI, and more extensive segmental and multifocal stenosis of the branches of both MCAs. It became clear that the patient was continuing to use cannabis. The SSRI and cannabis were stopped, and the headaches resolved.
- Six weeks after admission (C), repeat imaging showed bilateral cerebral infarction on FLAIR imaging, and resolution of the cerebral vasoconstriction.

immune inflammatory cannabis induced vasculopathy has been suggested in the report of a 36-year-old man presenting with bilateral ischemic strokes, and reduced caliber of the distal temporal branches of the left MCA, following an unusually heavy intake of cannabis (Mateo et al., 2005). These vascular changes were still present when the patient presented 1 year later, with further cerebral infarction. Wolff et al. (2013) suggested that the long duration of stenoses seen in patients with MIS was in favor of a drug-induced immunoallergic vasculitis (Wolff et al., 2013). However, there has never been any pathological evidence of cerebral vasculitis, with no infectious or noninfectious vasculitis seen in autopsies (two patients) or biopsy (one patient) of three adolescent men with cerebellar infarction, following cannabis use (Geller, Loftis, & Brink, 2004).

CONCLUSIONS

Cannabis is used by 11–27% of younger stroke patients. In recent years, the numbers of people with stroke using cannabis has been increasing, reflecting increased use in the wider community. Cannabis has been legalized for medicinal use in many US states, and the District of Columbia, and at the time of writing, can be bought legally for recreational use in Colorado and Washington State, Uruguay, and The

TABLE 51.4 Future Research Questions

Is there an association between cannabis use and stroke/TIA independent of tobacco?
Association independent of tobacco not shown to date
Confounded by the fact that most people who use cannabis also use tobacco
Are the reports of increasing cannabis use in stroke patients due to
A true increase in cannabis use?
Greater willingness of patients to volunteer cannabis use?
Greater willingness of clinicians to ask about cannabis use?
Does counselling stroke/TIA patients who have used cannabis reduce recurrent stroke?
Does cannabis lead to cerebral vasculitis?
Studies with biopsies required
Is multifocal intracranial stenosis simply a form of RCVS?
More studies with serial, high quality vascular imaging required

Netherlands. Use will further increase, as pressure to legalize cannabis elsewhere has an effect.

A causal link between cannabis and ischemic stroke, independent of tobacco, has not been proven, but is plausible. The high and increasing rates of cannabis use in people presenting with stroke makes it imperative that research is carried out to determine whether or not there is a link independent of other vascular risk factors (Table 51.4). This will require documentation of the timing, amount, and mode of cannabis use. Urine or blood should be tested, as questioning people is likely to obtain unreliable responses, and is prone to bias. Study design will be complicated by the fact cannabis is still, in most jurisdictions, illegal.

In the meantime, it is important for physicians caring for people with vascular disease to be aware of this potential association. Consideration should be given to screening younger stroke and TIA patients for cannabis, particularly where there are no other vascular risk factors found. Patients with positive tests could then be counseled about the association between cannabis and stroke, so that they are able to make informed decisions on future use.

MINI-DICTIONARY

Cardiac embolism Thrombi (blood clots) that form in the heart in people with atrial fibrillation, or after myocardial infarction (heart attack) that break off into the systemic circulation. These thrombi may block arteries in the brain or body, leading to ischemia and infarction of the tissue downstream to the blockage.
Infarction Death of tissue in the brain (cerebral) or heart muscle (myocardial) due to ischemia.
Intracerebral hemorrhage (ICH) Sudden onset of focal neurological deficit due to hemorrhage into brain parenchyma. In Caucasian populations, ICH accounts for 10–15% of all strokes.
Ischemia Reduced blood flow to the brain (cerebral ischemia), heart (coronary ischemia), or limbs (peripheral vascular ischemia).
Ischemic stroke Sudden onset of focal neurological deficit due to loss of blood supply to part of the brain, with evidence of cerebral infarction on brain imaging. In Caucasian populations, ischemic stroke accounts for 80–85% of all strokes.
Multifocal intracranial stenosis (MIS) Multifocal stenosis (narrowing) of intracranial arteries that may persist with continued cannabis exposure.
Reversible cerebral cannabis arteriopathy A term to describe the segmental and multifocal stenosis of arteries in patients presenting with stroke after using cannabis, which may be due to RCVS or MIS.
Reversible cerebral vasoconstriction syndrome (RCVS) A group of disorders where patients present with severe, often "thunderclap onset" headaches, with or without focal neurological deficits or seizures, and with reversible segmental and multifocal vasoconstriction (narrowing or occlusion) of cerebral arteries. Previously known as Call–Fleming syndrome.
Transient ischemic attack (TIA) Stroke symptoms and signs that usually disappear within minutes, with no evidence of infarction on brain imaging. TIAs may precede, and have the same causes, as ischemic stroke.
Vasculitis A disorder that damages arteries (arteritis) and veins by inflammation, leading to stenosis or occlusion of the affected vessel, and resulting in ischemia and infarction. In the brain, this is called cerebral vasculitis.

References

Aronow, W. S., & Cassidy, J. (1974). Effect of marihuana and placebo-marihuana smoking on angina pectoris. *New England Journal of Medicine*, *291*, 65–67.

Aronow, W. S., Kaplan, M. A., & Jacob, D. (1968). Tobacco: a precipitating factor in angina pectoris. *Annals of Internal Medicine*, *69*, 529–536.

Bal, S., Khurana, D., Lal, V., & Prabhakar, S. (2009). Posterior circulation stroke in a cannabis abuser. *Neurology India*, *57*, 91–92.

Barber, P. A., Roberts, S., Spriggs, D. A., & Anderson, N. E. (2014). Adverse cardiovascular, cerebrovascular, and peripheral vascular effects of marijuana: what cardiologists need to know. *American Journal of Cardiology*, *113*, 1086.

Barber, P. A., Pridmore, H. M., Krishnamurthy, V., Roberts, S., Spriggs, D. A., Carter, K. N., & Anderson, N. E. (2013). Cannabis, ischemic stroke, and transient ischemic attack: a case-control study. *Stroke*, *44*, 2327–2329.

Barnes, D., Palace, J., & O'Brien, M. D. (1992). Stroke following marijuana smoking. *Stroke*, *23*, 1381.

Calabrese, L. H., Dodick, D. W., Schwedt, T. J., & Singhal, A. B. (2007). Narrative review: reversible cerebral vasoconstriction syndromes. *Annals of Internal Medicine*, *146*, 34–44.

Charbonney, E., Sztajzel, J. M., Poletti, P. A., & Rutschmann, O. (2005). Paroxysmal atrial fibrillation after recreational marijuana smoking: another "holiday heart"? *Swiss Medical Weekly*, *135*, 412–414.

Cooles, P., & Michaud, R. (1987). Stroke after heavy cannabis smoking. *Postgraduate Medical Journal*, *63*, 511.

de Los Rios, F., Kleindorfer, D. O., Khoury, J., Broderick, J. P., Moomaw, C. J., Adeoye, O., Flaherty, M. L., Khatri, P., Woo, D., Alwell, K., Eilerman, J., Ferioli, S., & Kissela, B. M. (2012). Trends in substance abuse preceding stroke among young adults: a population-based study. *Stroke*, *43*, 3179–3183.

Desbois, A. C., & Cacoub, P. (2013). Cannabis-associated arterial disease. *Annals of Vascular Surgery*, *27*, 996–1005.

Disdier, P., Granel, B., Serratrice, J., Constans, J., Michon-Pasturel, U., Hachulla, E., Conri, C., Devulder, B., Swiader, L., Piquet, P., Branchereau, A., Jouglard, J., Moulin, G., & Weiller, P. J. (2001). Cannabis arteritis revisited—ten new case reports. *Angiology*, *52*, 1–5.

Ducros, A., Boukobza, M., Porcher, R. L., Sarov, M., Valade, D., & Bousser, M. -G. (2007). The clinical and radiological spectrum of reversible cerebral vasoconstriction syndrome. A prospective series of 67 patients. *Brain*, *130*, 3091–3101.

Dutta, T., Ryan, K., Cheng, Y., Kittner, S., & Cole, J. (2014). Marijuana use and the risk of ischemic stroke: The Stroke Prevention in Young Adults Study. *Neurology*, *82* (Suppl. 10), S55.003.

Finsterer, J., Christian, P., & Wolfgang, K. (2004). Occipital stroke shortly after cannabis consumption. *Clinical Neurology and Neurosurgery*, *106*, 305–308.

Fisher, B. A. C., Ghuran, A., Vadamalai, V., & Antonios, T. F. (2005). Cardiovascular complications induced by cannabis smoking: a case report and review of the literature. *Emergency Medicine Journal*, *22*, 679–680.

Garrett, C. P. O., Braithwaite, R. A., & Teale, J. D. (1977). Unusual case of tetrahydrocannabinol intoxication confirmed by radioimmunoassay. *British Medical Journal*, *2*, 166.

Geller, T., Loftis, L., & Brink, D. S. (2004). Cerebellar infarction in adolescent males associated with acute marijuana use. *Pediatrics*, *113*, e365–e370.

Herning, R. I., Better, W. E., Tate, K., & Cadet, J. L. (2001). Marijuana abusers are at increased risk for stroke. Preliminary evidence from cerebrovascular perfusion data. *Annals of the New York Academy of Sciences*, *939*, 413–415.

Herning, R. I., Better, W. E., Tate, K., & Cadet, J. L. (2005). Cerebrovascular perfusion in marijuana users during a month of monitored abstinence. *Neurology*, *64*, 488–493.

Jones, R. T. (2002). Cardiovascular system effects of marijuana. *Journal of Clinical Pharmacology*, *42*, 58S–63S.

Korantzopoulos, P., Liu, T., Papaioannides, D., Li, G., & Goudevenos, J. A. (2008). Atrial fibrillation and marijuana smoking. *International Journal of Clinical Practice*, *62*, 308–313.

Kosior, D. A., Filipiak, K. J., Stolarz, P., & Opolski, G. (2001). Paroxysmal atrial fibrillation following marijuana intoxication: a two-case report of possible association. *International Journal of Cardiology*, *78*, 183–184.

Lawson, T. M., & Rees, A. (1996). Stroke and transient ischaemic attacks in association with substance abuse in a young man. *Postgraduate Medical Journal*, *72*, 692–693.

Marinella, M. A. (2001). Stroke after marijuana smoking in a teenager with factor V Leiden mutation. *Southern Medical Journal*, *94*, 1217–1218.

Mateo, I., Pinedo, A., Gomez-Beldarrain, M., Basterretxea, J. M., & Garcia-Monco, J. C. (2005). Recurrent stroke associated with cannabis use. *Journal of Neurology, Neurosurgery and Psychiatry*, *76*, 435–437.

McCarron, M. O., & Thomas, A. M. (1997). Cannabis and alcohol in stroke. *Postgraduate Medical Journal*, *73*, 448.

Mesec, A., Rot, U., & Grad, A. (2001). Cerebrovascular disease associated with marijuana abuse: a case report. *Cerebrovascular Diseases*, *11*, 284–285.

Mittleman, M. A., Lewis, R. A., Maclure, M., Sherwood, J. B., & Muller, J. E. (2001). Triggering myocardial infarction by marijuana. *Circulation*, *103*, 2805–2809.

Mohan, H., & Sood, G. C. (1964). Conjugate deviation of the eyes after *Cannabis indica* intoxication. *British Journal of Ophthalmology*, *48*, 160–161.

Mouzak, A., Agathos, P., Kerezoudi, E., Mantas, A., & Vourdeli-Yiannakoura, E. (2000). Transient ischemic attack in heavy cannabis smokers—how 'safe' is it? *European Neurology*, *44*, 42–44.

Peyrot, I., Garsaud, A. M., Saint-Cyr, I., Quitman, O., Sanchez, B., & Quist, D. (2007). Cannabis arteritis: a new case report and a review of literature. *Journal of the European Academy of Dermatology and Venereology*, *21*, 388–391.

Phillips, M. C., Leyden, J. M., Chong, W. K., Kleinig, T., Czapran, P., Lee, A., Koblar, S. A., & Jannes, J. (2011). Ischaemic stroke among young people aged 15 to 50 years in Adelaide South Australia. *Medical Journal of Australia*, *195*, 610–614.

Renard, D., Taieb, G., Gras-Combe, G., & Labauge, P. (2012). Cannabis-related myocardial infarction and cardioembolic stroke. *Journal of Stroke and Cerebrovascular Diseases*, *21*, 82–83.

Singh, G. K. (2000). Atrial fibrillation associated with marijuana use. *Pediatric Cardiology*, *21*, 284.

Singh, N. N., Pan, Y., Muengtaweeponsa, S., Geller, T. J., & Cruz-Flores, S. (2012). Cannabis-related stroke: case series and review of literature. *Journal of Stroke and Cerebrovascular Diseases*, *21*, 555–560.

Thanvi, B. R., & Treadwell, S. D. (2009). Cannabis and stroke: is there a link? *Postgraduate Medical Journal*, *85*, 80–83.

Westover, A. N., Mcbride, S., & Haley, R. W. (2007). Stroke in young adults who abuse amphetamines or cocaine: a population-based study of hospitalized patients. *Archives of General Psychiatry*, *64*, 495–502.

White, D., Martin, D., Geller, T., & Pittman, T. (2000). Stroke associated with marijuana abuse. *Pediatric Neurosurgery*, *32*, 92–94.

Wolff, V., Armspach, J. P., Lauer, V., Rouyer, O., Bataillard, M., Marescaux, C, & Geny, B. (2013). Cannabis-related stroke: myth or reality? *Stroke*, *44*, 558–563.

Wolff, V., Lauer, V., Rouyer, O., Sellal, F., Meyer, N., Raul, J. S., Sabourdy, C., Boujan, F., Jahn, C., Beaujeux, R., & Marescaux, C. (2011). Cannabis use, ischemic stroke, and multifocal intracranial vasoconstriction: a prospective study in 48 consecutive young patients. *Stroke*, *42*, 1778-L1780.

Zachariah, S. B. (1991). Stroke after heavy marijuana smoking. *Stroke*, *22*, 406–409.

CHAPTER

52

Cannabis Smoking and the Lung

D.P. Tashkin

Division of Pulmonary and Critical Care, Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, United States

SUMMARY POINTS

- Nearly all systematic studies of the relationship between habitual cannabis smoking and chronic respiratory symptoms and/or lung function have consistently shown that cannabis is significantly associated with an increased prevalence of symptoms of chronic bronchitis (chronic cough and sputum, often with wheezing).
- On the other hand, the association with lung function impairment has been mixed, some studies showing no abnormalities in lung function in users of cannabis without tobacco, compared to the lung function of nonsmokers in either cross-sectional and/or longitudinal analysis, while others have suggested the presence of mild airflow obstruction; an exception is the finding of a modest, clinically insignificant reduction in specific airway conductance (increased resistance to airflow mainly in larger airways) in all three of the studies in which this measurement was made.
- The weight of evidence to date, therefore, does not suggest that regular cannabis use, in the absence of tobacco, is a significant risk factor for the development of chronic obstructive pulmonary disease.
- Habitual smoking of cannabis has been shown to be associated with evidence of microscopic injury to bronchial lining cells, including precancerous histopathologic alterations, and increased expression of molecular markers of pretumor progression.
- Several cases of lung cancer have been reported in relatively young patients with a history of heavy cannabis use, and some case-control studies have reported significantly positive associations with cannabis use, but these have been flawed by the inability to control for the confounding influence of tobacco, or small sample sizes among the heavier users, resulting in imprecise estimates of risk.
- In contrast, a large prospective cohort study and a case-control study that included large numbers of heavy cannabis users among both the cases and controls have failed to show any positive association with cannabis use, even heavy use; these negative findings need to be replicated.
- The immunosuppressive effect of Δ^9-tetrahydrocannabinol (THC), and the cannabis-associated bronchial epithelial injury that results in increased numbers of mucus-secreting cells, and the loss of ciliated bronchial epithelium, are likely to impair the lung's defense against infection.
- Case series and older observational studies suggest a possible association between cannabis use and both fungal and bacterial pneumonia, as well as pulmonary tuberculosis; however, large-scale epidemiologic studies have yet to be performed to test the significance of an association between cannabis and pneumonia.
- Several cases of pneumothorax and/or pneumomediastinum, as well as bullous lung disease, have been reported in mostly heavy users of cannabis, in association with variable lifetime cumulative tobacco smoking, but a cause-and-effect relationship with cannabis has not been established.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00060-0
Copyright © 2017 Elsevier Inc. All rights reserved.

KEY FACTS

- Regular smoking of cannabis (in the absence of tobacco) has been associated with symptoms of chronic bronchitis (cough and sputum) in most observational or community-based studies.
- Regular use of cannabis without tobacco has been variably associated with mild, if any, physiologic evidence of airflow obstruction but without any evidence of an abnormality in gas transfer (diffusing capacity).
- A majority of heavy habitual smokers of cannabis alone have widespread histopathological abnormalities involving the bronchial mucosa, including destruction of ciliated epithelial cells, and increased numbers of mucus-secreting surface epithelial cells, possibly accounting for the prevalent symptoms of chronic bronchitis.
- While cannabis smoke contains the same carcinogens found in tobacco and while some of the histopathological changes in the bronchial mucosa are considered precancerous, most well-controlled case-control studies have failed to find an association with lung cancer after controlling for tobacco.
- Some case series and older epidemiologic studies have suggested as association between cannabis use and fungal, bacterial, or mycobacterial infection, but well-designed epidemiologic studies are lacking.
- A few case series of pneumothorax/pneumomediastinum or bullous lung disease in smokers of cannabis (most often along with tobacco) have been reported, but a causal relationship has not yet been established.

LIST OF ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
CS	Smoker of cannabis only
CTS	Smoker of cannabis plus tobacco
D_LCO	Diffusing capacity of the lung for carbon monoxide
EGRF	Epidermal growth factor receptor
FEV_1	Forced expired volume in 1 second
FRC	Functional residual capacity
FVC	Forced vital capacity
HIV	Human immunodeficiency virus
HRCT	High resolution computed tomography
Ki-67	A cellular marker associated with cell proliferation
NS	Nonsmoker
OR	Odds ratio
PAHs	Polycyclic aromatic hydrocarbons
RV	Residual volume
SG_{aw}	Specific airway conductance
THC	Δ^9-Tetrahydrocannabinol
TLC	Total lung capacity
TS	Smoker of tobacco only

INTRODUCTION

Since the contents of cannabis smoke include an array of noxious and carcinogenic chemicals, similar to that found in tobacco smoke, there is understandable concern that habitual cannabis smoking might injure the lung in the same manner as tobacco smoking, and thereby predispose to the same pulmonary disorders associated with tobacco. The latter include simple chronic bronchitis, chronic obstructive pulmonary disease (chronic obstructive bronchitis and emphysema), lung cancer, and a heightened risk of pneumonia. This review will focus on the evidence concerning the potential associations of regular cannabis smoking, and the occurrence of these tobacco-related disorders, after adjusting for concomitant use of tobacco and other relevant risk factors. In addition, the evidence pertaining to other potential respiratory risks of cannabis use will be reviewed.

ACUTE EFFECTS OF CANNABIS ON AIRWAY DYNAMICS

In contrast to tobacco cigarette smoking, which causes temporary bronchial constriction mediated via a cholinergic reflex in response to irritants in the smoke (Nadel & Comroe, 1961), cannabis smoking produces acute bronchial dilatation that is directly proportional to the concentration of Δ^9-tetrahydrocannabinol (THC) (Tashkin, Shapiro, & Frank, 1973; Vachon, Fitzgerald, Solliday, Gould, & Gaensler, 1973). Smoked cannabis also causes bronchodilation in patients with asthma (Tashkin, Shapiro, & Frank, 1974) and, furthermore, reverses experimentally induced bronchospasm (Tashkin, Shapiro, Lee, & Harper, 1975). The mechanism of THC-induced bronchodilation involves stimulation of prejunctional cannabinoid-type 1 (CB1) receptors on efferent vagal nerves in the airway, resulting in inhibition of release of acetylcholine from these nerve endings, and of cholinergically mediated airway smooth muscle contraction (Calignano et al., 2000; Grassin-Delyle et al., 2014).

CHRONIC EFFECTS OF CANNABIS ON THE LUNG

Effects on Chronic Respiratory Symptoms

Several studies involving smokers and nonsmokers of cannabis and/or tobacco recruited from the general community have examined possible associations of cannabis alone, tobacco alone, cannabis plus tobacco, and

TABLE 52.1 Association of Regular Cannabis Use Alone With Chronic Respiratory Symptoms Compared With Nonsmokers of any Substance

Author	↑ Cough	↑ Sputum	↑ Wheeze	↑ Breathlessness
Bloom et al. (1987)	Numeric ↑	+	+	ns
Tashkin et al. (1987)	+	+	+	ns
Taylor et al. (2000)	Numeric ↑	Numeric ↑	Numeric ↑	Numeric ↑
Moore et al. (2004)	+	+	+	ns
Aldington et al. (2007)	+	+	+	NR
Tan et al. (2009)	No significant ↑ in symptoms consistent with COPD			

+, Significant increase compared with nonsmokers of any substance; ns, not significant; NR, not reported

no smoked substance, with self-reported chronic respiratory symptoms (chronic cough, sputum production, wheeze, chest tightness and shortness of breath) (Tashkin et al., 1987; Bloom, Kaltenborn, Paoletti, Camilli, & Lebowitz, 1987; Taylor, Poulton, Moffitt, Ramankutty, & Sears, 2000; Aldington et al., 2007; Moore, Augustson, Moser, & Budney, 2004; Tan et al., 2009) (Table 52.1). All but one of these studies showed a significant increase in symptoms of chronic bronchitis (chronic cough and/or sputum), along with wheeze, in smokers of cannabis alone (CS), compared with nonsmokers of any substance (NS); in two studies (Tashkin et al., 1987; Aldington et al., 2007) in which comparisons were made with smokers of tobacco alone (TS), the increase in these symptoms were compared to those noted in TS, without evidence of additive effects in the combined smokers of both cannabis and tobacco (CTS). Increases in chest tightness and shortness of breath in the CS, compared to NS, were variably observed. In one other study (Tan et al., 2009), a survey of a random sample of residents of Vancouver, Canada, an increase in the odds ratio (OR) for self-reported chronic respiratory symptoms was found for TS and CTS (but not for CS) versus NS. Taken together, the findings from these studies provide mostly consistent evidence of an association between cannabis use itself (apart from tobacco) and symptoms of chronic bronchitis.

An 8-year follow-up study of habitual smokers of cannabis demonstrated that CS who quit smoking cannabis subsequently experienced a significant reduction in their symptoms of cough, sputum, and wheeze, compared to those who continued to smoke, indicating that cannabis smoke-induced symptoms of chronic bronchitis are reversible (Tashkin et al., 2012). The latter possibility is supported by self-reported data from an internet survey of 6883 cannabis users which revealed that switching from smoking cannabis to using a vaporizer was followed by a reduction in self-reported respiratory symptoms; however, these results could have been influenced by reporting bias (Earleywine & Barnwell, 2007).

Two studies have examined the risk of cannabis for acute respiratory illnesses. Daily smokers of cannabis who did not smoke tobacco (n = 452), followed for ≥2 years in a health maintenance organization, had a small but significantly increased risk of outpatient visits for acute respiratory illnesses, compared to nonsmokers of either substance (n = 450) (relative risk 1.19; 95% CI 1.01–1.41) (Polen, Sidney, Tekawa, Sadler, & Friedman, 1993). In a convenience sample of nonsmokers and smokers of cannabis and/or tobacco, CS (n = 144) had a significantly higher incidence of episodes of acute bronchitis, compared to the nonsmokers (n = 99) ($p < 0.01$) (Tashkin et al., 1987).

Effects on Lung Function

Measurements of lung function were performed in most of the previously cited studies. In addition to these studies, lung function was also measured in a convenience sample of heavy, habitual cannabis smokers (Tashkin, Calvarese, Simmons, & Shapiro, 1980), a longitudinal extension of a birth cohort study already cited (Hancox et al., 2010; Taylor et al., 2000), and in another cohort study in which participants were followed for 20 years (Table 52.2). In two of the studies in which lung function was assessed cross-sectionally (Bloom et al., 1987; Taylor et al., 2000), a slight decrease was noted in the ratio of the forced expired volume in 1 s (FEV_1) to the forced vital capacity (FVC), suggesting possible early airflow obstruction in the CS, compared to the NS. In five other studies involving cross-sectional analysis, no association was found between spirometric evidence of airflow obstruction (as defined by a reduced FEV_1/FVC ratio) and cannabis, after adjusting for tobacco (Tashkin et al., 1980, 1987; Aldington et al., 2007; Tan et al., 2009; Hancox et al., 2010). Moreover, in one of the latter studies, a significant increase in FVC was noted in the CS (Hancox et al., 2010), consistent with possible stretching of the lung by the deep breathing maneuvers characteristic of cannabis smoking topography; thus, an elevated FVC could spuriously reduce the FEV_1/FVC ratio, so that the reduced FEV_1/FVC ratio noted in the two studies cited previously might not reflect the presence of true airflow obstruction. In longitudinal follow-up studies (Tashkin, Simmons, Sherrill, & Coulson, 1997,

TABLE 52.2 Association of Regular Cannabis Use Alone With Lung Function Abnormality Compared With Nonsmokers of any Substance

Author	FEV_1	FVC	FEV_1/ FVC	TLC	FRC	RV	SG_{aw}	D_LCO
Tashkin et al. (1980)	ns	ns	NR	ns	ns	NR	↓	ns
Bloom et al. (1987)	ns	NR	↓	NR	NR	NR	NR	NR
Tashkin et al. (1987)	ns	ns	ns	ns	ns	ns	↑	ns
Sherrill, Krzyzanowski, Bloom, and Lebowitz (1991)	ns[a]	ns[a]	ns[a]	NR	NR	NR	NR	NR
Taylor et al. (2000)	NR	NR	↓	NR	NR	NR	NR	NR
Moore et al. (2004)	NR	NR	ns	NR	NR	NR	NR	NR
Aldington et al. (2007)	ns	NR	ns	ns	ns	ns	↓	ns
Hancox et al. (2010)	ns	Trend to ↑	↑	ns	ns	Trend to ↑	↓	ns
Tan et al. (2009)	NR	NR	ns	NR	NR	NR	NR	NR
Pletcher et al. (2012)	↑[b]	↑[b]	NR	NR	NR	NR	NR	NR
Kempker, Honig, and Martin (2015)	ns[c]	ns[c]	ns[c]	NR	NR	NR	NR	NR

Definition of abbreviations: FEV_1, forced expired volume in 1 second; FVC, forced vital capacity; FEV_1/FVC, ratio of FEV_1 to FVC (the preferred measure for defining the presence of airflow obstruction); TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; SG_{aw}, specific airway conductance; D_LCO, single-breath diffusing capacity of the lung for carbon monoxide; ns, no significant difference from nonsmokers; NR, not reported; ↓, significant decrease compared to nonsmokers ($p < 0.05$); significant ↑ compared to nonsmokers ($p < 0.05$).

[a] *No significant ↓ in current cannabis smokers.*

[b] *Assessments over 20 years of follow-up; model includes lifetime exposure in joint-years.*

[c] *Among those with > 20 joint-years, significant ↓ in FEV_1/FVC but significant ↑ in FVC and a trend toward significant ↑ in FEV_1.*

Hancox et al., 2010; Pletcher et al., 2012), no significant association was found between cannabis use alone and the decline in FEV_1 with age, in contrast to an accelerated decline in lung function (characteristic of chronic obstructive pulmonary disease, COPD) associated with tobacco smoking. In one of these longitudinal studies (Pletcher et al., 2012), a nonsignificant trend toward decreases in FEV_1 over 20 years was noted only in the heaviest smokers of cannabis (50 joint-years), although the latter smokers comprised less than 1% of the study participants. In three studies in which airway resistance was measured with a body plethysmograph (Tashkin et al., 1980, 1987; Aldington et al., 2007), a modest increase in airway resistance (denoting some obstruction in the large central airways) was observed in the CS; however, this finding is probably of little clinical significance, given that the major site of obstruction in COPD is in the smaller, peripheral airways, and sensitive tests of small airways function have been found to be normal in CS (Tashkin et al., 1980, 1987). No physiologic evidence of pulmonary emphysema, for which the diffusing capacity of the lung for carbon monoxide (D_LCO) is a sensitive, if nonspecific, test, was found in the two studies in which the D_LCO was measured (Tashkin et al., 1987; Aldington et al., 2007). In summary, most published studies failed to find a positive association between cannabis use (in the absence of concomitant tobacco smoking) and the development of significant or clinically meaningful airflow obstruction or impairment in diffusing capacity, implying that regular use of cannabis, in contrast to tobacco, does not appear to be a significant risk factor for the development of COPD.

Given the similarity in the smoke contents of cannabis and tobacco, it is unclear why most studies fail to demonstrate evidence of significant airflow obstruction in habitual cannabis smokers, in contrast to consistently reported findings in regular tobacco smokers. It is unlikely that the acute bronchodilator effect of the THC within cannabis, in contrast to the acute bronchoconstrictor effect of tobacco, would account for this difference. A possible explanation is that the well-documented antiinflammatory effect of THC, including impairment of the activation of alveolar macrophages (the major immune effector cell residing in the air spaces) (see later), may counteract the proinflammatory effects of other components within the smoke, thus inhibiting the inflammatory cascade underlying the development of COPD in regular tobacco smokers.

Effects on Thoracic High-Resolution Computed Tomography

Aldington et al. (2007) obtained thoracic high-resolution computed tomography (HRCT) scans in 75 CS, 91 CTS, 92 TS, and 81 NS, and noted a small but significant increase in the percentage of CS and CTS with very

low levels of lung attenuation (suggesting enlarged air spaces) in the lung apices, compared to both NS and TS. However, no evidence of macroscopic emphysema was found in the CS.

Effects on Lung Pathology: Animal Studies

Only few long-term animal exposure studies have been reported. Cannabis smoke-inhaling dogs have been shown to develop evidence of bronchiolitis, and squamous metaplasia of the trachea (Roy, Magnan-Lapointe, Huy, & Boulet, 1976). Rats chronically exposed to the inhaled smoke of cannabis have developed a dose-related focal pneumonitis with some areas of early scarring (Fleischman, Hayden, Braude, & Rosenkrantz, 1975; Fleischman, Baker, & Rosenkrantz, 1979). It is noteworthy, however, that control animals in these same studies developed at least qualitatively similar histopathologic changes. In a study in which rhesus monkeys inhaled smoke from high or low amounts of cannabis (7 vs 2 days a week, respectively) or placebo (cannabis-extracted smoke) versus sham smoke, over 12 months, a higher frequency of several histopathological alterations (alveolitis, alveolar cell hyperplasia, and granulomatous inflammation) were found in the lungs of all smoking groups (irrespective of the cannabis amount), compared to the sham group (Fligiel et al., 1991). In another study, exposure of rats to comparable amounts of cannabis or tobacco smoke, for up to 1 year, failed to reveal any morphological or physiological evidence of emphysema in the cannabis-exposed animals, in contrast to the tobacco-exposed rats, which did develop emphysematous changes (Huber & Mahajan, 1987).

Effects on Human Bronchial Pathology

Videobronchoscopy was performed in 40 habitual smokers of cannabis and/or tobacco or nonsmokers (ages 20–49) to assess visual, as well as microscopic, evidence of airway injury and inflammation; the videotapes were blindly scored by an objective observer (Roth et al., 1998). Scores for visual changes of bronchitis (erythema, edema, increased secretions) were significantly higher in all of the smoking groups, compared to the nonsmokers. The visual changes correlated well with histopathologic evidence in endobronchial biopsies of vascular hyperplasia, submucosal edema, inflammatory cell infiltrates, and hyperplasia of mucus-secreting surface epithelial (goblet) cells. These findings indicate that regular cannabis smoking by young adults is associated with significant airway inflammation that is similar in frequency, type, and magnitude to that found in regular tobacco smokers.

Endoscopic biopsies of bronchial mucosa obtained in a total of 40 CS, 44 CTS, 31 TS, and 53 NS revealed significantly higher percentages of histopathologic alterations, including basal and goblet cell hyperplasia, squamous metaplasia, inflammation, cellular disorganization, and increased nuclear/cytoplasmic ratio in the CS that were comparable to the changes in the TS, with the suggestion of additive abnormalities in the combined smokers of both cannabis and tobacco (Fligiel et al., 1997). The increased numbers of goblet (mucus-secreting) cells, and the loss of ciliated bronchial epithelium replaced by basal, goblet and squamous cells, might explain the association between cannabis use and symptoms of chronic bronchitis; the combination of increased mucus production from the hyperplastic goblet cells, and the diminished capacity of the airways to clear the increased mucus, due to the loss of ciliated epithelium, leaves cough as an alternative mechanism to eliminate the increased mucus from the airways.

Effects on Alveolar Macrophage Number and Function

Bronchoalveolar lavage performed during bronchoscopy has revealed a threefold increase in the numbers of alveolar macrophages in both CS and TS, compared to NS, and a sixfold increase in CTS, as well as increases in neutrophils in all the smoking groups (Barbers, Gong, Tashkin, Oishi, & Wallace, 1987), documenting the impact of cannabis on lung inflammation, and the additive effects of combined smoking of both cannabis and tobacco. On the other hand, functional studies have revealed an impairment in both the phagocytic and microbicidal activity of alveolar macrophages in CS, but not TS (Baldwin et al., 1997). The mechanism of the cannabis-related impairment in alveolar macrophage bactericidal activity appears to be related to the immunosuppressive effect of THC that is mediated by cannabinoid type 2 (CB2) receptors on immune cells (Roth, Whittaker, Salehi, Tashkin, & Baldwin, 2004). THC-related immunosuppression leads to impairment in stimulated production by alveolar macrophages of proinflammatory cytokines (Roth et al., 2004) that are required during infection for upregulation of inducible nitric oxide synthase (Shay et al., 2003) that leads, in turn, to the production of nitric oxide as an effector molecule in bacterial killing. A consequence of THC-related inhibition of alveolar macrophage function is an impairment in the lung's defense against infection (see below).

RELATIONSHIP TO LUNG CANCER

A positive association of cannabis use with lung cancer is suggested by the following lines of evidence (Table 52.3). Cannabis smoke contains procarcinogenic chemicals, including the polycyclic aromatic hydrocarbons (PAHs), benzpyrene and benzanthracene, in concentrations not dissimilar to those found in tobacco

TABLE 52.3 Evidence Supporting a Positive Association Between Cannabis Use and Lung Cancer

- Presence of procarcinogens in the tar phase of mainstream cannabis smoke (eg, polycyclic aromatic hydrocarbons, including benz(a)pyrene and benz(a)anthracene) (Moir et al., 2008)
- Fourfold increase in lung deposition of insoluble smoke particulates (tar) from a single marijuana joint, compared to a single tobacco cigarette of the same weight (Wu et al., 1988)
- Precancerous histopathologic alterations in the bronchial epithelium of habitual cannabis smokers (Fligiel et al., 1997)
- Upregulation of molecular markers of pretumor progression (eg, EGFR and Ki-67) in the bronchial epithelium of habitual cannabis smokers (Barsky et al., 1998)
- Case series of an unexpectedly higher proportion of cannabis smokers (compared to the general population) among individuals diagnosed at an unusually young age with respiratory cancer (lung and upper aerodigestive tract cancers) (Sridhar et al., 1994; Fung et al., 1999)
- Case-control studies showing a significantly positive association of cannabis use with lung cancer (Berthiller et al., 2008—review of three separate North African studies; Aldington et al., 2008; Callaghan, Allebeck, & Sidorchuk, 2013)

smoke (Moir et al., 2008). While cannabis is generally smoked in lower amounts than tobacco, the prolonged breath-holding time during cannabis smoking, and the reduced rod filtration due to more loosely packed cannabis, leads to a fourfold increase in the respiratory deposition of tar, which contains the carcinogenic PAHs (Wu, Tashkin, Djahed, & Rose, 1988). Several of the histopathologic alterations within the bronchial epithelium of smokers of cannabis, even in the absence of tobacco, including squamous metaplasia, cellular atypia, and increased nuclear/cytoplasmic ratio, are considered to be premalignant (Fligiel et al., 1997; Auerbach, Stout, Hammond, & Garfinkel, 1961). Moreover, bronchial biopsies from habitual smokers of cannabis alone have shown significantly increased expression of molecular markers of pretumor progression, including EGRF (epidermal growth factor receptor) and Ki-67 (a nuclear proliferation protein), compared to nonsmokers (Barsky, Roth, Kleerup, Simmons, & Tashkin, 1998). Furthermore, several small case series have indicated an unusually high proportion of regular cannabis smokers among young persons (<40–45 years of age) with lung and upper airway cancer (Taylor, 1988; Sridhar et al., 1994; Fung, Gallagher, & Machtay, 1999), although such case series do not serve as evidence of causality.

A pooled analysis of three North African hospital-based case-control studies from Morocco, Tunisia, and Algeria (Berthiller et al., 2008) found a positive association of cannabis use with lung cancer, after adjustment for age, occupational exposure, and lifetime pack-years of tobacco smoking (not including the tobacco admixed with cannabis in the smoked product), with OR (95% CI) of 2.2 (1.1–4.5), 4.1 (1.8–9.0), and 2.0 (0.7–5.3), respectively. However, none of these studies could fully adjust for the confounding influence of tobacco, since cannabis is typically admixed with tobacco in the form of a "kiff," and smoked.

A population-based case-control study from New Zealand compared 79 lung cancer cases identified between 2001 and 2005 in individuals aged ≤55 years, with randomly selected controls matched for age (Aldington et al., 2008). OR for the association of cannabis use and lung cancer determined by multivariate logistic regression, with adjustments for age, gender, ethnicity, family history of lung cancer, and pack-years of tobacco, showed no difference between smokers of cannabis with a cumulative lifetime history of ≤10.5 joint-years, and nonsmokers (OR point estimates 0.3–0.9), but a significantly increased risk of lung cancer for cannabis smokers with >10.5 joint-years (OR 5.7; 95% CI 1.5–21.6). It is noteworthy, however, that the latter subgroup of heaviest cannabis smokers included only 14 cases and 4 controls; this very small sample size, therefore, likely led to imprecise and inflated estimates.

A possible association between cannabis and lung cancer has more recently been examined in a Swedish population-based cohort study of 49,321 men who had been questioned about tobacco and cannabis use during military conscription at age 18–20 years, and subsequently tracked for up to 40 years for incident lung cancer, using nationwide linked medical registries (Callaghan et al., 2013). Cox regression was used to assess relationships between lifetime use of cannabis *prior* to the time of conscription, and the risk of subsequent lung cancer, adjusted for cumulative tobacco use up to (but not beyond) the age of 18–20 years, chronic lung disease, and socioeconomic status. Whereas no association was found between smoking cannabis up to 50 times (lifetime use prior to the age of 18–20 years) and subsequent lung cancer incidence, a positive association was observed between lung cancer and smoking cannabis >50 times prior to conscription (hazard ratio 2.12; 95% CI 1.08–4.14). The major weakness of this study is the inability to adjust for true lifetime use of tobacco, including use during the ≤40-year follow-up period, as a crucially important confounder, or to take true lifetime use of cannabis into account.

Other evidence does not support a link between cannabis and lung cancer (Table 52.4). A retrospective cohort study of 64,855 enrollees, aged 15–49 years, in a health management program in California, United States, examined the association of self-reported cannabis use with cancer incidence over a mean of 8.6 years of follow-up (Sidney, Quesenberry, Friedman, & Tekawa, 1997). Ever- and current use of cannabis was not found to be associated with an increased risk of tobacco-related cancers, after adjustment for tobacco, alcohol use, and socioeconomic status in either men (relative risk 0.9; 95% CI 0.l7–1.2) or women (relative risk 1.0; 95% CI 0.8–1.1). A

TABLE 52.4 Evidence *Not* Supporting a Positive Association Between Cannabis Use and Lung Cancer

- Demonstration by several investigators of antitumoral effects of THC and other cannabinoids on a variety of malignancies (lung, glioma, thyroid, lymphoma, skin, pancreas, uterus, breath, prostate) in cell culture systems and animal models (reviewed in part by Bifulco, Laezza, Pisanti, & Gazzerro, 2006)
- Large retrospective cohort study failing to demonstrate an association between ever- or current use of cannabis, and any increased risk of lung or other tobacco-related cancers (relative risk ≤1.0) (Sidney et al., 1997)
- Large, prospective population-based case-control study failing to observe any positive association of cannabis use—even heavy, long-term use—with lung cancer (OR <1.0 for all quantity-duration categories of cannabis use), in contrast to a strong dose-response relationship for tobacco (Hashibe et al., 2006)

criticism of this study is that the enrollees were relatively young, and the follow-up period was relatively short, so that, over the period of observation, most of the cohort had not reached the age when cancer incidence is especially prominent, thereby limiting the power of the study to detect a statistically significant association between cannabis use and cancer.

The largest case-control study to estimate the effects of cannabis use on the risk of lung cancer was carried out in residents of Los Angeles county, aged 18–59 years (Hashibe et al., 2006). Over a 5-year period, 611 lung cancer cases and 1040 controls (matched on age, gender, and neighborhood as a surrogate for socioeconomic status) were enrolled and administered a detailed history of drug and alcohol use, occupational exposures, diet, and family history of cancer. Cases included 649 ever-smokers of cannabis, of whom 183 had a cumulative lifetime history of ≥10 joint-years, while controls included 557 ever-smokers of cannabis, of whom 112 had accumulated ≥10 joint-years of smoking. In unconditional logistic regression analyses, with age and gender as covariates, and adjustments for race, educational level, tobacco, and alcohol, no positive association was noted between any level of cannabis use (including ≥60 joint-years) and lung cancer incidence. Adjusted odds ratios (95% CI) ranged from 0.63 (0.46–0.87) for >0 to <1 joint-year to 0.62 (0.32–1.2) for ≥60 joint-years. In contrast, a strong dose-response relationship was observed between tobacco use and lung cancer: adjusted ORs ranged from 1.3 (0.96–1.8) for 1–20 pack-years, to 7.8 (5.5–11) for >30–40 pack-years, and 21 (12–32) for >40 pack years. While these findings argue against a link between cannabis use (when adjusted for concomitant tobacco smoking) and lung cancer, the study had limitations, including the large nonparticipation rate among individuals identified with lung cancer that could have led to selection bias, recall bias concerning how much cannabis was smoked, and errors in measuring the lifetime use of cannabis. Moreover, since the upper limit of the confidence intervals for the association of cannabis use and lung cancer exceeded 1.0, the possibility cannot be excluded that cannabis use might have contributed to the development of lung cancer in a small subgroup of cannabis smokers, particularly heavier users, who might have been unusually susceptible to the development of lung cancer for reasons other than cannabis (eg, genetic); however, these risks appear to be small, relative to the extensively documented hazards of tobacco smoking.

It is tempting to speculate on the reasons that might account for a null association between cannabis use (even heavy use) and lung cancer, in view of the fact that cannabis smoke contains carcinogens in amounts fairly comparable to those found in tobacco smoke. The generally smaller amounts of cannabis that are regularly smoked, compared to tobacco, do not appear to be entirely explanatory, since no dose-response relationship between cannabis use and lung cancer was observed in the large study of Hashibe et al. (2006), in contrast to the strong dose-response relationship observed for tobacco. A more likely explanation is the well-documented antitumoral effects of THC and other cannabinoids that have been demonstrated in both cell culture systems, and animal models of a variety of malignancies, as reviewed by Bifulco et al. (2006). These antitumoral effects (antimitogenic, proapoptotic, and antiangiogenetic) might be capable of counteracting the tumor-initiating or tumor-promoting effects of the carcinogens contained within the smoke of cannabis.

ASSOCIATION WITH LOWER RESPIRATORY INFECTION (TABLE 52.5)

THC-related impairment in the fungicidal and bactericidal activity of alveolar macrophages (the principal immune effector cell in the lung) (Baldwin et al., 1997; Shay et al., 2003) may compromise the lung's defense against microbial infection. Cannabis-related injury to the bronchial epithelium, manifested by loss of ciliated epithelial cells and hyperplasia of mucus-secreting goblet cells (Fligiel et al., 1997) can lead to further compromise of the lung's defense system by impairing mucociliary clearance, with accumulation of excess mucus in the airways that may serve as a substrate for microbes colonizing the airway.

As a consequence of cannabis-related impairment in the lung's defenses against infection, cannabis use could predispose to lower respiratory tract infection. Since cannabis has also been shown to be frequently contaminated with *Aspergillus fumigatus* (Kagen et al., 1983), and potentially pathogenic gram-negative bacteria (Ungerleider et al., 1982), introduction of these microorganisms into the lung by smoking provides an additional mechanism

TABLE 52.5 Evidence Implicating Cannabis Use as a Possible Risk Factor for Pneumonia

- Immunosuppressive properties of THC leading to inhibition of the phagocytic and microbicidal activity of alveolar macrophages, the primary immune effector cells in the lung responsible for the lung's defense against infection
- Destruction by regular cannabis smoking of ciliated epithelial cells in the bronchial mucosa, with replacement by mucus-secreting surface epithelial (goblet) cells, leading to excess mucus production and a diminished capacity to cleanse the airways of the mucus, which could serve as a substrate for infection
- Contamination of marijuana with *Aspergillus fumigatus* (Kagen, Kurup, Sohnle, & Fink, 1983) and potentially pathogenic gram-negative bacteria (Ungerleider, Andrysiak, Tashkin, & Gale, 1982)
- Case reports of *Aspergillus* pneumonia in cannabis smokers immunocompromised due to AIDS (Denning et al., 1991), chronic granulomatous disease (Chusid et al., 1975), bone marrow or renal transplantation (Hamadeh, Ardehali, Locksley, & York, 1988; Marks et al., 1996), chemotherapy for treatment of lung cancer (Sutton, Lum, & Torti, 1986)
- Acquisition of infection with tuberculosis by immunocompetent individuals sharing a cannabis bong with cases of active cavitary tuberculosis (Munckhof, Konstantinos, Wamsley, Mortlock, & Gilpin, 2003; Thu, Hayes, Miles, Tierney, & Foy, 2013)
- Older epidemiologic studies suggesting cannabis as an independent risk factor for opportunistic infection or bacterial pneumonia in HIV+ individuals (Newell et al., 1985; Tindall et al., 1988; Caiaffa et al., 1994). *However, an older AIDS cohort study has failed to implicate cannabis use as an independent risk factor for the development of an AIDS-defining opportunistic infection* (Kaslow et al., 1989)

whereby cannabis could increase the risk of pneumonia. This possibility is supported by case reports of *Aspergillus* pneumonia in smokers of cannabis who were immunocompromised by AIDS (Denning et al., 1991), chronic granulomatous disease (Chusid, Gelfiand, Nutter, & Fauci, 1975), bone marrow transplantation (Hamadeh et al., 1988), renal transplantation (Marks et al., 1996), or lung cancer treated with chemotherapy (Sutton et al., 1986). Although the underlying immuocompromised state could have been a factor contributing to opportunistic fungal infection in these patients, it is also possible that further immunosuppression by cannabis, particularly if contaminated by *Aspergillus*, could have added to the risk of fungal pneumonia. A report from Queensland, Australia, identified a cluster of five young men with cavitary tuberculosis (TB), among whom, along with their contacts, cannabis bong use was found to be common (Munckhof et al., 2003). Of the contacts, 114 were screened for TB, of whom 57 (50%) were TB skin-test positive. In multivariate logistic regression, sharing a cannabis water pipe with one of the cases of active cavitary TB was one of the only two variables associated with the risk of acquiring TB infection (OR 2.22; 95% CI 0.96–5.17). In a more recent report from Australia, a cluster of three additional cases of open cavitary TB infection was identified in individuals who smoked cannabis using a bong. Four of the five contacts who shared a bong with one of these cases (80%), and one of the two contacts who shared a bong with another case (50%) were found to be skin-test positive for TB (Thu et al., 2013). Sharing a bong with one of the cases of cavitary TB was associated with a more than sixfold increased risk of transmission (OR 6.5; 95% CI 01.4–30.4; $p = 0.016$). In these instances, however, it is not possible to determine whether the transmission of infection with tuberculosis resulted from close contact with the patients with cavitary TB and/or cannabis-related impairment in the lung's defenses against infection.

The results of a few older epidemiologic studies suggest that cannabis may be a significant independent risk factor for opportunistic pulmonary infection in HIV+ individuals (Newell et al., 1985; Tindall et al., 1988; Caiaffa et al., 1994). In contrast, however, preliminary analysis of data from the multicenter AIDS cohort study failed to find any evidence for a role of cannabis or other psychoactive drugs in accelerating immunodeficiency in HIV+ individuals (Kaslow et al., 1989). Further studies in immunocompromised individuals, over a longer period of observation, are required to resolve the controversy as to the true impact of cannabis use on pneumonia risk.

ASSOCIATION WITH PNEUMOTHORAX, PNEUMOMEDIASTINUM, AND BULLOUS LUNG DISEASE (TABLE 52.6)

A number of cases of pneumothorax and/or pneumomediastinum (dissection of air from the lung into the pleural and/or mediastinal space) have been reported in association with cannabis smoking (Miller et al., 1972; Mattox, 1976; Birrer & Calderon, 1984; Luque, Cavallaro, Torres, Emmanual, & Hillman, 1987; Herrejon Silvestre et al., 1992; Feldman, Sullivan, Passero, & Lewis, 1993; Hazouard et al., 2001; Goodyear et al., 2004; Beshay, Kaiser, Niedhart, Reymond, & Schmid, 2007). It has been hypothesized that the deep inhalation technique followed by a long breath-holding time characteristic of the cannabis smoking profile might explain this association, particu-

TABLE 52.6 Miscellaneous Potential Effects of Cannabis on the Lung

- High resolution computed tomography findings of significantly lower lung density (suggesting enlarged air spaces) in the lung apices of regular smokers of cannabis with or without tobacco. *However, no evidence of macroscopic emphysema was found in the cannabis smokers* (Aldington et al., 2007)
- Isolated cases of pneumothorax and/or pneumomediastinum in regular cannabis users
- Several cases of large lung bullae (pathologically enlarged air spaces—more than 1 cm—in the lung parenchyma) in mostly heavy smokers of cannabis along with varying amounts of tobacco

larly if the smoker performed a Valsalva maneuver during the breath-hold (Birrer & Calderon, 1984), potentially leading to alveolar rupture with leakage of air through the visceral pleural surface. Several cases of large lung bullae (pathologically enlarged air spaces in the lung parenchyma, measuring more than 1 cm) have been reported in smokers of mostly large amounts of cannabis, all of whom also smoked varying quantities of tobacco (Johnson, Smith, Morrison, Laszlo, & White, 2000; Thompson and White, 2002; Phan, Lau, & Li, 2005; Hii, Tam, Thompson, & Naughton, 2008). Since case series represent uncontrolled observations, and the prevalence of bullous lung disease in the general population is not known, a cause-and-effect relationship between cannabis use and lung bullae has not been established (Tan, Hatam, & Treasure, 2006).

MINI-DICTIONARY

Airflow obstruction Defined as an abnormally reduced ratio of FEV1 to FVC (ie, a ratio less than 70%).
Alveolar macrophages These are the most numerous cells residing in the distal respiratory tract, and serve as immune effector cells that are principally important for defending the lung against infection.
Bronchodilation Involves an increase in the diameter of the bronchial airways due to relaxation of airway smooth muscle.
Bronchoscopy A technique involving the insertion of a lighted fiber-optic scope through the larynx into the tracheobronchial tree, thereby allowing (1) visualization of the mucosal lining of the central airways (trachea and larger bronch), (2) the performance of bronchoalveolar lavage (instilling saline to rinse out cells and fluid from within the lung), and (3) the performance of biopsies of the bronchial tissue for microscopic examination.
Bullous lung disease Consists of one or more bullae, focally enlarged airspaces of >1 cm, within the lung parenchyma.
Chronic bronchitis Defined by the presence of cough and sputum on most days, for at least 3 months of the year, for 2 or more years; symptoms of chronic bronchitis do not necessarily imply the presence of chronic obstructive pulmonary disease.
Chronic obstructive pulmonary disease Consists of chronic obstructive bronchitis and emphysema, in varying proportions, and is characterized by the presence of significant airflow obstruction (as defined previously), which does not fully reverse to a normal FEV1/FVC ratio after administration of a bronchodilator (ie, the obstruction is not fully reversible).
Forced vital capacity in 1 second (FEV1) The maximum amount of air that can be exhaled in 1 s after a maximal inhalation.
Forced vital capacity The maximal amount of air than can be exhaled after a maximal inhalation.
Pneumothorax and pneumomediastinum Terms used to describe a leakage of air from the airspaces in the lung into the surrounding pleural space, either between the lung and the chest wall (pneumothorax) or the lung and the mediastinum, the structures between the two lungs (pneumomediastinum).
Pulmonary emphysema Defined by pathologically enlarged distal airspaces in the lung that can be quantitated by high-resolution computed tomography (HRCT) of the lung; the presence of pulmonary emphysema is also suggested by a significantly reduced diffusing capacity of the lung for carbon monoxide (D_LCO), a function of the rate of transfer of carbon monoxide from the distal airspaces in the lung (alveoli) to the blood in the alveolar capillaries.

References

Aldington, S., Harwood, M., Cox, B., Weatherall, M., Beckert, L., Hansell, A., & Beasley, R. (2008). Cannabis use and risk of lung cancer: a case-control study. *European Respiratory Journal*, *31*, 280–286.

Aldington, S., Williams, M., Nowitz, M., Weatherall, M., Pritchard, A., NcNaughton, A., & Beasley, R. (2007). Effects of cannabis on pulmonary structure, function and symptoms. *Thorax*, *62*, 1058–1063.

Auerbach, O., Stout, A. P., Hammond, E. C., & Garfinkel, L. (1961). Changes in bronchial epithelium in relation to cigarette smoking and its relation to lung cancer. *New England Journal of Medicine*, *265*, 253–267.

Baldwin, G. C., Tashkin, D. P., Buckley, D. M., Park, A. N., Dubinett, S. M., & Roth, M. D. (1997). Marijuana and cocaine impair alveolar macrophage function and cytokine production. *American Journal of Respiratory and Critical Care Medicine*, *156*, 1606–1613.

Barbers, R. G., Gong, H., Jr., Tashkin, D. P., Oishi, J., & Wallace, J. M. (1987). Differential examination of bronchoalveolar lavage cells in tobacco cigarette and marijuana smokers. *American Review of Respiratory Disease*, *135*, 1271–1275.

Barsky, S. H., Roth, M. D., Kleerup, E. C., Simmons, M., & Tashkin, D. P. (1998). Histopathologic and molecular alterations in bronchial epithelium in habitual smokers of marijuana, cocaine and/or tobacco. *Journal of the National Cancer Institute*, *90*, 1198–1200.

Berthiller, J., Straif, K., Boniol, M., Voirin, N., Benhaim-Luzon, V., Ben Ayoub, W., & Sasco, A. J. (2008). Cannabis smoking and risk of lung cancer in men. A pooled analysis of three studies in Maghreb. *Journal of Thoracic Oncology*, *3*, 1398–1403.

Beshay, M., Kaiser, H., Niedhart, D., Reymond, M. A., & Schmid, R. A. (2007). Emphysema and secondary pneumothorax in young adults smoking cannabis. *European Journal of Cardiothoracic Surgery*, *32*, 834–838.

Bifulco, M., Laezza, C., Pisanti, S., & Gazzerro, P. (2006). Cannabinoids and cancer: pros and cons of an antitumour strategy. *British Journal of Pharmacology*, *148*, 123–135.

Birrer, R. B., & Calderon, J. (1984). Pneumothorax, pneumomediastinum, and pneumopericardium following Valsalva's maneuver during marijuana smoking. *New York State Journal of Medicine*, *84*, 619–620.

Bloom, J. W., Kaltenborn, W. T., Paoletti, P., Camilli, A., & Lebowitz, M. D. (1987). Respiratory effects of non-tobacco cigarettes. *British Medical Journal*, *295*, 1516–1518.

Caiaffa, W. T., Vlahov, D., Graham, N. M. H., Astemborski, J., Solomon, L., Newson, K. E., & Munoz, A. (1994). Drug smoking, *Pneumocystis carinii* pneumonia, and immunosuppresion increase risk of bacterial pneumonia in human immunodeficiency virus-seropositive infection drug users. *American Review of Respiratory Disease*, *150*, 493–948.

Calignano, A., Katona, I., Desarnaud, F., Giuffrida, A., La Rana, G., Mackie, K., & Piomelli, D. (2000). Bidirectional control of airway responsiveness by endogenous cannabinoids. *Nature*, *408*, 96–101.

Callaghan, R. C., Allebeck, P., & Sidorchuk, A. (2013). Marijuana use and risk of lung cancer: a 40-year cohort study. *Cancer Causes and Control*, *24*, 1811–1820.

Chusid, M. J., Gelfiand, J. A., Nutter, C., & Fauci, A. S. (1975). Pulmonary aspergillosis, inhalation of contaminated marijuana smoke, chronic granulomatous disease. *Annals of Internal Medicine*, *82*, 682–683.

Denning, D. W., Follansbee, S. E., Scolaro, M., Norris, S., Edelstein, H., & Stevens, D. A. (1991). Pulmonary aspergillosis in the acquired immunodeficiency syndrome. *New England Journal of Medicine*, *324*, 654–662.

Earleywine, M., & Barnwell, S. S. (2007). Decreased respiratory symptoms in cannabis users who vaporize. *Harm Reduction Journal*, *4*, 11.

Feldman, A. L., Sullivan, J. T., Passero, M. A., & Lewis, D. C. (1993). Pneumothorax in polysubstance abusing marijuana and tobacco smokers: 3 cases. *Journal of Substance Abuse*, *5*, 183–186.

Fleischman, R. W., Baker, J. R., & Rosenkrantz, H. (1979). Pulmonary pathologic changes in rats exposed to marihuana smoke for 1 year. *Toxicology and Applied Pharmacology*, *47*, 557–566.

Fleischman, R. W., Hayden, D. W., Braude, M. C., & Rosenkrantz, H. (1975). Chronic marihuana inhalation toxicity in rats. *Toxicology and Applied Pharmacology*, *34*, 467–478.

Fligiel, S. E. G., Beals, T. F., Tashkin, D. P., Paule, M. G., Scallet, A. C., Ali, S. F., & Slikker, W., Jr. (1991). Marijuana exposure and pulmonary alterations in primates. *Pharmacology, Biochemistry and Behavior*, *40*, 637–642.

Fligiel, S. E. G., Roth, M. D., Kleerup, E. C., Barsky, S. H., Simmons, M. S., & Tashkin, D. P. (1997). Tracheobronchial histopathology in habitual smokers of cocaine, marijuana, and/or tobacco. *Chest*, *112*, 319–326.

Fung, M., Gallagher, C., & Machtay, M. (1999). Lung and aero-digestive cancers in young marijuana smokers. *Tumori*, *85*, 140–142.

Goodyear, K., Laws, D., & Turner, J. (2004). Bilateral spontaneous pneumothorax in a cannabis smoker. *Journal of the Royal Society of Medicine*, *97*, 435–436.

Grassin-Delyle, S., Naline, E., Buenestado, A., Faisy, C., Alvarez, J. -C., Salvator, H., & Devillier, P. (2014). Cannabinoids inhibit cholinergic contraction in human airways through prejunctional CB1 receptors. *British Journal of Pharmacology*, *171*, 2767–2777.

Hamadeh, R., Ardehali, A., Locksley, R. M., & York, M. K. (1988). Fatal aspergillosis associated with smoking contaminated marijuana in a marrow transplant recipient. *Chest*, *94*, 432–433.

Hancox, R. J., Poulton, R., Ely, M., Welch, D., Taylor, D. R., McLachlan, C. R., & Sears, M. R. (2010). Effects of cannabis on lung function: a population-based cohort study. *European Respiratory Journal*, *35*, 42–47.

Hashibe, M., Morgenstern, H., Cui, Y., Tashkin, D. P., Zhang, Z. -F., Cozen, W., Mack, T. M., & Greenland, S. (2006). Marijuana use and the risk of lower and upper aerodigestive tract cancers: results of a population-based case-control study. *Cancer Epidemiology, Biomarkers and Prevention*, *15*, 1829–1834.

Hazouard, E., Koninck, J. C., Attucci, S., Fauchier-Rolland, F., Brunereau, L., & Diot, P. (2001). Pneumothorax and pneumomediastinum caused by repeated Muller's maneuvers: complications of marijuana smoking. *Annals of Emergency Medicine*, *38*, 694–697.

Herrejon Silvestre, A., Blanquer Olivas, J., Simo Mompo, M., Ruiz Montalt, F., Nunez Sanchez, C., & Chiner Vives, E. (1992). Pneumothorax due to drug inhalation. *Anales Medicina Interna*, *9*, 137–139.

Hii, W. W., Tam, J. D. C., Thompson, B. R., & Naughton, M. T. (2008). Bullous lung disease due to marijuana. *Respirology*, *13*, 122–127.

Huber, G.L, & Mahajan, V.K. (1987). The comparative response of the lung to marihuana or tobacco smoke inhalation. In G., Chesher, P., Consroe, & R., Musty, (Eds.), Marijuana: An International Research Report. *Proceedings of Melbourne Symposium on Cannabis* September 2–4, 1987 (National Campaign Against Drug Abuse Monograph Series No. 7, pp. 19–24). Canberra: Australian Government Publishing Service.

Johnson, M. K., Smith, R. P., Morrison, D., Laszlo, G., & White, R. J. (2000). Large lung bullae in marijuana smokers. *Thorax*, *55*, 340–342.

Kagen, S. L., Kurup, V. P., Sohnle, P. C., & Fink, J. N. (1983). Marijuana smoking and fungal sensitization. *Journal of Allergy and Clinical Immunology*, *71*, 389–393.

Kaslow, R. A., Blackwelder, W. C., Ostrow, D. G., Yerg, D., Palenicek, J., Coulson, A. H., & Valdisem, R. O. (1989). No evidence for a role of alcohol or other psychoactive drugs in accelerating immunodeficiency in HIV-1-positive individuals. A report from the Multicenter AIDS Cohort Study. *American Medical Association Journal*, *261*, 3424–3429.

Kempker, J. A., Honig, E. G., & Martin, G. S. (2015). The effects of marijuana exposure on respiratory health in US adults. *Annals of the American Thoracic Society*, *12*, 135–141.

Luque, M. A. JIII, Cavallaro, D. L., Torres, M., Emmanual, P., & Hillman, J. V. (1987). Pneumomediastinum, pneumothorax, and subcutaneous emphysema after alternate cocaine inhalation and marijuana smoking. *Pediatric Emergency Care*, *3*, 107–109.

Marks, W. H., Florence, L., Lieberman, J., Chapman, P., Howard, D., Roberts, P., & Perkinson, D. (1996). Successfully treated invasive pulmonary aspergillosis associated with smoking marijuana in a renal transplant recipient. *Transplantation*, *61*, 1771–1783.

Mattox, K. L. (1976). Pneumomediastinum in heroin and marijuana users. *Journal of the American College of Emergency Physicians*, *5*, 26–28.

Miller, W. E., Spiekerman, R. E., & Hepper, N. G. (1972). Pneumomediastinum resulting from performing Valsalva maneuvers during marijuana smoking. *Chest*, *62*, 233–234.

Moir, L. D., Rickert, W. S., Levasseur, G., Larose, Y., Maertens, R., White, P., & Desjardins, S. (2008). A comparison of mainstream and sidestream marijuana and tobacco cigarette smoke produced under two machine smoking conditions. *Chemical Research in Toxicology*, *21*, 494–502.

Moore, B. A., Augustson, E. M., Moser, R. P., & Budney, A. J. (2004). Respiratory effects of marijuana and tobacco use in a U.S. sample. *Journal of General Internal Medicine*, *20*, 33–37.

Munckhof, W. J., Konstantinos, A., Wamsley, M., Mortlock, M., & Gilpin, C. (2003). A cluster of tuberculosis associated with use of a marijuana water pipe. *International Journal of Tuberculosis and Lung Disease*, *7*, 860–865.

Nadel, J. A., & Comroe, J. H., Jr. (1961). Acute effects of inhalation of cigarette smoke on airway conductance. *Journal of Applied Physiology*, *16*, 713–716.

Newell, G. R., Mansell, P. W., Wilson, M. B., Lynch, H. K., Spitz, M. R., & Hersh, E. M. (1985). Risk factor analysis among men referred for possible acquired immune deficiency syndrome. *Preventive Medicine*, *14*, 81–91.

Phan, T. D., Lau, K. K., & Li, X. (2005). Lung bullae and pulmonary fibrosis associated with marijuana smoking. *Australasian Radiology*, *49*, 411–414.

Pletcher, M. J., Vittinghoff, E., Kalhan, R., Larose, Y., Maertens, R., White, P., & Desjardins, S. (2012). Association between marijuana exposure and pulmonary function over 20 years. *American Medical Association Journal*, *307*, 173–181.

Polen, M. R., Sidney, S., Tekawa, I. S., Sadler, M., & Friedman, G. D. (1993). Health care use by frequent marijuana smokers who do not smoke tobacco. *Western Journal of Medicine*, *158*, 596–601.

Roth, M. D., Arora, A., Barsky, S. H., Kleerup, E. C., Simmons, M., & Tashkin, D. P. (1998). Airway inflammation in young marijuana and tobacco smokers. *American Journal of Respiratory and Critical Care Medicine*, *157*, 928–937.

Roth, M. D., Whittaker, K., Salehi, K., Tashkin, D. P., & Baldwin, G. C. (2004). Mechanisms for impaired effector function in alveolar macrophages from marijuana and cocaine smokers. *Journal of Neuroimmunology*, *147*, 82–86.

Roy, P. E., Magnan-Lapointe, F., Huy, N. D., & Boulet, M. (1976). Chronic inhalation of marijuana and tobacco smoke in dogs:pulmonary pathology. *Research Communications in Chemical Pathology and Pharmacology*, *14*, 305–317.

Shay, A. H., Choi, R., Whittaker, K., Salehi, K., Kitchen, C. M. R., Tashkin, D. P., & Baldwin, G. C. (2003). Impairment of antimicrobial activity and nitric oxide production in alveolar macrophages from smokes of marijuana and cocaine. *Journal of Infectious Diseases*, *187*, 700–704.

Sherrill, D. L., Krzyzanowski, M., Bloom, J. W., & Lebowitz, M. D. (1991). Respiratory effects of non-tobacco cigarettes: a longitudinal study in general population. *International Journal of Epidemiology*, *20*, 132–137.

Sidney, S., Quesenberry, C. P., Jr., Friedman, G. D., Tekawa, I. S., et al. (1997). Marijuana use and cancer incidence (California, United States). *Cancer Causes and Control*, *8*, 72272–72278.

Sridhar, K. S., Raub, W. A., Weatherby, N. L., Metsch, L. R., Surratt, H. L., Inciardi, J. A., & McCoy, C. B. (1994). Possible role of marijuana

smoking as a carcinogen in the development of lung cancer at a young age. *Journal of Psychoactive Drugs*, *26*, 1285–1288.

Sutton, S., Lum, B. L., & Torti, F. M. (1986). Possible risk of invasive pulmonary aspergillosis with marijuana use during chemotherapy for small cell lung cancer. *Drug Intelligence and Clinical Pharmacy*, *20*, 289–291.

Tan, C., Hatam, N., & Treasure, T. (2006). Bullous disease of the lung and cannabis smoking: insufficient evidence for a causative link. *Journal of the Royal Society of Medicine*, *99*, 77–80.

Tan, W. C., Lo, C., Jong, A., Xing, L., Fitzgerald, M. J., Vollmer, W. M., Buist, S. A., & Sin, D. D. (2009). Marijuana and chronic obstructive lung disease—a population-based study. *Canadian Medical Association Journal*, *180*, 814–820.

Tashkin, D. P., Calvarese, B. M., Simmons, M. S., & Shapiro, B. J (1980). Respiratory status of seventy-four habitual marijuana smokers. *Chest*, *78*, 699–706.

Tashkin, D. P., Coulson, A. H., Clark, V. A., Simmons, M., Bourque, L. B., Duann, S., & Gong, H. (1987). Respiratory symptoms and lung function in habitual heavy smokers of marijuana alone, smokers of marijuana and tobacco, smokers of tobacco alone, and nonsmokers. *American Review of Respiratory Disease*, *135*, 209–216.

Tashkin, D. P., Shapiro, B. J., & Frank, I. M. (1973). Acute pulmonary physiologic effects of smoked marijuana and oral Δ^9-tetrahydrocannabinol in healthy young men. *New England Journal of Medicine*, *289*, 336–341.

Tashkin, D. P., Shapiro, B. J., & Frank, I. M. (1974). Acute effects of smoked marijuana and oral Δ^9-tetrahydrocannabinol on specific airway conductance in asthmatic subjects. *American Review of Respiratory Disease*, *l09*, 420–428.

Tashkin, D. P., Shapiro, B. J., Lee, E. Y., & Harper, C. E. (1975). Effects of smoked marijuana in experimentally induced asthma. *American Review of Respiratory Disease*, *l12*, 377–386.

Tashkin, D. P., Simmons, M. S., Sherrill, D., & Coulson, A. H. (1997). Heavy habitual marijuana smoking does not cause an accelerated decline in FEV_1 with age. *American Journal of Respiratory and Critical Care Medicine*, *155*, 141–148.

Tashkin, D. P., Simmons, M., & Tseng, C. -H. (2012). Impact of changes in regular use of marijuana and/or tobacco on chronic bronchitis. *Journal of Chronic Obstructive Pulmonary Disease*, *9*, 367–374.

Taylor, D. R., Poulton, R., Moffitt, T. E., Ramankutty, P., & Sears, M. (2000). The respiratory effects of cannabis dependence in young adults. *Addiction*, *95*, 1669–1677.

Taylor, F. M. (1988). Marijuana as a potential respiratory tract carcinogen: a retrospective analysis of a community hospital population. *Southern Medical Journal*, *81*, 1213–1216.

Thompson, C. S., & White, R. J. (2002). Lung bullae and marijuana. *Thorax*, *57*, 563.

Thu, K., Hayes, M., Miles, S., Tierney, L., & Foy, A. (2013). Marijuana "bong" smoking and tuberculosis. *Internal Medicine Journal*, *43*, 456–458.

Tindall, B., Philpot, C. R., Cooper, D. A., Gold, J., Donovan, B., Penny, R., & Barnes, T. (1988). The Sydney AIDS project: development of acquired immunodeficiency syndrome in a group of HIV seropositive homosexual men. *Australian and New Zealand Journal of Medicine*, *18*, 8–15.

Ungerleider, J. T., Andrysiak, T., Tashkin, D. P., & Gale, R. P. (1982). Contamination of marihuana cigarettes with pathogenic bacteria—possible source of infection in cancer patients. *Cancer Treatment Reports*, *66*, 589–591.

Vachon, L., Fitzgerald, M. X., Solliday, N. H., Gould, I. A., & Gaensler, E. A. (1973). Single-dose effect of marijuana smoke: bronchial dynamics and respiratory-center sensitivity in normal subjects. *New England Journal of Medicine*, *288*, 985–989.

Wu, T. -C., Tashkin, D. P., Djahed, B., & Rose, J. E. (1988). Pulmonary hazards of smoking marijuana as compared with tobacco. *New England Journal of Medicine*, *288*, 347–351.

CHAPTER

53

Cannabis and Hepatic Injury

*S.A. Nada**, *O.M.E. Abdel-Salam***, *A.A. Sleem**

**Department of Pharmacology, National Research Centre, Dokki, Greater Cairo, Egypt
**Department of Toxicology and Narcotics, National Research Centre, Dokki, Greater Cairo, Egypt

SUMMARY POINTS

- The aim of this chapter is to summarize the findings pertaining to the effect of cannabis and/or different cannabinoids in the context of liver disease.
- Elevated serum liver enzymes is a frequent finding in chronic users of cannabis.
- Smoking cannabis is an important issue in liver transplantation because of the possible fungal infection, and immunity-related effects, of cannabinoids
- Smoking cannabis has been implicated in increasing fibrosis and steatosis in liver of patients infected with hepatitis C virus.
- The effect of cannabis and/or cannabinoids in the liver could be separated into hepatoprotective CB2 receptor mechanisms versus CB1 fibrogenic and steatosis enhancing mechanisms.
- CB1 antagonists or CB2 agonists might find utility in the treatment of liver steatosis, fibrosis and in improving the hemodynamic changes in advanced liver disease.

KEY FACTS OF FIBROSIS

- Fibrosis is a wound-healing process in response to chronic liver injury and inflammation, for example, in response to hepatitis B- and C-mediated liver disease.
- With repeated inflammation, excessive scar formation occurs, termed liver fibrosis.
- This will result in compression and death of liver cells, and shrinkage of the liver and liver failure.
- The attempted regeneration of the liver cells results in disorganized nodules (cirrhosis).
- Finally, the patient will require liver transplantation.

KEY FACTS OF HEPATITIS C VIRUS

- Hepatitis C is a major cause of chronic liver disease that affects more than 170 million people worldwide.
- The primary source of transmission is *via* blood transfusions, and sharing of nonsterilized needles and syringes (illicit drug injection).
- Results in slowly progressive liver fibrosis and hepatitis C are the most common indication for liver transplantation.
- Is an important cause of liver cancer.
- Currently, the standard treatment is injectable pegylated-interferon used in combination with ribavirin, with overall viral clearance rate of approximately 55%. Newer agents, such as sofosbuvir, added to this combination therapy are expected to achieve much higher eradication rates.

KEY FACTS OF FATTY LIVER

- Fatty liver results from the accumulation of fat within the liver cells (steatosis).
- Causes include alcohol, hepatitis C virus, diabetes mellitus, obesity, excessive caloric intake, or drugs.
- It is a common form of chronic liver disease, and a frequent cause of abnormal liver enzymes.
- It is reversible, but if the causative agent is not removed, it might result in inflammation, fibrosis, or the development of cirrhosis.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00062-4
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

Δ^9-THC	Δ^9-Tetrahydrocannabinol
Δ^9-THCV	Δ^9-Tetrahydrocannabivarin
2-AG	2-Arachidonoylglycerol
ACEA	Arachidonyl-2-chloroethylamide
AEA	*N*-Arachidonoylethanolamine (anandamide)
CCl_4	Carbon tetrachloride
CB1	Cannabinoid receptor subtype 1
CB2	Cannabinoid receptor subtype 2
ConA	Concanvalin-A
FAAH	Fatty acid amide hydrolase
HCV	Hepatitis C virus
HSCs	Hepatic stellate cells
IL-6	Interleukin-6
NF-κB	Nuclear factor κB
TGF-β1	Hepatic transforming growth factor-beta 1
TNF-α	Tumor necrosis factor-alpha
TRPV1	Transient receptor potential vanilloid type-1

INTRODUCTION

The discovery of the cannabinoid receptors and their endogenous ligands have paved the way toward our understanding of the involvement of the endocannabinoid system in the regulation of different physiological and pathological processes in the body. Among these, the effect of cannabis/cannabinoids on the liver has received particular interest. There is evidence that the endocannabinoid system is activated in liver disease. In human active cirrhotic liver, cannabinoid subtype 2 receptors (CB2) were expressed in hepatic fibrogenic cells. CB2 receptors were also detected in cultured hepatic myofibroblasts and in activated hepatic stellate cells (HSCs) (Julien et al., 2005). In liver samples from patients with alcoholic liver disease, CB1 expression was restricted to areas with advanced fibrosis only (Patsenker et al., 2011). In patients with primary biliary cirrhosis, CB1 receptors were upregulated in hepatocytes and biliary epithelial cells, while CB2 were expressed in hepatocytes and in cholangiocytes (Floreani et al., 2010). Circulating levels of anandamide were increased in plasma of cirrhotic patients (Fernández-Rodriguez et al., 2004).

HUMAN STUDIES

Smoking cannabis is common in patients with chronic liver disease, including those who are potential candidates for liver transplantation (Hézode et al., 2005; Coffman, 2008; Ranney et al., 2009). In patients who were potential liver transplant, marijuana users were significantly younger, more likely to be male, and have hepatitis C (Ranney et al., 2009). The effect of smoking cannabis on liver disease is somewhat controversial. In HIV/AIDS patients undergoing retroviral therapy, there was no statistically significant change in liver enzymes in dronabinol and/or marijuana users, followed up for 12 months (Whitfield, Bechtel, & Starich, 1997). Other studies suggested that smoking cannabis possibly damages the liver. Borini, Guimarães, and Borini (2004) found that chronic marijuana usage, on its own or in association with other drugs of abuse, was associated with hepatomegaly, splenomegaly, and slightly elevated alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities in serum. Other researchers also observed increased alanine aminotransferase and gamma glutamyltransferase activities, total bile acids, and bilirubin in sera of chronic marijuana smokers with no history of liver disease (Toson, 2011).

Liver steatosis is a common finding in patients with hepatitis C virus (HCV) infection, and is an independent risk factor associated with severe hepatic fibrosis (Cholet et al., 2004). Smoking cannabis has been implicated in accelerating liver fibrosis and steatosis severity in untreated patients infected with HCV (Hézode et al., 2005, 2008; Ishida et al., 2008). Hézode et al. (2005) found that cannabis use was independently related to increased fibrosis progression rate, and to the severity of fibrosis (on liver biopsy). Compared with nondaily cannabis users, daily use was significantly associated with moderate to severe fibrosis. The study included 270 patients (14% were occasional users, and 33% were daily users) (Hézode et al., 2005). In another study, on 315 patients (12.4% were occasional users, and 24.1% were daily users), marked steatosis (on liver biopsy) was more frequent in daily cannabis users, compared with occasional users and nonusers (Hézode et al., 2008). In subjects with chronic HCV infection, Ishida et al. (2008) found that daily compared with nondaily cannabis use was significantly associated with moderate to severe fibrosis (fibrosis score on biopsy). The study comprised 204 persons (13.7% were daily users, and 45.1% occasional users, within prior 12 months). Thus daily cannabis smoking emerged as a novel independent predictor of fibrosis and steatosis severity during chronic HCV infection, necessitating cessation of cannabis in these subjects (Hézode et al., 2005, 2008; Ishida et al., 2008).

More recently, however, Brunet et al. (2013) reported no adverse effect for cannabis on liver fibrosis (AST-to-platelet ratio), or disease progression in HIV/HCV coinfected individuals. 53% of patients had smoked marijuana in the past 6 months (a median of 7 joints/week), while 40% smoked daily (38% used injection drugs at baseline, and about half the participants used alcohol, among whom 15% reported alcohol abuse at baseline). Each 10 additional joints/week smoked resulted in 33% increase in the risk of progression to clinically diagnosed cirrhosis, or 13% increase in cirrhosis and end stage liver

disease. This association was attenuated after lagging marijuana exposure to 6–12 months before the diagnosis.

Beneficial effects for cannabis in liver disease have also been reported. Thus, smoking cannabis was found to improve the tolerability to antiviral therapy with interferon-α (IFN-α) plus ribavirin (Sylvestre, Clements, & Malibu, 2006). The standard treatment for HCV is the combination of pegylated IFN-α and ribavirin. This regimen, however, is associated with significant side effects resulting in a discontinuation of significant proportion of patients. Flu-like symptoms, hematological toxicity, elevated transaminases, nausea, fatigue, depressive symptoms, and psychiatric sequelae, are the most frequently encountered (Sleijfer et al., 2005). Sylvestre et al. (2006) found that modest cannabis users were more likely to adhere to the antiviral treatment, compared with nonusers. This resulted in an increase in the duration of time that patients remain on therapy, and in more sustained virological response (54%), compared with nonusers (18%). In patients (n = 3) with cholestatic liver disease and intractable pruritus that has failed conventional (and unconventional) remedies, the situation improved following treatment with Δ^9-THC (Marinol: 5 mg at bedtime). The antipruritic effect of Marinol lasted only for 4–6 h, suggesting the need for more frequent dosing. Nevertheless, sleep improved, and patients were able to resume their work (Neff et al., 2002).

Marijuana use in liver transplant recipients is not uncommon. In these patients, contamination of fungal spores in cannabis might increase the risk of developing aspergillosis. Thus, in many transplant centers, the use of cannabis in liver transplant patients is discouraged (McGuire et al., 2009). Interestingly, in one study, the use of marijuana had no effect on survival rate in potential liver transplant candidates; patients who did and did not use marijuana had similar survival rates (Ranney et al., 2009).

ANIMAL STUDIES

Ethanol-Induced Hepatic Damage

In alcohol-induced fatty liver in mice, there is evidence to suggest a protective role of CB2 receptors in contrast to a fibrogenic and steatosis enhancing effect for CB1 receptors. Thus, Osei-Hyiaman et al. (2005) observed that high-fat diet intake in mice increased liver anandamide, CB1 density, and de novo fatty acid synthesis. Moreover, treatment with a CB1 agonist increased the hepatic gene expression of the lipogenic transcription factor sterol response element-binding protein-1c (SREBP-1c), and its targets acetyl-CoA carboxylase-1 and fatty acid synthase, as well as de novo fatty acid synthesis in the liver or in isolated hepatocytes. The effect of high fat diet on fatty acid synthesis could be blocked by CB1 receptor antagonist rimonabant. Thus, anandamide acting peripherally on hepatic CB1 is involved in diet-induced obesity. Mice fed high-sucrose, high-fat diet and then treated with the rimonabant exhibited reduced fat mass, insulin levels, and liver triglycerides. Rimonabant restored serum adiponectin levels, induced expression of scavenger receptor class B type I, and hepatic lipase genes. In visceral fat, rimonabant also counteracted upregulation of genes involved in transport, synthesis, oxidation, and release of fatty acids (Jourdan et al., 2010).

$CB1^{-/-}$ mice or hepatocyte-specific CB1 knockout mice were resistant to ethanol-induced steatosis and the increase in lipogenic gene expression. Ethanol feeding resulted in increased hepatic expression of CB1 receptors, and upregulation of the endocannabinoid 2-arachidonoylglycerol (2-AG) in HSCs, the main fibrogenic cell type in the liver. Thus, it was suggested that HSCs release 2-AG, leading to paracrine stimulation of hepatocyte CB1 receptors that mediates ethanol-induced steatosis (Jeong et al., 2008). In vitro, 2-AG and Δ^9-THC induced CB1 mRNA expression in HSCs, whereas CB1 blockage with rimonabant inhibited HSCs proliferation, and downregulated mRNA expression of fibrosis mediated genes PCα1(I), TIMP-1, and MMP-13 (Patsenker et al., 2011).

Other studies confirmed that ethanol effect was attenuated in CB1 knockout mice (Patsenker et al., 2011; Trebicka et al., 2011). In contrast, $CB2^{-/-}$ mice ethanol intake exhibited more steatosis and inflammation, compared with wild-type animals and $CB1^{-/-}$ mice. $CB2^{-/-}$ mice showed marked HSC activation and collagen production (Trebicka et al., 2011; Louvet et al., 2011). Moreover, in alcohol-induced fatty liver in mice, stimulation of CB2 receptors with JWH-133 reduced hepatocyte steatosis (Louvet et al., 2011). CB2 receptor stimulation reduced alcohol-induced inflammation by regulating Kupffer cell polarization, thereby reducing hepatocyte steatosis *via* paracrine interactions between Kupffer cells and hepatocytes (Louvet et al., 2011).

In fatty immortalized human hepatocytes, De Gottardi et al. (2010), however, found that not only the CB1 receptor agonist ACEA, but also the CB2 receptor agonist AM1241 increased lipid accumulation of oleic acid-treated fatty hepatocytes. The cannabinoid receptor agonists altered the expression of key enzymes of lipid synthesis and transport (fatty acid synthase, carnitine palmitoyltransferase 1, and lecithin-cholesterol acetyltransferase).

ISCHEMIA-REPERFUSION INJURY

Bátkai et al. (2007a) found that hepatic ischemia-reperfusion (I/R) injury was associated with markedly increased hepatic levels of anandamide and 2-AG (originating from hepatocytes, Kupffer, and endothelial cells). This increase in tissue endocannabinoids correlated with

the degree of hepatic damage and with serum TNF-α, MIP-1α, and MIP-2 levels. JWH133 (CB2 agonist) protected against I/R damage by decreasing oxidative stress, inflammatory cell infiltration, tissue and serum inflammatory cytokines. Moreover, $CB2^{-/-}$ mice showed increased I/R-induced tissue damage, and proinflammatory phenotype. The study suggested a role for endocannabinoids in liver I/R injury, and also indicated a protective role for CB2 receptors (Table 53.1).

Similarly, the CB2 receptor agonist HU-308, given prior to I/R, attenuated tissue injury, lipid peroxidation, neutrophil infiltration, DNA fragmentation, and caspase 3 activity. Serum and tissue TNF-α, MIP-1α, and MIP-2 levels decreased as well. HU-308 given after the ischemic episode also protected against liver damage (Rajesh et al., 2007). When given before ischemia, HU-910, a novel CB2 receptor agonist attenuated hepatic proinflammatory chemokines (CCL3 and CXCL2) and TNF-α level. HU-910 also decreased neutrophil infiltration, intercellular adhesion molecule-1, oxidative stress, and cell death. HU-910 effects were independent of cannabinoid receptors, since the agonist was also protective in CB2 knockout mice, and its effects were not prevented by CB1/2 antagonists in vitro (Horváth et al., 2012).

Attenuation of I/R injury was also obtained by the prior administration of Δ^8-tetrahydrocannabivarin (Δ^8-THCV). This synthetic cannabinoid exerts antiinflammatory effects *via* CB2 receptors. In liver tissue, oxidative stress, chemokines (CCL3 and CXCL2, TNF-α), intercellular adhesion molecule 1, neutrophil infiltration, caspase activity, and DNA fragmentation were all decreased by Δ^8-THCV. The cannabinoid also showed protective effects, when given at the beginning of reperfusion (Bátkai et al., 2012).

The nonpsychotropic cannabidiol was also found to decrease liver inflammation, oxidative/nitrosative stress, and cell death in mouse I/R injury, independently of cannabinoid receptors. The action of cannabidiol involves decreased NF-κB activation, and TNF-α production from Kupffer cells. It also decreased the adhesion molecule expression in liver sinusoidal endothelial cells, and attachment of human neutrophils to the activated endothelium (Mukhopadhyay et al., 2011).

JZL184 is an inhibitor of 2-AG inactivation by monoacylglycerol lipase. The latter hydrolyzes 2-AG to generate the arachidonic acid precursor pool for prostaglandin production. When given to mice, JZL184 suppressed inflammation and oxidative stress and protected against hepatic I/R injury. Increased endocannabinoid signaling *via*

TABLE 53.1 Effect of Cannabinoids on Liver Damage Induced by Ethanol

Experimental model	Cannabinoid	Effect	Suggested mechanism/CB receptor involvement	References
Mice fed low-fat, liquid ethanol diet	$CB1^{-/-}$ mice or hepatocyte-specific CB1 knockout mice	↓ Steatosis ↓Lipogenic gene expression	↓ Release of 2-AG from hepatic stellate cells leading to ↓ paracrine stimulation of hepatocyte CB1 receptors	Jeong et al. (2008)
Mice treated with thioacetamide/ethanol	$CB1^{-/-}$ mice	↓ Steatosis	CB1 receptor mediated	Patsenker et al. (2011)
Mice fed an ethanol diet	$CB1^{-/-}$ mice	↓ Steatosis		Trebicka et al. (2011)
Mice fed an ethanol diet	$CB2^{-/-}$ mice	↑ Steatosis and inflammation	Sterol regulatory element binding protein 1c (SREBP-1c) mediated steatosis ↑ Activation of hepatic stellate cells (CB1 receptor mediated)	Trebicka et al. (2011)
Mice fed an ethanol diet	JWH-133 (CB2 agonist)	↓ Steatosis	↑ Hepatic expression of macrophage HO-1 (paracrine interactions between Kupffer cells and hepatocytes/CB2 receptor mediated)	Louvet et al. (2011)
Mice fed an ethanol diet	$CB2^{-/-}$ mice	↑ Steatosis		Louvet et al. (2011)
In vitro fatty immortalized human hepatocytes	Arachidonyl-2-chloroethylamide/ACEA (CB1 receptor agonist)	↑ Steatosis	Regulate the expression of fatty acid synthase, carnitine palmitoyltransferase 1 and lecithin-cholesterol acetyltransferase	De Gottardi, Spahr, Ravier-Dall'Antonia, and Hadengue (2010)
	AM1241 (CB2 receptor agonist)	↑ Steatosis		

↑, Increase; ↓, decrease.

CB2 and reduced production of eicosanoids in the liver were the likely proposed mechanisms (Cao et al., 2013).

CARBON TETRACHLORIDE-INDUCED HEPATIC DAMAGE

In mice, CB2 was induced in the nonparenchymal cell fraction by carbon tetrachloride (CCl_4) (Teixeira-Clerc et al., 2010), while CB1 and CB2 receptor expression was increased in CCl_4-treated cirrhotic rats (Giannone et al., 2012). Teixeira-Clerc et al. (2010) found that the CB2 agonist JWH-133 reduced liver injury, and accelerated liver regeneration. In contrast, $CB2^{-/-}$ mice showed accelerated liver injury and delayed liver regeneration, in response to CCl_4. These mice exhibited decreased iNOS and TNF-α expression, lower levels of hepatic IL-6 messenger RNA, and increased matrix metalloproteinase 2 (MMP-2) activity. Treatment with a nitric oxide donor partially reversed these changes (Teixeira-Clerc et al., 2010). In rats with CCl_4-induced cirrhosis, treatment with rimonabant reduced fibrosis. The likely underlying mechanisms are induction of CB2 receptor expression, and suppression of profibrogenic and inflammatory mediators. CB1 antagonists might thus be an efficient antifibrotic therapeutic modality, even in advanced liver disease (Giannone et al., 2012) (Table 53.2).

In our studies we used total extract from *Cannabis sativa*, consisting mainly of Δ^9-THC. The effect of the whole plant differs from that of Δ^9-THC, in view of its content of other cannabinoids, terpenoids, and flavonoids (McPartland & Russo, 2001). The administration of only cannabis for 2 days had no significant effect on serum liver enzymes, or the oxidative status of the liver (tissue levels of malondialdehyde, reduced glutathione or nitric oxide). In rats intoxicated with CCl_4 (or acetaminophen), however, cannabis at 5 or 10 mg/kg (expressed as Δ^9-THC) significantly increased serum liver enzymes, liver oxidative stress, histological tissue damage, and decreased DNA content of liver cells (Abdel-Salam et al., 2013). Cannabis given to rats at high dose of 20 mg/kg for 1 month increased liver DNA fragmentation, caspase-3 positively stained cells, and was associated with fibrosis, vacuolar degeneration, and cellular infiltration in the portal area (Abdel-Salam, Youness, & Shaffee). Thus, a whole cannabis extract induced liver damage and exacerbated other liver toxicants – findings which are in line with observations in some human studies (Borini et al., 2004; Hézode et al., 2005).

Autoimmune Hepatitis

Autoimmune hepatitis can be induced in mice by the administration of concanvalin-A (ConA). In this model, Δ^9-THC, anandamide, as well as increasing endogenous endocannabinoid levels *via* fatty acid amide hydrolase inhibitors given after challenge with ConA, were able to decrease liver enzymes and tissue damage. Δ^9-THC or anandamide suppressed inflammatory cytokines, and Δ^9-THC increased the number of T regulatory cells in

TABLE 53.2 Effect of Cannabinoids on Liver Damage Induced by Ischemia/Reperfusion (I/R) Injury

Experimental model	Cannabinoid	Effect	Suggested mechanism/CB receptor involvement	References
In vivo mouse model of hepatic I/R	JWH133 (CB2 receptor agonist)	↓ Liver injury	↓ Oxidative stress and inflammation (CB2 receptor mediated)	Bátkai et al. (2007b)
In vivo mouse model of hepatic I/R	$CB2^{-/-}$ mice	↑ Liver injury		Bátkai et al. (2007b)
In vivo mouse model of hepatic I/R	HU-308 (CB2 receptor agonist)	↓ Liver injury	↓ Oxidative stress and inflammation Antiapoptotic activity (CB2 receptor mediated)	Rajesh et al. (2007)
In vivo mouse model of segmental hepatic I/R	HU-910 (CB2 receptor agonist)	↓ Liver injury	↓ Oxidative stress and inflammation (cannabinoid receptor-independent)	Horváth et al. (2012)
In vivo mouse model of hepatic I/R	Δ^8-THCV (CB2 receptor agonist)	↓ Liver injury	↓ Oxidative stress and inflammation Antiapoptotic activity (CB2 receptor mediated)	Bátkai et al. (2012)
In vivo mouse model of hepatic I/R	Cannabidiol	↓ Liver injury	↓ Oxidative/nitrative stress and ↓ inflammation (cannabinoid receptor-independent)	Mukhopadhyay et al. (2011)
In vivo mouse model of hepatic I/R	JZL184 (inhibitor of 2-arachidonoyl-glycerol inactivation by monoacylglycerol lipase)	↓ Liver injury	↓ Oxidative stress and inflammation ↓ Eicosanoids (CB2 receptor-dependent)	Cao et al. (2013)

↑, Increase; ↓, decrease.

liver. Δ^9-THC effect was reversed by both CB1 and CB2 antagonists (Hegde et al., 2008). Cannabidiol also inhibited liver inflammation when given after ConA challenge in mice. Cannabidiol decreased proinflammatory cytokines and tissue neutrophil infiltration. The mechanism is likely to involve induction of myeloid-derived suppressor cells, and consequently suppression of T cell proliferation *via* TRPV1 receptors (Hegde, Nagarkatti, & Nagarkatti, 2011). The study thus links TRPV1 receptors and cannabidiol in suppression of autoimmune hepatitis. In liver disease, where immunity has a role in the pathogenesis of liver injury, Schwabe & Siegmund (2005) suggested that the immune-modulatory effects of cannabis ingredients are likely to affect disease progression and fibrogenesis in chronic hepatitis C, by suppressing antiviral immunity.

Effect of Cannabinoids on Liver Fibrosis

CB2 receptors are expressed in cultured hepatic myofibroblasts and in activated HSCs, and CB2 receptor stimulation resulted in growth inhibition and apoptosis (Julien et al., 2005). Studies indicated that endocannabinoids are upregulated during liver fibrogenesis, and these might act as an antifibrogenic agent in the liver by selectively inducing cell death in activated HSCs, but not in hepatocytes (Siegmund et al., 2005, 2007, 2013; Wojtalla et al., 2012) (Table 53.3). Thus, anandamide blocked the proliferation and, at high concentrations, induced cell death of activated HSCs in primary culture and in vivo. Anandamide-mediated HSCs death was caspase independent, and showed typical features of necrosis such as rapid ATP depletion. This effect of anandamide depended on membrane cholesterol, and was associated with increased intracellular Ca^{++} and reactive oxygen species (attenuated by glutathione or Ca^{++} chelation) (Siegmund et al., 2005). The endocannabinoid 2-AG might act as an antifibrogenic agent in the liver by inducing cell death in activated HSCs (but not primary hepatocytes). The effect was mediated through increased mitochondrial release of reactive oxygen species, and was independent of cannabinoid receptors (Siegmund et al., 2007).

Moreover, Wojtalla et al. (2012) demonstrated that, in culture-activated primary murine or human HSCs, incubation with another endocannabinoid, that is, *N*-arachidonoyl dopamine (NADA), resulted in HSCs death. The effect of NADA was independent of cannabinoid receptors CB1 and CB2, or transient receptor potential cation channel subfamily V, member 1. Increased reactive oxygen species formation in HSCs was the likely mechanism since NADA-induced death decreased by treatment with glutathione. On the other hand, primary hepatocytes were resistant to NADA or anandamide, probably due to their high content of glutathione (Wojtalla et al., 2012). It was also suggested that the ability of hepatocytes to resist the AEA-induced injury is due to their higher expression of the AEA-degrading enzyme fatty acid amide hydrolase (FAAH) in contrast to HSCs. Interestingly, adenoviral mediated FAAH overexpression was found to exert protective actions against

TABLE 53.3 Effect of Cannabinoids on Liver Damage Induced by Carbon-Tetrachloride (CCl_4)

Experimental model	Cannabinoid	Effect	Suggested mechanism/CB receptor involvement	References
Mice treated with CCl_4	JWH133 (CB2 receptor agonist)	↓ Liver injury ↑ Liver regeneration	Paracrine mechanisms involving hepatic myofibroblasts (CB2 receptor mediated)	Teixeira-Clerc et al. (2010)
Mice treated with CCl_4	CB2 $^{-/-}$ mice	↑ Liver injury Delayed liver regeneration	↓ iNOS and ↓ IL-6 mediated decrease in matrix metalloproteinase 2 activity	Teixeira-Clerc et al. (2010)
Rats with CCl_4-induced cirrhosis	Rimonabant (CB1 antagonist)	↓ Liver injury	↓ Profibrogenic and inflammatory mediators (mediated by ↑ CB2 receptor expression)	Giannone et al. (2012)
Rats treated with CCl_4	Cannabis sativa extract	↑ Liver injury	—	Abdel-Salam, Metwaly, Sleem, Morsy, and Sharaf (2013)
Rats with CCl_4-induced cirrhosis	AM251 (CB1 antagonist)	↑ Mean blood pressure ↑ Cardiac systolic function	—	Bátkai et al. (2007a)
Rats with CCl_4-induced cirrhosis	Rimonabant (CB1 antagonist)	↓ Liver fibrosis ↓ Ascites	CB1 receptor antagonism	Domenicali et al. (2009)

↑, Increase; ↓, decrease; iNOS, inducible nitric oxide synthase; IL-6, interleukin-6.

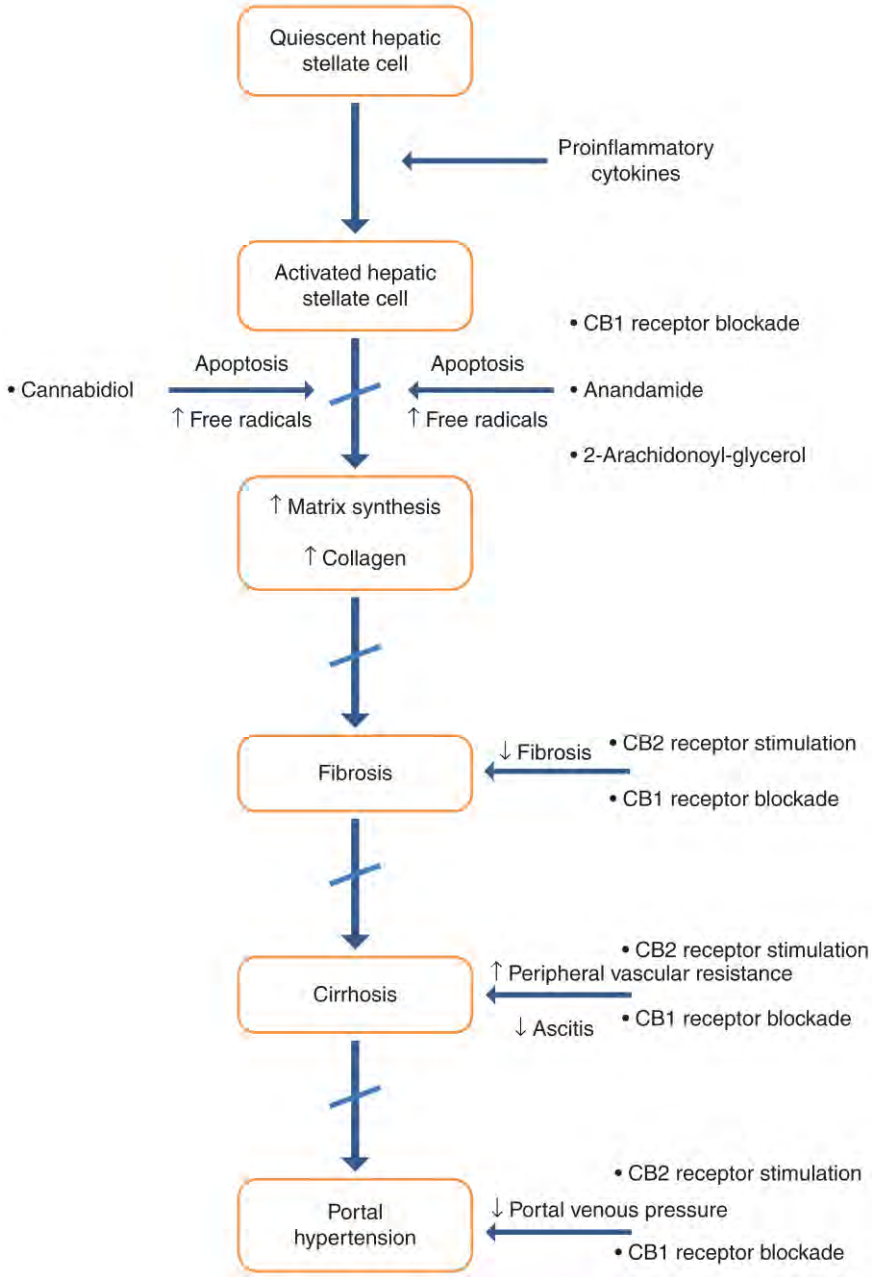


FIGURE 53.1 Effect of cannabinoids on the progression of liver disease. Cannabinoids and/or cannabinoid receptor stimulation modulate the progression of liver disease at different stages.

2-AG induced death in HSCs. Moreover, hepatocytes pretreated with FAAH inhibitor, $FAAH^{-/-}$ hepatocytes, or hepatocytes depleted of glutathione were susceptible to 2-AG induced death (Siegmund et al., 2013) (Fig. 53.1).

It has been suggested that CB1 and CB2 receptors exert opposing effects on liver fibrogenesis; CB1 receptors enhance fibrosis (Yang et al., 2007a; Trebicka et al., 2011) while CB2 mediate hepatic protective and antifibrogenic effects (Julien et al., 2005; Bátkai et al., 2007b; Muñoz-Luque et al., 2008). Mice lacking CB2 receptors showed enhanced liver fibrosis following chronic CCl_4 treatment (Julien et al., 2005). Moreover, hepatic CB1 receptor blockade with rimonabant (SR141716A) decreased the wound healing response to acute liver injury, and inhibited the progression to fibrosis in chronic models of liver injury. CB1 receptor antagonism lowered hepatic TGF-β1, reduced the accumulation of fibrogenic cells in the liver, and inhibited the growth of hepatic myofibroblasts (Teixeira-Clerc et al., 2006). Selective suppression of CB1 expression using lentivirus-mediated small interfering RNA was associated with decreased HSCs proliferation, and extracellular matrix production. It also meliorated dimethylnitrosamine-induced hepatic fibrosis. This occurred through reversal of epithelial-to-mesenchymal

TABLE 53.4 Effect of Cannabinoids on Hepatic Stellate Cells (HSCs)

Cannabinoid	Effect	Suggested mechanism/CB receptor involvement	References
2-AG (endocannabinoid) and Δ^9-THC	Induced CB1 mRNA expression in HSCs in culture	—	Patsenker et al. (2011)
Rimonabant (selective CB1 receptor antagonist)	Inhibited HSCs proliferation Downregulated mRNA expression of fibrosis mediated genes	—	Patsenker et al. (2011)
Anandamide	Induced HSCs death in HSCs primary culture-activated and in vivo-activated HSCs	↑ Reactive oxygen species formation ↑ Intracellular Ca(2+) (cannabinoid-receptor-independent mechanism)	Siegmund, Uchinami, Osawa, Brenner, and Schwabe (2005)
N-arachidonoyl dopamine (NADA) (endocannabinoid)	Induced HSCs death in culture-activated primary murine or human HSCs	↑ Reactive oxygen species formation (cannabinoid-receptor-independent mechanism)	Wojtalla et al. (2012)
2-AG (endocannabinoid) Culture-activated HSCs	Induced HSCs death (apoptotic) in culture-activated HSCs	↑ Reactive oxygen species (cannabinoid-receptor-independent mechanism)	Siegmund et al. (2007)
Cannabidiol	Induced death (apoptosis) of activated HSCs in culture	Endoplasmic reticulum stress response (cannabinoid receptor-independent mechanism)	Lim, Devi, and Rozenfeld (2011)

transition of HSCs. In contrast, the CB1 antagonist AM251 was without effect on epithelial-mesenchymal transitions of HSCs (Chen et al., 2012) (Table 53.4).

Muñoz-Luque et al. (2008) investigated the effect of daily treatment with CB2 receptor-selective agonist JWH-133 in cirrhotic rats with ascites. Mean arterial pressure increased following CB2 stimulation with JWH-133. In the liver tissue, there were decreased inflammatory infiltrates, and reduced number of activated stellate cells and decreased fibrosis. JWH-133 resulted in decreased alpha-smooth muscle actin and collagen I, and increased MMP-2 in hepatic tissue. Thus CB1 receptor antagonists or CB2 receptor agonists might find utility in the treatment of established liver fibrosis. There is evidence also that CB1 antagonism could reverse fibrosis, with this effect of CB1 receptor involving the induction of CB2 receptor expression (Giannone et al., 2012).

Finally, cannabidiol was shown to induce endoplasmic reticulum-mediated apoptosis in activated HSCs (but not in quiescent cells) independently of cannabinoid receptors (Lim et al., 2011).

CANNABINOIDS AND HEMODYNAMIC CHANGES IN LIVER CIRRHOSIS

The endocannabinoid system has been implicated in the systemic vasodilatation, hyperdynamic circulation, and cardiac muscle abnormalities that occur in advanced liver cirrhosis. Anandamide induces marked vasodilatation in isolated vascular preparations (White et al., 2001; Zygmunt et al., 1999) and these involved the activation of vanilloid receptors, since they were blocked by the vanilloid receptor antagonist capsazepine (Zygmunt et al., 1999). Another endocannabinoid, that is, virodhamine, caused endothelium-dependent relaxation of the rat isolated small mesenteric artery (Hoi & Hiley, 2006). In rats with CCl_4-induced cirrhosis, anandamide tissue levels increased in the heart, while both 2-AG and anandamide were markedly elevated in liver tissue (Bátkai et al., 2007a). Cirrhotic mesenteric arteries showed an overexpression of CB1 receptor associated with increased relaxation response to anandamide, which was suppressed by combinations of vascular Ca(2+)-dependent K(+) channel blockers (Yang et al., 2007b).

Ros et al. (2002) found that in cirrhotic rats with ascites (but not in control rats), the administration of a CB1 receptor antagonist significantly increased arterial pressure and peripheral resistance. Anandamide was detected in monocytes of cirrhotic animals, and this induced fall in blood pressure in recipient rats. It is likely that, in cirrhotic rats with ascites, monocytes are activated to produce anandamide that, in turn, contributes to arterial hypotension. The endocannabinoid system participates in the extreme mesenteric vasodilation, portal hypertension, and systemic hypotension present in advanced liver cirrhosis. Anandamide produced a drop in systemic vascular resistance, and decreased mean arterial pressure and the compensatory rise in cardiac output. Anandamide also increased portal venous flow and pressure, and decreased mesenteric vascular resistance.

These effects were partially prevented by the CB1 antagonist SR-141716A (Garcia, Járai, Mirshahi, Kunos, & Sanyal, 2001). Anandamide increased portal perfusion pressure in normal as well as in cirrhotic liver of bile duct-ligated (BDL) rats. In cirrhotic livers, anandamide increased intrahepatic resistance. These effects were associated with increased cysteinyl leukotrienes and thromboxane(2) production. The anandamide-induced increase in intrahepatic resistance was attenuated by the cyclooxygenase inhibitor indomethacin, as well as by a thromboxane A(2) synthase inhibitor (Yang et al., 2006). In BDL rats, the specific CB1 receptor antagonist AM251, given for 1 week, resulted in a decrease in portal venous pressure, intrahepatic resistance, and in superior mesenteric artery blood flow. The hepatic levels of TGF-β1, 5-lipoxygenase and cyclooxygenase-2, and the eicosanoids thromboxane B2 and cysteinyl leukotrienes were decreased, along with decreased hepatic collagen deposition (Yang et al., 2007b).

Gaskari et al. (2005) provided data suggesting a role for increased local neuronal production of endocannabinoids via a CB1 responsive pathway in cirrhotic cardiomyopathy. The authors observed that in isolated left ventricular papillary muscle from BDL cirrhotic rats, the CB1 antagonist AM251 restored the blunted response to beta-adrenergic stimulation. Increasing endogenous anandamide by the reuptake inhibitors VDM11 and AM404 enhanced muscle relaxation, which was completely blocked by AM251 and pertussis toxin. In rats with CCl_4-induced cirrhosis (but not in control rats), the CB1 antagonist AM251 acutely (intravenously) increased mean blood pressure, and the indexes of cardiac systolic function. CB1 receptor antagonists might therefore be of value to contractile function in cirrhotic cardiomyopathy (Bátkai et al., 2007a). In rats with CCl_4-induced cirrhosis and ascites, CB1 antagonism with rimonabant decreased ascites formation and volume, improved sodium balance, and decreased liver fibrosis. Rimonabant increased mean arterial pressure, peripheral vascular resistance, and renal blood flow. Improvement in systemic and renal hemodynamics, as well as antifibrotic effect, have accounted for the ascites reducing effect of CB1 antagonism in experimental cirrhosis (Domenicali et al., 2009).

In BDL cirrhotic rats, the CB2 agonists JWH-015 or JWH-133 decreased portal venous pressure, alleviated portosystemic shunting, and mesenteric vascular density. These effects were mediated via CB2 receptors (abolished by the CB2 antagonist AM630). JWH-015 decreased mesenteric COX-1 and COX-2 messenger RNA expressions, eNOS protein expressions, and decreased intrahepatic angiogenesis and fibrosis (Huang et al., 2012). Data suggested a modulatory effect for CB2 agonists (JWH-133 and GPla) on Kupffer cells activation. In BDL rats whose Kupffer cells were activated by zymosan or LPS, these CB2 agonists attenuated portal pressure. JWH-133 attenuated thromboxane B2 production following Kupffer cells activation, and increased heme oxygenase-1 expression. The inhibition of heme oxygenase-1 enhanced portal hypertension, thus heme oxygenase-1 induction by JWH-133 plays an important role (Steib et al., 2013) (Table 53.5).

TABLE 53.5 Summary of the Effect of Cannabinoid CB1 and CB2 Receptor Stimulation or Antagonism in the Liver

CB1 receptor activation	References
• ↑ De novo fatty acid synthesis in the liver or in isolated hepatocytes	Osei-Hyiaman et al. (2005)
• Induced CB1 mRNA expression in HSCs	Patsenker et al. (2011)
• ↑ Hepatocyte steatosis	De Gottardi et al. (2010)
CB1 receptor antagonism	
• Blocked the effect of high fat diet on fatty acid synthesis in liver	Osei-Hyiaman et al. (2005)
• ↓ Liver fibrosis	Teixeira-Clerc et al. (2006)
• ↓ Liver fibrosis • ↓ TGF-β1	Giannone et al. (2012)
• ↑ Arterial blood pressure • ↑ Peripheral vascular resistance	Ros et al. (2002)
• ↓ Portal venous pressure • ↓ Intrahepatic resistance • ↓Hepatic TGF-β1 • ↓Hepatic collagen	Yang et al. (2007b)
• Improved cirrhotic cardiomyopathy	Gaskari et al. (2005)
• ↑ Arterial blood pressure • Improved cirrhotic cardiomyopathy	Bátkai et al. (2007a)
• ↓ Liver fibrosis • ↓ Ascites formation	Domenicali et al. (2009)
CB2 receptor activation	
• ↓ Oxidative stress • ↓ Inflammatory cell infiltration • ↓ TNF-α	Bátkai et al. (2007a)
• ↓ Adhesion of human neutrophils to sinusoidal endothelial cells	Bátkai et al. (2007a); Rajesh et al. (2007)
• ↓ Inflammatory infiltrate • ↓ Activated HSCs • ↓ Hepatic collagen • ↓ Liver fibrosis	Muñoz-Luque et al. (2008)
• ↓ Caspase 3 activity • ↓ Lipid peroxidation • ↓ Neutrophil infiltration • ↓ TNF-α	Rajesh et al. (2007)
• ↑ Hepatocyte steatosis	De Gottardi et al. (2010)

TABLE 53.5 (*cont.*)

CB1 receptor activation	References
• ↓ Liver injury induced • ↑ Liver regeneration	Teixeira-Clerc et al. (2010)
• ↓ Hepatocyte steatosis • ↓ NF-κB activity • ↑ Hepatic expression of heme oxygenase-1 • Induced heme oxygenase-1 expression in cultured macrophages	Louvet et al. (2011)
• ↓ Oxidative stress • ↓ Neutrophil infiltration • ↓ TNF-α • ↓Inter-cellular adhesion molecule-1	Horváth et al. (2012)
• ↓ Portal venous pressure	Huang et al. (2012)
• ↓ Portal venous pressure • ↓ Kupffer cell activation	Steib et al. (2013)

↑, increase; ↓, decrease; HSCs, hepatic stellate cells; NF-κB, nuclear factor kappa-B; TNF-α, tumor necrosis factor-alpha, TGF-β1, hepatic transforming growth factor-beta1.

MINI-DICTIONARY

Ascites The accumulation of fluid within the abdominal cavity in liver cirrhosis.
Autoimmune hepatitis Liver damage caused by perturbed immunoregulation resulting in autoimmune attack on liver.
Cirrhosis The formation of diffuse fibrosis and regenerative nodules giving the liver nodular, irregular appearance. It is the end stage of different disease processes affecting the liver.
Hepatic stellate cell The cell which derives hepatic wound healing and fibrosis. In the normal human liver, stellate cells store vitamin A. In response to oxidative stress and various inflammatory signals, these cells become activated, and synthesize and secrete extracellular matrix proteins.
Kupffer cell The resident immune cell of the liver. Kupffer cell activation during liver injury is an important step in progression of liver tissue damage.
Steatosis The accumulation of lipid droplets in liver cells.
Sustained virological response A measure of virus eradication. It is the absence of virus nucleic acid in serum at the end of treatment and 6 months later.

References

Abdel-Salam, O. M. E., Youness, E. R., & Shaffee, N. (2014). Biochemical, immunological, DNA and histopathological changes caused by *Cannabis sativa* in the rat. *Journal of Neurology and Epidemiology*, *2*, 6–16.

Abdel-Salam, O. M. E., Metwaly, S., Sleem, A. A., Morsy, F. A., & Sharaf, H. A. (2013). Cannabis sativa exacerbates hepatic injury caused by acetaminophen or carbon tetrachloride in rats. *Comparative Clinical Pathology*, *22*, 209–218.

Bátkai, S., Mukhopadhyay, P., Harvey-White, J., Kechrid, R., Pacher, P., & Kunos, G. (2007a). Endocannabinoids acting at CB1 receptors mediate the cardiac contractile dysfunction in vivo in cirrhotic rats. *American Journal of Physiology*, *293*, H1689–H1695.

Bátkai, S., Osei-Hyiaman, D., Pan, H., El-Assal, O., Rajesh, M., Mukhopadhyay, P., Hong, F., Harvey-White, J., Jafri, A., Haskó, G., Huffman, J. W., Gao, B., Kunos, G., & Pacher, P. (2007b). Cannabinoid-2 receptor mediates protection against hepatic ischemia/reperfusion injury. *FASEB Journal*, *21*, 1788–1800.

Bátkai, S., Mukhopadhyay, P., Horváth, B., Rajesh, M., Gao, R. Y., Mahadevan, A., Amere, M., Battista, N., Lichtman, A. H., Gauson, L. A., Maccarrone, M., Pertwee, R. G., & Pacher, P. (2012). Δ8-Tetrahydrocannabivarin prevents hepatic ischaemia/reperfusion injury by decreasing oxidative stress and inflammatory responses through cannabinoid CB2 receptors. *British Journal of Pharmacology*, *165*, 2450–2461.

Borini, P., Guimarães, R. C., & Borini, S. B. (2004). Possible hepatotoxicity of chronic marijuana usage. *Sao Paulo Medical Journal*, *122*, 110–116.

Brunet, L., Moodie, E. E. M., Rollet, K., Cooper, C., Walmsley, S., Potter, M., & Klein, M. B. (2013). Marijuana smoking does not accelerate progression of liver disease in HIV–hepatitis C coinfection: a longitudinal cohort analysis. *Clinical Infectious Diseases Advance*, *57*(5), 663–670.

Cao, Z., Mulvihill, M. M., Mukhopadhyay, P., Xu, H., Erdélyi, K., Hao, E., Holovac, E., Haskó, G., Cravatt, B. F., Nomura, D. K., & Pacher, P. (2013). Monoacylglycerol lipase controls endocannabinoid and eicosanoid signaling and hepatic injury in mice. *Gastroenterology*, *144*, 808.e15–817.e15.

Chen, S. W., Wu, B. Y., Xu, S. P., Fan, K. X., Yan, L., Gong, Y., Wen, J. B., & Wu, D. H. (2012). Suppression of CB1 cannabinoid receptor by lentivirus mediated small interfering RNA ameliorates hepatic fibrosis in rats. *PLoS One*, *7*, e50850.

Cholet, F., Nousbaum, J. B., Richecoeur, M., Oger, E., Cauvin, J. M., Lagarde, N., Robaszkiewicz, M., & Gouarou, H. (2004). Factors associated with liver steatosis and fibrosis in chronic hepatitis C patients. *Gastroenterology and Clinical Biology*, *28*, 272–278.

Coffman, K. L. (2008). The debate about marijuana usage in transplant candidates: recent medical evidence on marijuana health effects. *Current Opinion in Organ Transplantation*, *13*, 189–195.

De Gottardi, A., Spahr, L., Ravier-Dall'Antonia, F., & Hadengue, A. (2010). Cannabinoid receptor 1 and 2 agonists increase lipid accumulation in hepatocytes. *Liver International*, *30*, 1482–1489.

Domenicali, M. l., Caraceni, P., Giannone, F., Pertosa, A. M., Principe, A., Zambruni, A., Trevisani, F., Croci, T., & Bernardi, M. (2009). Cannabinoid type 1 receptor antagonism delays ascites formation in rats with cirrhosis. *Gastroenterology*, *137*, 341–349.

ElSohly, M. A., & Slade, D. (2005). Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life Sciences*, *78*, 539–548.

Fernández-Rodriguez, C. M., Romero, J., Petros, T. J., Bradshaw, H., Gasalla, J. M., Gutiérrez, M. L., Lledó, J. L., Santander, C., Fernández, T. P., Tomás, E., Cacho, G., & Walker, J. M. (2004). Circulating endogenous cannabinoid anandamide and portal, systemic and renal hemodynamics in cirrhosis. *Liver International*, *24*, 477–483.

Floreani, A., Lazzari, R., Macchi, V., Porzionato, A., Variola, A., Colavito, D., Leon, A., GuidoM, Baldo, V., De Caro, R., & Bergasa, N. V. (2010). Hepatic expression of endocannabinoid receptors and their novel polymorphisms in primary biliary cirrhosis. *Journal of Gastroenterology*, *45*, 68–76.

Garcia, N., Jr., Járai, Z., Mirshahi, F., Kunos, G., & Sanyal, A. J. (2001). Systemic and portal hemodynamic effects of anandamide. *American Journal of Physiology*, *280*, G14–G20.

Gaskari, S. A., Liu, H., Moezi, L., Li, Y., Baik, S. K., & Lee, S. S. (2005). Role of endocannabinoids in the pathogenesis of cirrhotic cardiomyopathy in bile duct-ligated rats. *British Journal of Pharmacology*, *146*, 315–323.

Giannone, F. A., Baldassarre, M., Domenicali, M., Zaccherini, G., Trevisani, F., Bernardi, M., & Caraceni, P. (2012). Reversal of liver fibrosis by the antagonism of endocannabinoid CB1 receptor in a rat model of CCl(4)-induced advanced cirrhosis. *Laboratory Investigations*, *92*, 384–395.

Hegde, V. L., Nagarkatti, P. S., & Nagarkatti, M. (2011). Role of myeloid-derived suppressor cells in amelioration of experimental autoimmune hepatitis following activation of TRPV1 receptors by cannabidiol. *PLoS One*, *6*, e18281.

Hegde, V. L., Hegde, S., Cravatt, B. F., Hofseth, L. J., Nagarkatti, M., & Nagarkatti, P. S. (2008). Attenuation of experimental autoimmune hepatitis by exogenous and endogenous cannabinoids: involvement of regulatory T cells. *Molecular Pharmacology*, *74*, 20–33.

Hézode, C., Roudot-Thoraval, F., Nguyen, S., Grenard, P., Julien, B., Zafrani, E. S., Pawlotsky, J. M., Dhumeaux, D., Lotersztajn, S., & Mallat, A. (2005). Daily cannabis smoking as a risk factor for progression of fibrosis in chronic hepatitis C. *Hepatology*, *42*, 63–71.

Hézode, C., Zafrani, E. S., Roudot-Thoraval, F., Costentin, C., Hessami, A., Bouvier-Alias, M., Medkour, F., Pawlostky, J. M., Lotersztajn, S., & Mallat, A. (2008). Daily cannabis use: a novel risk factor of steatosis severity in patients with chronic hepatitis C. *Gastroenterology*, *134*, 432–439.

Hoi, P. M., & Hiley, C. R. (2006). Vasorelaxant effects of oleamide in rat small mesenteric artery indicate action at a novel cannabinoid receptor. *British Journal of Pharmacology*, *147*, 560–568.

Horváth, B., Magid, L., Mukhopadhyay, P., Bátkai, S., Rajesh, M., Park, O., Tanchian, G., Gao, R. Y., Goodfellow, C. E., Glass, M., Mechoulam, R., & Pacher, P. (2012). A new cannabinoid CB2 receptor agonist HU-910 attenuates oxidative stress, inflammation and cell death associated with hepatic ischaemia/reperfusion injury. *British Journal of Pharmacology*, *165*, 2462–3478.

Huang, H. C., Wang, S. S., Hsin, I. F., Chang, C. C., Lee, F. Y., Lin, H. C., Chuang, C. L., Lee, J. Y., Hsieh, H. G., & Lee, S. D. (2012). Cannabinoid receptor 2 agonist ameliorates mesenteric angiogenesis and portosystemic collaterals in cirrhotic rats. *Hepatology*, *56*, 248–258.

Ishida, J. H., Peters, M. G., Jin, C., Louie, K., Tan, V., Bacchetti, P., & Terrault, N. A. (2008). Influence of cannabis use on severity of hepatitis C disease. *Clinical Gastroenterology and Hepatology*, *6*, 69–75.

Jeong, W. I., Osei-Hyiaman, D., Park, O., Liu, J., Bátkai, S., Mukhopadhyay, P., Horiguchi, N., Harvey-White, J., Marsicano, G., Lutz, B., Gao, B., & Kunos, G. (2008). Paracrine activation of hepatic CB1 receptors by stellate cell-derived endocannabinoids mediates alcoholic fatty liver. *Cell Metabolism*, *7*, 227–235.

Jourdan, T., Djaouti, L., Demizieux, L., Gresti, J., Vergès, B., & Degrace, P. (2010). CB1 antagonism exerts specific molecular effects on visceral and subcutaneous fat and reverses liver steatosis in diet-induced obese mice. *Diabetes*, *59*, 926–934.

Julien, B., Grenard, P., Teixeira-Clerc, F., Van Nhieu, J. T., Li, L., Karsak, M., Zimmer, A., Mallat, A., & Lotersztajn, S. (2005). Antifibrogenic role of the cannabinoid receptor CB2 in the liver. *Gastroenterology*, *128*, 742–755.

Lim, M. P., Devi, L. A., & Rozenfeld, R. (2011). Cannabidiol causes activated hepatic stellate cell death through a mechanism of endoplasmic reticulum stress-induced apoptosis. *Cell Death and Disease*, *2*, e170.

Louvet, A., Teixeira-Clerc, F., le Chobert, M. -N., Deveaux, V., Pavoine, C., Zimmer, A., Pecker, F., Mallat, A., & Lotersztajn, S. (2011). Cannabinoid CB2 receptors protect against alcoholic liver disease by regulating Kupffer cell polarization in mice. *Hepatology*, *54*, 1217–2122.

Mechoulam, R., & Gaoni, Y. (1967). The absolute configuration of delta-1-tetrahydrocannabinol, the major active constituent of hashish. *Tetrahedron Letters*, *12*, 1109–1111.

McGuire, B. M., Rosenthal, P., Brown, C. C., Busch, A. M. H., et al. (2009). Long-term management of the liver transplant patient: recommendations for the primary care doctor. *American Journal of Transplantation*, *9*, 1988–2003.

McPartland, J. M., & Russo, E. B. (2001). Cannabis and cannabis extracts: greater than the sum of their parts? *Journal of Cannabis Therapeutics*, *3/4*, 103–132.

Mukhopadhyay, P., Rajesh, M., Horváth, B., Bátkai, S., Park, O., Tanchian, G., Gao, R. Y., Patel, V., Wink, D. A., Liaudet, L., Haskó, G., Mechoulam, R., & Pacher, P. (2011). Cannabidiol protects against hepatic ischemia/reperfusion injury by attenuating inflammatory signaling and response, oxidative/nitrative stress, and cell death. *Free Radical Biology and Medicine*, *50*, 1368–1381.

Muñoz-Luque, J., Ros, J., Fernández-Varo, G., Tugues, S., Morales-Ruiz, M., Alvarez, C. E., Friedman, S. L., Arroyo, V., & Jiménez, W. (2008). Regression of fibrosis after chronic stimulation of cannabinoid CB2 receptor in cirrhotic rats. *Journal of Pharmacology and Experimental Therapeutics*, *324*, 475–483.

Neff, G. W., O'Brien, C. B., Reddy, K. R., Bergasa, N. V., Regev, A., Molina, E., Amaro, R., Rodriguez, M. J., Chase, V., Jeffers, L., & Schiff, E. (2002). Preliminary observation with dronabinol in patients with intractable pruritus secondary to cholestatic liver disease. *American Journal of Gastroenterology*, *97*, 2117–2119.

Osei-Hyiaman, D., DePetrillo, M., Pacher, P., Liu, J., Radaeva, S., Bátkai, S., Harvey-White, J., Mackie, K., Offertáler, L., Wang, L., & Kunos, G. (2005). Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *Journal of Clinical Investigations*, *115*, 1298–1305.

Patsenker, E., Stoll, M., Millonig, G., Agaimy, A., Wissniowski, T., Schneider, V., Mueller, S., Brenneisen, R., Seitz, H. K., Ocker, M., & Stickel, F. (2011). Cannabinoid receptor type I modulates alcohol-induced liver fibrosis. *Molecular Medicine*, *17*, 1285–1294.

Rajesh, M., Pan, H., Mukhopadhyay, P., Bátkai, S., Osei-Hyiaman, D., Haskó, G., Liaudet, L., Gao, B., & Pacher, P. (2007). Cannabinoid-2 receptor agonist HU-308 protects against hepatic ischemia/reperfusion injury by attenuating oxidative stress, inflammatory response, and apoptosis. *Journal of Leukocyte Biology*, *82*, 1382–1389.

Ranney, D. N., Acker, W. B., Al-Holou, S. N., Ehrlichman, L., Lee, D. S., Lewin, S. A., Nguyen, C., Peterson, S. F., Sell, K., Kubus, J., Reid, D., & Englesbe, M. J. (2009). Marijuana use in potential liver transplant candidates. *American Journal of Transplantation*, *9*, 280–285.

Ros, J., Clària, J., To-Figueras, J., Planagumà, A., Cejudo-Martín, P., Fernández-Varo, G., Martín-Ruiz, R., Arroyo, V., Rivera, F., Rodés, J., & Jiménez, W. (2002). Endogenous cannabinoids: a new system involved in the homeostasis of arterial pressure in experimental cirrhosis in the rat. *Gastroenterology*, *122*, 85–93.

Schwabe, R. F., & Siegmund, S. V. (2005). Potential role of CB2 receptors in cannabis smokers with chronic hepatitis C. *Hepatology*, *42*, 975–976.

Siegmund, S. V., Uchinami, H., Osawa, Y., Brenner, D. A., & Schwabe, R. F. (2005). Anandamide induces necrosis in primary hepatic stellate cells. *Hepatology*, *41*, 1085–1095.

Siegmund, S. V., Wojtalla, A., Schlosser, M., Zimmer, A., & Singer, M. V. (2013). Fatty acid amide hydrolase but not monoacyl glycerol lipase controls cell death induced by the endocannabinoid 2-arachidonoyl glycerol in hepatic cell populations. *Biochemical and Biophysical Research Communications*, *437*, 48–54.

Siegmund, S. V., Qian, T., de Minicis, S., Harvey-White, J., Kunos, G., Vinod, K. Y., Hungund, B., & Schwabe, R. F. (2007). The endocannabinoid 2-arachidonoyl glycerol induces death of hepatic stellate cells via mitochondrial reactive oxygen species. *FASEB Journal*, *21*, 2798–2806.

Sleijfer, S., Bannink, M., Van Gool, A. R., Kruit, W. H., & Stoter, G. (2005). Side effects of interferon-alpha therapy. *Pharmacy World and Science*, *27*, 423–431.

Steib, C. J., Gmelin, L., Pfeiler, S., Schewe, J., Brand, S., Göke, B., & Gerbes, A. L. (2013). Functional relevance of the cannabinoid receptor 2—heme oxygenase pathway: a novel target for the attenuation of portal hypertension. *Life Sciences*, *93*, 543–551.

Sylvestre, D. L., Clements, B. J., & Malibu, Y. (2006). Cannabis use improves retention and virological outcomes in patients treated for hepatitis C. *European Journal of Gastroenterology and Hepatology*, *18*, 1057–1063.

Teixeira-Clerc, F., Belot, M. P., Manin, S., Deveaux, V., Cadoudal, T., Chobert, M. N., Louvet, A., Zimmer, A., Tordjmann, T., Mallat,

A., & Lotersztajn, S. (2010). Beneficial paracrine effects of cannabinoid receptor 2 on liver injury and regeneration. *Hepatology*, *52*, 1046–1059.

Teixeira-Clerc, F., Julien, B., Grenard, P., Tran Van Nhieu, J., Deveaux, V., Li, L., Serriere-Lanneau, V., Ledent, C., Mallat, A., & Lotersztajn, S. (2006). CB1 cannabinoid receptor antagonism: a new strategy for the treatment of liver fibrosis. *Nature Medicine*, *12*, 671–676.

Toson, E (2011). Impact of marijuana smoking on liver and sex hormones: correlation with oxidative stress. *Nature and Science*, *9*, 76–87.

Trebicka, J., Racz, I., Siegmund, S. V., Cara, E., Granzow, M., Schierwagen, R., Klein, S., Wojtalla, A., Hennenberg, M., Huss, S., Fischer, H. -P., Heller, J., Zimmer, A., & Sauerbruch, T. (2011). Role of cannabinoid receptors in alcoholic hepatic injury: steatosis and fibrogenesis are increased in CB2 receptor-deficient mice and decreased in CB1 receptor knockouts. *Liver International*, *31*, 860–870.

White, R., Ho, W. S., Bottrill, F. E., Ford, W. R., & Hiley, C. R. (2001). Mechanisms of anandamide-induced vasorelaxation in rat isolated coronary arteries. *British Journal of Pharmacology*, *134*, 921–929.

Whitfield, R. M., Bechtel, L. M., & Starich, G. H. (1997). The impact of ethanol and Marinol/marijuana usage on HIV + /AIDS patients undergoing azidothymidine, azidothymidine/dideoxycytidine, or dideoxyinosine therapy. *Alcoholism: Clinical and Experimental Research*, *21*, 122–127.

Wojtalla, A., Herweck, F., Granzow, M., Klein, S., Trebicka, J., Huss, S., Lerner, R., Lutz, B., Schildberg, F. A., Knolle, P. A., Sauerbruch, T., Singer, M. V., Zimmer, A., & Siegmund, S. V. (2012). The endocannabinoid N-arachidonoyl dopamine (NADA) selectively induces oxidative stress-mediated cell death in hepatic stellate cells but not in hepatocytes. *American Journal of Physiology*, *302*, G873–G887.

Yang, Y. Y., Lin, H. C., Huang, Y. T., Lee, T. Y., Hou, M. C., Wang, Y. W., Lee, F. Y., & Lee, S. D. (2006). Roles of anandamide in the hepatic microcirculation in cirrhotic rats. *American Journal of Physiology*, *290*, G328–G334.

Yang, Y. Y., Lin, H. C., Huang, Y. T., Lee, T. Y., Hou, M. C., Wang, Y. W., Lee, F. Y., & Lee, S. D. (2007a). Role of Ca2+-dependent potassium channels in in vitro anandamide-mediated mesenteric vasorelaxation in rats with biliary cirrhosis. *Liver International*, *27*, 1045–1055.

Yang, Y. Y., Lin, H. C., Huang, Y. T., Lee, T. Y., Hou, M. C., Wang, Y. W., Lee, F. Y., & Lee, S. D. (2007b). Effect of chronic CB1 cannabinoid receptor antagonism on livers of rats with biliary cirrhosis. *Clinical Science*, *112*, 533–542.

Zygmunt, P. M., Petersson, J., Andersson, D. A., Chuang, H., Sørgård, M., Di Marzo, V., Julius, D., & Högestätt, E. D. (1999). Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature*, *400*, 452–457.

CHAPTER

54

Cannabis Allergy: More Than a Bad Trip

A.L. Van Gasse, V. Sabato, M.M. Faber, C.H. Bridts, D.G. Ebo

Faculty of Medicine and Health Science, Department of Immunology-Allergology-Rheumatology, University of Antwerp, Antwerp, Belgium

SUMMARY POINTS

- An allergy is defined as an immune-pathological, mostly IgE-mediated, reaction that ultimately results into a hypersensitivity reaction toward one or more foreign compounds, that is, an allergen.
- The exact prevalence of cannabis allergy is not known, it seems to be an increasing problem in the past few years.
- Active use, as well as passive exposure to cannabis could lead to sensitization to cannabis allergens, and eventually to cannabis allergy if this sensitization is of clinical relevance.
- The clinical manifestations of cannabis allergy can vary from mild to life-threatening reactions, often depending on the route of exposure.
- Sensitization to cannabis could also lead to secondary allergies, such as allergies to fruit and vegetables, designated as the "cannabis-fruit/vegetable syndrome," but also to *Hevea* latex, cereals, tobacco, and alcoholic beverages, such as beer and wine.
- At present, diagnosis generally rests upon history, skin testing, and an IgE test with an industrial hemp variety.
- Recently, the basophil activation test (BAT) has been proven to be a reliable additional diagnostic.
- For the time being, there is no cure for cannabis-related allergies. Strict avoidance of cannabis and other allergens implicated in the individual cross-reactivity syndrome is imperative.

KEY FACTS OF CONTACT DERMATITIS

- Contact dermatitis is a delayed hypersensitivity reaction mediated by T cells, instead of antibodies (Coombs & Gell, 1963).
- Although cannabis related-allergies are generally IgE-mediated, a delayed T cell mediated reaction is also possible (Williams et al., 2008).
- Contact dermatitis is characterized by a local inflammation of the skin due to skin contact with a substance originating from outside the body, for example, an allergen or irritant.
- A red and itchy rash can be seen as clinical manifestation.
- Diagnosis of delayed hypersensitivity reactions is made by placing the contact allergens on the surface of the skin, and subsequently evaluating the skin after 24, 48, and 72 h; a method known as a patch test.
- The treatment comprises strict avoidance of skin contact with the culprit allergens, but when the contact dermatitis had already arisen, local corticosteroid creams can be used.

LIST OF ABBREVIATIONS

Act d	*Actinidia deliciosa* (kiwi fruit)
Ara h	*Arachis hypogaea* (peanut)
ATP	Adenosine triphosphate
BAT	Basophil activation test
Bet v	*Betula verrucosa* (birch)
Can s	*Cannabis sativa*
CCD	Cross-reactive carbohydrate determinants
Cit s	*Citrus sinensis* (orange)

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00063-6
Copyright © 2017 Elsevier Inc. All rights reserved.

Cor a	*Corylus avellana* (hazelnut)
CRD	Component resolved diagnosis
Cup a	*Cupressus arizonica* (cypress native to the southwest of north America)
DC	Dendritic cell (professional antigen presenting cell)
FEIA	Fluorescent enzyme immunoassay
Hev b	*Hevea brasiliensis* (natural rubber tree)
IL	Interleukin
Lyc e	*Lycopersicom esculentum* (tomato)
Mal d	*Malus domestica* (apple)
MALT	Mucosa associated lymphoid tissue
Mus a	*Musa acuminate* (banana)
Ns-LTP	Nonspecific lipid transfer protein
OAS	Oral allergy syndrome
Phl p	*Phleum pratense* (Timothy grass)
PR	Pathogenesis-related
Pru av	*Prunus avium* (cherry)
Pru p	*Prunus persica* (peach)
RuBisCo	Ribulose-1,5-biphosphate carboxylase/oxygenase
sIgE	Specific immunoglobulin E
Th	T helper
TLP	Thaumatin-like protein
Tri a	*Triticum aestivum* (wheat)
Vit v	*Vitis vinifera* (grape)

INTRODUCTION

The main function of the immune system is to provide defense against pathogenic microorganisms and toxins. Although immune responses are regulated in order to avoid excessive responses, and development of autoimmune conditions, potential harmful immune-pathologic reactions can occur. "Allergy" is a typical example of such an immune-pathological reaction in which an undesired immune response is mounted against a harmless foreign compound.

In the literature, allergies to food, aeroallergens, Hymenoptera venoms, and medicines are well described. In contrast, mainly by their illegal nature, little is known about allergy to illicit drugs and narcotics (Anselme, Metz-Favre, & de Blay, 2011; Swerts et al., 2014). This chapter focuses on the current knowledge and management of cannabis-related allergies.

THE ALLERGIC REACTION

An "allergy" is defined as an immune-pathological reaction that results into a hypersensitivity reaction to one or more foreign compounds, that is, an allergen. According to the underlying humoral or cellular pathomechanisms and clinical manifestations, allergic reactions are classified in four classes, as initially described by Coombs and Gell (1963). Describing these immune pathologic reactions is beyond the scope of this review that focuses on IgE-dependent cannabis-related allergies.

THE IgE-MEDIATED REACTION

Principles

Primary IgE-Mediated Allergy

Exposure to foreign proteins or products (antigens and allergens) predominantly occurs via skin and mucosae, where immunologic and nonimmunologic mechanisms prevent allergens entering the body and/or eliciting immune responses. However, some allergenic components evade these surveillance mechanisms and will encounter resident specialized antigen-presenting dendritic cell (DC) populations. After uptake of allergens, these DCs migrate to the T-cell zones of secondary lymphoid organs (eg, lymph nodes, mucosa associated lymphoid tissue, and spleen) and present antigenic peptides to naïve T helper (Th)-cells, that will proliferate and polarize into interleukin (IL)-4/IL-13 producing Th2 cells (Fig. 54.1A). These Th2 cytokines trigger immunoglobulin isotype switching, and the synthesis of allergen-specific IgE (sIgE) antibodies by B cells and plasma cells. These sIgE antibodies bind to effector cells, mainly tissue-resident mast cells and peripheral blood basophils. Upon re-exposure to the sensitizing allergen, the allergen that triggered the immune system to produce sIgE antibodies can cross-link at least two adjacent sIgE antibodies bound to the surface high-affinity IgE receptor (FcεRI), inducing the effector cells to release a broad variety of mediators, such as histamine, leukotrienes, prostaglandins, and cytokines. These mediators are responsible for the symptoms of the allergic reaction (Table 54.1). Most of the time, allergens triggering IgE-mediated reactions are glycoproteins (Kumar & Bhatia, 2013). Allergens are not recognized as a whole component by the immune system. There are only specific regions of the allergen that elicit the immune response. These specific regions are called B-cell epitopes, as they are complementary to the allergen-binding site of the corresponding specific antibodies (Kumar, Verma, Das, & Dwivedi, 2012).

Secondary IgE-Mediated Allergy (Cross-Reactivity)

Unlike primary IgE-mediated allergy, in which the allergenic determinants responsible for direct sensitization of the immune system and subsequent triggering of effector cells are the same, secondary IgE-mediated allergies are acquired indirectly, that is, via immunologic cross-reactivity. This immunologic cross-reactivity occurs when an antigen (or allergen) triggers the production

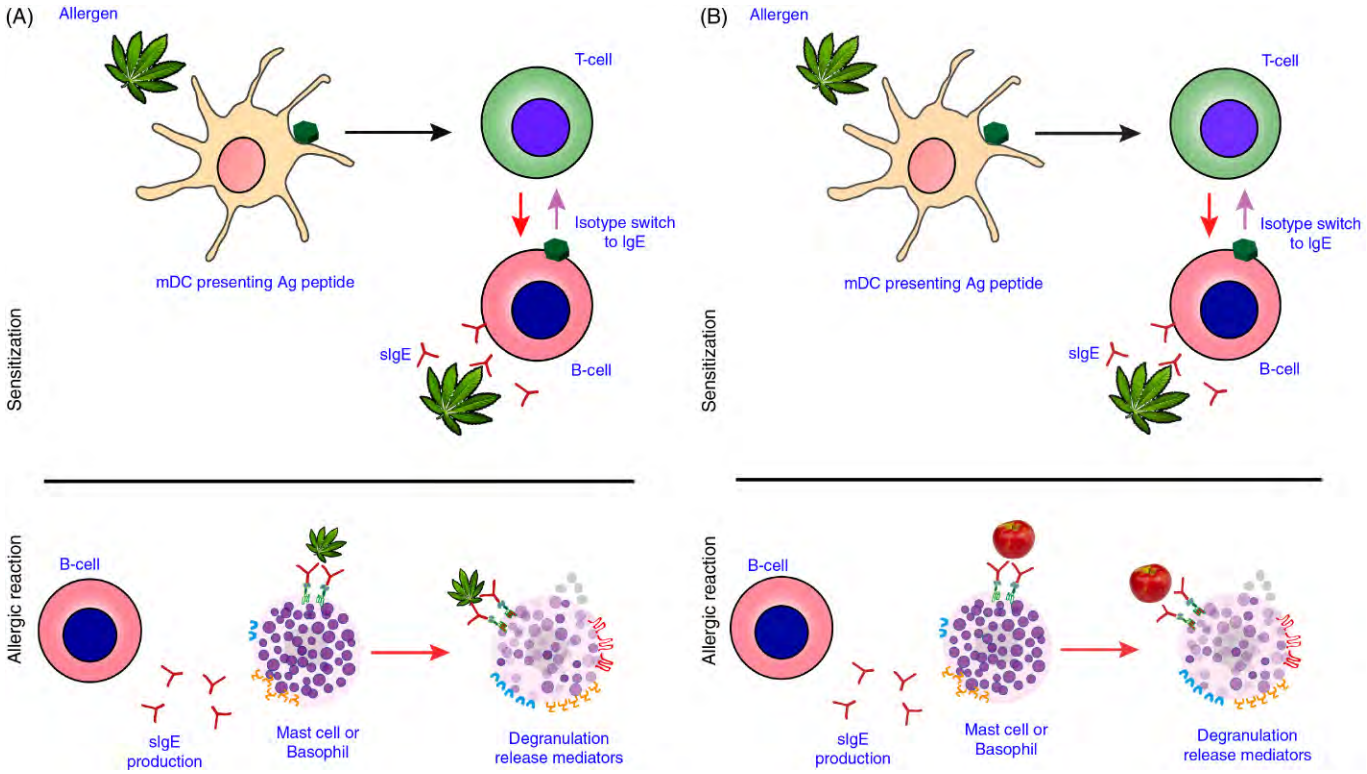


FIGURE 54.1 (A) Pathogenesis of a primary (genuine) IgE-mediated allergy against cannabis. The epitopes responsible for sensitization and subsequent triggering of the effector cells, that is, basophils and mast cells, are identical. (B) Pathogenesis of a secondary IgE-mediated (cross-) allergy. The allergenic determinants responsible for initial sensitization are different from the allergenic determinants that trigger mast cells and basophils. In this particular example, the sensitizing allergen is cannabis, the cross-reacting allergen is apple.

TABLE 54.1 IgE-Mediated Allergy: Clinical Manifestations

Skin	Pruritus (itching) Erythema Nonspecific rash Wheals Urticaria Angioedema
Eyes	Conjunctivitis (tearing, itching, redness)
Nose	Rhinitis (runny nose, blocked nose, sneezing, itching)
Bronchial–pulmonary	Laryngeal edema Bronchospasm Cough Tachypnea
Intestinal	Cramps Nausea, vomiting Diarrhea
Cardiovascular	Tachycardia Hypotension (collapse) Shock
Death	

of sIgE antibodies that can bind to structural similar or identical epitopes and components present in allergens originating from taxonomically related and distant sources (Fig. 54.1B). Noticeably, these cross-reactive antibodies are not of clinical relevance per se, that is, induce cross-allergy between the allergens originating from the various sources. Depending on the presence or absence of clinical manifestations, cross-reactive allergenic components can roughly be divided into three groups.

A first group of cross-reactive components comprises structures and epitopes frequently having clinical significance. This means that these allergenic components often trigger a cross-allergy. In our regions, the best-known example of cross-allergy is the birch pollen-related food syndrome (Ebo & Stevens, 2001). In this syndrome, patients are initially sensitized to Bet v 1, the major allergenic component from birch pollen (*Betula verrucosa*), and can subsequently display a broad cross-allergy to plant-derived foods, mainly pit and stone fruit, tree nuts, soy, and carrot. In this birch pollen-related food syndrome, along with their respiratory symptoms upon exposure to birch pollen, these patients will complain of symptoms in the oral cavity and throat, also known as an oral allergy syndrome (OAS) after ingestion of the different

foods involved. Another example, predominantly observed in southern Europe, is the "ns-LTP syndrome" governed by peach. In this syndrome, sensitization to Pru p 3, the nonspecific lipid transfer protein (ns-LTP) of peach (*Prunus persica*), entails the risk for severe allergic reactions, such as anaphylaxis, to multiple plant-derived foods (Van Winkle & Chang, 2014).

A second group of cross-reactive components includes allergenic structures that seldom evoke clinical manifestations, such as profilins (Ebo, Hagendorens, Bridts, De Clerck, & Stevens, 2004). This means that patients sensitized to profilins will predominantly display a broad in vitro cross-reactivity, but only a minority of these patients will present a clinically relevant cross-allergy. The most prevalent profilin-related cross-allergies are allergies to banana and melon, secondary to grass pollen allergy.

Finally, a third group of cross-reactive components comprises the cross-reactive carbohydrate determinants (CCD). These immunogenic, nonmammalian carbohydrate moieties are ubiquitous in plant kingdom and invertebrates (eg, hymenoptera venom), but sIgE antibodies raised against CCD are generally believed not to cause clinical symptoms (Ebo et al., 2004; Ebo et al., 2002).

CANNABIS ALLERGY

Prevalence

Allergic reactions to certain members of the *Cannabaceae* family, such as industrial hemp and hop (*Humulus lupulus*) have repeatedly been described (Herzinger, Schopf, Przybilla, & Rueff, 2011; Spiewak, Gora, & Dutkiewicz, 2001; Williams, Thompstone, & Wilkinson, 2008). In contrast, reports of IgE-mediated reactions to cannabis remain rare. A possible explanation for this low number of reports probably relates to the illegal status of cannabis use, which makes the patients reluctant to report their abuse and associated allergic symptoms (Nayak et al., 2013). Today, no information is available about the prevalence of cannabis allergy. However, it seems to be an increasing problem since a few years (Van Gasse, Sabato, Bridts, & Ebo, 2014).

Cannabis Allergens

To date, the allergenic composition of *Cannabis sativa* (Can s), the plant from which proteins can be extracted, remains relatively elusive (Nayak et al., 2013). Larramendi et al. (2013) found that six different bands with a molecular weight of 10, 14, 20, 35, 38, and 60 kDa were recognized by the individual patients' sera. The 10-kDa IgE-binding band was already described in other reports (de Larramendi et al., 2008; Gamboa et al., 2007; Tanaka, Degawa, Kawata, Hayashi, & Shoyama, 1998), and probably corresponds to Can s 3, the nonspecific lipid transfer protein (ns-LTP) of *C. sativa* (Gamboa et al., 2007) that belongs to the pathogenesis-related proteins (PR)-14 group. Pathogenesis-related proteins are plant host proteins induced specifically in response to pathological situations, such as an infection by fungi, bacteria, viruses, and other means of stress (Van Loon, 1999). The 38-kDa band corresponds with a thaumatin-like protein (TLP) that belongs to the PR-5 family (Larramendi et al., 2013). Although in the study of Larramendi et al. (2013) no homology was found between the 14-kDa band and any known allergen, this band probably corresponds with a profilin (de Larramendi et al., 2008).

In an American study of Nayak et al. (2013), 23 kDa and 50 kDa seem to be the most prominent bands. The 23-kDa band corresponds with "oxygen-evolving enhancer protein 2," an enzyme involved in the photosynthesis. The 50-kDa band corresponds with the heavy chain subunit of ribulose-1,5-biphosphate carboxylase/oxygenase. Other possible allergens identified by Nayak et al. (2013) are glyceraldehyde-3-phosphate and adenosine triphosphate synthase. Finally, they observed that CCDs might be the cause of some IgE reactivity. Unlike the European studies, in this American study no ns-LTP sequences were identifiable, and no IgE reactivity to this panallergen was demonstrable, even though IgE reactivity at approximately 10 kDa was observed in two patients. In contrast to the European series, most of the American patients did not suffer from a cannabis-related cross-reactivity syndrome as is described later. The most important cannabis allergens are displayed in Table 54.2.

Routes of Exposure/Sensitization

Possible ways of exposure and sensitization to allergens are so called *routes of exposure*. Regarding cannabis, multiple routes of exposure/sensitization might exist.

First, people may be exposed to cannabis through active use, such as smoking, vaporizing, chewing, oral ingestion, or intravenous use of the drug. Second, cutaneous contact could be a possible route. This could be important in cannabis growers, for example. An alternative route of exposure and sensitization could be passive by proxy when allergens become airborne or are transferred via contact. Therefore, children can also be exposed to cannabis allergens, for example, through parental smoking (Ebo et al., 2013).

Finally, it should be kept in mind that *C. sativa* is an anemophilous plant that produces wind-borne pollen, in case of a male plant. The pollen can be transported over extreme distances (Swerts et al., 2014). For example, in Nebraska, where industrial *C. sativa* is cultivated, Can s pollen account for 36% of the total pollen count during mid- to late-August (Stokes, Hartel, Ford, & Casale, 2000). In European countries, such as France (Anselme et al., 2011), Spain (Mayoral, Calderon, Cano, & Lombardero, 2008),

TABLE 54.2 Cannabis Allergens

Molecular weight (kDa)	Allergen	Function	Homologs (not exhaustive)	References
9	Can s 3	Ns-LTP (PR-14)	Pru p 3, Mal d 3, Cor a 8, Hev b 12, Ara h 9	de Larramendi et al. (2008); Ebo et al. (2013); Gamboa et al. (2007); Larramendi et al. (2013); Metz-Favre et al. (2011)
14	Profilin	Cytoskeleton	Bet v 2, Phl p 12	de Larramendi et al. (2008); Gamboa et al. (2007)
23	Oxygen-evolving enhancer protein	Photosynthesis		Nayak et al. (2013)
38	TLP (thaumatin-like)	PR-5	Act d 2, Mal d 2, Mus a 4, Pru av 2, Cup a 3	Larramendi et al. (2013)
50	Can s RuBisCo	Photosynthesis		Nayak et al. (2013)

Adapted from Van Gasse et al. (2014).

and Italy (Torre et al., 2007), quite similar observations were made. Noticeably, only female (nonpollinating) plants are cultivated for illicit use. Therefore, it is unlikely that abusers of cannabis, who grow their own plants, are becoming sensitized to marihuana through pollen.

CLINICAL MANIFESTATIONS

Genuine Cannabis Allergy

Symptoms of cannabis allergy can vary from mild to life-threatening reactions that generally relate to the route of exposure.

First, respiratory reactions, such as rhinoconjunctivitis, asthma, and palpebral angioedema have been described. These reactions predominantly occur when cannabis is consumed by smoking or vaporizing (Ebo et al., 2013; Swerts et al., 2014; Van Gasse et al., 2014). Respiratory symptoms may also arise from passive exposure to cannabis smoke, or inhalation of *C. sativa* pollen. However, the latter is unlikely to induce sensitization or trigger symptoms in patients growing their own plants.

Handling of Can s plants may lead to contact urticaria (Majmudar, Azam, & Finch, 2006; Williams et al., 2008) and contact dermatitis (Williams et al., 2008). Nevertheless, urticaria and angioedema may also occasionally be triggered by cannabis allergens that became airborne (Swerts et al., 2014).

At last, anaphylaxis can result from ingestion of hempseed (Stadtmauer, Beyer, Bardina, & Sicherer, 2003) and from drinking marihuana tea (Tessmer et al., 2012).

Cross-Reactivity Syndrome

Patients with IgE-mediated cannabis allergy can display distinct sensitization profiles with sensitization toward Can s 3 to be frequent in Europe and almost absent in the United States. Nonspecific lipid transfer PR-14 proteins are panallergens ubiquitously present throughout the plant kingdom (Egger, Hauser, Mari, Ferreira, & Gadermaier, 2010). Consequently, sensitization to Can s 3 could be an explanation for the high variety of secondary plant-derived food allergies seen in European patients with a cannabis allergy. This (sometimes extensive) cross-reactivity between cannabis and plant-derived food has been described in the study by Ebo et al. (2013), and was recently designated as the "cannabis-fruit/vegetable syndrome" (Van Gasse et al., 2014). In our case-control series (Ebo et al., 2013), 10 out of 12 patients with a documented cannabis allergy were sensitized to different ns-LTPs, including Pru p 3, the ns-LTP of peach (*P. persica*). The food allergies most commonly implicated in the cannabis-fruit/vegetable syndrome were allergies to peach, banana, apple, cherry, nuts, tomato, and occasionally citrus fruits, such as orange and grapefruit. Important to note is that these allergic reactions were more severe than the OAS generally observed in food allergy related to sensitization to Bet v 1(Ebo & Stevens, 2001). This could be explained by the fact that ns-LTPs resist to gastroduodenal proteolysis (Cavatorta et al., 2010; Wijesinha-Bettoni et al., 2010) and thermal processing (Matejkova et al., 2009; Sancho et al., 2005; Scheurer et al., 2004). It should also be kept in mind that, in addition to plant food allergies, sensitization to Can s 3 might also explain cross-reactions to *Hevea* latex (Beezhold et al., 2003; Faber et al., 2015; Rihs et al., 2006), alcoholic beverages such as beer and wine (Asero, Mistrello, Roncarolo, Amato, & van Ree, 2001; Jegou, Douliez, Molle, Boivin, & Marion, 2000) and tobacco (*Nicotinia tabaccum*) (Carnes et al., 2013).

Fig. 54.2 gives a nonexhaustive overview of this cannabis-fruit/vegetable syndrome.

As already exemplified in Table 54.2, thaumatin-like proteins (TLP), belonging to the PR-5 family, constitute

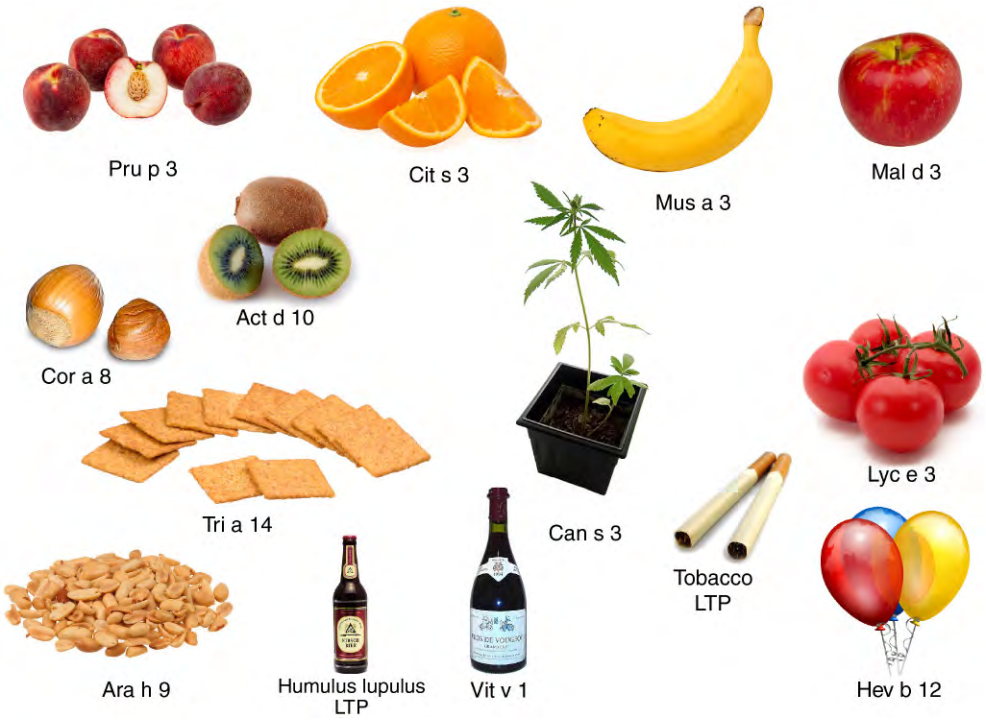


FIGURE 54.2 **The cannabis-fruit/vegetable syndrome and other cross-allergies.** Nonspecific lipid transfer proteins (ns-LTPs) are ubiquitously present in the plant kingdom. Consequently, sensitization to Can s 3, the ns-LTP from *C. sativa*, could lead to a broad variety of cross-reactions. Cross-reactive substances displayed in the figure: cherry (*Prunus avium*), tangerine (*Citrus reticulata*), orange (*Citrus sinensis*), peach (*P. persica*), apple (*M. domestica*), tomato (*Lycopersicom esculentum*), hazelnut (*Corylus avellana*), walnut (*Juglans regia*), banana (*Musa acuminate*), wheat (*Triticum aestivum*), latex (*Hevea brasiliensis*), tobacco and alcoholic beverages, such as wine (grapes: *Vitis vinifera*) and beer (common hop: *Humulus lupulus*). *Source: Adapted from Van Gasse et al. (2014).*

another group of components that might explain cross-reactivity between cannabis and plant-derived food in European patients (Larramendi et al., 2013). These TLPs can be found in pollen and different foods, such as NP24 proteins from tomato (*Solanum lycopersicon*) (Sharma, Singh, Singh, Gaur, & Arora, 2011), Cup a 3 from cypress (*Cupressus arizonica*) (Breiteneder, 2004), Act d 2 from kiwi fruit (*Actinidia deliciosa*) (Bublin et al., 2004), and Mal d 2 from apple (*Malus domestica*) (Hsieh, Moos, & Lin, 1995). Like ns-LTP hitherto, sensitization to TLP has been only observed in south European patients (Larramendi et al., 2013).

DIAGNOSIS

At present, diagnosis of a cannabis allergy rests upon a history, skin testing, and a sIgE antibody test with the industrial hemp variety. However, as will emerge from the following paragraphs, correct diagnosis of cannabis allergy is not always straightforward.

History Taking

Although a thorough anamnesis is essential for correct diagnosis, it appears that history is frequently pieced together from inadequate description and recall by patients reluctant to admit or simply denying illicit drug abuse. In a series of 50 patients with a cannabis allergy who attended our outpatients' clinic, none was consulted because of respiratory or cutaneous symptoms upon exposure to the drug. Actually, all had their diagnosis established because of a plant-derived food and/or vegetable allergy. History might also be misleading because of misinterpretation or misconception of symptoms related to active or passive exposure.

Confirmatory Testing

Skin Prick Test

The principles of skin prick tests (SPT) are displayed in Fig. 54.3. Briefly, a fresh or processed allergen extract is placed on the skin (mostly of the forearm), and pricked with a lancet or similar device. The SPT is considered positive when a wheal with a diameter of at least 3 mm appears. In each test, there also has to be performed a positive (histamine) and negative (saline buffer solution) control to check for skin anergy and dermographism, respectively.

For the time being, sensitization and allergy to cannabis is almost exclusively studied or documented by prick-prick skin testing using a broad variety of raw materials,

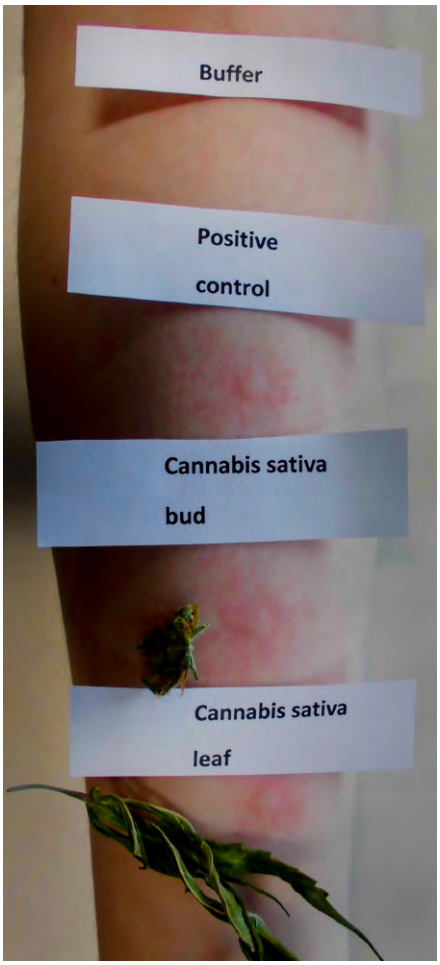


FIGURE 54.3 **Principle of the skin prick test.** Two skin prick tests with *C. sativa* were performed: the first one with the bud and the second one with the leaves of the *C. sativa* plant. In addition, also a positive (histamine) and negative (saline buffer solution) control were performed. The two prick tests with *C. sativa* were positive as a wheal from more than 3 mm appeared.

such as *C. sativa* leaves, buds, and flowers (de Larramendi et al., 2008; Ebo et al., 2013; Gamboa et al., 2007; Larramendi et al., 2013; Metz-Favre, Pauli, Bessot, & De Blay, 2011; Nayak et al., 2013; Stadtmauer et al., 2003; Tessmer et al., 2012) (Figure 54.3). Needless to say that, at this moment, this approach is flawed by many issues, and is virtually impossible to standardize because of unpredictable variations in composition, and potential contaminations with other allergens of the raw material.

Measurement of Total and Allergen-Specific IgE Antibodies

Quantification of total IgE is a prerequisite in order to allow correct interpretation of sIgE, mainly because of unspecific binding to the solid-phase in case of elevated titers. However, quantification of total IgE is not informative for identification of the culprit allergen (Laske, Bunikowski, & Niggemann, 2003).

Today, sIgE is usually measured using the fluorescent enzyme immunoassay ImmunoCAP (Thermofisher Scientific, Uppsala, Sweden). As displayed in Fig. 54.4 (right panels), the antigen (or allergen) of interest is covalently coupled to a solid phase. When sIgE antibodies that recognize this antigen are present in the patients' serum, these antibodies will bind the antigen to form an antigen-antibody immune complex. Subsequently, nonbound sIgE is rinsed away, and an enzyme-conjugated antihuman-IgE antibody is added. This so-called secondary antibody will bind the sIgE-antigen complex. Finally, after washing, a substrate is added. This substrate will be metabolized by the bounded enzyme producing a quantifiable fluorescence. In traditional assays, sIgE results of $\geq$ 0.35 kUA/L are considered positive. However, some issues should be kept in mind for correct interpretation of sIgE results. First, none of the available sIgE assays demonstrates an absolute predictive value. A negative sIgE result does not preclude allergy. Alternatively, a positive sIgE result should always be interpreted with prudence, because it is not per se an indication of the presence of an allergic disease, but can merely reflect cross-sensitization.

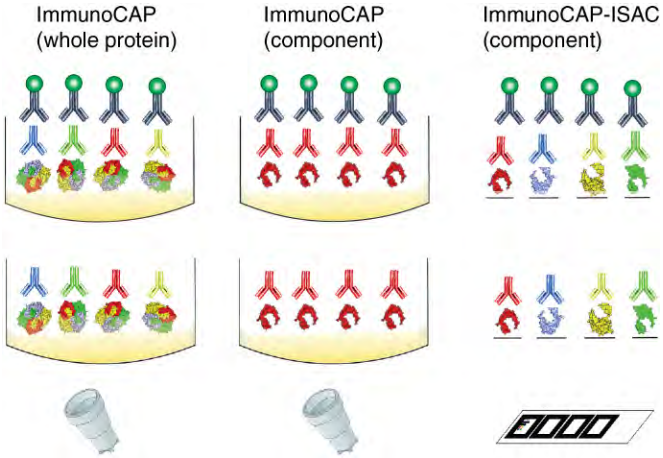


FIGURE 54.4 **Principle of measurement of sIgE.** In traditional ImmunoCAP (left panels), the antigen (a heterogeneous protein extract containing different components) of interest is coupled on a CNBr-activated cellulose solid phase. sIgE antibodies in the patients' serum recognizing the antigen will bind to form a so-called antigen-antibody immune complex. Subsequently, an enzyme-conjugated antihuman-IgE antibody is added. This secondary antibody will bind the antigen-antibody immune complex. Finally, a substrate is added that will be metabolized to produce a quantifiable fluorescence. In component-resolved diagnosis (CRD), two techniques are frequently used. First in a singleplex assay single native or recombinant components (eg, proteins or peptide components) (De Knop et al., 2010; Ebo et al., 2012; Valenta et al., 1999) are coupled to a solid phase as described previously (center panels) and detection of sIgE is exactly the same as in the traditional technique. In the multiplex assay, single native and/or recombinant components are spotted on a polymer coated slide (right panels), sIgE from the patient blood sample bind to the components and these antibodies are detected with a fluorochrome labeled anti-IgE. Finally, the reaction is scanned in a laser scanner and results are evaluated by image analysis software.

This concern relates mainly to the fact that traditional sIgE assays use heterogeneous protein extract-based allergens from different sources. As already exemplified in the section *Secondary IgE-Mediated Allergy*, ubiquitous cross-reactive structures, such as CCD, present on glycoproteins of plants and hymenoptera venom, and profilins present in plant allergens can easily elicit positive, but clinically irrelevant serology with a broad spectrum of cross-reactivity, including *C. sativa* (Ebo et al., 2013). In our series, three out of eight pollen allergic patients, without cannabis allergy, demonstrated a false positive industrial hemp sIgE test (Ebo et al., 2013).

During the last two decades, advances in biochemistry and molecular biology enabled the characterization, cloning, and recombinant production of relevant allergenic components and epitope-emulating peptides, allowing quantification of sIgE antibodies to these components or sequential epitopes; this is a method known as component resolved diagnosis (CRD) (Fig. 54.4, center and left panels). In contrast to traditional sIgE tests, CRD does not rely upon whole extract preparations, but upon single native or recombinant components (eg, proteins or peptide components) (De Knop et al., 2010; Ebo, Hagendorens, Bridts, & Stevens, 2012; Valenta et al., 1999). Briefly, CRD involves unique marker components to study the genuine sensitization of patients toward a particular allergen, and the presence of sIgE antibodies to cross-reactive components (eg, profilins and CCD) that point to cross-reactivity. CRD not only enables to discriminate between genuine allergy and cross-reactivity, but also allows to establish individual predictive sensitization profiles.

Unfortunately, at present, no *C. sativa* components are readily available, but Can s 3 has been cloned recently (Rihs et al., 2014). However, multiplex CRD using the ISAC 112 can help to confirm sensitization toward ns-LTP as an important cause of the cannabis-fruit/vegetable syndrome (Ebo et al., 2013).

Basophil Activation Test

In 1991, the discovery of the basophil degranulation marker, CD63 (Knol, Mul, Jansen, Calafat, & Roos, 1991), induced the development of a novel method to quantify allergen-specific in vitro activated basophils (BAT). This flow cytometric technique relies upon identification and quantification of alterations of particular surface markers such as CD63/CD107a and CD203c (Fig. 54.5). At present, this technique has proven to be a reliable diagnostic aid for various allergies, such as allergy to food, inhalant allergens, medicines, hymenoptera venom, and most recently also cannabis.

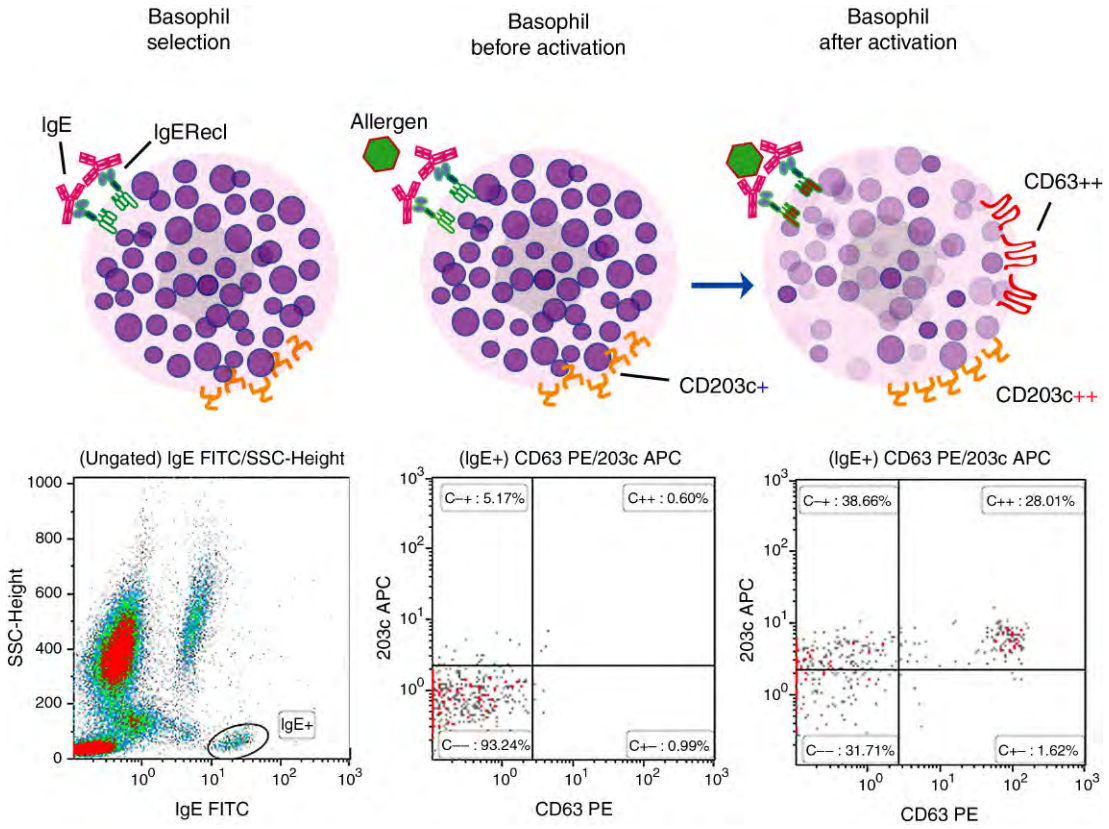


FIGURE 54.5 **Principle of the basophil activation test (BAT).** The allergen of interest is added to a blood sample of the patient. When the allergen is able to cross-link at least two specific IgE molecules bound on the surface of the basophil, activation of the basophil occurs. This activation leads to an upregulation of activation markers that can be measured using flow cytometry. Important surface markers are CD63 and CD203c.

TREATMENT

For the time being, there is no cure for cannabis allergy or for the cannabis-fruit/vegetable syndrome. Therefore, avoidance measures are of utmost importance. These measures comprise an absolute stop of further abuse of the drug and eventually also avoidance of exposures to allergens implicated in the individual cross-reactivity syndrome.

NATURAL HISTORY

The natural history of a cannabis allergy is unknown. Nevertheless, from an own study we observed that, although the patient stopped cannabis exposure, secondary food allergies can still increase.

MINI-DICTIONARY

Allergen/antigen A foreign compound that is recognized by the immune system. An allergen/antigen should be regarded as a collection of different epitopes (antigenic determinants) that can trigger specific humoral and/or cellular immune responses.
Anaphylaxis A generalized allergic reaction to an allergen, for example, foods, latex, drugs, or insect venoms. It is an acute and potentially life-threatening condition.
Angioedema An abrupt deep dermal, subcutaneous, or mucosal swelling. Pain and tenderness are often present, whereas itching is not obligatory.
Autoimmune condition A disorder due to an abnormal immune response against healthy body tissue.
Cross-reactivity/cross-allergy The in vitro reaction between an antigen and antibody due to sensitization to a different but structurally similar antigen (sensitizing antigen). A cross-reaction, however, is not always clinically relevant, but can merely represent an in vitro phenomenon. In cross-allergy, cross-reactivity is clinically relevant, that is, the patient will suffer from a reaction to both the sensitizing and cross-reacting antigen. The principles of cross-reactivity are displayed in Fig. 54.1B.
Cytoskeleton A network of microtubules, microfilaments, and intermediate filaments that gives structure to a cell.
Hymenoptera An order of insects comprising honeybees, bumblebees, wasps, and ants.
ISAC 112 A microarray technique enabling the measurement of 112 allergen components at the same time.
Native compound A substance, chemically processed or not, of natural origin (source material).
Panallergen A native compound, mostly a protein, that is widely distributed in nature, and often leads to cross-reactivity.
Photosynthesis A chemical process in which (green) plants and other photosynthetic organisms use the energy of the sun to transform carbon dioxide and water into oxygen and glucose.
Primary prevention Interventions that attempt to avoid the development of a certain condition.
Recombinant compound A substance produced by altering genetic material in a living organism, that is, genetic engineering.
Rhinoconjunctivitis An inflammation of the mucosa of the nose and eyes. It is characterized by itching of the eyes and nose, a runny nose, nasal congestion, sneezing, and red eyes.
Skin anergy The skin is not able to respond to any stimulus.
Urticaria A skin rash characterized by red or pale elevated wheals associated with severe itching. It is also known as hives.

References

Anselme, A., Metz-Favre, C., & de Blay, F. (2011). Allergie aux stupéfiants. *Revue française d'allergologie*, *51*, 548–552, In French.

Asero, R., Mistrello, G., Roncarolo, D., Amato, S., & van Ree, R. (2001). A case of allergy to beer showing cross-reactivity between lipid transfer proteins. *Annals of Allergy, Asthma and Immunology*, *87*(1), 65–67.

Beezhold, D. H., Hickey, V. L., Kostyal, D. A., Puhl, H., Zuidmeer, L., van Ree, R., & Sussman, G. L. (2003). Lipid transfer protein from *Hevea brasiliensis* (Hev b 12), a cross-reactive latex protein. *Annals of Allergy, Asthma and Immunology*, *90*(4), 439–445.

Breiteneder, H. (2004). Thaumatin-like proteins—a new family of pollen and fruit allergens. *Allergy*, *59*(5), 479–481.

Bublin, M., Mari, A., Ebner, C., Knulst, A., Scheiner, O., Hoffmann-Sommergruber, K., Radauer, C., et al. (2004). IgE sensitization profiles toward green and gold kiwifruits differ among patients allergic to kiwifruit from 3 European countries. *Journal of Allergy and Clinical Immunology*, *114*(5), 1169–1175.

Carnes, J., de Larramendi, C. H., Ferrer, A., Huertas, A. J., Lopez-Matas, M. A., Pagan, J. A., Pena, M., et al. (2013). Recently introduced foods as new allergenic sources: sensitisation to Goji berries (*Lycium barbarum*). *Food Chemistry*, *137*(1–4), 130–135.

Cavatorta, V., Sforza, S., Aquino, G., Galaverna, G., Dossena, A., Pastorello, E. A., & Marchelli, R. (2010). In vitro gastrointestinal digestion of the major peach allergen Pru 3, a lipid transfer protein: molecular characterization of the products and assessment of their IgE binding abilities. *Molecular Nutrition Food Research*, *54*(10), 1452–1457.

Coombs, R. R. A., & Gell, P. G. H. (1963). The classification of allergic reactions underlying disease. In P. G. H. Gell, & R. R. A. Coombs (Eds.), *Clinical aspects of immunology*. Oxford, UK: Blackwell.

De Knop, K. J., Bridts, C. H., Verweij, M. M., Hagendorens, M. M., De Clerck, L. S., Stevens, W. J., & Ebo, D. G. (2010). Component-resolved allergy diagnosis by microarray: potential, pitfalls, and prospects. *Advances in Clinical Chemistry*, *50*, 87–101.

de Larramendi, C. H., Carnes, J., Garcia-Abujeta, J. L., Garcia-Endrino, A., Munoz-Palomino, E., Huertas, A. J., Ferrer, A., et al. (2008). Sensitization and allergy to *Cannabis sativa* leaves in a population of tomato (*Lycopersicon esculentum*)-sensitized patients. *International Archives of Allergy and Immunology*, *146*(3), 195–202.

Ebo, D. G., Hagendorens, M. M., Bridts, C. H., de Clerck, L. S., & Stevens, W. J. (2004). Sensitization to cross-reactive carbohydrate determinants and the ubiquitous protein profilin: mimickers of allergy. *Clinical and Experimental Allergy*, *34*(1), 137–144.

Ebo, D. G., Hagendorens, M. M., Bridts, C. H., & Stevens, W. J. (2012). In vitro diagnosis of IgE-mediated allergy: breakthroughs in the last decade. *Expert Reviews in Clinical Immunology*, *8*(1), 9–11.

Ebo, D. G., Lechkar, B., Schuerwegh, A. J., Bridts, C. H., De Clerck, L. S., & Stevens, W. J. (2002). Validation of a two-color flow cytometric assay detecting in vitro basophil activation for the diagnosis of IgE-mediated natural rubber latex allergy. *Allergy*, *57*(8), 706–712.

Ebo, D. G., & Stevens, W. J. (2001). IgE-mediated food allergy—extensive review of the literature. *Acta Clinica Belgica*, *56*(4), 234–247.

Ebo, D. G., Swerts, S., Sabato, V., Hagendorens, M. M., Bridts, C. H., Jorens, P. G., & De Clerck, L. S. (2013). New food allergies in a European non Mediterranean region: is *Cannabis sativa* to blame? *International Archives of Allergy and Immunology*, *161*(3), 220–228.

Egger, M., Hauser, M., Mari, A., Ferreira, F., & Gadermaier, G. (2010). The role of lipid transfer proteins in allergic diseases. *Current Allergy and Asthma Reports*, *10*(5), 326–335.

Faber, M., Van Gasse, A., Sabato, V., Hagendorens, M. M., Bridts, C. H., De Clerck, L. S., & Ebo, D. G. (2014). Marihuana allergy: beyond the joint. *Journal of Investigational Allergy and Clinical Immunology*, *25*(1), 70–72.

Gamboa, P., Sanchez-Monge, R., Sanz, M. L., Palacin, A., Salcedo, G., & Diaz-Perales, A. (2007). Sensitization to *Cannabis sativa* caused by a novel allergenic lipid transfer protein Can s 3. *Journal of Allergy and Clinical Immunology*, *120*(6), 1459–1460.

Herzinger, T., Schopf, P., Przybilla, B., & Rueff, F. (2011). IgE-mediated hypersensitivity reactions to cannabis in laboratory personnel. *International Archives of Allergy and Immunology*, *156*(4), 423–426.

Hsieh, L. S., Moos, M., Jr., & Lin, Y. (1995). Characterization of apple 18 and 31 kd allergens by microsequencing and evaluation of their content during storage and ripening. *Journal of Allergy and Clinical Immunology*, *96*(6 Pt 1), 960–970.

Jegou, S., Douliez, J. P., Molle, D., Boivin, P., & Marion, D. (2000). Purification and structural characterization of LTP1 polypeptides from beer. *Journal of Agricultural and Food Chemistry*, *48*(10), 5023–5029.

Knol, E. F., Mul, F. P., Jansen, H., Calafat, J., & Roos, D. (1991). Monitoring human basophil activation via CD63 monoclonal antibody 435. *Journal of Allergy and Clinical Immunology*, *88*(3 Pt 1), 328–338.

Kumar, S., Verma, A. K., Das, M., & Dwivedi, P. D. (2012). Molecular mechanisms of IgE mediated food allergy. *International Immunopharmacology*, *13*(4), 432–439.

Kumar, Y., & Bhatia, A. (2013). Immunopathogenesis of allergic disorders: current concepts. *Expert Reviews in Clinical Immunology*, *9*(3), 211–226.

Larramendi, C. H., Lopez-Matas, M. A., Ferrer, A., Huertas, A. J., Pagan, J. A., Navarro, L. A., & Carnes, J. (2013). Prevalence of sensitization to *Cannabis sativa*. Lipid-transfer and thaumatin-like proteins are relevant allergens. *International Archives of Allergy and Immunology*, *162*(2), 115–122.

Laske, N., Bunikowski, R., & Niggemann, B. (2003). Extraordinarily high serum IgE levels and consequences for atopic phenotypes. *Annals of Allergy, Asthma and Immunology*, *91*(2), 202–204.

Majmudar, V., Azam, N. A., & Finch, T. (2006). Contact urticaria to *Cannabis sativa*. *Contact Dermatitis*, *54*(2), 127.

Matejkova, M., Zidkova, J., Zidek, L., Wimmerova, M., Chmelik, J., & Sklenar, V. (2009). Investigation of thermal denaturation of barley nonspecific lipid transfer protein 1 (ns-LTP1b) by nuclear magnetic resonance and differential scanning calorimetry. *Journal of Agricultural and Food Chemistry*, *57*(18), 8444–8452.

Mayoral, M., Calderon, H., Cano, R., & Lombardero, M. (2008). Allergic rhinoconjunctivitis caused by *Cannabis sativa* pollen. *Journal of Investigational Allergology and Clinical Immunology*, *18*(1), 73–74.

Metz-Favre, C., Pauli, G., Bessot, J. C., & De Blay, F. (2011). Molecular allergology in practice: an unusual case of LTP allergy. *European Annals of Allergy and Clinical Immunology*, *43*(6), 193–195.

Nayak, A. P., Green, B. J., Sussman, G., Berlin, N., Lata, H., Chandra, S., Beezhold, D. H., et al. (2013). Characterization of *Cannabis sativa* allergens. *Annals of Allergy, Asthma and Immunology*, *111*(1), 32–37.

Rihs, H. P., Armentia Medina, A., Lobin, W., Sander, I., Brüning, T., Raulf-Heimsoth, M., & Varga, R. (2014). A recombinant lipid transfer protein from *Cannabis sativa*: IgE-binding properties in patients with symptoms to Cannabis. *Clinical and Translational Allergy*, *4*(Suppl 2), 57.

Rihs, H. P., Rueff, F., Lundberg, M., Rozynek, P., Barber, D., Scheurer, S., Raulf-Heimsoth, M., et al. (2006). Relevance of the recombinant lipid transfer protein of *Hevea brasiliensis*: IgE-binding reactivity in fruit-allergic adults. *Annals of Allergy, Asthma and Immunology*, *97*(5), 643–649.

Sancho, A. I., Rigby, N. M., Zuidmeer, L., Asero, R., Mistrello, G., Amato, S., Mills, E. N., et al. (2005). The effect of thermal processing on the IgE reactivity of the non-specific lipid transfer protein from apple, Mal d 3. *Allergy*, *60*(10), 1262–1268.

Scheurer, S., Lauer, I., Foetisch, K., San Miguel Moncin, M., Retzek, M., Hartz, C., Vieths, S., et al. (2004). Strong allergenicity of Pru av 3, the lipid transfer protein from cherry, is related to high stability against thermal processing and digestion. *Journal of Allergy and Clin Immunology*, *114*(4), 900–907.

Sharma, P., Singh, A. K., Singh, B. P., Gaur, S. N., & Arora, N. (2011). Allergenicity assessment of osmotin, a pathogenesis-related protein, used for transgenic crops. *Journal of Agricultural and Food Chemistry*, *59*(18), 9990–9995.

Spiewak, R., Gora, A., & Dutkiewicz, J. (2001). Work-related skin symptoms and type I allergy among eastern-Polish farmers growing hops and other crops. *Annals of Agricultural and Environmental Medicine*, *8*(1), 51–56.

Stadtmauer, G., Beyer, K., Bardina, L., & Sicherer, S. H. (2003). Anaphylaxis to ingestion of hempseed (*Cannabis sativa*). *Journal of Allergy and Clinical Immunology*, *112*(1), 216–217.

Stokes, J. R., Hartel, R., Ford, L. B., & Casale, T. B. (2000). Cannabis (hemp) positive skin tests and respiratory symptoms. *Annals of Allergy and Asthma Immunology*, *85*(3), 238–240.

Swerts, S., Van Gasse, A., Leysen, J., Faber, M., Sabato, V., Bridts, C. H., Ebo, D. G., et al. (2014). Allergy to illicit drugs and narcotics. *Clinical and Experimental Allergy*, *44*, 307–318.

Tanaka, H., Degawa, M., Kawata, E., Hayashi, J., & Shoyama, Y. (1998). Identification of Cannabis pollens using an allergic patient's immunoglobulin E and purification and characterization of allergens in Cannabis pollens. *Forensic Science International*, *97*(2-3), 139–153.

Tessmer, A., Berlin, N., Sussman, G., Leader, N., Chung, E. C., & Beezhold, D. (2012). Hypersensitivity reactions to marijuana. *Annals of Allergy, Asthma and Immunology*, *108*(4), 282–284.

Torre, F. D., Limonta, A., Molinari, A., Masala, E., Vercelloni, S., & Torre, E. D. (2007). Cannabaceae pollen in the atmosphere of Brianza, Northen Italy. *European Annals of Allergy and Clinical Immunology*, *39*(1), 9–11.

Valenta, R., Lidholm, J., Niederberger, V., Hayek, B., Kraft, D., & Gronlund, H. (1999). The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clinical and Experimental Allergy*, *29*(7), 896–904.

Van Gasse, A. L., Sabato, V., Bridts, C. H., & Ebo, D. G. (2014). L'allergie au cannabis: bien plus qu'un voyage stupéfiant. *Revue Francaise d'Allergologie*, *54*(3), 144–147.

Van Loon, L. C. (1999). Occurrence and properties of plant pathogenesis-related proteins. In S. K. Datta, & S. Muthukrishnan (Eds.), *Pathogenesis-related proteins in plants* (pp. 1–19). Boca Raton, FL: CRC Press LLC.

Van Winkle, R. C., & Chang, C. (2012). The biochemical basis and clinical evidence of food allergy due to lipid transfer proteins: a comprehensive ceview. *Clinical Reviews of Allergy and Immunology*, *46*(3), 211–224.

Wijesinha-Bettoni, R., Alexeev, Y., Johnson, P., Marsh, J., Sancho, A. I., Abdullah, S. U., Mills, E. N., et al. (2010). The structural characteristics of nonspecific lipid transfer proteins explain their resistance to gastroduodenal proteolysis. *Biochemistry*, *49*(10), 2130–2139.

Williams, C., Thompstone, J., & Wilkinson, M. (2008). Work-related contact urticaria to *Cannabis sativa*. *Contact Dermatitis*, *58*(1), 62–63.

CHAPTER

55

Marijuana and Breastfeeding

M.G. Hill, K.L. Reed

The University of Arizona, Tucson, AZ, United States

SUMMARY POINTS

- Maternal marijuana use does result in presence of THC and its metabolites in the breastmilk.
- The concentration of THC in breastmilk, after different levels of use, has been inadequately studied.
- Marijuana use does not seem to inhibit milk letdown in postpartum women.
- There is not sufficient evidence to confirm immediate physical harm to the neonate breastfeeding from a woman who uses marijuana.
- There is some evidence that there are long-term effects of neonatal exposure to marijuana in certain areas of reasoning. However, there is not sufficient evidence to conclude that overall Intelligence Quotient is affected in human offspring.

KEY FACTS ABOUT MARIJUANA AND BREASTFEEDING

- Women who use marijuana during breastfeeding do produce milk with marijuana and its metabolites present in detectable quantities. It is not known how much of the drug is absorbed in the gastrointestinal tract of the infant.
- There is a lack of evidence pertaining to outcomes of children exposed exclusively to marijuana (and not other drugs), and exclusively through breast milk (and not in utero). This makes clinical recommendations difficult.

LIST OF ABBREVIATIONS

SIDS	Sudden infant death syndrome
THC	Tetrahydrocannabinol

INTRODUCTION

Marijuana is the most frequently used illicit drug in many areas of the world. In the United States, Colorado and Washington legalized in 2014 the commercial sale and use of marijuana. Studies from multiple countries have suggested that the rate of use is approximately 5–20% in pregnant and breastfeeding women (Scragg et al., 2001; Fried, Watkinson, Grant, & Knights, 1990; Wallace, Burns, Gilmour, & Hutchinson, 2007). An internet search regarding marijuana and breastfeeding yields many confidently written, yet contradictory opinions on the benefits and risks of this activity. There remain gaps in understanding of the clinical utility and the risk of marijuana use in pregnant and breastfeeding women. This chapter focuses on what is known about the effects of marijuana use in this population.

ROUTES OF MATERNAL INGESTION

Marijuana can be inhaled or ingested. The inhalation route is the most common way for marijuana to be accessed (Huestis, 2007). Inhalation yields varying amounts of absorbed marijuana (Huestis, 2007). Absorption of active drug appears to be dependent on multiple factors. One of these is the use of smoking aids. For example, the smoking of a "joint" (rolled cigarette of marijuana, often combined with tobacco) allows a larger proportion of the THC (tetrahydrocannabinol) to be released into the surrounding environment between breaths taken by

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00064-8
Copyright © 2017 Elsevier Inc. All rights reserved.

the user, whereas a "bong" device ensures that a larger portion of the smoke produced from the marijuana is collected in a canister for inhalation. There is also some evidence that users more experienced in smoking marijuana inhale a greater proportion of smoke from a standardized amount of marijuana (Huestis, 2007). This may be related to breathing techniques and tolerance to the irritation of smoke on the airways (Huestis, 2007).

Oral intake of marijuana is another common route of administration. Users will frequently cook leaves and buds of the marijuana plant inside foods, wherein the baking process releases the active ingredients from the marijuana. In some populations, marijuana tea is more common. Resins of marijuana can also be produced for human consumption. The effects of marijuana occur later after oral ingestion than with inhalation, as complete absorption takes several hours (Sharma, Murthy, & Bharath, 2012).

With the advent of medical marijuana, methods for dosing including rectal administration and patch administration are now also being investigated. The commonly marketed form of synthetic medication is dronabinol (Marinol) for oral administration. Several synthetic compounds are accessible to patients, some of which stimulate the endocannabinoid system, producing effects on appetite without producing the same significant effects on mental state that marijuana is known for (Fride, Bregman, & Kirkham, 2005). These are not the focus of this chapter, though their use is becoming increasingly common.

FACTORS THAT INFLUENCE INITIATION AND CONTINUATION OF BREASTFEEDING

Women tend to make the decision to breastfeed before pregnancy or during pregnancy (Kools, Thijs, & de Vries, 2005). One large survey addressing breastfeeding attitudes illustrated that women make decisions based on their own understanding of the benefits of breastfeeding, as well as other factors (work commitments, convenience) that may make formula feeding a better option for their situation (Kools et al., 2005). The success of breastfeeding seems not to be related to the age of the mother or her level of education (Kools et al., 2005). Most women do not make their decision about method of feeding based upon physician or midwife advice (Kools et al., 2005). In fact, there is evidence that the introduction of intensive postpartum clinical support in women using drugs does not modify parental behavior. A study from Australia found that home visits for "at risk" women did not increase the rates of continuation of breastfeeding; the control group breastfed for longer, in spite of the fact that the home visit group arguably had more breastfeeding support (Bartu, Sharp, Ludlow, & Doherty, 2006).

The strongest predictor for initiation and continuation of breastfeeding is previous experience with breastfeeding (Kools et al., 2005). Women who have previously breastfed an infant are much more likely to be successful in initiating and continuing breastfeeding (Kools et al., 2005). Women who have never had an infant, or who have previously fed their children with formula, are less likely to breastfeed (Kools et al., 2005). Use of marijuana does not seem to heavily influence the decision of most women as to method of newborn feeding. Survey-based research has indicated that many women who use marijuana and cigarettes will decrease or stop use in pregnancy (Fried et al., 1990). However, some will then increase use again after delivery. Theoretically, women who choose to breastfeed may be doing so at a time when they are increasing the use of marijuana.

MARIJUANA USE, MILK PRODUCTION, AND WILLINGNESS TO BREASTFEED

A concern cited regarding the use of marijuana in breastfeeding women is that it may delay the production of breastmilk. This is poorly studied in humans.

One study identified changes in prolactin levels in nonpregnant human females in response to marijuana inhalation (Mendelson, Mello, & Ellingboe, 1985). This experiment included 16 females who served as their own controls. Serum prolactin levels were measured in the follicular and luteal phases of the menstrual cycle, after inhalation of a marijuana cigarette or a placebo cigarette. Intriguingly, the patients had no difference in prolactin levels in the follicular phase in response to marijuana inhalation, but did have a decrease in prolactin levels in the luteal phase of the cycle (Mendelson et al., 1985). The implication is that marijuana may cause a decrease in prolactin levels in lactating women. However, as stated, this study was performed on women who were not pregnant or breastfeeding, who were having menstrual cycles. Additionally, other important hormones for the production of milk in humans, such as oxytocin, were not studied.

There are several longitudinal studies assessing the continuing health of mother–infant dyads with marijuana exposure both during pregnancy and breastfeeding (Astley & Little, 1990; Dreher, Nugent, & Hudgins, 1994; Hayes, Lampart, Dreher, & Morgan, 1991; Tennes et al., 1985). The results of marijuana use on infant development will be discussed later, however, the very existence of these studies confirms that milk production and letdown does occur in these women. Additionally, studies from multiple countries show equivalent rates of breastfeeding initiation and continuation in mothers using and abstaining from marijuana. One study

from Colorado followed 62 infants who were breastfed (Tennes et al., 1985). 27 infants had been exposed to marijuana through the mother's breastmilk. The age of weaning in infants was no different between groups (Tennes et al., 1985). This suggests that marijuana use probably does not significantly impair a mother's physiologic ability to nurse an infant.

Some authors suggest that marijuana use leads women to become apathetic, sedated, and less alert to the needs of their infants (Liston, 1998), or that the drug causes immediate adverse effects similar to an intoxication in the infant (Garry et al., 2009). These claims have a poor scientific basis, and seem to be rooted in the bias that people using marijuana have impaired functioning.

The effects of marijuana on infant appetite are unknown. The anorexia associated with multiple medical conditions has been treated with marijuana with encouraging results (Fride et al., 2005). Animal studies suggest that stimulation of the endocannabinoid system increases suckling behavior in juveniles (Fride et al., 2005). These findings indicate that marijuana exposure during infancy likely does not cause a decrease in appetite. Multiple cohorts reporting on marijuana exposure of the fetus and infant have not shown slower growth (an indication of decreased milk intake) in newborns (Dreher et al., 1994; Hayes et al., 1991; Tennes et al., 1985). Likewise, overall growth into the teen years does not seem to be affected by early life exposure to marijuana. However, the lifelong effects of appetite manipulation in the newborn via the endocannabinoid system are unknown.

LEVELS OF NEONATAL EXPOSURE THROUGH BREASTFEEDING

In spite of many resources touting the risks of marijuana to the breastfed infant, there is a concerning lack of scientific studies related to this subject. An important publication on the subject detailed the maternal serum, maternal breast milk, and neonatal fecal THC concentrations and the metabolites of THC; 11-OH-THC and 9-carboxy-THC (Perez-Reyes & Wall, 1982). This 1982 publication had two subjects.

The first subject used marijuana via a pipe once per day. Levels of THC in the milk of this subject were 105 ng/mL. The metabolites of THC were not detected in her milk. Additionally, the urine testing of her infant was also negative for the metabolite 9-carboxy-THC.

The second subject in this study had heavier use of marijuana, with inhalation via a pipe seven times per day. Her milk level of THC was 340 ng/mL, the metabolite 11-OH -THC was found at a level of 4 ng/mL. The second metabolite, 9-carboxy-THC was absent. The urine of her infant also tested negative for the metabolite 9-carboxy-THC (Table 55.1).

TABLE 55.1 Concentrations of THC and THC Metabolites in Human Milk

Concentration	Subject 1 (1 pipe per day)	Subject 2 (7 pipes per day)
Milk concentration of THC	105 ng/mL	340 ng/mL
Milk concentration of 11-OH-THC	Not detected	4 ng/mL
Milk concentration of 9-carboxy-THC	Not detected	Not detected

Unsatisfied with these results, the investigators requested that the second subject, with heavier use, provide serum, milk, and infant fecal samples for comparison. On the second testing of this subject, the investigators discovered that THC levels were higher in the infant's feces (347 ng in the total fecal sample), as compared to the maternal plasma (7.2 ng/mL) or milk (60.3 ng/mL). There were also higher levels of the metabolites (Table 55.2). The authors assessed the proportional increases in THC and its metabolites found in the neonatal feces (compared to the maternal milk), and concluded that the neonates were metabolizing and excreting the drug into the feces.

Though this was important work, the leaps in thinking based on these two clinical cases are problematic. This research has been used as justification for statements that marijuana "concentrates" in breastmilk, and is accumulated in the neonate.

The publication of only two clinical cases with only one parent–infant pair being studied is insufficient investigation on which to base practice recommendations. It is curious that the authors chose to report concentrations as ng/mL in the maternal serum and milk, but as "total fecal sample" for levels in the neonate. The authors do not comment on the volume of the fecal sample provided, or whether it was from a single bowel movement of from several samples. Obviously, the larger the sample from which a "total level" was obtained, the larger the amount of active and metabolized drug. Additionally, the presence of THC and its metabolites in infant feces does not necessarily imply absorption by the infant, just that it ingested breastmilk that contained these substances. Another confounding variable is that this infant may very well have inhaled second hand smoke from the mother's intake of marijuana via a pipe seven times per day, and excreted the metabolites

TABLE 55.2 Levels of THC in Mother and Neonate

	Maternal plasma	Maternal milk	Neonatal feces
Level of THC	7.2 ng/mL	60.3 ng/mL	347 ng (total fecal sample)

via the feces. Lastly, more than 65% of marijuana is excreted in the feces (Huestis, 2007). Hence, a fecal sample should not be compared to a serum or urine sample, as the marijuana is preferentially excreted into the feces. It is likely that the maternal feces would have had a higher concentration of the THC and metabolites, had it been tested.

The levels of THC absorbed by infants via breastfeeding with THC present in the maternal milk, as well as the amount the typical infant is exposed to via secondhand smoke, are important topics of future study. This is a challenging area, considering that many women may be reticent to allow their and their infant's participation in such a study for fear of loss of custody of their children, loss of work, or inferior medical treatment because of physician bias resulting from their participation. The current public bias seems to remain that these parents are not "doing the best" by their children (discussed in the next section). However, more evidence is needed to further clarify infant exposure and outcomes following ingestion of marijuana during breastfeeding.

MARIJUANA AND SUDDEN INFANT DEATH SYNDROME

There is an established role of the use of cigarettes (not just by the mother, but cigarette use in the home) in cases of sudden infant death syndrome (SIDS) (Schoendorf & Kiely, 1992). Studies assessing use of marijuana and SIDS risk are inevitably confounded by the concurrent use of tobacco, as marijuana is most often inhaled via smoking, and marijuana smokers are more likely than controls to also use cigarettes. In addition to this, there is also the confounding introduced by polydrug and alcohol use, in most studies assessing the use of marijuana during breastfeeding and risk of SIDS.

One study from New Zealand assessed the use of marijuana in breastfeeding with the outcome of SIDS being investigated (Scragg et al., 2001). 393 cases (patients who used marijuana) and 1592 controls (patients who did not use marijuana) were included. This study found an increased risk of SIDS in patients who used marijuana, when results were adjusted for use of tobacco and ethnicity of the study population OR 2.23 (1.39–3.57). After the multivariate analysis was employed, which adjusted for additional factors, the results were less compelling, with the confidence interval crossing 1.0 – OR of 1.55 (0.87–2.75).

Even if the results of this study were accepted without further investigation, the risk of using marijuana and SIDS occurrence is equivalent to the use of tobacco during breastfeeding. Additionally, the importance of recall bias in a case control study should be considered. It is possible that women who had experienced the loss of a child would have already performed their own analysis of the likely reason this may have happened. Self-blame for the death of an infant may lead to more complete reporting of drug use in the group of women who had lost a child to SIDS than those in the group who had living children.

Studies pertaining to smoking and breastfeeding have indicated that infants of women who smoke are exposed to the smoke (and chemicals contained within the smoke) of the mother, whether the mother chooses to breastfeed or formula feed (Shulte-Hobein, Schwartz-Bickenbach, Abt, Plum, & Nau, 1992). Cotinine levels (easily detected in the urine of women and infants exposed to cigarette smoke) remain elevated in infants of women who smoke, irrespective of whether they were breast or formula fed (Shulte-Hobein et al., 1992). Multiple authors and organizations have recommended that women who smoke tobacco should breastfeed (Australian Breastfeeding Association, 2014; Dorea, 2007). This mode of feeding is recommended during for the first year of life, and provides infants with nutritional and immunologic benefits (D'Apolito, 2013; Hauck, Thompson, Tanabe, Moon, & Vennemann, 2011; Ip et al., 2007). The "protection" of their infant afforded by abstaining from breastfeeding is a false one, in which the infant remains exposed to the second hand smoke and the smoke particles on the mothers skin, hair, and clothes, without the protections provided by breastfeeding (Australian Breastfeeding Association, 2014).

A concerning phenomenon is the recommendation put forward by multiple clinicians and academic societies that women who use marijuana should not breastfeed. A recommendation to feed with formula because of maternal use of marijuana should be undertaken with caution. The clinician should consider the overwhelming evidence of the benefits of breastfeeding, and the lack of evidence that breastfeeding in the setting of marijuana use risks immediate harm to the infant. There is a lack of evidence that neonatal exposure to smoke from marijuana in conjunction with formula feeding is superior to breastfeeding while using marijuana.

SHORT AND LONG-TERM EFFECTS ON THE NEONATE

Studies pertaining to the neonatal and childhood outcomes in the breastfed baby exposed to marijuana have two important sources of confounding. These are the influence of prenatal marijuana exposure (women who used marijuana during pregnancy in addition to during breastfeeding), and the use of substances other than marijuana. The other substances are most commonly cigarettes, alcohol, and cocaine.

Two large cohorts, the MHPCD (Maternal Health Practices and Child Development Study) and the OPPS (Ottawa Prenatal Prospective Study) are of interest. Each is a prospective cohort, the first from the United States, and the second from Canada. These studies did not look only at marijuana use during breastfeeding. Women were asked about drug use during pregnancy, as well as after a pregnancy. The results of these studies suggest that there is no effect of marijuana use on motor development or intelligence quotient, after adjustments are made for confounding variables. However, there was some evidence that the children of heavy users had a detrimental effect in the area of visual perception and reasoning. This difference was not dramatic between those exposed and unexposed in the cohorts, and was the only effect found after a battery of developmental tests (Fried, 1995). When growth was assessed in the OPPS, there was a difference in head circumference in early teenage years. This finding, in isolation, is difficult to interpret (Fried, Watkinson, & Gray, 1999).

There was one prospective cohort from Jamaica in which children were exposed to marijuana in a more culturally accepted pattern of use (Dreher et al., 1994; Hayes et al., 1991). Women in this cohort smoked marijuana, and also drank marijuana tea (Dreher et al., 1994). Specific information on feeding practices after delivery was not reported. However, it is likely that in rural Jamaica, most of these infants were breastfed, and that their mothers continued their daily consumption in the socially accepted practice for that region. In the first month of life, the infants of mothers who used marijuana had better outcomes, and their mothers seemed to transition to their new role with more ease (Dreher et al., 1994). This may have been related to the fact that the mothers who used marijuana also seemed to have more financial control in their lives (investigators reported that marijuana use in this population was associated with a higher average income) (Dreher et al., 1994). In a 5 years' follow-up, there were no detrimental effects in the marijuana-exposed group in this cohort (Hayes et al., 1991).

A cohort from Colorado also showed no adverse effects in children exposed to marijuana in utero and during breastfeeding (Tennes et al., 1985). However, the length of assessment did not exceed 1 year.

Lastly, a report exists detailing poorer motor development at the age of 1 year in infants exposed to marijuana during breastfeeding than controls (Astley & Little, 1990). This cohort included 68 mother–infant dyads per group. However, only 14 infants were exposed only during breastfeeding (the other 54 infants were exposed both during the pregnancy and breastfeeding). Additionally, the mothers in the marijuana exposure group were three times more likely to use cocaine, both during pregnancy and lactation, and had a lower average income than the controls. Considering these factors, a conclusion should not be drawn that the isolated use of marijuana is solely responsible for these effects.

LEGAL CONCERNS

Pregnancy and motherhood expose women to legal vulnerability. The use of marijuana, while legal or decriminalized in many parts of the world, can still cause a significant disadvantage to a woman in relation to her rights. The use of drugs, including marijuana, can lead to investigation of a family by social services, even if no concerns about parental behavior exist, and can also be discussed during custody proceedings. Some regions of the United States require that physicians and healthcare workers report any illicit drug use to child protective services. This requirement persists, in spite of a lack of evidence of immediate harm related to the use of marijuana while breastfeeding.

Health care workers should be aware of the laws that exist within their geographic region pertaining to reporting of substance use. If serum or urine screening tools are used, they should be employed only with the informed consent of patients. Ideally, patients should be made aware that there are very few instances in which the use of marijuana will impact their immediate health or the health of their child. However, a positive finding on screening may create legal concerns for the patient. Physicians may choose to opt for screening that excludes detection of marijuana, seeing the evidence regarding harm is inconsistent.

Likewise, the neonate should not be tested for illicit substances without the express consent of the parents. Testing in the absence of parental consent is unethical, opens family units to legal costs (both financial and emotional), and damages the doctor–patient relationship. Healthcare workers should be cognizant that screening of women and infants has historically been biased toward ethnic minorities and those with socioeconomic disadvantage. Focusing on nonconsensual testing of these populations serves to further increase the divide between the advantaged and disadvantaged, and the perception of victimization by these groups.

CONCLUSIONS

There is a lack of evidence regarding the relative benefits and risks of marijuana use during breastfeeding. Marijuana is illegal in many areas of the world, and its use remains socially unacceptable in other areas, making study of the effects of the drug during lactation difficult.

The most common mode of marijuana dosing is via inhalation, often mixed with tobacco. Considering the known health risks of smoke exposure to the neonate,

TABLE 55.3 Benefits of Breastfeeding

Nutritionally complete for newborns
Provides immunologic protection
Encourages time for bonding between mother and infant
Eliminates need to purchase supplies for infant feeding

TABLE 55.4 Effects of Marijuana Use During Pregnancy and Breastfeeding

Potential adverse effects of marijuana use while pregnant and breastfeeding	Supporting evidence
Maternal sedation and negligence	Opinion only
Failure of milk letdown	Opinion only
Neonatal sedation and intoxication	Opinion only
Sudden infant death syndrome	Risk may be equivalent to infants of mothers who smoke tobacco
Abnormal infant growth	Several prospective cohorts found no significant difference in infant growth

including sudden infant death syndrome and respiratory infections, it is optimal that women not smoke marijuana or tobacco during breastfeeding. This being said, breastfeeding offers many benefits, and formula feeding does not protect the infant from the dangers of maternal smoking. Rather it allows for continued smoke exposure without the immunologic, nutritional, and financial benefits of breastfeeding. If a woman is going to smoke tobacco, she should be encouraged to continue breastfeeding. There is a lack of evidence to suggest that the recommendation to a mother who uses marijuana should be any different (Tables 55.3 and 55.4).

References

Astley, S. J., & Little, R. E. (1990). Maternal marijuana use during lactation and infant development at one year. *Neurotoxicology and Teratology*, *12*(2), 161–168.

Bartu, A., Sharp, J., Ludlow, J., & Doherty, D. A. (2006). Postnatal home visiting for illicit drug-using mothers and their infants: a randomised controlled trial. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, *46*(5), 419–426.

Breastfeeding and Smoking. Australian Breastfeeding Association. Malvern, VIC. Available from https://www.breastfeeding.asn.au/bfinfo/breastfeeding-and-smoking; 2014.

D'Apolito, K. (2013). Breastfeeding and substance abuse. *Clinical Obstetrics and Gynecology*, *56*(1), 202–211.

Dorea, J. G. (2007). Maternal smoking and infant feeding: breastfeeding is better and safer. *Maternal and Child Health Journal*, *11*(3), 287–291.

Dreher, M. C., Nugent, K., & Hudgins, R. (1994). Prenatal marijuana exposure and neonatal outcomes in Jamaica: an ethnographic study. *Pediatrics*, *93*(2), 254–260.

Fride, E., Bregman, T., & Kirkham, T. C. (2005). Endocannabinoids and food intake: newborn suckling and appetite regulation in adulthood. *Experimental Biology and Medicine*, *230*(4), 225–234.

Fried, P. A. (1995). Prenatal exposure to marihuana and tobacco during infancy, early and middle childhood: effects and an attempt at synthesis. In *Toxicology in transition* Archives of Toxicology, (Vol. 17) (pp. 233–260). Berlin, Heidelberg, Germany: Springer-Verlag.

Fried, P. A., Watkinson, B., Grant, A., & Knights, R. M. (1990). Changing patterns of soft drug use prior to and during pregnancy: a prospective study. *Drug and Alcohol Dependence*, *6*(5), 323–343.

Fried, P. A., Watkinson, B., & Gray, R. (1999). Growth from birth to early adolescence in offspring prenatally exposed to cigarettes and marijuana. *Neurotoxicology and Teratology*, *21*(5), 513–525.

Garry, A., Rigourd, V., Amirouche, A., Fauroux, V., Aubry, S., & Serreau, R. (2009). Cannabis and breastfeeding. *Journal of Toxicology*, 2009, 596149.

Hauck, F. R., Thompson, J. M. D., Tanabe, K. O., Moon, R. Y., & Vennemann, M. M. (2011). Breastfeeding and reduced risk of sudden infant death syndrome: a meta-analysis. *Pediatrics*, *128*(1), 103–110.

Hayes, J. S., Lampart, R., Dreher, M. C., & Morgan, L. (1991). Five-year follow-up of rural Jamaican children whose mother used marijuana during pregnancy. *West Indian Medical Journal*, *40*(3), 120–123.

Huestis, M. A. (2007). Human cannabinoid pharmacokinetics. *Chemistry and Biodiversity*, *4*(8), 1770–1804.

Ip, S., Chung, M., Raman, G., Chew, P., Magula, N., DeVine, D., Trikalinos, T., & Lau, J. (2007). *Breastfeeding and maternal and infant health outcomes in developed countries. Evidence Report/Technology Assessment (Full Rep) Issue 153* (pp. 1–186). (Rockville, MD: Agency for Healthcare Research and Quality, Prepared by Tufts-New England Medical Center Evidence-Base Practice Center, Under Contract No. 290-02-0022). AHRQ Publication 07-E007.

Kools, E. J., Thijs, C., & de Vries, H. (2005). The behavioral determinants of breast-feeding in The Netherlands: predictors for the initiation of breast-feeding. *Health Education and Behavior*, *32*(6), 809–824.

Liston, J. (1998). Breastfeeding and the use of recreational drugs—alcohol, caffeine, nicotine and marijuana. *Breastfeeding Review*, *6*(2), 27–30.

Mendelson, J. H., Mello, N. K., & Ellingboe, J. (1985). Acute effects of marihuana smoking on prolactin levels in human females. *Journal of Pharmacology and Experimental Therapeutics*, *232*(1), 220–222.

Perez-Reyes, M., & Wall, M. E. (1982). Presence of delta-9-tetrahydrocannabinol in human milk. *New England Journal of Medicine*, *307*(13), 819–820.

Schoendorf, K. C., & Kiely, J. L. (1992). Relationship of sudden infant death syndrome to maternal smoking during and after pregnancy. *Pediatrics*, *90*(6), 905–908.

Scragg, R. K., Mitchell, E. A., Ford, R. P., Thompson, J. M., Taylor, B. J., & Stewart, A. W. (2001). Maternal cannabis use in the sudden death syndrome. *Acta Paediatrica*, *90*(1), 57–60.

Sharma, P., Murthy, P., & Bharath, M. M. (2012). Chemistry, metabolism, and toxicology of cannabis: clinical implications. *Iranian Journal of Psychiatry*, *7*(4), 149–156.

Shulte-Hobein, B., Schwartz-Bickenbach, D., Abt, S., Plum, C., & Nau, H. (1992). Cigarette smoke exposure and development of infants throughout the first year of life: influence of passive smoking and nursing on cotinine levels in breast milk and infant's urine. *Acta Paediatrica*, *81*(6–7), 550–557.

Tennes, K., Avitable, N., Blackard, C., Boyles, C., Hassoun, B., Holmes, L., & Kreye, M. (1985). Marijuana: prenatal and postnatal exposure in the human. *NIDA Research Monograph Series*, *59*, 48–60.

Wallace, C., Burns, L., Gilmour, S., & Hutchinson, D. (2007). Substance use, psychological distress and violence among pregnant and breastfeeding Australian women. *Australian and New Zealand Journal of Public Health*, *31*(1), 51–56.

CHAPTER

56

Hypocretins/Orexins and Addiction: Role in *Cannabis* Dependence

Á. Flores, R. Maldonado, F. Berrendero

Department of Experimental and Health Sciences, Laboratory of Neuropharmacology, School of Health and Life Sciences, Pompeu Fabra University, Barcelona, Spain

SUMMARY POINTS

- Hypocretins/orexins are hypothalamic neuropeptides that regulate physiological processes, such as energy balance, stress and arousal, sleep/wake cycle, and reward.
- The hypocretinergic system modulates the addictive properties of several drugs of abuse (ie, alcohol, nicotine, opioids, psychostimulants, and cannabinoids).
- Anatomical, biochemical and functional cross-talks exist between hypocretinergic and endocannabinoid systems, the latter being the main target of cannabis compounds.
- The hypocretin receptor 1 is necessary for the rewarding properties of the synthetic cannabinoid WIN55,212-2.
- Chronic exposure to THC or WIN55,212-2 affects the activity of hypocretin neurons.
- The dopaminergic transmission in the mesolimbic system is disrupted if the hypocretin receptor 1 is not present.

KEY FACTS OF DRUG SELF-ADMINISTRATION PARADIGMS

- Drug self-administration is a form of operant conditioning used for assessing drug-seeking and drug-taking behavior.
- This method allows us to directly evaluate the reinforcing properties of a drug, and hence to predict its rewarding (and presumably addictive) effects.
- Drug self-administration protocols are performed in operant conditioning chambers, generally equipped with two levers (or holes), one active and one inactive, distinguishable for the presence of a cue light above the active one.
- A correct lever-pressing or nose-poking response results in the contingent presentation of reward: the delivery of an intravenous drug infusion by a syringe pump.
- Different reinforcement schedules can be employed, depending on the type of response to be evaluated:
  - Fixed-ratio (FR). A determined number of operant responses are required to dispense one unit of reinforcer, that is, FR5 requires 5 active lever-presses to receive one drug infusion. Commonly used during the acquisition of drug-taking.
  - Progressive ratio (PR). The number of operant responses required to receive one reinforcer are gradually increased. This schedule provides information about the motivation to obtain the drug, since it reveals at which amount of effort the subject ceases drug self-administration.
- Drug self-administration allows the study of diverse stages present in the addictive behavior:
  - Initial acquisition of drug-taking behavior.
  - Extinction of this behavior during the absence of the drug.
  - Reinstatement of drug-seeking after exposure to stress, associated context/cues, or the drug itself.
- Drug self-administration is considered to be the most reliable model of addiction and to have a high predictive value.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00065-X
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

2-AG	2-arachidonoylglycerol
cAMP	Cyclic adenosine monophosphate
CB1	Cannabinoid receptor 1
CB2	Cannabinoid receptor 2
CNS	Central nervous system
DAG	Diacylglycerol
DMH	Dorsomedial hypothalamus
GABA	γ-Aminobutyric acid
HcrtR1	Hypocretin receptor 1
HcrtR2	Hypocretin receptor 2
HcrtRs	Hypocretin receptors
IP3	Inositol triphosphate
LH	Lateral hypothalamus
MAP	Mitogen-activated protein
mRNA	Messenger ribonucleic acid
NAc	Nucleus accumbens
PFA	Perifornical area
THC	Δ^9-Tetrahydrocannabinol
VTA	Ventral tegmental area

THE HYPOCRETIN/OREXIN SYSTEM

Hypocretin-1/orexin-A and hypocretin-2/orexin-B are two neuropeptides proteotically cleaved from the same precursor, prepro-hypocretin/prepro-orexin (de Lecea et al., 1998; Sakurai et al., 1998). Two closely related G protein-coupled receptors that respond to hypocretins have been cloned, hypocretin or orexin receptor 1 (HcrtR1/OXR1) and hypocretin or orexin receptor 2 (HcrtR2/OXR2) (Fig. 56.1). Hypocretin-1 binds to both receptors with similar affinity, whereas hypocretin-2 binds to HcrtR2 with ten-fold greater affinity than to HcrtR1. The intracellular signaling pathways that mediate the effects of hypocretins have been intensively investigated, but remain still to be completely elucidated. Stimulation of both hypocretin receptors (HcrtRs) leads to a prominent increase in intracellular Ca^{2+} concentrations through the activation of Gq proteins, followed by phospholipase and subsequent protein kinase C (PKC) stimulation (Xu, Yang, Ward, Gao, & Liu, 2013). The Ca^{2+} elevation induced by hypocretin receptor activation explains the commonly reported neuroexcitatory nature of hypocretin peptides on the brain. In addition, hypocretins have been reported to activate the MAP kinase pathway (Xu et al., 2013) (Fig. 56.2).

In the central nervous system (CNS), hypocretin expression is restricted to a few thousand neurons in some particular regions of the hypothalamus: the perifornical area (PFA), the dorsomedial hypothalamus (DMH), and the dorsal and lateral hypothalamus (LH) (Peyron et al., 1998). Although hypocretin-containing neurons represent a relatively small number of cells, their projections are widely distributed throughout the brain (Peyron et al., 1998) (Fig. 56.1). The widespread extension of the hypocretin system in the CNS is in agreement with the variety of physiological functions of hypocretin peptides that includes energy homeostasis, behavioral arousal, sleep/wake cycles, and emotional regulation (Li, Hu, & de Lecea, 2014). Moreover, numerous studies demonstrate a role for hypocretins in reward-seeking and addiction (Plaza-Zabala, Maldonado, & Berrendero, 2012). Consistent with this, hypocretin neurons send significant efferent projections to structures related to addiction, such as the paraventricular nucleus of the thalamus, the septal nuclei, the bed nucleus of the stria terminalis, the anterior and central amygdaloid nuclei, and the ventral tegmental area (VTA). Disperse axons are also found

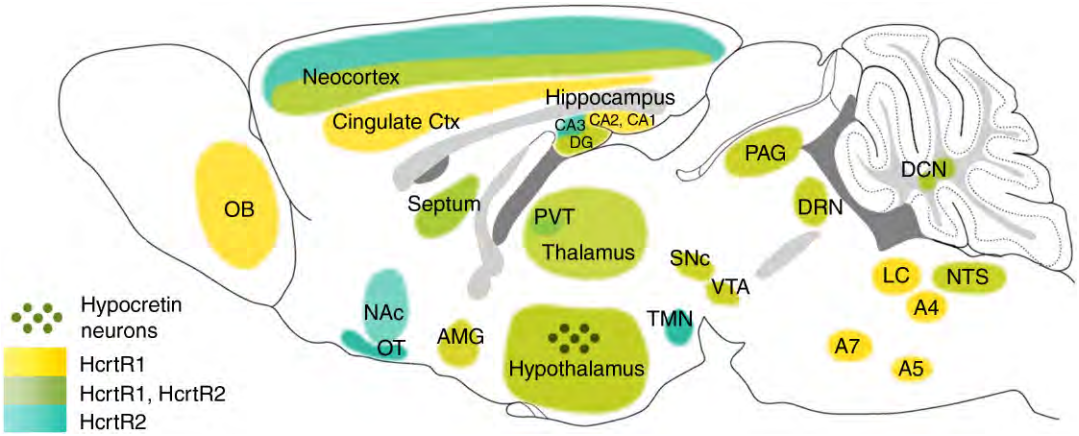


FIGURE 56.1 **Hypocretinergic/orexinergic system distribution in the brain.** Schematic representation of the main areas expressing HcrtR1 and HcrtR2 in the mouse brain and location of hypocretin/orexin neurons. *A4*, *A5*, *A7*, pons cell groups; *AMG*, amygdala; *Ctx*, cortex; *DCN*, deep cerebellar nuclei; *DG*, dentate gyrus; *DRN*, dorsal raphe nucleus; *LC*, locus coeruleus; *NAc*, nucleus accumbens; *NTS*, nucleus of the solitary tract; *OB*, olfactory bulb; *OT*, olfactory tubercle; *PAG*, periaqueductal gray; *PVT*, paraventricular nucleus of thalamus; *SNc*, substantia nigra pars compacta; *TMN*, tuberomammillary nucleus; *VTA*, ventral tegmental area. *Source: Adapted with permission from Flores et al. (2013).*

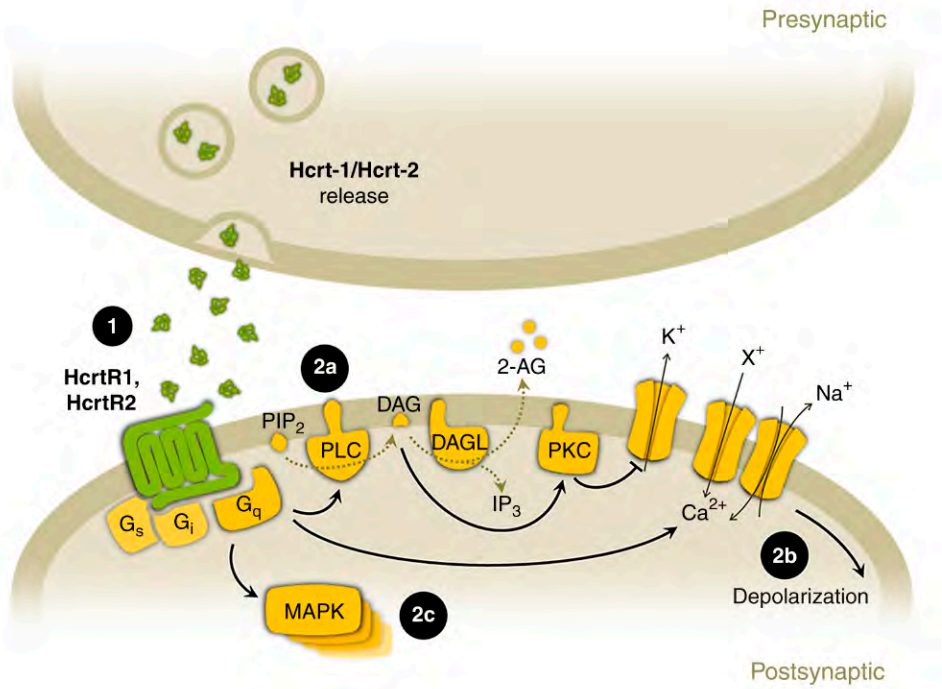


FIGURE 56.2 **Hypocretin-mediated synaptic signaling.** (1) Hypocretins are released from presynaptic terminals and stimulate postsynaptic HcrtR1 and HcrtR2. (2) HcrtR activation is mainly associated with Gq-protein stimulation, but it can activate also other G-protein subtypes. The key downstream outcomes from HcrtR activation and subsequent Gq-protein stimulation are: (2a) activation of PLC activity, and preceding DAG and 2-AG synthesis; (2b) membrane depolarization due to the modulation of K^+ channels, nonspecific cationic channels and $Na^+/Ca2^+$exchanger; and (2c) activation of protein kinase cascades such as the MAPK pathway. *PIP_2*, phosphatidylinositol bisphosphate; *DAG*, diacylglicerol; *2-AG*, 2-arachidonoylglycerol; *PLC*, phospholipase C; *DAGL*, diacylglycerol lipase; *MAPK*, mitogen-activated protein kinase; *Hcrt-1*, hypocretin-1; *Hcrt-2*, hypocretin-2; *PKC*, protein kinase C; *X^+*, unspecific cation. *Source: Adapted with permission from Flores et al. (2013).*

throughout the cortex and the medial part of the nucleus accumbens (NAc) (Peyron et al., 1998). Moreover, hypocretin-1 injected into the VTA increases dopamine in the NAc (España et al., 2010), suggesting that these neuropeptides play a role in the regulation of the dopaminergic mesocorticolimbic system and reward learning.

ROLE OF THE HYPOCRETIN/OREXIN SYSTEM IN THE ADDICTIVE PROPERTIES OF DRUGS OF ABUSE

As mentioned previously, the hypocretin system plays a crucial role in the regulation of the addictive properties of several major drugs of abuse (Fig. 56.3). Hypocretin transmission regulates the primary reinforcing effects of opioids, nicotine, and alcohol (Plaza-Zabala et al., 2012). Recent evidence also suggests a role for these neuropeptides in cannabinoid reward (Flores, Maldonado, & Berrendero, 2014). However, in the case of psychostimulants, an involvement of the hypocretin system is revealed only when effort requirement to obtain the drug is high (España et al., 2010). The differential participation of hypocretins in the reinforcing properties of psychostimulants could be explained by the different action upon the mesolimbic system. Thus, opioids, nicotine, alcohol, and cannabinoids increase dopaminergic cell firing in the VTA, whereas psychostimulants directly inhibit dopamine uptake in the NAc. These differences suggest that the VTA might be a crucial site of action for hypocretins to mediate the rewarding effects of different drugs of abuse (Aston-Jones, Smith, Moorman, & Richardson, 2009). Therefore, behavioral effects that depend on increased VTA dopaminergic activation (opioid/alcohol/nicotine/cannabinoid reinforcement) would be attenuated by HcrtR1 antagonist because they may require hypocretin transmission. However, behaviors that are independent of the activation of VTA (primary cocaine reinforcement) could avoid this critical site of actions of hypocretins. Moreover, hypocretins regulate the reinstatement of drug-seeking behaviors induced by drug-associated cues, as described for cocaine, alcohol, heroin, and nicotine. Stress-induced reinstatement of cocaine and alcohol is also mediated by hypocretin transmission (Khoo & Brown, 2014). As a whole, these data suggest that HcrtR1 antagonists might be of potential interest for the treatment of drug addiction (Figure 56.3). The possible usefulness of HcrtR2 antagonists should be considered cautiously, since this receptor seems to be mainly involved in the regulation of sleep-wake cycle.

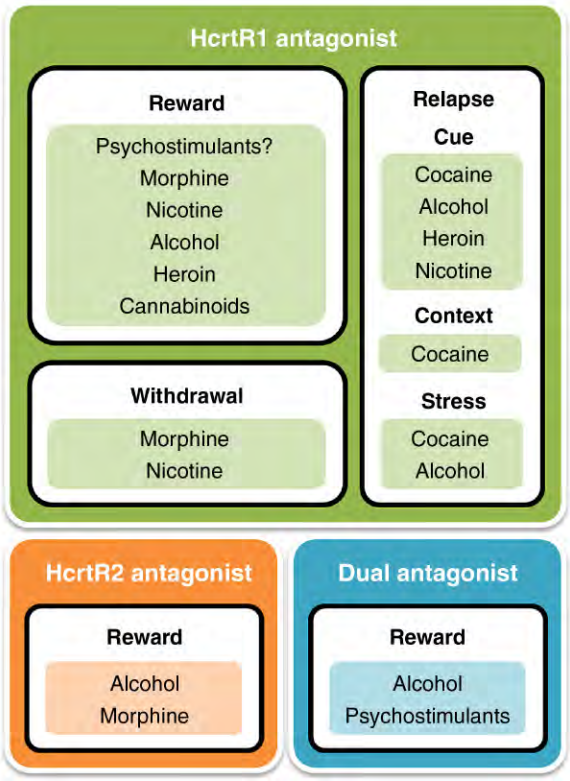


FIGURE 56.3 Potential therapeutic utility of hypocretin/orexin receptor antagonists. Diagram showing the different stages of drug addiction in which hypocretin/orexin receptor antagonists constitute potential therapeutic tools. *Source: Adapted with permission from Plaza-Zabala et al. (2012).*

NEUROBIOLOGICAL MECHANISMS OF CANNABINOID DEPENDENCE

The neurochemical processes by which cannabinoid addiction is developed are similar to those reported for other drugs of abuse. The endocannabinoid system is the leading site of action for the rewarding and pharmacological responses induced by cannabinoids. In fact, this system fulfils a general modulatory effect on the reward circuitry, participating in the rewarding and addictive properties of all classical drugs of abuse.

CB1 receptors are abundantly expressed in the diverse regions of the brain reward system, including the VTA, the NAc, the prefrontal cortex, and the amygdala. However, it is known that VTA dopaminergic neurons are unlikely to express CB1 receptors (Julian et al., 2003). Indeed, CB1 receptors present in the VTA are located on presynaptic glutamatergic and GABAergic neurons. Endocannabinoids modulate the excitatory and inhibitory synaptic inputs into the VTA, acting as a retrograde messenger. Thus, the activation of CB1 receptors in the VTA present in GABAergic interneurons, or in glutamatergic terminals mainly from prefrontal cortex neurons, would remove, respectively, these inhibitory or excitatory inputs on dopaminergic neurons. The final effect on the modulation of VTA dopaminergic activity by endocannabinoids would depend on the functional balance between these GABAergic and glutamatergic inputs (Maldonado, Valverde, & Berrendero, 2006; Fattore, Fadda, Spano, Pistis, & Fratta, 2008). Accordingly, exogenous cannabinoid agonists stimulate the activity of mesencephalic dopaminergic neurons by altering this balance. Both Δ^9-Tetrahydrocannabinol (THC) and the synthetic cannabinoid WIN55,212-2 enhance the firing rate and bursting activity of dopaminergic neurons in the VTA, subsequently enhancing dopamine release in terminal regions, such as the NAc and the prefrontal cortex (Chen et al., 1990; Tanda, Pontieri, & Di Chiara, 1997), a fact that has been associated to their reinforcing properties.

Parallel dopamine-independent mechanisms in the development of cannabinoid addiction may also be modulated by the endocannabinoid system. For instance, CB1 receptors present in the prefrontal cortex might explain the involvement of the endocannabinoid system in the motivation to seek the drug, since this brain area integrates sensory information, emotional processing, and hedonic experience. Besides, reinstatement of drug-seeking behavior is probably influenced by the endocannabinoid system because of its capacity to modulate synaptic plasticity, consolidating the reward-driven behavior required to establish addictive processes (Maldonado et al., 2006).

In summary, endocannabinoid and dopaminergic systems are crucially involved in the neurobiological mechanisms underlying the addictive effects of the main classical drugs of abuse, including cannabinoids. Likewise, other neurochemical systems have also been involved in the addictive processes induced by cannabinoids, including endogenous opioids, GABA, glutamate, monoamines, and several neuropeptides (Maldonado, Berrendero, Ozaita, & Robledo, 2011). Lately, hypocretins have been reported to contribute also to cannabinoid dependence, revealing a new potential therapeutic target for the treatment of this disorder.

INTERACTION BETWEEN HYPOCRETIN/OREXIN AND ENDOCANNABINOID SYSTEMS

In later years, research has shown the possible existence of a cross talk between hypocretinergic and endocannabinoid systems. Although few investigations have analyzed this interaction, emerging neuroanatomical and biochemical evidences strongly suggest the existence of such cross-modulation (Flores, Maldonado, & Berrendero, 2013). Hence, CB1 and HcrtRs show an overlapping distribution in several areas of the CNS (Marcus et al., 2001; Mackie, 2005).

The common expression of HcrtRs and CB1 within the entire hypothalamus denotes an important modulation of energy homeostasis, and neuroendocrine and autonomic functions by both hypocretin and endocannabinoid systems. They are also abundant along the mesocorticolimbic system, denoting their regulation of reward processing and addiction. Likewise, the mutual involvement of these neuromodulators in the control of anxiety-like responses, sleep/wake cycle, and nociception is supported by the presence of HcrtRs and CB1 within diverse brainstem nuclei. However, HcrtRs location among different neuronal populations and therefore direct synaptic connections between HcrtRs and CB1, are not well defined yet. On the other hand, the recent detection of multifocal CB2 expression in the brain opens a new range of possibilities for hypocretin–cannabinoid interaction.

CB1 and CB2, as well as HcrtR1 and HcrtR2, are classified as rhodopsin-like G-protein coupled receptors. Although most cellular signals triggered upon cannabinoid receptor activation differ from those initiated following the stimulation of hypocretin receptor, it seems that cannabinoid and HcrtRs share diverse signaling pathways (Demuth & Molleman, 2006; Kukkonen, 2013). CB1 and CB2 receptors are coupled to the Gi/o family of G-proteins and, subsequently, inhibit adenylyl cyclase activity, and decrease cAMP levels. In contrast, HcrtRs are mainly associated with Gq-proteins, inducing the activation of phospholipase C that produces the second messengers DAG and IP3. Additionally, stimulation of both HcrtRs has been suggested to modulate adenylyl cyclase activity by coupling other G-proteins, in certain experimental conditions. One of the most relevant differences between hypocretin and cannabinoid cellular signaling is the effect on transmembrane potential as a consequence of their divergent modulation of effector ion channels triggered upon receptor stimulation. Thus, CB1 activation triggers the plasmatic membrane repolarization that results in neurotransmitter release inhibition, whereas HcrtRs stimulation induces membrane depolarization, facilitating the formation of action potentials. On the other hand, the main cellular pathway activated by both cannabinoid and hypocretinergic stimulation is the phosphorilation and activation of the MAP kinase cascade that regulates neuronal gene expression and synaptic plasticity. Additionally, recent in vitro and in vivo studies report that HcrtR1 stimulation can lead to 2-arachidonoylglycerol (2-AG) release activating CB1 receptors in nearby cells, since DAG produced upon phospholipase C stimulation is used by diacylglycerol lipase as a substrate for 2-AG synthesis (Turunen, Jäntti, & Kukkonen, 2012). This hypocretin-induced endocannabinoid release might be a relevant mechanism by which hypocretins could mediate synaptic inhibition in certain conditions.

CB1 receptors have a significant propensity to make homo- and heteromeric complexes. Indeed, the existence of CB1-HcrtR1 and CB1-HcrtR2 heteromers in diverse in vitro models has been demonstrated by electron microscopy colocalization, and fluorescence and bioluminiscence resonance energy transfer (FRET and BRET) imaging studies (Hilairet, Bouaboula, Carrière, Le Fur, & Casellas, 2003; Ellis, Pediani, Canals, Milasta, & Milligan, 2006). The functional impact of this heteromerization has been associated with HcrtRs and CB1 receptor trafficking, as well as cross-agonism/-antagonism phenomena. However, whether HcrtR-CB1 heteromers actually exist within the CNS tissue, and if they are indeed of physiological relevance, remains to be further elucidated.

So far, various functional studies have revealed the existence of a mutual regulation between the hypocretin and endocannabinoid systems, mainly regarding the areas of nociception, appetite, and reward. Thus, systemic administration of the CB1 antagonist AM251 reverses the antinociceptive effect of hypocretin-1 microinjection into the periaqueductal gray, during the hot-plate test in rats (Ho et al., 2011). Similarly, it has been suggested that hypocretin-1 exerts its orexigenic action through CB1 receptor activation, since pretreatment with a nonanorectic dose of the CB1 antagonist rimonabant blocks the orexigenic effect of hypocretin-1 administered by intracerebroventricular route in rats (Crespo, Gómez de Heras, Rodríguez de Fonseca, & Navarro, 2008). In the same line, it has been shown that conditioned place preference induced by chemical stimulation of the LH, which depends on HcrtR1 activation in the VTA, is reduced in a dose-dependent manner by previous intra-VTA administration of rimonabant (Taslimi, Haghparast, Hassanpour-Ezatti, & Safari, 2011). Moreover, coadministration of effective doses of both HcrtR1 and CB1 antagonists into the VTA reduces conditioned place preference in a nonadditive manner, suggesting that these receptors regulate this effect by a common mechanism (Taslimi et al., 2011). This goes in line with the idea that hypocretins and endocannabinoids may share a common site of action for reward modulation, since primary rewarding effects of drugs of abuse that depend on increased VTA dopaminergic activity, such as opioids, nicotine, and alcohol, require both hypocretinergic transmission and presence of CB1 receptors. A possible explanation for this cannabinoid-mediated effect of hypocretins is the synthesis and release of 2-AG upon HcrtR1 activation, mentioned previously. However, this mechanism has been only confirmed to occur in the periaqueductal gray (Ho et al., 2011), and in the dorsal raphe nucleus (Haj-Dahmane & Shen, 2005), and further studies will be necessary to better understand the interaction between the endocannabinoid and hypocretin systems in the regulation of the reward circuit.

ROLE OF HYPOCRETINS/OREXINS IN CANNABINOID DEPENDENCE

Despite the evidence supporting the hypocretin-cannabinoid cross talk in the CNS and the undeniable regulation of the addictive properties of several drugs of abuse by the hypocretinergic system, there is little information regarding the role of hypocretins in cannabinoid dependence. The main data available are focused on the rewarding properties of cannabinoids, whereas other relevant aspects of cannabinoid dependence, such as relapse and withdrawal, remain unexplored.

The initiation of cannabis addiction has been related to its capacity to induce rewarding effects. There are several predictive animal models to study responses related to the rewarding effects produced by cannabinoids. Among these paradigms, drug self-administration procedures currently represent the most reliable models of drug consumption in humans and have a high predictive value by directly evaluating the reinforcing properties of the drug (Maldonado et al., 2011). Recent studies have revealed persistent cannabinoid self-administration under different experimental conditions. Thus, intravenous self-administration of THC has been described in squirrel monkeys (Justinová, Tanda, Redhi, & Goldberg, 2003). Although operant responding for self-infused THC has not been consistently demonstrated in rodents, intravenous self-administration of the synthetic and short half-life cannabinoid agonist WIN55,212-2 has been reported in rats and mice (Solinas et al., 2007; Mendizábal, Zimmer, & Maldonado, 2006).

A recent study has investigated the contribution of hypocretins to the reinforcing properties of cannabinoids, by using the intravenous WIN55,212-2 self-administration paradigm in mice (Flores et al., 2014). In this study, animals were trained to self-administer the synthetic cannabinoid during 12 days, and the role of HcrtRs was evaluated by diverse pharmacological and genetic approaches (Fig. 56.4). The study showed that a chronic treatment with the HcrtR1 antagonist SB334867 during the acquisition phase of WIN55,212-2 self-administration reduced the number of animals reaching the acquisition criteria for this operant response. Moreover, acute SB334867 administration, after a successful acquisition of WIN55,212-2 self-administration, impaired the reinforcing properties of this synthetic cannabinoid. An acute HcrtR1 antagonist injection also reduced the maximal effort required to obtain a WIN55,212-2 infusion in a progressive ratio schedule session, suggesting a decrease in the motivation for the drug. On the contrary, the administration of the HcrtR2 antagonist TCSOX229 did not modify WIN55,212-2 self-administration in any of the reinforcement schedules tested. These data point to a specific role for HcrtR1 in the modulation of the reinforcing properties of cannabinoids. In fact, HcrtR2 appears to be of minor relevance in the regulation of the rewarding properties of drugs of abuse, although both hypocretin receptor subtypes are abundant in brain areas relevant in reward processing, such as the VTA, NAc, and bed nucleus of the stria terminalis (Plaza-Zabala et al., 2012). In agreement with the pharmacological data previously mentioned, HcrtR1 knockout mice showed an impaired WIN55,212-2 self-administration behavior in comparison with wild-type animals (Flores et al., 2014). Thus, the acquisition of the operant behavior was successfully reached by a minor percentage of mutant mice. The amount of WIN55,212-2 self-infused by these knockout animals was reduced, as well. Mice lacking HcrtR1 also showed diminished motivation for the cannabinoid, since their maximal effort to obtain a WIN55,212-2 infusion was reduced, when compared to wild-type mice in a progressive ratio schedule session. It is worth mentioning that the impairment of WIN55,212-2 self-administration was not attributable to other unspecific effects, such as locomotion alteration or possible learning deficits in the acquisition of an operant behavior, since none of the treatments or genetic manipulations altered operant responding for a natural reward such as water. As a whole, these behavioral data suggest that the primary reinforcing properties of cannabinoids, as well as motivation to obtain the drug are modulated by HcrtR1 signaling (Flores et al., 2014).

Little information is available regarding the possible changes of the hypocretinergic activity in response to a chronic cannabinoid exposure. It has been recently shown that chronic THC administration during the adolescent period of male rats decreases hypothalamic prepro-hypocretin mRNA levels in adulthood (Llorente-Berzal et al., 2013). Interestingly, hypothalamic prepro-hypocretin mRNA levels remain unaffected in adult female rats receiving THC during adolescence (Llorente-Berzal et al., 2013). This sexual dimorphism results are interesting, since female rats also acquire stable WIN55,212-2 self-administration more rapidly and at higher rates than males (Fattore et al., 2007). Thus, lower hypocretin levels could be related to phenotypes less vulnerable to cannabinoid dependence. On the other hand, it has been observed that hypocretin-1 expression in peripheral blood cells is reduced in cannabis-dependent smokers, when compared to nicotine-dependent smokers and nonsmokers (Rotter et al., 2012). However, this information should be carefully interpreted, since peripheral hypocretin mRNA levels do not necessarily reflect the situation in certain areas of the CNS. Moreover, these differences could be related to peripheral effects of THC, and not to the central modulation of cannabinoid dependence (Flores et al., 2013).

Operant yoked-control models of drug self-administration are essential to understanding whether neurochemical changes are attributable to the effects of drug

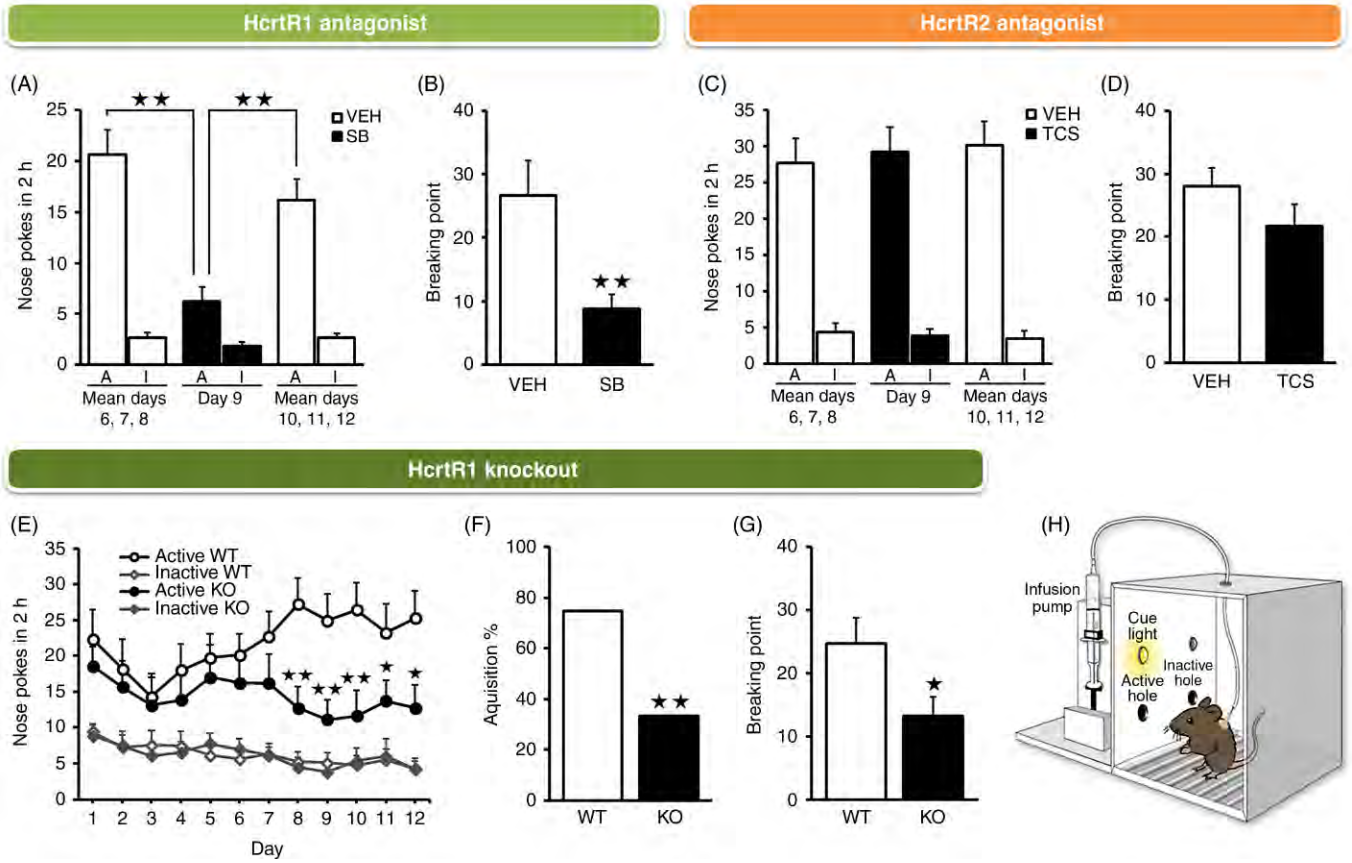


FIGURE 56.4 Effects of HcrtR1 and HcrtR2 pharmacological blockade or HcrtR1 gene deletion in WIN55,212-2 self-administration. HcrtR1 antagonization or gene deletion reduces operant WIN55,212-2 self-administration, and motivation to obtain the drug, whereas HcrtR2 pharmacological blockade has no effect on this behavioral response. In the pharmacological approach (A–D) mice were trained to self-administer the synthetic cannabinoid WIN55,212-2 under fixed ratio 1 (FR1) until stable behavior was acquired. SB334867 (10 mg/kg, intraperitoneal) or TCSOX229 (10 mg/kg, intraperitoneal) was administered 30 min before a FR1 session on day 9, or before the progressive ratio (PR) session. (A, C) Number of active and inactive responses displayed after acute (A) SB334867 or (C) TCSOX229 pretreatment on day 9, and during the 3 days before (mean days 6–8), and after (mean days 10–12) these pharmacologic challenges. (B, D) Breaking-point values achieved in the PR schedule after acute (B) SB334867 or (D) TCSOX229 pretreatment. In the genetic approach (E–G) HcrtR1 knockout and wild-type mice were trained to self-administer WIN55,212-2 during 12 days, and underwent a PR session on day 13. The time course (E) and the percentage of acquisition (F) of WIN55,212-2 self-administration in wild-type and knockout mice is shown, as well as the breaking-point values achieved in the PR schedule (G). (H) Schematic diagram of operant chambers where drug self-administration procedures are performed. Values are represented as mean + SEM. *SB*, SB334867; *VEH*, vehicle; *TCS*, TCSOX229; *WT*, wild-type; *KO*, HcrtR1 knockout; *A*, active hole; *I*, inactive hole; ★, $p < 0.05$; ★★, $p < 0.01$ when compared to the control group. *Source: Adapted with permission from Flores et al. (2014).*

seeking, or to the pharmacologic actions of the drug. Interestingly, contingent (active) and noncontingent (passive) WIN55,212-2 self-administration have been reported to modulate differently the activation of hypocretin neurons (Flores et al., 2014). Thus, immunofluorescence studies showed that contingent self-administration of the cannabinoid induced an increase in the percentage of hypocretin cells expressing FosB/ΔFosB in the LH, a transcription factor considered a marker of neuronal activity (Fig. 56.5). Notably, hypocretin cells were not activated in mice receiving passive infusions of the cannabinoid, suggesting that the recruitment of hypocretin cells within the LH is mainly due to operant seeking for the reinforcing effects of this drug. Moreover, these neurons were not recruited due to the learning process inherent to the acquisition of an operant behavior, since operant conditioning maintained by water did not modify the activation of hypocretin cells within the LH. In contrast, operant WIN55,212-2 self-administration had no apparent effect on FosB/ΔFosB expression in DMH/PFA hypocretin neurons, although mice receiving passive infusions of the drug showed a lower activation of these cells, in comparison with those receiving the drug contingently (Fig. 56.5). Activation of hypocretin cells located within the LH, but not those in the DMH/PFA, has been related also with the exposure to other drugs of abuse, such as morphine (Richardson & Aston-Jones, 2012) and ethanol (Lawrence, Cowen, Yang, Chen, & Oldfield, 2006). In fact, it has been proposed the existence of a functional dichotomy between the diverse

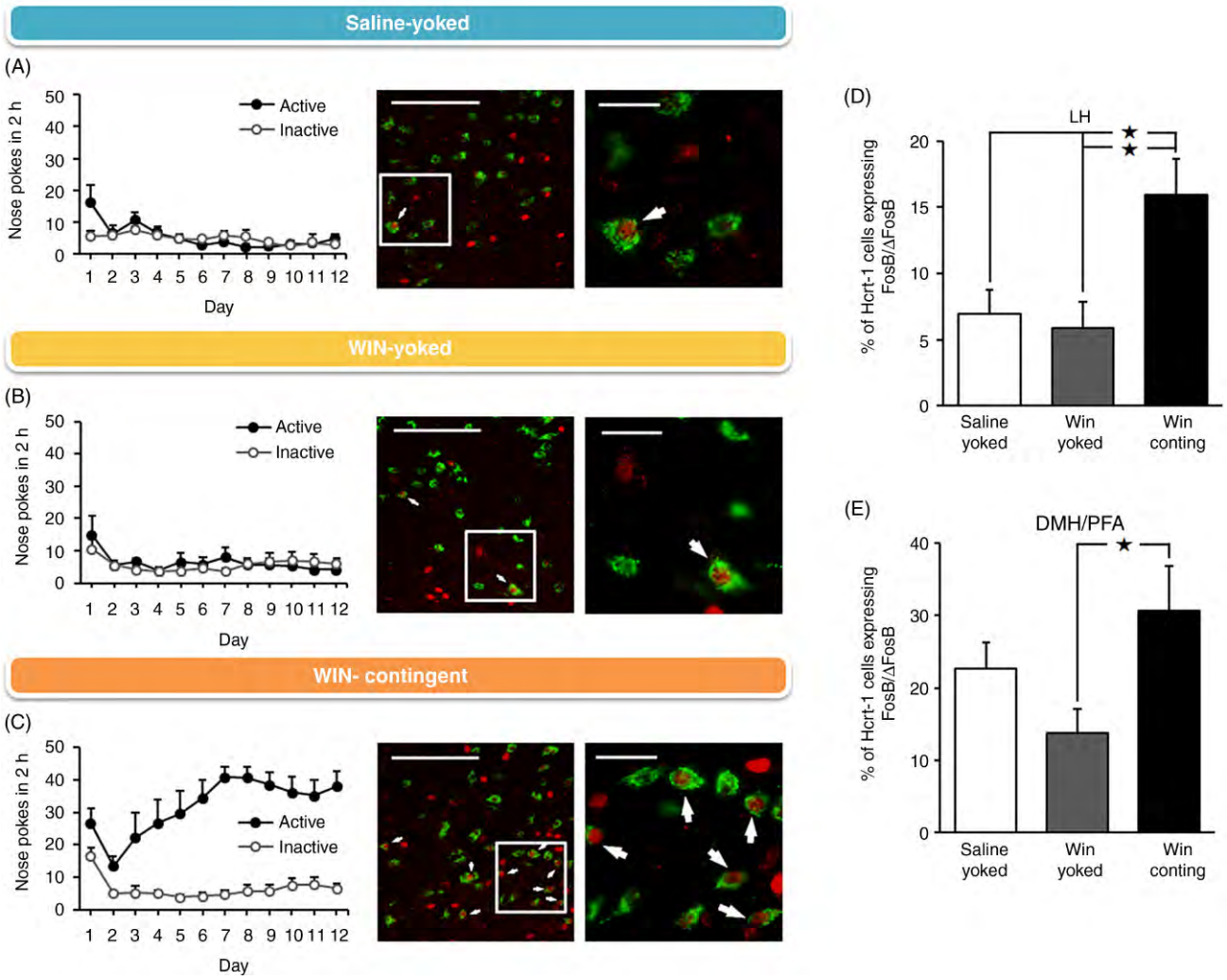


FIGURE 56.5 **Hypocretin/orexin neurons in the lateral hypothalamus are activated during contingent, but not noncontingent, WIN55,212-2 self-administration.** Double-label immunofluorescence of FosB/ΔFosB with hypocretin-1 was performed in the lateral as well as dorsomedial and perifornical hypothalamic area of mice that had underwent a contingent/noncontingent WIN55,212-2 self-administration paradigm during 12 days. (A–C) Operant responding on the active and inactive holes in mice receiving (A) passive saline infusions (saline-yoked mice), (B) passive intravenous WIN55,212-2 infusions (WIN-yoked mice), and (C) contingent intravenous WIN55,212-2 infusions (WIN-contingent). Each group is illustrated with their corresponding representative images of sections of the LH obtained via fluorescence microscopy. Arrowheads indicate FosB/ΔFosB positive hypocretin-1 expressing neurons. The scale bar represents 100 μm in left-side images and 25 μm in right-side images. (D,E) Percentage of FosB/ΔFosB positive hypocretin-1 expressing neurons after contingent/noncontingent WIN55,212-2 self-administration in the LH (D) and the DMH/PFA (E). Values are represented as mean + SEM. *LH*, lateral hypothalamus; *DMH/PFA*, dorsomedial and perifornical area; *WIN*, WIN55,212-2; *, $p < 0.05$ between groups. *Source: Adapted with permission from Flores et al. (2014).*

hypocretin neurons subpopulations, being those located in the LH mainly involved in reward processing, whereas those located in the DMH/PFA are more related with regulation of arousal and stress (Harris & Aston-Jones, 2006). However, some divergent findings suggest that further studies are required to support a differentiated role for each hypocretin cell subpopulation.

As previously mentioned, the modulation of VTA dopaminergic transmission by hypocretins seems to be especially important in the regulation of reward processing, particularly in drug seeking behavior (Mahler, Smith, Moorman, Sartor, & Aston-Jones, 2012). Accordingly, cannabinoid-induced release of dopamine in the NAc, which correlates with the rewarding effects of the drug, seems to be modulated by HcrtR1 signaling. Thus, increased extracellular dopamine levels in the NAc, induced by an acute systemic injection of THC, was observed in wild-type mice but not in HcrtR1 knockout animals, as revealed by in vivo microdialysis studies (Fig. 56.6) (Flores et al., 2014). Although the neuronal mechanism remains unclear at present, it seems that cannabinoid exposure could activate hypocretin transmission in the VTA. The subsequent activation of HcrtR1 in the VTA might be contributing to the rewarding

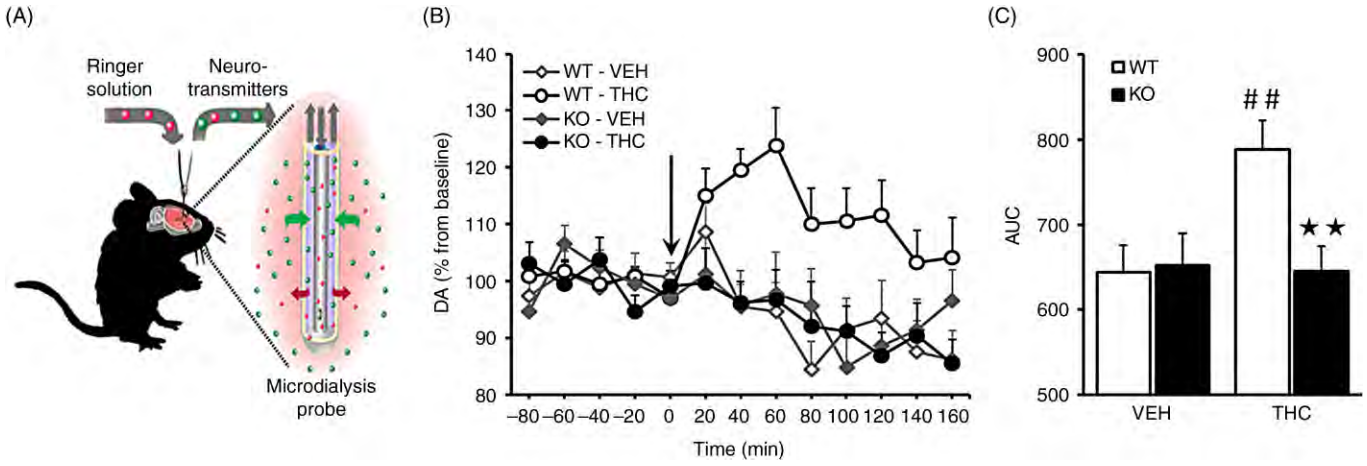


FIGURE 56.6 **THC-induced enhancement in dopamine extracellular levels in the nucleus accumbens is blocked in HcrtR1 knockout mice.** (A) Schematic diagram of in vivo microdialysis principles. (B) Time course of basal and stimulated levels of dopamine (percentage from baseline) in the nucleus accumbens. Dialysates were collected 80 min before and 160 min after a challenge injection of THC (0.3 mg/kg, intraperitoneal; arrow at time 0) or vehicle in wild-type and HcrtR1 knockout mice. (C) Area under the curve (AUC) values for levels after THC or vehicle injection (from 0 to 160 min) for the different groups of mice. Values are represented as mean+SEM. *DA*, dopamine; *WT*, wild-type; *KO*, knockout; *VEH*, vehicle; **, $p < 0.01$ between genotypes; $^{\#\#}$, $p < 0.01$ between treatments. *Source: Adapted with permission from Flores et al. (2014).*

properties of cannabinoids through the modulation of the dopamine mesolimbic pathway.

In summary, hypocretin transmission at HcrtR1 is a critical component involved in the reinforcing and motivational properties of cannabinoids. Therefore, HcrtR1 antagonists could represent an interesting pharmacologic tool for cannabis dependence in humans, as this is a major clinical need devoid of available treatment at the present moment.

MINI-DICTIONARY

Conditioned place preference Behavioral paradigm used to determine the rewarding or aversive effects of a drug, performed in a two-compartment box. Mice are administered with a drug in one of the compartments, and with vehicle in the other compartment, during several days. The time spent in each compartment during the test day gives an idea of the reward or aversion induced by the drug.

Fluorescence and bioluminescence resonance energy transfer (FRET and BRET) In vitro technique used to determine if two components are close enough to form a complex, based on the distance at which different types of energy is able to be transferred from one to another.

G-protein coupled receptor Receptor located in the cellular membrane that activates an intracellular pathway through stimulation of a G protein.

Heteromer A complex formed by different types of subunits. In this case, this complex is formed by diverse receptors, and shows features distinct from those of its components.

In vivo microdialysis Technique used to measure in vivo the amount of a determined compound (eg, a neurotransmitter) released to the extracellular space, in a small volume of tissue (eg, a defined brain structure). It requires the implantation of a probe containing a semipermeable membrane that will be crossed by small solutes by passive diffusion.

Mesocorticolimbic system Group of brain structures involved in the processing of reward and hedonic experiences, including the ventral tegmental area in the brainstem, and its target structures, such as nucleus accumbens, prefrontal cortex, amygdala, and bed nucleus of stria terminalis.

Reinforcement A term used in operant conditioning to refer to a stimulus that increases the probability that a response will occur. For instance, in an attempt to increase the likelihood of a behavior occurring in the future, an operant response is associated by the presentation of a reward.

Synaptic plasticity Group of processes that confer to a synapse the ability to strengthen or weaken its capacity of transmission.

References

Aston-Jones, G., Smith, R. J., Moorman, D. E., & Richardson, K. A. (2009). Role of lateral hypothalamic orexin neurons in reward processing and addiction. *Neuropharmacology*, *56*(Suppl 1), 112–121.

Chen, J. P., Paredes, W., Li, J., Smith, D., Lowinson, J., & Gardner, E. L. (1990). Delta 9-tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. *Psychopharmacology*, *102*, 156–162.

Crespo, I., Gómez de Heras, R., Rodríguez de Fonseca, F., & Navarro, M. (2008). Pretreatment with subeffective doses of Rimonabant attenuates orexigenic actions of orexin A-hypocretin 1. *Neuropharmacology*, *54*, 219–225.

de Lecea, L., Kilduff, T. S., Peyron, C., Gao, X., Foye, P. E., Danielson, P. E., Fukuhara, C., Battenberg, E. L., Gautvik, V. T., Bartlett, F. S., 2nd., Frankel, W. N., van den Pol, A. N., Bloom, F. E., Gautvik, K. M., & Sutcliffe, J. G. (1998). The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 322–327.

Demuth, D. G., & Molleman, A. (2006). Cannabinoid signalling. *Life Sciences*, *78*, 549–563.

Ellis, J., Pediani, J. D., Canals, M., Milasta, S., & Milligan, G. (2006). Orexin-1 receptor-cannabinoid CB1 receptor heterodimerization

results in both ligand-dependent and -independent coordinated alterations of receptor localization and function. *Journal of Biological Chemistry*, *281*, 38812–38824.

España, R. A., Oleson, E. B., Locke, J. L., Brookshire, B. R., Roberts, D. C., & Jones, S. R. (2010). The hypocretin-orexin system regulates cocaine self-administration via actions on the mesolimbic dopamine system. *European Journal of Neuroscience*, *31*, 336–348.

Fattore, L., Spano, M. S., Altea, S., Angius, F., Fadda, P., & Fratta, W. (2007). Cannabinoid self-administration in rats: sex differences and the influence of ovarian function. *British Journal of Pharmacology*, *152*, 795–804.

Fattore, L., Fadda, P., Spano, M. S., Pistis, M., & Fratta, W. (2008). Neurobiological mechanisms of cannabinoid addiction. *Molecular and Cellular Endocrinology*, *286*, S97–S107.

Flores, Á., Maldonado, R., & Berrendero, F. (2013). Cannabinoid-hypocretin cross-talk in the central nervous system: what we know so far. *Frontiers in Neuroscience*, *7*, 256.

Flores, Á., Maldonado, R., & Berrendero, F. (2014). The hypocretin/orexin receptor-1 as a novel target to modulate cannabinoid reward. *Biological Psychiatry*, *75*, 499–507.

Haj-Dahmane, S., & Shen, R. Y. (2005). The wake-promoting peptide orexin-B inhibits glutamatergic transmission to dorsal raphe nucleus serotonin neurons through retrograde endocannabinoid signaling. *Journal of Neuroscience*, *25*, 896–905.

Harris, G. C., & Aston-Jones, G. (2006). Arousal and reward: a dichotomy in orexin function. *Trends in Neurosciences*, *29*, 571–577.

Hilairet, S., Bouaboula, M., Carrière, D., Le Fur, G., & Casellas, P. (2003). Hypersensitization of the Orexin 1 receptor by the CB1 receptor: evidence for cross-talk blocked by the specific CB1 antagonist, SR 141716. *Journal of Biological Chemistry*, *278*, 23731–23737.

Ho, Y. C., Lee, H. J., Tung, L. W., Liao, Y. Y., Fu, S. Y., Teng, S. F., Liao, H. T., Mackie, K., & Chiou, L. C. (2011). Activation of orexin 1 receptors in the periaqueductal gray of male rats leads to antinociception via retrograde endocannabinoid (2-arachidonoylglycerol)-induced disinhibition. *Journal of Neuroscience*, *31*, 14600–14610.

Julian, M. D., Martin, A. B., Cuellar, B., Rodriguez De Fonseca, F., Navarro, M., Moratalla, R., & Garcia-Segura, L. M. (2003). Neuroanatomical relationship between type 1 cannabinoid receptors and dopaminergic systems in the rat basal ganglia. *Neuroscience*, *119*, 309–318.

Justinová, Z., Tanda, G., Redhi, G. H., & Goldberg, S. R. (2003). Self-administration of delta9-tetrahydrocannabinol (THC) by drug naive squirrel monkeys. *Psychopharmacology*, *169*, 135–140.

Khoo, S. Y., & Brown, R. M. (2014). Orexin/hypocretin based pharmacotherapies for the treatment of addiction: DORA or SORA? *CNS Drugs*, *28*, 713–730.

Kukkonen, J. P. (2013). Physiology of the orexinergic/hypocretinergic system: a revisit in 2012. *American Journal of Physiology—Cell Physiology*, *304*, C2–C32.

Lawrence, A. J., Cowen, M. S., Yang, H. J., Chen, F., & Oldfield, B. (2006). The orexin system regulates alcohol-seeking in rats. *British Journal of Pharmacology*, *148*, 752–759.

Li, J., Hu, Z., & de Lecea, L. (2014). The hypocretins/orexins: integrators of multiple physiological functions. *British Journal of Pharmacology*, *171*, 332–350.

Llorente-Berzal, A., Puighermanal, E., Burokas, A., Ozaita, A., Maldonado, R., Marco, E. M., & Viveros, M. P. (2013). Sex-dependent psychoneuroendocrine effects of THC and MDMA in an animal model of adolescent drug consumption. *PLoS One*, *8*, e78386.

Mackie, K. (2005). Distribution of cannabinoid receptors in the central and peripheral nervous system. *Handbook of Experimental Pharmacology*, *168*, 299–325.

Mahler, S. V., Smith, R. J., Moorman, D. E., Sartor, G. C., & Aston-Jones, G. (2012). Multiple roles for orexin/hypocretin in addiction. *Progress in Brain Research*, *198*, 79–121.

Maldonado, R., Valverde, O., & Berrendero, F. (2006). Involvement of the endocannabinoid system in drug addiction. *Trends in Neurosciences*, *29*, 225–232.

Maldonado, R., Berrendero, F., Ozaita, A., & Robledo, P. (2011). Neurochemical basis of cannabis addiction. *Neuroscience*, *181*, 1–17.

Marcus, J. N., Aschkenasi, C. J., Lee, C. E., Chemelli, R. M., Saper, C. B., Yanagisawa, M., & Elmquist, J. K. (2001). Differential expression of orexin receptors 1 and 2 in the rat brain. *Journal of Comparative Neurology*, *435*, 6–25.

Mendizábal, V., Zimmer, A., & Maldonado, R. (2006). Involvement of kappa/dynorphin system in WIN 55 212-2 self-administration in mice. *Neuropsychopharmacology*, *31*, 1957–1966.

Peyron, C., Tighe, D. K., van den Pol, A. N., de Lecea, L., Heller, H. C., Sutcliffe, J. G., & Kilduff, T. S. (1998). Neurons containing hypocretin (orexin) project to multiple neuronal systems. *Journal of Neuroscience*, *18*, 9996–10015.

Plaza-Zabala, A., Maldonado, R., & Berrendero, F. (2012). The hypocretin/orexin system: implications for drug reward and relapse. *Molecular Neurobiology*, *45*, 424–439.

Richardson, K. A., & Aston-Jones, G. (2012). Lateral hypothalamic orexin/hypocretin neurons that project to ventral tegmental area are differentially activated with morphine preference. *Journal of Neuroscience*, *32*, 3809–3817.

Rotter, A., Bayerlein, K., Hansbauer, M., Weiland, J., Sperling, W., Kornhuber, J., & Biermann, T. (2012). Orexin A expression and promoter methylation in patients with cannabis dependence in comparison to nicotine-dependent cigarette smokers and nonsmokers. *Neuropsychobiology*, *66*, 126–133.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R. M., Tanaka, H., Williams, S. C., Richardson, J. A., Kozlowski, G. P., Wilson, S., Arch, J. R., Buckingham, R. E., Haynes, A. C., Carr, S. A., Annan, R. S., McNulty, D. E., Liu, W. S., Terrett, J. A., Elshourbagy, N. A., Bergsma, D. J., & Yanagisawa, M. (1998). Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*, *92*, 573–585.

Solinas, M., Scherma, M., Fattore, L., Stroik, J., Wertheim, C., Tanda, G., Fratta, W., & Goldberg, S. R. (2007). Nicotinic alpha 7 receptors as a new target for treatment of cannabis abuse. *Journal of Neuroscience*, *27*, 5615–5620.

Tanda, G., Pontieri, F. E., & Di Chiara, G. (1997). Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. *Science*, *276*, 2048–2050.

Taslimi, Z., Haghparast, A., Hassanpour-Ezatti, M., & Safari, M. S. (2011). Chemical stimulation of the lateral hypothalamus induces conditioned place preference in rats: involvement of OX1 and CB1 receptors in the ventral tegmental area. *Behavioral Brain Research*, *217*, 41–46.

Turunen, P. M., Jäntti, M. H., & Kukkonen, J. P. (2012). OX1 orexin/hypocretin receptor signaling through arachidonic acid and endocannabinoid release. *Molecular Pharmacology*, *82*, 156–167.

Xu, T. -R., Yang, Y., Ward, R., Gao, L., & Liu, Y. (2013). Orexin receptors: multi-functional therapeutic targets for sleeping disorders, eating disorders, drug addiction, cancers and other physiological disorders. *Cellular Signalling*, *25*, 2413–2423.

CHAPTER

57

Regulatory Role of Cannabinoids for Skin Barrier Functions and Cutaneous Inflammation

T. Tüting, E. Gaffal

Laboratory of Experimental Dermatology, Department of Dermatology and Allergy, University Hospital of the Friedrich-Wilhelm-University Bonn, Bonn, Germany

SUMMARY POINTS

- This chapter focuses on the role of endogenous and exogenous cannabinoids for the regulation of skin inflammation in allergic contact dermatitis and atopic dermatitis.
- The endogenous cannabinoid system with its receptors, lipid ligands, and enzymes for their synthesis and degradation can be found in human skin.
- Endogenous and exogenous cannabinoids have been shown to downregulate allergic inflammation in the skin. In particular, CB1 receptors on keratinocytes help limit inflammation, and promote the homeostatic regeneration of epidermal integrity.
- These insights have important implications for the future development of strategies to harness cannabinoids for the treatment of inflammatory skin diseases.

KEY FACTS OF ALLERGIC CONTACT DERMATITIS AND ATOPIC DERMATITIS

- Allergic contact dermatitis occurs in about 11% of women and 5% of men in industrialized countries, and is one of the most common occupational diseases.
- Contact allergy remains for a lifetime. Treatment includes the local application of steroids. Furthermore, contact to known allergens should be avoided.
- Up to 10% of children and 1–3% of adults in industrialized countries are affected by atopic dermatitis. Genetic factors play an important role in the pathogenesis of this inflammatory skin disease.
- Patients with atopic dermatitis often also suffer from allergic asthma, and they tend to develop food allergies.
- Common treatments of atopic eczema include skin moisturizers, topical and systemically application of steroids, topical immunomodulators (eg, Tacrolimus, Pimecrolimus), and UV-light therapy.

LIST OF ABBREVIATIONS

ACD	Allergic contact dermatitis
CB	Cannabinoid
CCL, CXCL	Chemokine subfamilies
CHS	Contact hypersensitivity
Cnr	Gene ID for cannabinoid receptor
DNFB	2,4-Dinitro-1-fluorobenzene
ECS	Endogenous cannabinoid system
FITC	Fluorescein isothiocyanate
IFN	Interferon
IgE	Immunoglobulin E
IL	Interleukin
KC	Keratinocyte

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00067-3
Copyright © 2017 Elsevier Inc. All rights reserved.

NOD	Nucleotide-binding oligomerization domain
PEA	Palmitoylethanolamide
TGF	Transforming growth factor
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TSLP	Thymic stromal lymphopoietin

THE SKIN AS A BARRIER ORGAN

With a size of approximately 1.5–2.0 m^2, the skin is the largest organ of the human body. Similar to lung and intestinal mucosal tissue, it forms a barrier between the organism and its surrounding environment that protects from harmful physical, chemical, or biological influences such as UV radiation, toxic substances, or pathogenic microorganisms (outside-in barrier). In addition, our skin regulates body temperature and prevents excessive loss of water and electrolytes (inside-out barrier). The skin can be divided into three layers: the epidermis (outer layer), the dermis, and the subcutaneous tissue (hypodermis). The epidermis is a stratified squamous epithelium which constantly renews itself under physiological and pathophysiological conditions, and forms the first physical and immunological barrier. It mostly consists of keratinocytes which are arranged in four layers: the stratum basale (basal cell layer), spinosum, granulosum, and corneum. The basal cell layer contains undifferentiated keratinocytes attached to the basal membrane by keratin filaments (keratin 5, keratin 14). The stratum spinosum is named after the spine-like shape of keratinocytes in this part of the epidermis. Here, keratinocytes slowly begin to differentiate, keratin 5 and 14 are replaced by the differentiation-specific keratins 1 and 10 (Fuchs & Green, 1980; Waseem et al., 1999). Lamellar bodies, which contain lipids and hydrolytic enzymes, can now be detected. The stratum granulosum is characterized by basophilic keratohyalin granules found in further differentiating keratinocytes. They contain profilaggrin, loricrin, and keratin filaments which are important components for the formation of the cornified cell envelope, and are crucial for cell stability. Finally, keratinocytes undergo a specialized form of programmed cell death to form the stratum corneum. This outermost layer consists of dead lipid-depleted keratinocytes (corneocytes) surrounded by a lipid matrix predominantly made of ceramides and cholesterol (Houben, De Paepe, & Rogiers, 2007; Kalinin, Kajava, & Steinert, 2002). This structure is also known as "bricks" and "mortar." It helps to protect the skin from water loss, and prevents the penetration of environmental toxins, allergens, and pathogens.

Keratinocytes not only maintain a physical barrier, but also play a central role in the regulation of immunological barrier functions in the epidermis. Damage to the skin leads to the secretion of numerous cytokines and chemokines such as TNF, IL1, IL8, IL18, CXCL2, CXCL10, and CCL2. These mediators activate local immune cells and endothelial cells, initiate antigen-specific immune responses, and recruit various immune cells into the skin (Albanesi, 2010; Cataisson et al., 2006; Ryckman, Vandal, Rouleau, Talbot, & Tessier, 2003; Wetzler, Kampfer, Pfeilschifter, & Frank, 2000). On the surface of keratinocytes, there are numerous cytokine and chemokine receptors which modulate inflammatory immune responses. Their activation also regulates the proliferation and differentiation of keratinocytes. In addition, keratinocytes secrete antimicrobial peptides (AMPs), such as beta-defensins and cathelicidin, which prevent the invasion of microbes by direct killing of pathogens, and activation of the innate immune system. Furthermore, they are able to recognize invading viruses, parasites, bacteria, and fungi in the skin through Toll-like (TLR) and NOD-like receptors. Perturbations in the physical and immunological functions of keratinocytes are of major importance for the development of allergic inflammatory responses in the skin (Fig. 57.1).

THE ENDOGENOUS CANNABINOID SYSTEM IN KERATINOCYTES

The endogenous cannabinoid system (ECS) with its specific receptors, endogenous ligands, and enzymes for their synthesis and degradation can be found in human and murine skin (Maccarrone et al., 2003; Stander, Reinhardt, & Luger, 2006). The CB1 receptor is expressed on keratinocytes in the stratum spinosum and stratum granulosum of the epidermis, and can be found in differentiated cells of the hair follicle. CB2 receptors were detected in undifferentiated cells of the stratum basale, and in hair follicles (Stander, Schmelz, Metze, Luger, & Rukwied, 2005). Cultured immortalized and normal human keratinocytes (HaCaT, NHEK) also express cannabinoid receptors, and possess a fully active biochemical machinery to metabolize endogenous cannabinoids. The ECS of the skin plays an important role for the growth and differentiation of keratinocytes. The endogenous cannabinoid anandamide inhibits the differentiation of keratinocytes in vitro by downregulating the expression of epidermal structure proteins like keratin 1, keratin 10, and transglutaminase 5 in a CB1-dependent way (Paradisi, Pasquariello, Barcaroli, & Maccarrone, 2008). Plant-derived cannabinoids like Δ^9-tetrahydrocannabinol (THC) exert an antiproliferative effect on human keratinocytes and NHEKs in vitro (Wilkinson & Williamson, 2007). The pharmacological blockade of CB1 receptors on human keratinocytes induces an increased production of inflammatory mediators like IL8 and CCL2/MCP1.

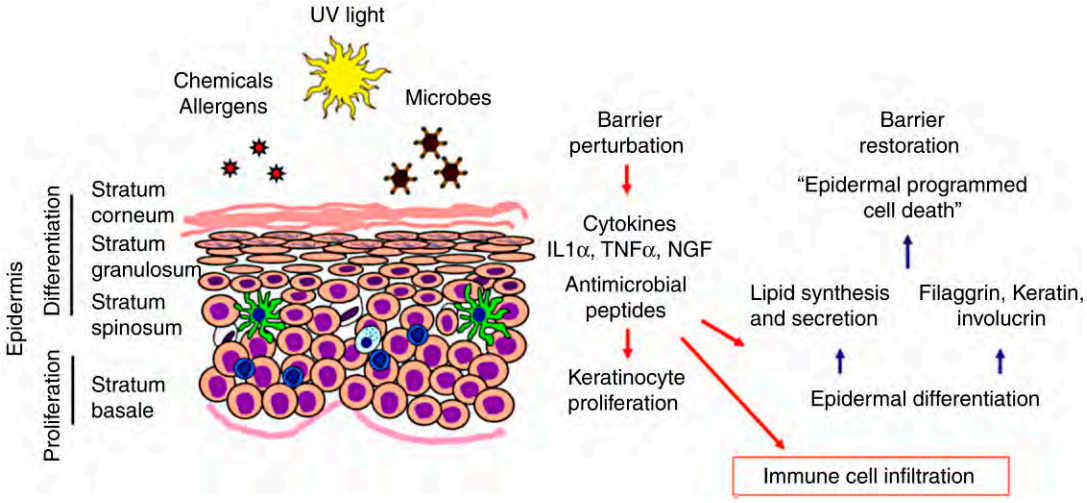


FIGURE 57.1 **Regulation of epidermal barrier functions.** Schematic diagram of the epidermis. It mostly consists of keratinocytes, organized in four layers: the stratum basale, spinosum, granulosum, and corneum. Basal keratinocytes proliferate, and slowly move to the upper layers of the skin. During this time, they differentiate and undergo a defined "epidermal" programmed cell death to form the outermost lipid-rich corneal layer that protects the body from chemicals, and prevents excessive water loss. Through the secretion of various cytokines, chemokines, and antimicrobial peptides, keratinocytes also actively participate in the regulation of immune responses in the skin.

ALLERGIC CONTACT DERMATITIS

The clinical term "allergic contact dermatitis" describes an inflammatory response of the skin after contact to certain highly reactive chemicals with a low molecular weight, which can penetrate the skin barrier. Key findings about the underlying pathophysiology were obtained with the help of experimental mouse models for contact sensitization ("contact hypersensitivity" or CHS). Here, obligate contact sensitizers like 2,4-dinitro-1-fluorobenzene (DNFB) are commonly used to induce contact hypersensitivity. Allergic contact dermatitis develops in three phases. In the sensitization phase, chemicals react with epidermal proteins to form so-called haptens, and stimulate the innate immune system via TLRs (Kaplan, Igyarto, & Gaspari, 2012; Schmidt et al., 2010), as well as the inflammasome (Watanabe et al., 2007) on resident skin cells. The production of proinflammatory mediators (TNF, IL1, IL18) leads to the activation of cutaneous dendritic cells (DC) and Langerhans cells. They take up the haptenized proteins and migrate to the draining lymph nodes. Here, they present the allergen to antigen-specific naive CD8+ and CD4+ T-lymphocytes which get activated, and differentiate toward Th1/Th17 phenotypes. In the effector phase, repeated exposure to the hapten leads to the recruitment of allergen-specific T lymphocytes in the skin. They secrete IL17 and IFNg which induce the production of proinflammatory mediators including CXCL1, CXCL2, CXCL10, and CCL2 by keratinocytes and endothelial cells. These inflammatory mediators support the cutaneous infiltration of neutrophil granulocytes and macrophages. Together with keratinocytes, they initially amplify the infiltration with effector cells (Engeman, Gorbachev, Kish, & Fairchild, 2004; Kish, Li, & Fairchild, 2009; Kish, Gorbachev, Parameswaran, Gupta, & Fairchild, 2012). In this inflammatory environment, keratinocytes initiate a regenerative proliferative response which contributes to the restoration of epidermal barrier functions. When the individual is no longer exposed to the allergen, inflammation gradually decreases in the resolution phase. Now, regulatory CD4 + T cells secrete cytokines such as IL-10 and TGF which downregulate proinflammatory mediators, and inhibit further activation of dendritic cells, neutrophils, and macrophages (Cavani et al., 2000; Cavani, De Pità, & Girolomoni, 2007; Grimbaldeston, Nakae, Kalesnikoff, Tsai, & Galli, 2007).

THE ENDOGENOUS CANNABINOID SYSTEM ATTENUATES CONTACT ALLERGIC INFLAMMATION

Using the model of experimental contact allergy, we were able to show for the first time that the endogenous cannabinoid system participates in the regulation of allergic skin inflammation. Mice with a genetic deletion of CB1 and CB2 receptors ($Cnr1/2^{-/-}$ mice) display stronger signs of inflammation, including increased allergic ear swelling and elevated infiltration of inflammatory immune cells, when compared with wild-type animals. The pharmacological blockade of CB receptors using specific antagonists (SR141716 and SR144528) also resulted in a gain of allergic ear swelling in wild-type mice. In contrast, mice genetically lacking enzymes that degrade endocannabinoids ($FAAH^{-/-}$ mice) displayed

reduced signs of allergic ear swelling. A whole-genome mRNA expression analysis of contact allergic inflamed ear tissue revealed a significantly increased expression of the proinflammatory chemokine CCL8/MCP2 in Cnr1/2$^{-/-}$ mice, when compared to wild-type animals. Interestingly, especially CB1 receptors seemed to play a central role in modulating cutaneous inflammation since Cnr1$^{-/-}$ animals developed an allergic reaction that was nearly as strong as that observed in Cnr1/2$^{-/-}$ mice (Karsak et al., 2007).

CB1 RECEPTORS ON KERATINOCYTES DOWNREGULATE THE SECRETION OF PROINFLAMMATORY CHEMOKINES IN CONTACT ALLERGIC INFLAMMATION

CB1 receptors are primarily expressed in the central nervous system (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990). Here, they are important for the sensation of pain (Zimmer, Zimmer, Hohmann, Herkenham, & Bonner, 1999), the extinction of aversive memories (Marsicano et al., 2002), and for protection against excessive neuronal activity (Marsicano et al., 2003). However, in the last years, evidence has accumulated that CB1 receptors also modulate protective and inflammatory processes in peripheral organs. In an experimental model of inflammatory bowel disease, the activation of CB1 receptors on peripheral nerve fibers resulted in a reduction of gastrointestinal inflammation, and inflammation-induced hypermotility (Massa et al., 2004). In addition to their functions in the central and peripheral nervous system, CB1 receptors are also involved in the regulation of T-cell-specific immune responses. In a murine model for multiple sclerosis (experimental autoimmune encephalitis, EAE), it could be shown that the activation of neuronal CB1 receptors attenuated T-cell-mediated inflammation in the brain (Maresz et al., 2007). These observations suggest that CB1 receptors are expressed in peripheral tissues, and their activation protects against excessive T-cell-dependent inflammatory responses.

To further evaluate the role of CB1 receptors for the regulation of contact allergic inflammation, we generated mice with a tissue-specific knockout of CB1 receptors on peripheral sensory neurons (SNS-CB1$^{-/-}$), on immune cells (LysM-CB1$^{-/-}$), and on keratinocytes (KC-CB1$^{-/-}$). In the model of experimental contact allergy, only KC-Cnr1$^{-/-}$ animals developed an allergic inflammation almost as strong as observed in Cnr1$^{-/-}$ and Cnr1/2$^{-/-}$ animals. Keratinocytes play a central role in the regulation of T-cell-dependent immune responses in the skin. They are attacked by infiltrating T-lymphocytes, which leads to an increased production of proinflammatory cytokines and chemokines, and initiates the recruitment of myeloid immune cells, especially of neutrophils. This initially amplifies the inflammatory response, but also contributes to the repair and regeneration of epidermal barrier functions (Albanesi, 2010; Gaffal et al., 2013a; Wittmann & Werfel, 2006). In our model, the infiltration of myeloid immune cells into inflamed ear tissue and the proinflammatory chemokine CCL8/MCP2 were also significantly increased in KC-Cnr1$^{-/-}$ mice, in comparison to wild-type animals. In the skin, CCL8 is secreted by epidermal keratinocytes and fibroblasts in an IFNγ-dependent manner, in the context of a Th1-driven inflammatory response (Struyf et al., 1998). CCL8 increases the recruitment of myeloid immune cells (Zaidi et al., 2011) and Th2 type lymphocytes into the skin (Islam et al., 2011). In vitro studies on primary keratinocyte cultures confirmed that CB1 receptor-deficient cells produce higher levels of CCL8 after stimulation with IFNγ. Additionally, CCL8 was chemotactic for bone marrow-derived macrophages (Gaffal et al., 2013a). Taken together, these results provide the first in vivo evidence for the importance of CB1 receptors on epidermal keratinocytes for the regulation of T-cell-dependent immune responses in the skin.

ATOPIC DERMATITIS ASSOCIATED WITH IMPAIRED BARRIER FUNCTIONS

Atopic dermatitis (atopic eczema) is a chronic inflammatory skin disease. Early genetic linkage studies found an association of atopic eczema with polymorphisms in genes for immunoregulatory proteins which control IgE receptor synthesis, IL-4 production, and IL-4 receptor synthesis. These findings support the so-called "inside-out" hypothesis, where the genetic predisposition to an enhanced Th2-dependent inflammatory response leads to impaired epidermal barrier functions. But geneticists also discovered an association with polymorphisms in genes for epithelial structural proteins, such as filaggrin or claudin, which are important for composing and maintaining the epidermal barrier. Thus, a genetically induced barrier defect in the epidermis also promotes the development of atopic inflammation ("outside-in" hypothesis) (Barnes, 2010; Eyerich & Novak, 2013).

The unaffected skin of patients with atopic dermatitis can already show differences to healthy skin, and contains increased numbers of mast cells, and discrete perivascular T-cell infiltrates secreting Th2 cytokines, such as IL4 and IL13. Disruption of the epidermal barrier caused by allergens, scratching, or microbial toxins in the acute phase of atopic eczema initiates the secretion of inflammatory mediators like IL1, TNF, and GM-CSF through dendritic cells, mast cells, and epidermal keratinocytes. These mediators upregulate adhesion molecules on endothelial cells, which in turn, support the infiltration of immune cells. Skin lesions in the early

phase of atopic inflammation are mainly characterized by the presence of CD4+ Th2 cells, as well as increased IL4, IL5, and IL13 levels. The cytokine "thymic stromal lymphopoietin" (TSLP) produced by keratinocytes additionally supports the development of a Th2-mediated inflammation through its effect on dendritic cells and mast cells in the skin. Chemokines like CCL5/RANTES, CCL13/MCP4, and CCL11/eotaxin also induce the recruitment of eosinophils and macrophages into the skin. In the chronic phase of atopic eczema, the initially Th2-dominated inflammation is replaced by a Th1-, Th17-, and Th22-dominated immune cell infiltrate. The exact molecular mechanisms which control this conversion are not fully understood. Centrally important cytokines are IL12 and IL18, secreted by activated epidermal dendritic cells and macrophages. The germ colonization of the skin also plays an important role. In chronic skin lesions, the number of dermal macrophages is significantly increased, and IgE-bearing Langerhans cells and activated dendritic cells can be found in the epidermis. Compared to acute eczema, IL5, IL12, and IFNγ levels in the skin are elevated (Boguniewicz & Leung, 2011; Homey, Steinhoff, Ruzicka, & Leung, 2006). Different experimental mouse models helped to elucidate the pathophysiology of atopic dermatitis. One possibility to induce atopic-like inflammation in mice is the repetitive epicutaneous application of the contact sensitizer fluorescein isothiocyanate (FITC), which induces a Th2-dependent immune response in the skin, and increases serum IgE levels. In transgenic mouse models, proinflammatory mediators like TSLP or IL-4 are overexpressed in the skin to investigate their importance for the development of atopic eczema. Furthermore, there are models in which mice spontaneously develop the symptoms of atopic eczema. These include the so-called "flaky-tail" mice with a spontaneous null mutation in the filaggrin gene, resulting in impaired epidermal barrier functions (Dearman & Kimber, 2000; Fallon et al., 2009; Jin, He, Oyoshi, & Geha, 2009; Oyoshi, Murphy, & Geha, 2009; Spergel, Mizoguchi, Oettgen, Bhan, & Geha, 1999; Takeshita, Yamasaki, Akira, Gantner, & Bacon, 2004).

CB1 RECEPTORS ON KERATINOCYTES PARTICIPATE IN THE REGULATION OF ATOPIC-LIKE INFLAMMATION

Using the experimental model for Th2-driven, atopic-like contact allergic inflammation against FITC, we discovered that CB1 receptors in keratinocytes are also important for the preservation of physical barrier functions. We found that $Cnr1^{-/-}$ and KC-$Cnr1^{-/-}$ mice show increased transepidermal water loss and delayed repair responses, following epidermal barrier disruption, when compared to wild-type animals. These findings were associated with an elevated epidermal penetration of FITC, and reduced expression of epidermal structural proteins such as filaggrin and involucrin in the inflamed skin. Furthermore, $Cnr1^{-/-}$ and KC-$Cnr1^{-/-}$ mice showed increased allergic skin inflammation, and significantly higher mRNA levels for IL4, TSLP, and CCL8, when compared to wild-type controls. $Cnr1^{-/-}$ and KC-$Cnr1^{-/-}$ mice also displayed enhanced infiltration with eosinophils into FITC-challenged ear skin. In vitro studies with cultured primary keratinocytes revealed that CB1 receptor deficient cells produced higher levels of TSLP under basal and inflammatory conditions (Gaffal, Glodde, Jakobs, Bald, & Tüting, 2014). Similar results have independently been observed by another group studying skin barrier repair responses in CB1 receptor-deficient mice (Roelandt et al., 2012). Using electron microscopy, these investigators found that the naïve skin of $Cnr1^{-/-}$ mice already shows alterations in the composition of the stratum corneum lipid bilayer. The fact that endogenous cannabinoids also regulate the expression of genes for epidermal differentiation in human keratinocytes in a CB1-dependent manner further supports the important role of the endogenous cannabinoid system in the regulation of epidermal barrier functions (Pasquariello, Oddi, Malaponti, & Maccarrone, 2009) (Fig. 57.2).

THERAPEUTIC POTENTIAL OF CANNABINOIDS FOR THE TREATMENT OF ALLERGIC SKIN DISEASES

Due to their antiinflammatory potential, cannabinoids have been considered for the treatment of inflammatory skin diseases (Klein, 2005; Kupczyk, Reich, & Szepietowski, 2009; Nagarkatti, Pandey, Rieder, Hegde, & Nagarkatti, 2009). We found that the systemic and local administration of the CB receptor agonist Δ^9-tetrahydrocannabinol (THC) can significantly reduce inflammatory responses in the model of DNFB-induced contact hypersensitivity. THC not only decreased allergic ear swelling and the number of infiltrating immune cells in inflamed ear tissue in vivo, but also the secretion of IFNγ-induced proinflammatory chemokines CXCL10 and CCL8 by keratinocytes, in vitro. Migration experiments with macrophages using supernatants from IFNγ-activated keratinocyte cultures supported our hypothesis that the THC-mediated inhibition of chemokine production decreases the recruitment of myeloid immune cells (Gaffal, Cron, Glodde, & Tuting, 2013b; Karsak et al., 2007). Similar observations were reported using the endogenous cannabinoid-like lipid palmitoylethanolamide. Palmitoylethanolamide was shown to reduce the secretion of proinflammatory chemokines in human keratinocytes in vitro, and diminish the signs of acute dermatitis in vivo (Petrosino et al., 2010). In a clinical study in

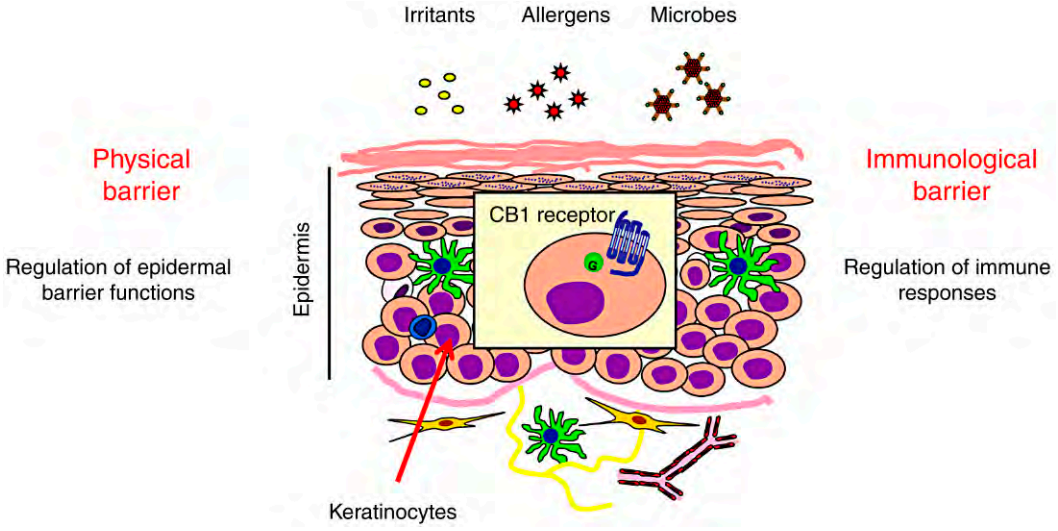


FIGURE 57.2 **Role of the endocannabinoid system in epidermal barrier functions.** Cannabinoid-1 receptors in keratinocytes help to maintain physical and immunological barrier functions of the skin.

patients with chronic pruritus, a strong itch-relieving effect was described after local application of palmitoylethanolamide (Stander et al., 2006). Taken together, these findings open up new possibilities for the topical treatment of inflammatory skin diseases with cannabinoids and other antiinflammatory lipids.

MINI-DICTIONARY

Allergic contact dermatitis Clinical term for the inflammatory response of the skin after repeated contact to sensitizing chemicals which can be found in cosmetics, jewelry, or clothes. Affected patients develop local redness, itching, edema, and blisters. Typical locations are hands, feet, eyelids, and lips.

Atopic dermatitis Chronic inflammatory skin disease characterized by the occurrence of relapsing eczema, chronic dry skin, severe itching, and an increased tendency to develop cutaneous infections.

Chemokines Chemical messengers attracting immune cells to areas of inflammation (chemotaxis).

Contact hypersensitivity Experimental model for allergic contact dermatitis.

Keratinocytes Predominant cell type of the epidermis with important immunological and physical barrier functions.

References

Albanesi, C. (2010). Keratinocytes in allergic skin diseases. *Current Opinion in Allergy and Clinical Immunology*, *10*, 452–456.

Barnes, K. C. (2010). An update on the genetics of atopic dermatitis: scratching the surface in 2009. *Journal of Allergy and Clinical Immunology*, *125*, 16–29.

Boguniewicz, M., & Leung, D. Y. (2011). Atopic dermatitis: a disease of altered skin barrier and immune dysregulation. *Immunology Reviews*, *242*, 233–246.

Cataisson, C., Pearson, A. J., Tsien, M. Z., Mascia, F., Gao, J. L., Pastore, S., et al. (2006). CXCR2 ligands and G-CSF mediate PKCalpha-induced intraepidermal inflammation. *Journal of Clinical Investigation*, *116*, 2757–2766.

Cavani, A., De Pità, O., & Girolomoni, G. (2007). New aspects of the molecular basis of contact allergy. *Current Opinion in Allergy and Clinical Immunology*, *7*, 404–408.

Cavani, A., Nasorri, F., Prezzi, C., Sebastiani, S., Albanesi, C., & Girolomoni, G. (2000). Human CD4+ T lymphocytes with remarkable regulatory functions on dendritic cells and nickel-specific Th1 immune responses. *Journal of Investigative Dermatology*, *114*, 295–302.

Dearman, R. J., & Kimber, I. (2000). Role of CD4(+) T helper 2-type cells in cutaneous inflammatory responses induced by fluorescein isothiocyanate. *Immunology*, *101*, 442–451.

Engeman, T., Gorbachev, A. V., Kish, D. D., & Fairchild, R. L. (2004). The intensity of neutrophil infiltration controls the number of antigen-primed CD8 T cells recruited into cutaneous antigen challenge sites. *Journal of Leukocyte Biology*, *76*, 941–949.

Eyerich, K., & Novak, N. (2013). Immunology of atopic eczema: overcoming the Th1/Th2 paradigm. *Allergy*, *68*, 974–982.

Fallon, P. G., Sasaki, T., Sandilands, A., Campbell, L. E., Saunders, S. P., Mangan, N. E., et al. (2009). A homozygous frameshift mutation in the mouse Flg gene facilitates enhanced percutaneous allergen priming. *Nature Genetics*, *41*, 602–608.

Fuchs, E., & Green, H. (1980). Changes in keratin gene expression during terminal differentiation of the keratinocyte. *Cell*, *19*, 1033–1042.

Gaffal, E., Cron, M., Glodde, N., Bald, T., Kuner, R., Zimmer, A., et al. (2013a). Cannabinoid 1 receptors in keratinocytes modulate proinflammatory chemokine secretion and attenuate contact allergic inflammation. *Journal of Immunology*, *190*, 4929–4936.

Gaffal, E., Cron, M., Glodde, N., & Tuting, T. (2013b). Anti-inflammatory activity of topical THC in DNFB-mediated mouse allergic contact dermatitis independent of CB1 and CB2 receptors. *Allergy*, *68*, 994–1000.

Gaffal, E., Glodde, N., Jakobs, M., Bald, T., & Tuting, T. (2014). Cannabinoid 1 receptors in keratinocytes attenuate fluorescein isothiocyanate-induced mouse atopic-like dermatitis. *Experimental Dermatology*, *23*, 401–406.

Grimbaldeston, M. A., Nakae, S., Kalesnikoff, J., Tsai, M., & Galli, S. J. (2007). Mast cell-derived interleukin 10 limits skin pathology in contact dermatitis and chronic irradiation with ultraviolet B. *Nature Immunology*, *8*, 1095–1104.

Homey, B., Steinhoff, M., Ruzicka, T., & Leung, D. Y. (2006). Cytokines and chemokines orchestrate atopic skin inflammation. *Journal of Allergy and Clinical Immunology*, *118*, 178–189.

Houben, E., De Paepe, K., & Rogiers, V. (2007). A keratinocyte's course of life. *Skin Pharmacology and Physiology*, *20*, 122–132.

Islam, S. A., Chang, D. S., Colvin, R. A., Byrne, M. H., McCully, M. L., Moser, B., et al. (2011). Mouse CCL8, a CCR8 agonist, promotes atopic dermatitis by recruiting IL-5+ T(H)2 cells. *Nature Immunology*, *12*, 167–177.

Jin, H., He, R., Oyoshi, M., & Geha, R. S. (2009). Animal models of atopic dermatitis. *Journal of Investigative Dermatology*, *129*, 31–40.

Kalinin, A. E., Kajava, A. V., & Steinert, P. M. (2002). Epithelial barrier function: assembly and structural features of the cornified cell envelope. *BioEssays*, *24*, 789–800.

Kaplan, D. H., Igyarto, B. Z., & Gaspari, A. A. (2012). Early immune events in the induction of allergic contact dermatitis. *Nature Reviews Immunology*, *12*, 114–124.

Karsak, M., Gaffal, E., Date, R., Wang-Eckhardt, L., Rehnelt, J., Petrosino, S., et al. (2007). Attenuation of allergic contact dermatitis through the endocannabinoid system. *Science*, *316*, 1494–1497.

Kish, D. D., Gorbachev, A. V., Parameswaran, N., Gupta, N., & Fairchild, R. L. (2012). Neutrophil expression of Fas ligand and perforin directs effector CD8 T cell infiltration into antigen-challenged skin. *Journal of Immunology*, *189*, 2191–2202.

Kish, D. D., Li, X., & Fairchild, R. L. (2009). CD8 T cells producing IL-17 and IFN-gamma initiate the innate immune response required for responses to antigen skin challenge. *Journal of Immunology*, *182*, 5949–5959.

Klein, T. W. (2005). Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nature Reviews Immunology*, *5*, 400–411.

Kupczyk, P., Reich, A., & Szepietowski, J. C. (2009). Cannabinoid system in the skin—a possible target for future therapies in dermatology. *Experimental Dermatology*, *18*, 669–679.

Maccarrone, M., Di Rienzo, M., Battista, N., Gasperi, V., Guerrieri, P., Rossi, A., et al. (2003). The endocannabinoid system in human keratinocytes. Evidence that anandamide inhibits epidermal differentiation through CB1 receptor-dependent inhibition of protein kinase C, activation protein-1, and transglutaminase. *Journal of Biological Chemistry*, *278*, 33896–33903.

Maresz, K., Pryce, G., Ponomarev, E. D., Marsicano, G., Croxford, J. L., Shriver, L. P., et al. (2007). Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB1 on neurons and CB2 on autoreactive T cells. *Nature Medicine*, *13*, 492–497.

Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., et al. (2003). CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science*, *302*, 84–88.

Marsicano, G., Wotjak, C. T., Azad, S. C., Bisogno, T., Rammes, G., Cascio, M. G., et al. (2002). The endogenous cannabinoid system controls extinction of aversive memories. *Nature*, *418*, 530–534.

Massa, F., Marsicano, G., Hermann, H., Cannich, A., Monory, K., Cravatt, B. F., et al. (2004). The endogenous cannabinoid system protects against colonic inflammation. *Journal of Clinical Investigation*, *113*, 1202–1209.

Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., & Bonner, T. I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature*, *346*, 561–564.

Nagarkatti, P., Pandey, R., Rieder, S. A., Hegde, V. L., & Nagarkatti, M. (2009). Cannabinoids as novel anti-inflammatory drugs. *Future Medical Chemistry*, *1*, 1333–1349.

Oyoshi, M. K., Murphy, G. F., & Geha, R. S. (2009). Filaggrin-deficient mice exhibit TH17-dominated skin inflammation and permissiveness to epicutaneous sensitization with protein antigen. *Journal of Allergy and Clinical Immunology*, *124*, 485–493, 493.

Paradisi, A., Pasquariello, N., Barcaroli, D., & Maccarrone, M. (2008). Anandamide regulates keratinocyte differentiation by inducing DNA methylation in a CB1 receptor-dependent manner. *Journal of Biological Chemistry*, *283*, 6005–6012.

Pasquariello, N., Oddi, S., Malaponti, M., & Maccarrone, M. (2009). Regulation of gene transcription and keratinocyte differentiation by anandamide. *Vitamins and Hormones*, *81*, 441–467.

Petrosino, S., Cristino, L., Karsak, M., Gaffal, E., Ueda, N., Tuting, T., et al. (2010). Protective role of palmitoylethanolamide in contact allergic dermatitis. *Allergy*, *65*, 698–711.

Roelandt, T., Heughebaert, C., Bredif, S., Giddelo, C., Baudouin, C., Msika, P., et al. (2012). Cannabinoid receptors 1 and 2 oppositely regulate epidermal permeability barrier status and differentiation. *Experimental Dermatology*, *21*, 688–693.

Ryckman, C., Vandal, K., Rouleau, P., Talbot, M., & Tessier, P. A. (2003). Proinflammatory activities of S100: proteins S100A8, S100A9, and S100A8/A9 induce neutrophil chemotaxis and adhesion. *Journal of Immunology*, *170*, 3233–3242.

Schmidt, M., Raghavan, B., Muller, V., Vogl, T., Fejer, G., Tchaptchet, S., et al. (2010). Crucial role for human Toll-like receptor 4 in the development of contact allergy to nickel. *Nature Immunology*, *11*, 814–819.

Spergel, J. M., Mizoguchi, E., Oettgen, H., Bhan, A. K., & Geha, R. S. (1999). Roles of TH1 and TH2 cytokines in a murine model of allergic dermatitis. *Journal of Clinical Investigation*, *103*, 1103–1111.

Stander, S., Reinhardt, H. W., & Luger, T. A. (2006). Topical cannabinoid agonists. An effective new possibility for treating chronic pruritus. *Hautarzt*, *57*, 801–807 (in German).

Stander, S., Schmelz, M., Metze, D., Luger, T., & Rukwied, R. (2005). Distribution of cannabinoid receptor 1 (CB1) and 2 (CB2) on sensory nerve fibers and adnexal structures in human skin. *Journal of Dermatological Science*, *38*, 177–188.

Struyf, S., Van, C. E., Paemen, L., Put, W., Lenaerts, J. P., Proost, P., et al. (1998). Synergistic induction of MCP-1 and -2 by IL-1beta and interferons in fibroblasts and epithelial cells. *Journal of Leukocyte Biology*, *63*, 364–372.

Takeshita, K., Yamasaki, T., Akira, S., Gantner, F., & Bacon, K. B. (2004). Essential role of MHC II-independent CD4+ T cells, IL-4 and STAT6 in contact hypersensitivity induced by fluorescein isothiocyanate in the mouse. *International Immunology*, *16*, 685–695.

Waseem, A., Dogan, B., Tidman, N., Alam, Y., Purkis, P., Jackson, S., et al. (1999). Keratin 15 expression in stratified epithelia: downregulation in activated keratinocytes. *Journal of Investigative Dermatology*, *112*, 362–369.

Watanabe, H., Gaide, O., Petrilli, V., Martinon, F., Contassot, E., Roques, S., et al. (2007). Activation of the IL-1beta-processing inflammasome is involved in contact hypersensitivity. *Journal of Investigative Dermatology*, *127*, 1956–1963.

Wetzler, C., Kampfer, H., Pfeilschifter, J., & Frank, S. (2000). Keratinocyte-derived chemotactic cytokines: expressional modulation by nitric oxide in vitro and during cutaneous wound repair in vivo. *Biochemical and Biophysical Research Communications*, *274*, 689–696.

Wilkinson, J. D., & Williamson, E. M. (2007). Cannabinoids inhibit human keratinocyte proliferation through a non-CB1/CB2 mechanism and have a potential therapeutic value in the treatment of psoriasis. *Journal of Dermatological Science*, *45*, 87–92.

Wittmann, M., & Werfel, T. (2006). Interaction of keratinocytes with infiltrating lymphocytes in allergic eczematous skin diseases. *Current Opinion in Allergy and Clinical Immunology*, *6*, 329–334.

Zaidi, M. R., Davis, S., Noonan, F. P., Graff-Cherry, C., Hawley, T. S., Walker, R. L., et al. (2011). Interferon-gamma links ultraviolet radiation to melanomagenesis in mice. *Nature*, *469*, 548–553.

Zimmer, A., Zimmer, A. M., Hohmann, A. G., Herkenham, M., & Bonner, T. I. (1999). Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proceedings of the National Academy of Sciences of the United States of America*, *96*, 5780–5785.

Page left intentionally blank

CHAPTER

e8

Cannabis and Oral Health: Deleterious Effects on Periodontitis and Dental Implants

G. Nogueira-Filho

School of Health Sciences, University Salvador, Laureate International Universities, Salvador, Bahia, Brazil

SUMMARY POINTS

- *Cannabis* smoke decreases bone filling around titanium implants.
- *Cannabis* smoke impairs cancellous bone formation.
- *Cannabis* does not affect the cortical bone around the implants.
- *Cannabis* smoke increases bone loss in teeth with induced periodontitis.
- *Cannabis* smoke had no effect on bone physiology in periodontally healthy teeth.
- The deleterious impact of *Cannabis* on bone healing represents a new concern for dental implants success/failure.
- There is a lack of evidence to prove the role of *Cannabis* as an etiologic risk factor for periodontitis.
- Further investigations are necessary to determine whether smoking *Cannabis* can be considered a real threat to oral health.

KEY FACTS

- Cannabis sativa can enhance an established inflammatory response in periodontitis sites
- Cannabis sativa can enhance bone loss around dental implants
- Cannabis sativa is not able to initiate disease at the gingival and bone levels

LIST OF ABBREVIATIONS

CB1	Cannabinoid receptor-1
CB2	Cannabinoid receptors-2
CBD	Cannabidiol
D9-THC	D9-tetrahydrocannabinol
IFNg	Interferon gamma
IL-1	Interleukin 1
IL-6	Interleukin 6
IL-10	Interleukin 10
NK cells	Natural killer cells
Th1	T helper 1 lymphocyte
Th2	T helper 2 lymphocyte
THC	Tetrahydrocannabinol
TNFa	Tumor necrosis factor alpha

INTRODUCTION

Cannabis sativa (marijuana) is a plant that contains more than 60 types of cannabinoids produced by small glands on its leaf surface (Grotenhermen, 2004). *Cannabis* and its cannabinoids can affect the immunological human functions (Peeke, Jones, & Stone, 1976). Cannabinoids seem to attenuate the production of some inflammatory mediators such as interferon-g (IFNg), tumor necrosis factor-a (TNFa), interleukin (IL)-1, IL-6, and IL-10 (Klein, 2005; Massi, Vaccani, & Parolaro, 2006). Cannabinoids activate specific cell-surface cannabinoid receptors-1 and -2 (CB1 and CB2, respectively), which are normally engaged by a family of endogenous ligands so-called

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00057-0
Copyright © 2017 Elsevier Inc. All rights reserved.

the endocannabinoids (Do, McKallip, Nagarkatti, & Nagarkatti, 2004). Compounds that bind these receptors induce cannabimimetic responses in vivo and in vitro. Indeed, recent evidence indicates that cannabinoids might influence vascular inflammatory diseases in several ways such mediating vasodilatation particularly in lipopolysaccharide (LPS) induced infectious diseases (Grotenhermen, 2004). The cannabinoids can also reduce the immune response, and impair the body defense against viral, bacterial, and protozoa's infections (Do et al., 2004).

Experimental models have been used for host response evaluation in cultures of cells exposed to tetrahydrocannabinol (THC), and in human populations of declared consumers of cannabis. These studies suggest that the cannabinoids can affect the resistance of the host by modifying the primary and secondary immune system, especially the function of lymphocyte T and B, as well as the NK cells and macrophages (Klein, 2005). Moreover, cannabinoids can act as immunomodulators by modifying the production and function of cytokines in the acute phase of the inflammatory process in the phases T helper 1 (Th1) and T helper 2 (Th2). Animal and cell experiments have demonstrated that THC exerts complex effects on cellular and humoral immunity. THC was shown to modulate the immune response of T lymphocytes. It suppressed the proliferation of and Th2 cytokines (Grotenhermen, 2004).

Cannabinoids can influence vascular inflammatory diseases (Powles, te Poele, & Shamash, 2005) in several ways, such as mediating vasodilatation, particularly in LPS-induced diseases. Studies (Davies, Sornberger, & Huber, 1979; Idris, van't Hof, & Greig, 2005; Ofek et al., 2006) have also indicated the association between bone loss and the use of cannabis. Considering bone metabolism, the suspect of cannabis effect was confirmed by molecular studies that demonstrated a negative impact on bone physiology by cannabinoids (Grotenhermen, 2004; Klein, 2005; Tam, Ofek, & Fride, 2006).

Nakajima, Furuichi, and Biswas (2006) suggested a possible role of cannabinoids in periodontitis by reporting expression of cannabinoid receptors (CB1 and CB2) in periodontal tissues, followed by an increase in anandamide, an agonist of the cannabinoid receptor derived from arachidonic acid. Many oral problems have been associated with the use of cannabis, including xerostomia, with predisposition to dental caries and periodontitis, severe gingivitis, oral mucosal disease, abnormal stress response on administration of adrenalin-containing local anesthetics, leukoedema, and increased prevalence of opportunistic microorganisms such as *Candida albicans* (Darling, Arendorf, & Coldrey, 1990; Versteeg, Slot, van der Velden, & van der Weijden, 2008; Veitz-Keenan & Spivakovsky, 2011; Nogueira-Filho et al., 2011; Kayal, Elias, Alharthi, Demyati, & Mandurah, 2014).

Considering the apparent lack of scientific information regarding the effects of cannabis on oral bones, and its impact in oral inflammation, our research group has developed an animal model to study second hand cannabis smoke in rats (Fig. e8.1). So far, we have summarized the main findings of two studies published recently in dental journals (Nogueira-Filho, Cadide, & Rosa, 2008; Nogueira-Filho et al., 2011) that reflect our impressions about the possible relationship between cannabis and the health of oral bones around teeth, and also on the bones around endosseous titanium implants.

STUDIES

STUDY 1. Cannabis sativa smoke inhalation decreases bone filling around titanium implants: a histomorphometric study in rats (Nogueira-Filho et al., 2008)

In this first study, we aimed to investigate if second hand cannabis smoke could impair bone formation and repair around prototypes of dental implants to be inserted in rat tibia (Figs. e8.2 and e8.3).

Thirty Wistar rats were used in this study. After anesthesia, the tibiae surface was exposed, and one screw-shaped titanium implant was placed bilaterally. The animals were randomly assigned to one of the following groups: CTRL (control; $n = 15$), and MSI (cannabis smoke inhalation 8 min/day; $n = 15$), as illustrated in Fig. e8.1. Urine samples were obtained to detect the presence of THC for both groups. After 60 days, the animals were killed. The degree of bone-to-implant contact and the bone area within the limits of the threads of the implant were measured in the cortical (zone A) and cancellous bone (zone B). The experiment finished with 23 animals, instead of 30 (CTRL/$n = 15$, and MSI/$n = 8$), as seven rats died during the experiment because of respiratory failure provoked by intermittent cannabis smoke inhalation. THC was detected in urine samples from all animals from MSI group ($n = 8$). Negative THC detection was found in all samples from control animals. Also, according to the Brazilian Research Protocol for Illegal Drugs, all human researchers that worked directly with the cannabis smoke experiments had also their urine checked. All their samples were negative to THC (Table e8.1), confirming the effectiveness of their personal protective equipment used during cannabis handling and smoke exposure.

This study demonstrated that cannabis smoke inhalation had a deleterious impact on cancellous bone healing around titanium implants by reducing bone filling and bone-to-implant contact inside the implant threads, while no effect was observed in the cortical bone (Figs. e8.2 and e8.3). The negative effects of cigarette smoking on periodontitis and around titanium implants have been demonstrated by the authors (Nogueira-Filho, Froes Neto, & Casati, 2004; Nogueira-Filho, Rosa, Cesar-Neto, Tunes, & Tunes, 2007), where cigarette smoking seems to reduce bone capacity to formation and/or increasing

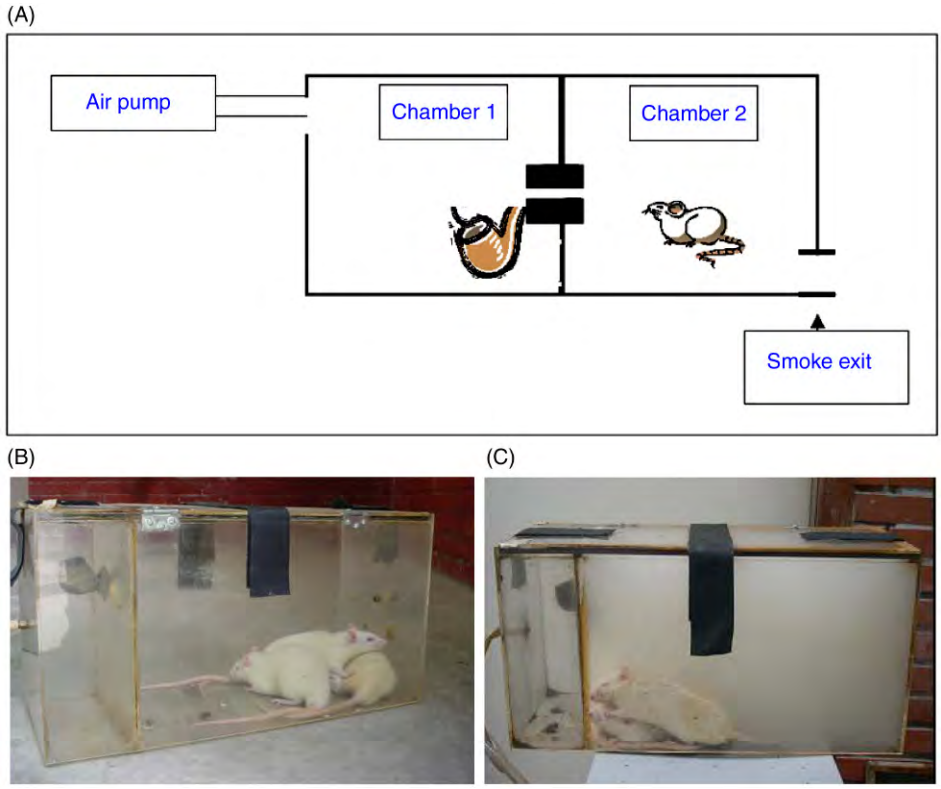


FIGURE e8.1 Illustrations of the cannabis smoke-generating apparatus (A), before (B) and during smoke exposure (C). (A) Depiction of the cannabis smoke apparatus; (B) animals inside the apparatus before cannabis smoke exposure; (C) animals inside the apparatus during smoke exposure. This figure shows an illustration of the cannabis smoke apparatus (A) and the actual smoke exposure (B and C) according to the cannabis smoke exposure protocol.

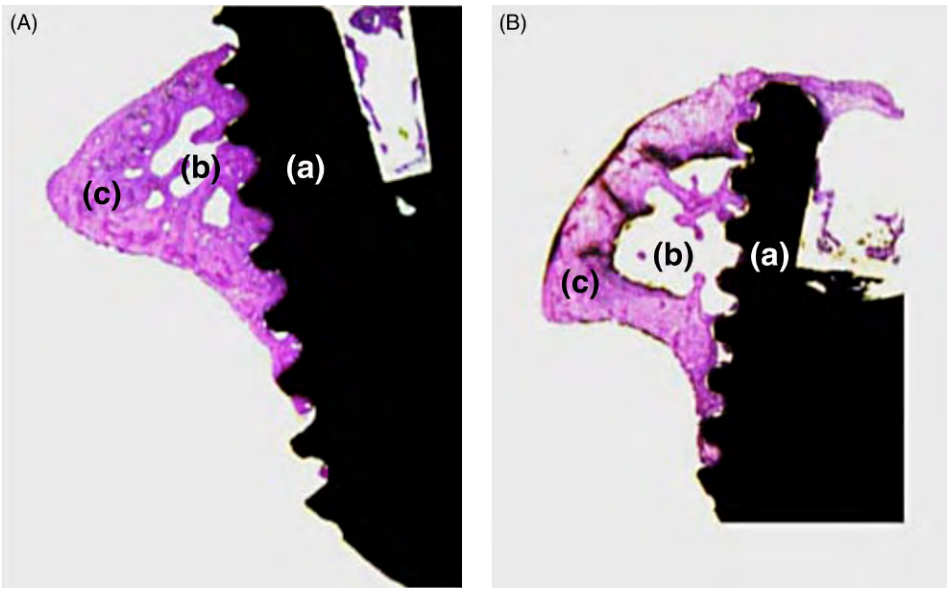


FIGURE e8.2 Photomicrographs illustrating bone healing in the implant surface (Toluidine blue; original magnification = 4×). These photomicrographs (4×) correspond to histological sections from titanium implants inserted in the rat tibias. (A) Represents control rats, whereas (B) represents cannabis-exposed rats. The *black* zone (a) corresponds to the titanium implant screw; the inner *pink* zone (b) corresponds to newly formed bone in direct contact to the implant surface while the outer *pink* zone (c) corresponds to original bone. *Source: Data are from Nogueira-Filho et al. (2008).*

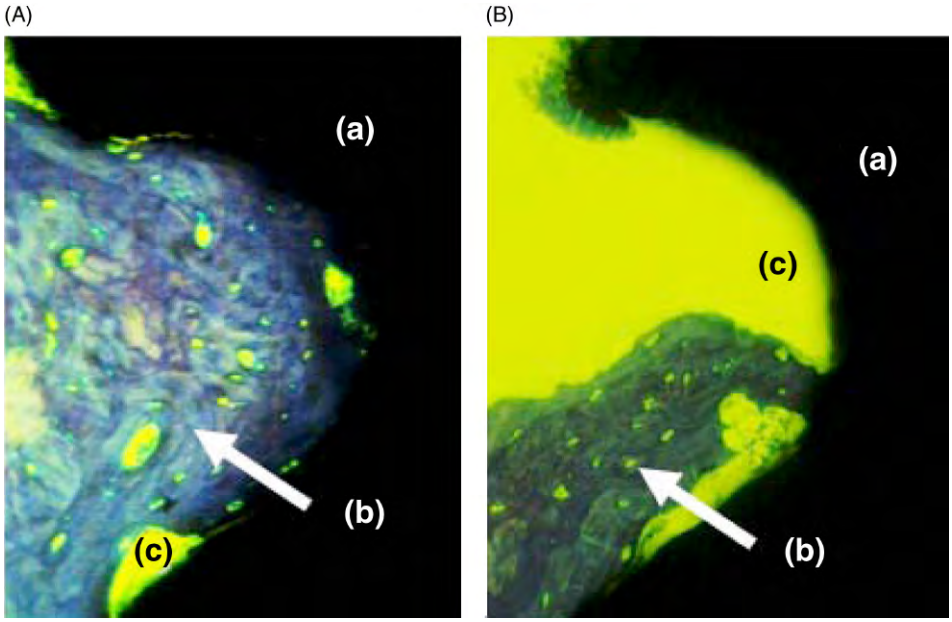


FIGURE e8.3 Photomicrographs illustrating bone healing in the implant surface (Toluidine blue; original magnification = 100×). These photomicrographs (100×) correspond to histological sections from titanium implants inserted in the rat tibias. (A) Represents control rats, whereas (B) represents cannabis-exposed rats. The *black* zone (a) corresponds to the titanium implant screw; the *blue* zone (b) corresponds to newly formed bone in direct contact to the thread surface of the implant, while the empty zone (c) corresponds to lack of bone-to-implant contact, that is, no direct bone formation on the implant surface. *Source: Data are from Nogueira-Filho et al. (2008).*

TABLE e8.1 Tetrahydrocannabinol (THC) Detection in Urine Samples

Animals	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
CTRL	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
MSI[a]	+	+	+	+	+	+	+	+							
RES	1	2	3	4											
	–	–	–	–											

This table shows THC presence (+) or absence (–) in urine collected from rats exposed to cannabis smoke (MSI), control rats (CTRL), and human researchers (RES) that worked with the cannabis apparatus (as shown in Fig. e8.1).
[a]*Seven animals from MSI died during experiment due respiratory complications.*
Data are from Nogueira-Filho et al. (2008).

resorption. Despite the limitations of the present study, it is the first one, to our knowledge, that demonstrates the negative impact of *Cannabis sativa* smoke on bone healing around titanium implants.

According to Tam et al. (2006), bone mass and shape are determined by continuous remodeling consisting of the concerted and balanced action of osteoclasts (the bone resorbing cells), and osteoblasts (the bone forming cells). Firstly, immune cells express endocannabinoids, and cannabinoid receptors (CB1 and CB2) at the surface of immune cells are activated after infection or immune stimulation. The second general principle is that the main immune targets of cannabinoid-based drugs involve the suppression of cytokines and cell-mediated immunity, through cannabinoid receptor-dependent and -independent mechanisms. The results of the present study clearly demonstrated that cannabis smoke could decrease bone mass around titanium implants in the cancellous zone. As a matter of fact, to explain such phenomenon, some observations might be further addressed considering the CB1/CB2 receptor system of the cannabinoids.

Bone is densely innervated by sympathetic fibers (Mach, Rogers, & Sabino, 2002). These fibers release norepinephrine, thus potently mediating central signals that restrain bone formation, and stimulate bone resorption (Schlicker & Kathmann, 2001). Because CB1 is expressed in such nerve fibers elsewhere, its presence in bone sympathetic nerve fibers was further explored, indicating also the presence of CB1 receptors in sympathetic fibers that innervate the cancellous bone (Elefteriou, Ahn, & Takeda, 2005).

On the other hand, CB2 receptors are located principally in immune cells, among them leukocytes, in the spleen, and tonsils. Immune cells also express CB1

receptors, but there is markedly more mRNA for CB2 than CB1 receptors in the immune system. One of the functions of CB receptors in the immune system is modulation of cytokine release. Activation of the CB1 receptor produces cannabis-like effects on psyche and circulation, while activation of the CB2 receptor does not. CB2 is expressed in the vast majority of hematopoietic cells, including macrophages, and attenuates immune responses. CB2 is expressed in osteoblasts, osteocytes, and osteoclasts. It was found that osteoclasts, cells derived from the monocyte-macrophage lineage, have high levels of CB2 mRNA (Ofek et al., 2006). Specific cannabinoids as the CB2 are also characterized by increased activity of osteoblasts (bone-forming cells), increased osteoclast (bone-resorbing cell) numbers, and a markedly decreased number of osteoblast precursors.

Our results were not able to present any disturbance in the cortical bone around the implants placed in the rats that inhaled the cannabis smoke. Interestingly, this phenomenon could be explained by the presence of a CB2-specific agonist that does not have any psychotropic effects enhancing endocortical osteoblast number and activity. Cannabis smoke inhalation somehow seems to impair the healing of the cancellous bone, probably because of a THC effect in inhibiting CB2 expression on osteoblasts and, consequently, reducing bone formation.

In the same way, the reduced bone-to-implant contact values in the cannabis group may also suggest the occurrence of a restraining in the osteoclastogenesis, apparently because of the inhibition of the proliferation of osteoclast precursors, and receptor activator of NF-κB ligand expression in bone marrow-derived osteoblasts/stromal cells (Nakajima et al., 2006). Endocannabinoids could regulate the periodontal inflammation through NF-κB pathway inhibition. The endocannabinoid system seems to participate in bone physiology by regulating bone mass, bone loss, and osteoclast activity by signaling via the peripheral CB2 expressed in bone, and its effect decreased the bone healing around the implants in the experimental rats from cannabis group (Idris et al., 2005).

As long as it is well established that titanium implant success is a reality, and only a few situations, such as cigarette smoking, may cause implant failure, this study was a hallmark as the first in the literature to raise the concern about the consumption of cannabis as a possible risk factor in order to explain some of dental implant failures in patients that not always inform, during anamnesis, that they are marijuana consumers. Nevertheless, further epidemiological and molecular studies are mandatory to confirm such suspect.

STUDY 2. Marijuana smoke inhalation increases bone loss during ligature-induced periodontitis (Nogueira-Filho et al., 2011)

In this second study, we aimed to investigate if second hand cannabis smoke could increase bone inflammation around teeth with induced periodontal inflammation, in a rat model.

30 male Wistar rats were used in the study. A ligature was placed around one of the mandible first molars (ligated teeth) of each animal, and they were then randomly assigned to one of two groups: CTRL (control; $n = 15$), or MSI (marijuana smoke inhalation for 8 min/day; $n = 15$), as illustrated in Fig. e8.1. Urine samples were obtained to detect the presence of THC. After 30 days, the animals were killed, and decalcified sections of the tooth furcation area were obtained and evaluated according to the following histometric parameters: bone area, bone density, and bone loss.

Seven rats in the MSI group died during the experimental period, probably because of respiratory failure provoked by intermittent cannabis smoke inhalation; however, no further investigations were performed to confirm the cause of death. At the end of the 30 days of cannabis inhalation, the remaining animals from the MSI group presented abnormal behavior, as noted by erratic movements in the cage, and excessive food ingestion. The teeth ligatures that promoted periodontitis were kept in place in all 23 animals that completed the experimental period, and served as a retention device for oral microorganisms.

This study demonstrated that cannabis smoke inhalation increased bone loss in the furcation area of teeth with induced periodontitis in rats, whereas no effect was observed in periodontally healthy sites, confirmed by the histomorphometric analysis that showed the highest values of bone loss into the furcation area from cannabis-exposed teeth (Fig. e8.4). Despite the studies by Davies et al. (1979) on the effect of marijuana inhalation on alveolar macrophages, by the use of a smoke exposure chamber, to the best of our knowledge, this is the first study evaluating the influence of cannabis smoke on healthy periodontal tissues, and during experimental periodontitis.

Though our results and the biologic plausibility that cannabis use could be considered a possible risk factor for periodontitis, some limitations of the present study have to be emphasized. Some animals from the cannabis group probably died because of respiratory difficulties because the smoke that was produced looked very dense and dusty, and may not be comparable to the doses inhaled by human cannabis consumers. Also, regardless of our interesting results, contradictory findings may be observed in other studies. Conversely, Napimoga, Benatti, and Lima (2009) demonstrated experimentally that the administration of cannabidiol (CBD), a cannabinoid component from *Cannabis* that does not induce psychotomimetic effects, and possess antiinflammatory properties, significantly inhibited bone loss in experimental periodontitis in rats. The study by Napimoga et al. (2009) suggests that such

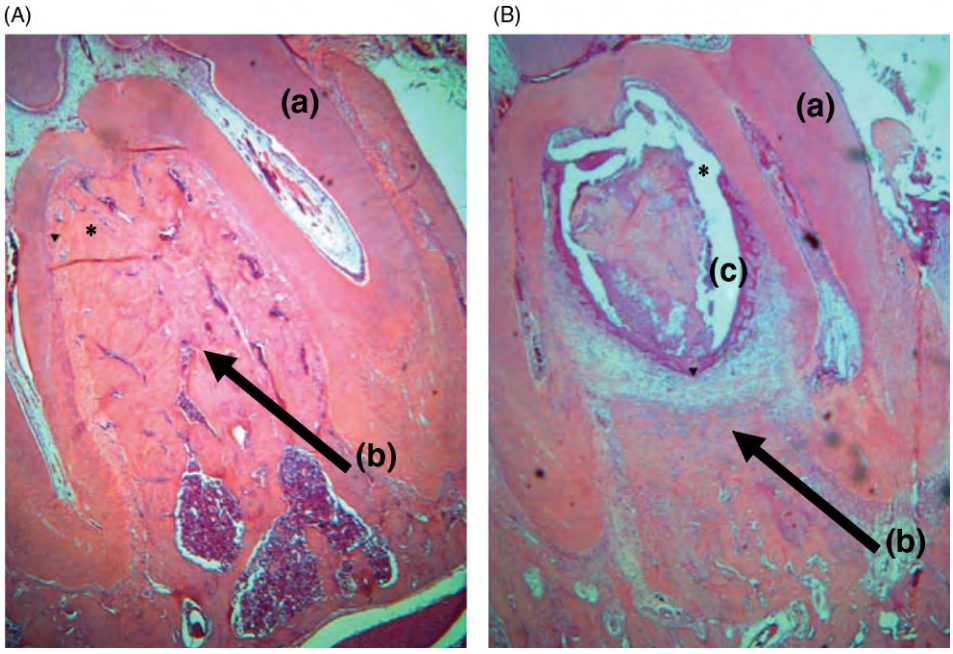


FIGURE e8.4 Photomicrographs illustrating the furcation region of a rat molar showing no bone loss in the control group with no cannabis smoke exposure (hematoxylin and eosin; magnification = 10×). These photomicrographs correspond to histological sections from the rat teeth. (A) Represents control rats, whereas (B) represents cannabis-exposed rats. For (A) and (B), the zone (a) corresponds to the tooth root, and zone (b) corresponds to the furcation area filled with original bone. For (B), the zone (c) corresponds to furcation bone loss due the cannabis smoke exposure. *Source: Data are from Nogueira-Filho et al. (2011).*

cannabis antiinflammatory effect might be related to drug-induced reduction of bone-related molecules, as receptor activator of NF-κB and receptor activator of NF-κB ligand expression, neutrophil infiltration, and cytokine production at gingival tissues.

Although both studies (Napimoga et al., 2009; Nogueira-Filho et al., 2011) used the same experimental model (ligature-induced periodontitis in rats), a direct comparison between the studies has to be taken with caution. Napimoga et al. (2009) tested just cannabidiol, one of the compounds of *Cannabis* smoke, whereas the study by Nogueira-Filho et al. (2011) evaluated whole *Cannabis* smoke inhalation. Interestingly, cannabis leaves, like tobacco leaves, contain more than 400 compounds, but *Cannabis* presents at least 60 cannabinoids. The noncannabinoid constituents are similar to tobacco (except for nicotine), which carry systemic health risks, and have histopathologic effects similar to those of tobacco smoke. Realistically, in the study by Napimoga et al. (2009) only cannabidiol was administered via intraperitoneal injections in the rats; whereas the animals in the study by Nogueira-Filho et al. (2011) inhaled the cannabis smoke itself. These methodological differences may explain, at least in part, the contradictory results of these two interesting studies.

Two relevant clinical studies have reported the effects of cannabis usage on periodontal tissues. In a prospective cohort study, Thomson, Poulton, and Broadbent (2008) found *Cannabis* smoking to be an independent risk factor for periodontal disease. Contradictorily, López and Baelum (2009) found no evidence associating the use of cannabis with periodontal diseases. The human populations of each investigation may be considered the most important difference, when comparing both studies. The study by Thomson et al. (2008) evaluated only adult patients (~32 years old); however, López and Baelum (2009) evaluated younger patients (~12–21 years old). Notwithstanding this, it is well recognized that in such clinical studies it is difficult to control the frequency, amount, duration, and mode of administration of cannabis in all individuals included in epidemiological evaluations. Personal risk factors, including age, oral hygiene, general health, concurrent tobacco smoking, and multidrug use, may also act as confounding factors, making even more difficult the identification of the specific influence of *Cannabis* on periodontitis.

FINAL CONSIDERATIONS

Our studies propose a methodology widely used to evaluate the influence of cigarette smoke on living tissues, in order to study the impact of *Cannabis* smoke on ligature-induced periodontitis, and on endosseous implants. Cigarette and *Cannabis* smoke are quite similar regarding composition, which may result in similar biologic effects. Cigarette smoking can reduce bone capacity

of formation or increase its resorption. Similarly, the present results demonstrate that *Cannabis* smoke might also alter bone pathophysiologic patterns. Cannabinoids may suppress important biologic pathways related to inflammatory processes, whereas nicotine, a key tobacco constituent, promotes these pathways. However, the biologic pathway by which *Cannabis* smoke exerts its effects on periodontal tissues and bone around dental implants is still not totally understood.

Because the healthy groups did not present changes in the bone levels, the greater bone loss found in the *Cannabis* groups for periodontitis sites, and around titanium implants, might be probably related to impaired immune function during the process of bone repair, or even the activation of specific receptors that might increase bone destruction. In this regard, some speculation has arisen about the endocannabinoid system that seems to play a role in the regulation of bone metabolism. The suspicion of a *Cannabis* effect on bone metabolism was confirmed by some molecular studies that demonstrated a negative impact by cannabinoids on bone physiology. Mice lacking either of the cannabinoid receptors CB1 or CB2 have abnormal bone phenotypes because cannabinoid receptor agonists and inverse agonists reduce bone loss by presenting direct effects on both bone-resorbing cells (osteoclasts) and bone-forming cells (osteoblasts) in vitro. Nevertheless, conflicting results have been reported with regard to the effects of *Cannabis* on the regulation of bone mass via bone resorption and osteoclast function. THC has also demonstrated a direct impact on immune cell activity, leading to immunosuppressive effect on macrophages, natural killer cells, and T and B-lymphocytes.

Undoubtedly, it is wise to consider the fact that many individuals have or will have contact with *Cannabis* as a recreational drug, or as a daily basis usage. The question remains as to how far this *Cannabis* smoking habit may disrupt the bone homeostasis in the oral cavity.

MINI-DICTIONARY

Bone loss Bone loss is the breakdown of bone through a process also called bone resorption, in which bone volume and bone density are decreased physiologically or because of a disease.

Bone resorption Bone resorption is the destruction of bone tissues that promotes bone loss, that is, a decrease in bone mass and bone density.

Furcation region Furcation region of a tooth corresponds to the area filled by bone in between the roots. It is generally found in multiradicular teeth in the oral cavity.

Hematoxylin and eosin (H&E) stain H&E is a common histological staining method for tissues, in which the cell nuclei are stained in a deep blue with hematoxylin, while the cell cytoplasm is stained in a pink color after the counterstaining with eosin.

Periodontal disease Periodontal disease is an inflammation of gingiva and oral bones around teeth. It is called gingivitis when only the gingiva is affected by inflammation, whereas periodontitis involves destruction of alveolar-supporting bone around teeth.

Trabecular bone Trabecular bone is a synonymous with the cancellous bone. It is one of two types of bone tissue in vertebrates.

Xerostomia This is a condition in which there is an extreme decrease of salivary flow in the oral cavity, promoting mouth dryness. It is usually due to physiological and/or pathological decrease of saliva production by the salivary glands present in the oral cavity.

Acknowledgments

The authors thank The National Council for Scientific and Technological Development (CNPq), a foundation linked to the Ministry of Science and Technology (MCT), for supporting this research (#grant 308114/2004-3), The Federal Police Department of Brazil, Public Security Secretary and The Health State Secretary authorities for providing legal use of *Cannabis sativa* samples in experimental protocols.

References

Darling, M. R., Arendorf, T. M., & Coldrey, N. A. (1990). Effect of cannabis use on oral candidal carriage. *Journal of Oral Pathology and Medicine*, *19*, 319–321.

Davies, P., Sornberger, G. C., & Huber, G. L. (1979). Effects of experimental marijuana and tobacco smoke inhalation on alveolar macrophages. A comparative stereologic study. *Laboratory Investigation*, *41*, 220–223.

Do, Y., McKallip, R. J., Nagarkatti, M., & Nagarkatti, P. S. (2004). Activation through Cannabinoid receptors 1 and 2 on dendritic cells triggers NF-kappaB-dependent apoptosis: novel role for endogenous and exogenous cannabinoids in immunoregulation. *Journal of Immunology*, *173*, 2373–2382.

Elefteriou, F., Ahn, J. D., Takeda, S., et al. (2005). Leptin regulation of bone resorption by the sympathetic nervous system and CART. *Nature*, *24*, 514–520.

Grotenhermen, F. (2004). Pharmacology of cannabinoids. *Neurology Endocrinology Letters*, *25*, 14–23.

Idris, A. I., van't Hof, R. J., Greig, I. R., et al. (2005). Regulation of bone mass, bone loss and osteoclast activity by cannabinoid receptors. *Nature Medicine*, *11*, 774–779.

Kayal, R. A., Elias, W. Y., Alharthi, K. J., Demyati, A. K., & Mandurah, J. M. (2014). Illicit drug abuse affects periodontal health status. *Saudi Medicine Journal*, *35*, 724–728.

Klein, T. W. (2005). Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nature Reviews of Immunology*, *5*, 400–411.

López, R., & Baelum, V. (2009). Cannabis use and destructive periodontal diseases among adolescents. *Journal of Clinical Periodontology*, *36*, 185–189.

Mach, D. B., Rogers, S. D., Sabino, M. C., et al. (2002). Origins of skeletal pain: sensory and sympathetic innervation of the mouse femur. *Neuroscience*, *113*, 155–166.

Massi, P., Vaccani, A., & Parolaro, D. (2006). Cannabinoids, immune system and cytokine network. *Current Pharmaceutical Design*, *12*, 3135–3146.

Nakajima, Y., Furuichi, Y., Biswas, K. K., et al. (2006). Endocannabinoid, anandamide in gingival tissue regulates the periodontal inflammation through NF-kappaB pathway inhibition. *FEBS Letters*, *23*, 613–619.

Napimoga, M. H., Benatti, B. B., Lima, F. O., et al. (2009). Cannabidiol decreases bone resorption by inhibiting RANK/RANKL expression and pro-inflammatory cytokines during experimental periodontitis in rats. *International Immunopharmacology*, *9*, 216–222.

Nogueira-Filho, G. R., Cadide, T., Rosa, B. T., et al. (2008). Cannabis sativa smoke inhalation decreases bone filling around titanium implants: a histomorphometric study in rats. *Implant Dentistry*, *17*, 461–470.

Nogueira-Filho, G. R., Froes Neto, E. B., Casati, M. Z., et al. (2004). Nicotine effects on alveolar bone changes induced by occlusal trauma: a histometric study in rats. *Journal of Periodontology*, *75*, 348–352.

Nogueira-Filho, G. R., Rosa, B. T., Cesar-Neto, J. B., Tunes, R. S., & Tunes, U. daR. (2007). Low- and high-yield cigarette smoke inhalation potentiates bone loss during ligature-induced periodontitis. *Journal of Periodontology*, *78*, 730–735.

Nogueira-Filho, G. R., Todescan, S., Shah, A., Rosa, B. T., Tunes, U. R., & Cesar Neto, J. B. (2011). Impact of cannabis sativa (marijuana) smoke on alveolar bone loss: a histometric study in rats. *Journal of Periodontology*, *82*, 1602–1607.

Ofek, O., Karsak, M., Leclerc, N., et al. (2006). Peripheral cannabinoid receptor, CB2, regulates bone mass. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 696–701.

Peeke, S. C., Jones, R. T., & Stone, G. C. (1976). Effects of practice on marijuana-induced changes in reaction time. *Psychopharmacology*, *48*, 159–163.

Powles, T., te Poele, R., Shamash, J., et al. (2005). Cannabis-induced cytotoxicity in leukemic cell lines: the role of the cannabinoid receptors and the MAPK pathway. *Blood*, *105*, 1214–1221.

Schlicker, E., & Kathmann, M. (2001). Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends in Pharmacology Science*, *22*, 565–572.

Tam, J., Ofek, O., Fride, E., et al. (2006). Involvement of neuronal cannabinoid receptor CB1 in regulation of bone mass and bone remodeling. *Molecular Pharmacology*, *70*, 786–792.

Thomson, W. M., Poulton, R., Broadbent, J. M., et al. (2008). Cannabis smoking and periodontal disease among young adults. *Journal of the American Medicine Association*, *299*, 525–531.

Veitz-Keenan, A., & Spivakovsky, S. (2011). Cannabis use and oral diseases. *Evidence Based Dentistry*, *12*, 38–41.

Versteeg, P. A., Slot, D. E., van der Velden, U., & van der Weijden, G. A. (2008). Effect of cannabis usage on the oral environment: a review. *International Journal of Dental Hygiene*, *6*, 315–320.

CHAPTER

e9

Does Cannabis Use Increase the Risk of Developing Cancer in Humans?

R.C. Callaghan[*,**,†], *M. Verdichevski*[*], *T.M. Fyfe*[*], *J.M. Gatley*[*,**,†]

*Northern Medical Program, University of Northern British Columbia, Prince George, BC, Canada
**Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada
†Human Brain Lab, Centre for Addiction and Mental Health, Toronto, ON, Canada

SUMMARY POINTS

- Using a systematic process to identify all eligible studies published from 2004–14, our chapter attempts to answer the following question: "Does marijuana use increase the risk of developing cancer in humans?"
- Seven scientific databases were searched, and the review process identified 19 eligible studies.
- Eligible studies yielded inconsistent results in relation to marijuana use and the development of head and neck cancer, or lung cancer.
- Few eligible studies included sufficient numbers of long-term marijuana users with substantial joint-years of usage (eg, >10 joint-years) and, as a result, a possible connection between long-term and heavy marijuana use and lung cancer or head and neck cancers cannot be ruled out.
- Based on the three available studies, the scant evidence suggests a potential relation between marijuana use and increased risk of nonseminoma testicular germ cell cancers.

KEY FACTS OF LUNG CANCER

- Globally, lung cancer accounts for the biggest proportion of cancer-related mortality.

KEY FACTS OF TESTICULAR CANCER

- Testicular germ cell tumors (TGCTs) are responsible for 95% of testicular tumors.
- TGCTs are classified as either seminomas or nonseminomas.

KEY FACTS OF HEAD AND NECK CANCER

- Globally, head and neck cancers are among the top ten causes of male cancer mortality.
- About 90% of head and neck cancers are squamous cell carcinomas of the head and neck (SCCHNs).

LIST OF ABBREVIATIONS

HNC	Head and neck cancer
TCC	Transitional cell cancer
TGCT	Testicular germ cell tumours
SCCHN	Squamous cell carcinomas of the head and neck

INTRODUCTION

Marijuana is produced and consumed in virtually every country, thus facilitating its status as the most widely used illicit drug worldwide (UNODC, 2014). According to the

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00061-2
Copyright © 2017 Elsevier Inc. All rights reserved.

2014 World Drug Report (UNODC, 2014), approximately 180 million people—or 3.8% of the global population—consumed marijuana in the past year, and it is likely that these numbers are due, in part, to the common perception that its use confers minimal health risks (Berg et al., 2015).

Globally, a range of nations, including countries of the European Union, Australia, and the Americas have recently implemented or proposed reforms to the ways in which they control marijuana use, thereby departing from traditional approaches of criminal prohibition that have dominated throughout most of the 20th century (Room, Fischer, Hall, Lenton, & Reuter, 2010). In light of the global debate about marijuana decriminalization/legalization, as well as its widespread global use, it is critically important to assess the impact of marijuana smoking on human health, especially on the development of cancer. Despite the current lack of data on the prevalence of marijuana use by method of intake, smoking is presumed to be the most common form of consumption (National Institute on Drug Abuse, 2014).

There are a number of good reasons to think that marijuana smoking may facilitate the development of cancer, especially for those cancers identified as directly attributable to tobacco use (Centers for Disease Control and Prevention, 2013). Tobacco smoking is the leading cause of lung cancer (Alberg, Ford, Samet, & American College of Chest Physicians, 2007), and marijuana and tobacco smoke contain many of the same potent carcinogens (Maertens et al., 2009)—but with marijuana-smoke condensates being even more cytotoxic and mutagenic (Maertens et al., 2009). In comparison to tobacco smoking, marijuana smoking is usually associated with deeper inhalation, longer breath-holding times, use of unfiltered marijuana cigarettes (joints), and relatively greater delivery of tar (a known carcinogen) to the lungs (Mehra, Moore, & Crothers, 2006). In addition, studies employing bronchial biopsies have demonstrated that marijuana users not only manifest airway inflammation, but also histopathological and/or molecular changes indicative of precancerous bronchial activity (Tashkin, Baldwin, Sarafian, Dubinett, & Roth, 2002).

In view of the similarity of marijuana smoke to that of tobacco, our narrative review focuses on the following question: "Does marijuana use increase the risk of developing cancer in humans?" Given that the last major systematic review attempting to answer this question was published a decade ago by Hashibe et al. (2005), we provide a narrative assessment of all subsequent articles published from 2004–14.

HOW WERE ALL RELEVANT STUDIES IDENTIFIED?

A Master's-level university librarian (TF) conducted a systematic search of the following databases from the beginning date of the database to Jun. 2014: Medline OvidSP, Embase OvidSP, LILACS, ERIC EBSCO, PsycInfo EBSCO, Web of Science ISI, and ProQuest Dissertations and Theses. The searches incorporated keywords and subject headings for "cannabis," "marijuana," and "cancer" (see Fig. e9.1 for more details).

WHAT WERE THE INCLUSION/EXCLUSION CRITERIA FOR ELIGIBLE STUDIES?

Articles were eligible for our review if they included all of the following items:

1. A cancer diagnosis;
2. Cannabis use assessment;
3. A risk parameter for estimating relation between cannabis use and cancer outcome; and
4. Original estimates or original data.

In addition, the manuscript must have been written in English, conducted in humans, and published between 2004 and 2014.

WHAT WERE THE STEPS IN THE IDENTIFICATION PROCESS OF ELIGIBLE STUDIES?

The search process yielded 878 potentially eligible articles, and the removal of duplicates produced 478 unique papers. The second author (MV) screened the abstracts and text of each of the 478 articles remaining, and identified all those meeting the criteria for potential inclusion. References of included articles were hand-searched by the second author (MV) and considered for inclusion. This screening process identified 21 articles, which the second author (MV) and the lead author (RCC) then reviewed. Agreement was reached on all articles for inclusion to occur, and 19 articles were chosen for our review (Tables e9.1–e9.3).

NARRATIVE ASSESSMENT OF ELIGIBLE STUDIES

The identified articles can be grouped into three main cancer types—lung, head and neck, and testicular germ cell—as well as a miscellaneous category. Within each grouping, a critical analysis of each publication will be presented, followed by an overarching conclusion at the end of the paper.

LUNG CANCER

Critical Analysis of Callaghan, Allebeck, and Sidorchuk (2013)

The only cohort study included in our review (Callaghan et al., 2013) presents the potential association

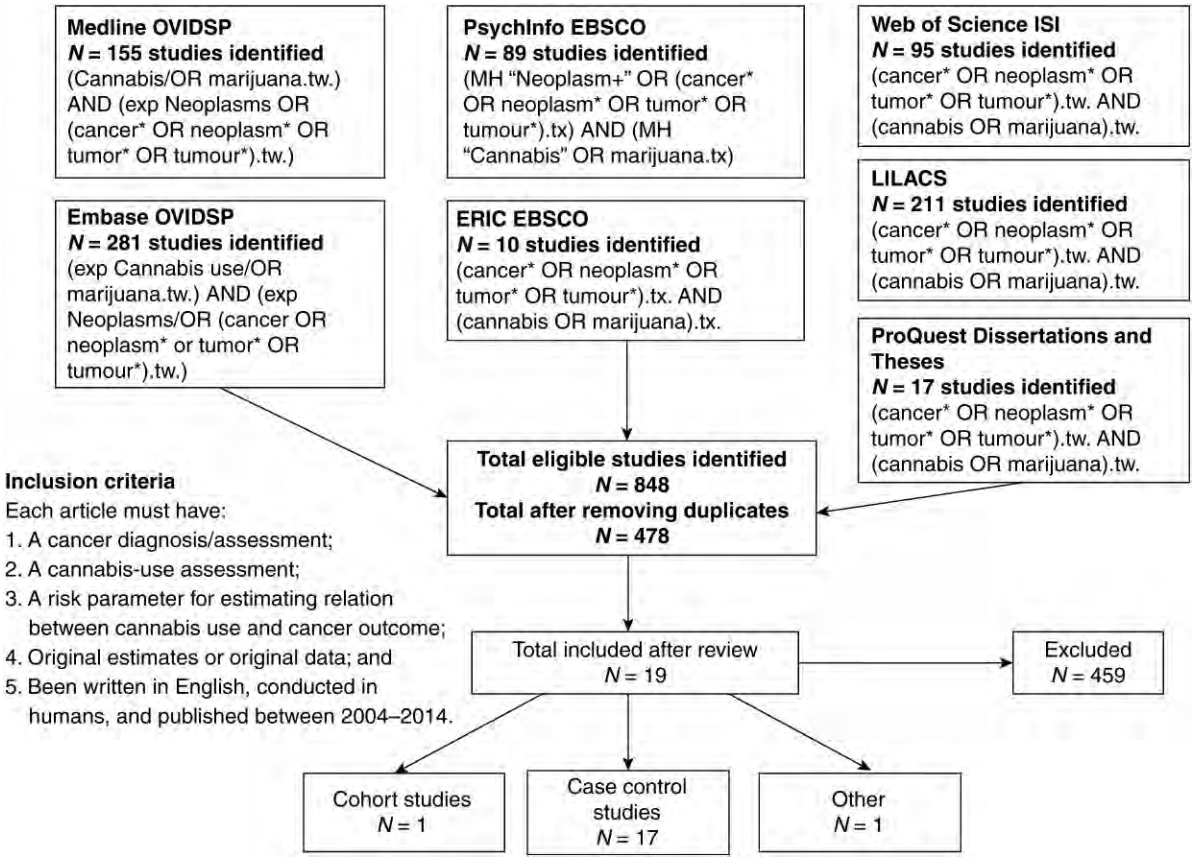


FIGURE e9.1 Flowchart outlining the systematic identification process of eligible studies. The flow chart illustrates how studies were systematically selected for our review.

between marijuana use in late adolescence/early adulthood and the development of lung cancer over a 40-year follow-up period. The sample comprised male Swedish military conscripts (n = 49,321), aged 18–20, who were inducted into military service in 1969–70. Most of those who smoked marijuana had also smoked tobacco, with approximately 91% of the "heavy" marijuana smokers (defined as those using more than 50 times during lifetime) also indicating at least some tobacco use. Heavy marijuana use was associated with more than a twofold increased risk of lung cancer, an association that remained even after adjustment for tobacco and alcohol use, respiratory conditions at conscription, and socioeconomic status (HR = 2.12; 95% CI = 1.08–4.14). It is important to note that no information on the use of marijuana or tobacco is available for the period preceding conscription or following it, and there is a strong possibility of residual confounding by tobacco use, in relation to the study's main findings.

Critical Analysis of Hashibe et al. (2006)

In one of the best designed studies in the field, researchers investigated the association between marijuana use and lung cancer in a large Southern Californian case-control study [611 lung-cancer cases; 1040 controls (Hashibe et al., 2006)]. Despite the study's strength of having a large number of heavy marijuana smokers as participants (defined in this study as >10 joint years, n = 85 for cases, n = 115 for controls), after adjustment for several confounders, including cigarette smoking and alcohol consumption, no evidence of significant relations between joint-years of marijuana smoking and lung cancer were evident. In the subgroup analyses of marijuana users who were not tobacco users, the results showed similar patterns. The findings ran counter to the authors' initial hypotheses, and the researchers suggested that their results may have been affected by selection bias in choosing controls, or error in measuring lifetime exposure/confounder histories.

Critical Analysis of Aldington et al. (2008a)

A population-based case control study was conducted in New Zealand (Aldington et al., 2008a), with 79 cases and 324 controls aged 55 years and younger being administered face-to-face questionnaires regarding possible risk factors for lung cancer. Although marijuana smoking (lifetime use of 20 or more joints) was not associated with increased risk of lung cancer, those in

TABLE e9.1 Key Characteristics of Eligible Cohort Studies Included in the Review

Study location/ period	Cohort description	Exposure assessment	Type of cancer	Prevalence of cannabis use	Exposure categories	No. of cases	Crude HR (95% CI)	Tobacco-adjusted HR (95% CI)	Fully adjusted HR (95% CI)
Callaghan et al. (2013). Sweden. Baseline data collected in 1969–70, and participants were tracked until 2009	Men aged 18–20 years, surveyed during military conscription (n = 49, 321)	Nonanonymous questionnaires for conscription to compulsory military service	Lung cancer	*Never (ref) 81.9%* *Ever 10.5%* Once 2.5% 2–4 times 3.0% 5–10 times 1.7% 11–50 times 1.5% >50 times 1.7% No answer 7.7% No answer 7.6%	*Never (ref)* *Ever* Once 2–4 times 5–10 times 11–50 times >50 times No answer No answer	40,397 5156 1213 1485 853 742 831 3800 3768 Cancer cases Total: 189 Never: 141 Ever: 38 Heavy: 10	1.0 1.90 (1.30–2.75) 2.07 (1.06–4.06) 0.95 (0.39–2.33) 1.02 (0.32–3.20) 2.69 (1.26–5.74) 3.72 (1.96–7.06) – –	1.0 1.25 (0.85–1.83) 1.48 (0.75–2.91) 0.65 (0.26–1.58) 0.66 (0.21–2.09) 1.68 (0.78–3.62) 2.24 (1.17–4.29)	1.0 1.25 (0.84–1.87) 1.52 (0.77–3.01) 0.66 (0.27–1.62) 0.68 (0.21–2.16) 1.68 (0.77–3.66) 2.12 (1.08–4.14)

The table presents the key characteristics of cohort studies (n = 1) selected for the systematic review.

TABLE e9.2 Key Characteristics of Eligible Cases-Control Studies Included in the Review

Study location/ period	Type(s) of cancer	Characteristics of cases	Characteristics of controls	Exposure assessment	Prevalence of cannabis use	Exposure categories (JY = joint years[a])	Hazard ratio (HR)/Odds ratio (OR)/Risk ratio (RR), 95% Confidence Interval (CI)	Adjustment for potential confounders
Hashibe et al. (2006). Los Angeles County, CA, USA. Study took place in 1999–2004	Lung; upper aerodigestive tract (UAT) cancer	1212 incident cancer cases, 62% males, identified by the rapid ascertainment system of the Cancer Surveillance Program for Los Angeles County. Most cases (95%) were histologically confirmed, remainder were confirmed by MRI, CAT scan, or other methods. Participation rates were 39% for lung cancer, 54% for oral cancer, 45% for pharyngeal cancer, 42% for laryngeal cancer, and 35% for esophageal cancer	1040 controls with no lung or UAT cancer recruited from the same neighborhoods as controls, 60% males, matched to cases on age, gender, and neighborhood. Participation rate of eligible controls was 72%	Standardized questionnaire	Smoked cannabis Hashish/hash oil *Controls* Ever 54% ≥10 JY 11% *Controls* Ever 54% ≥10 JY 15%	*Adjusted OR among cigarette users* *Oral cancer* Never <0 to <1 JY 1 to <1 0 JY 10 to <30 JY 30 to <60 JY ≥60 *Pharyngeal cancer* Never <0 to <1 JY 1 to <10 JY 10 to <30 JY ≥30 *Laryngeal cancer* Never <0 to <1 JY 1 to <10 JY 10 to <30 JY 30 to <60 JY ≥60 *Esophageal cancer* Never <0 to <1 JY 1 to <10 JY 10 to <30 JY ≥30 *Lung cancer* Never <0 to <1 JY 1 to <10 JY 10 to <30 JY 30 to <60 JY ≥60 *Adjusted OR among never cigarette users* *Oral cancer* Never <0 to <1 JY 1 to <10 JY ≥10 *Pharyngeal cancer* Never Ever *Laryngeal cancer* Never Ever *Esophageal cancer* Never Ever *Lung cancer* Never 0 to <1 JY ≥1 JY	 1 1.1 (0.74–1.5) 1.1 (0.65–1.7) 0.92 (0.48–1.7) 0.88 (0.38–2.0) 1.1 (0.56–2.1) 1 0.67 (0.37–1.2) 0.71 (0.30–1.7) 0.39 (0.10–1.5) 0.57 (0.20–1.6) 1 0.81 (0.42–1.6) 0.42 (0.15–1.2) 0.91 (0.33–2.5) 0.71 (0.19–2.7) 0.84 (0.28–2.5) 1 0.71 (0.41–1.2) 0.77 (0.36–1.6) 0.44 (0.15–1.3) 0.53 (0.22–1.3) 1 0.63(0.46–0.87) 0.71 (0.46–1.1) 0.56 (0.31, 1.0) 0.82 (0.38–1.7) 0.62 (0.32–1.2) 1 0.93 (0.53–1.6) 1.5 (0.68–3.5) 1.8 (0.69–4.7) 1 0.92 (0.41–2.1) 1 1.2(0.26–5.5) 1 0.79 (0.30–2.1) 1 0.44 (0.21–0.92) 1.1 (0.48–2.6)	OR among cigarette users adjusted for age, gender, race/ ethnicity, education, drink-years, tobacco use (ever vs. never) and pack-years. OR among never cigarette users adjusted for age, gender, race/ ethnicity, education, drink-years

Voirin et al. (2006). Tunis, Tunisia. Subjects enrolled Mar. 2000–Feb. 2003 at the same hospital	Lung cancer	Hospitalized sample of 149 males with incident lung cancer, diagnosed by histologic or cytologic examination, except for two cases diagnosed only radiographically	Hospitalized sample of 188 males, diagnosed with other most non-malignant conditions	Questionnaire administered by a trained physician	*Cases* Never 80% Past use 20% *Controls* Never 94% Past use 6%	*Adjusted OR* Never Past use [a]No subjects reported current use	1 4.1 (1.9–9.0)	Adjusted for age, occupational exposure, duration of tobacco smoking
Aldington et al. (2008a). New Zealand. Subjects diagnosed between Jan. 2001 and Jul. 2005	Lung cancer	79 cases with incident and prevalent lung cancer, diagnosed in the previous 5 years, identified from hospital databases and the New Zealand Cancer Registry, all ≤55 years old. Of 102 cases contacted the participation rate was 77%	324 controls (493 potential controls were contacted with a participation rate of 66%) were randomly selected from the electoral roll and frequency-matched to subjects in 5-year age groups, as well as district healthboards	Questionnaires administered by trained interviewers. Individuals who had ever smoked ≥20 marijuana joints were administered another more detailed questionnaire	*Cases* Nonsmoker (<20 joints) 73% Smoker (≥20 joints) 27% *Controls* Nonsmoker (<20 joints) 88% Smoker (≥20 joints) 12%	*Adjusted RR* *Cannabis use* Nonsmoker Smoker (≥20 joints) *JY* Nonsmoker First tertile (<1.39 JY) Second tertile (1.39–10.5 JY) Third tertile (>10.5 JY)	 1 1.2 (0.5–2.6) 1 0.3 (0.1–1.7) 0.5 (0.1–2.0) 5.7 (1.5–21.6)	Adjusted for age, sex, ethnicity, family history of lung cancer, pack-years of cigarette smoking, and joint-years of cannabis use

(*Continued*)

TABLE e9.2 Key Characteristics of Eligible Cases-Control Studies Included in the Review (*cont.*)

Study location/ period	Type(s) of cancer	Characteristics of cases	Characteristics of controls	Exposure assessment	Prevalence of cannabis use	Exposure categories (JY = joint years[a])	Hazard ratio (HR)/Odds ratio (OR)/Risk ratio (RR), 95% Confidence Interval (CI)	Adjustment for potential confounders
Berthiller et al. (2008). Three pooled studies: Tunisia, where study was conducted between Mar. 2000 and Feb. 2003[b]; Morocco, where study was conducted between Jan. 1996 and Jan. 1998; and Algeria, where study was conducted between Mar. 2003 and Jan. 2004	Lung cancer	In Morocco, 118 primary incident lung cancer cases diagnosed radiologically, with 77 cases histologically confirmed. Hospital-based sample of 100% males (females were excluded from analysis due to small number). In Algeria, 167 histologically or cytologically confirmed cases of primary incident lung cancer. Hospital-based sample of 100% males. A total of 430 cases were included in the pooled analysis	In Tunisia, 188 cancer-free controls hospitalized in the same hospital as subjects for other conditions. 100% males. In Morocco, 235 cancer-free controls hospitalized in the same hospital as subjects for other conditions were matched to subjects by age, sex, and place of residence. In Algeria, 340 cancer-free controls hospitalized in the same hospital as subjects for other conditions were matched to subjects by age and place of residence. Hospital-based sample of 100% males. A total of 755 controls were included in the pooled analysis	Questionnaires administered by trained physician	*Tunisia* *Cases* Never 80% Past use 20% *Controls* Never 94% Past use 6% *Morocco* *Cases* Never 77% Past use 23% *Controls* Never 92% Past use 8% *Algeria* *Cases* Never 94% Past use 6% *Controls* Never 97% Past use 3%	*Adjusted OR* *Marijuana use, age adjusted* Never Ever *Marijuana use, multivariate adjusted* Never Ever *Combined exposure* *Tobacco* / *Cannabis* Never / Never Former / Never Former / Ever Current / Never Current / Ever	 1 3.3 (2.2–5.1) 1 2.4 (1.5–3.7) 1 4.1 (2.3–7.5) 11.9 (5.3–26.8) 11.3 (6.2–20.5) 18.4 (8.2–41.6)	Adjustments made for age, occupational exposure, country, and tobacco use in categories of use (never, former, <25 years, 35–35 years, >35 years)

Zhang et al. (2015). Pooled analysis of six studies conducted in the United States, Canada, United Kingdom, and New Zealand. Study periods ranged from 1999 to 2013	Lung	2159 cases pooled from 6 case-control studies within the International Lung Consortium. Cases included hospital-based, family and clinic-based, and population-based matching factors. Studies considered primary, incident and histologically confirmed lung cancer cases	2985 controls pooled from 6 case-control studies in the United States, Canada, the United Kingdom, and New Zealand within the International Lung Consortium	Study-specific questionnaires	*Cases* Nonhabitual smoker 90% Habitual smoker 10% *Controls* Nonhabitual smoker 89% Habitual smoker 11%	*Adjusted OR* *Status* Nonhabitual smoker Habitual smoker *Intensity (joints/day)* Nonhabitual smoker <1 ≥1 Continuous *Duration, years* Nonhabitual smoker >0 to <20 ≥20 Continuous *Joint-years* *Ages of start, years* Nonhabitual smoker (<1) 1 to <10 ≥10 Continuous *Joint-years* *Ages of start, years* Nonhabitual smoker (<1) >18 ≤18	 1 1.03 (0.51–2.08) 1 1.33 (0.61–2.93) 0.49 (0.11–2.25) 1.08 (0.91–1.30) 1 0.89 (0.39–2.00) 1.64 (0.45–6.00) 0.97 (0.93–1.01) 1 1.26 (0.57–2.75) 0.54 (0.12–2.55) 1.00 (0.93–1.07) 1 1.25 (0.47–3.29) 0.85 (0.32–2.31)	Study-specific models were adjusted for age, sex, race, highest education, tobacco smoking status and tobacco-smoking pack-years.
Aldington et al. (2008b). New Zealand. Cases were diagnosed between Jan. 2001 and Jul. 2005	Head and neck (lip, base of tongue, tongue, floor of mouth, palate, mouth, tonsil, oropharynx, hypopharynx, pharynx, nasal cavities, unspecified, larynx)	75 prevalent and incident cancer cases, 75% males, ≤55 years of age, identified from the New Zealand Cancer Registry. A total of 106 potential cases were contacted, and participation =76%	319 controls, 45% males, were randomly selected from the electoral roll and were frequency matched in 5-year age groups	Questionnaires administered by trained interviewers		*Adjusted RR* *Never* *Ever* *Joint years* None First tertile Second tertile Third tertile	 1 1 (0.5–2.3) 1 0.4 (0.1–2.2) 1.2 (0.3–4.2) 1.6 (0.5–5.2)	Adjusted for age, sex, ethnicity, alcohol consumption, income, and pack-years of cigarette smoking.

(*Continued*)

TABLE e9.2 Key Characteristics of Eligible Cases-Control Studies Included in the Review (*cont.*)

Study location/ period	Type(s) of cancer	Characteristics of cases	Characteristics of controls	Exposure assessment	Prevalence of cannabis use	Exposure categories (JY = joint years[a])	Hazard ratio (HR)/Odds ratio (OR)/Risk ratio (RR), 95% Confidence Interval (CI)	Adjustment for potential confounders
Gillison et al. (2008). Baltimore, OR, United States. Cases were identified between May 2000 and Jun. 2006	Head and neck squamous cell carcinoma (HNSCC)	240 cases of incident HNSCCs at the outpatient otolaryngology clinic at Johns Hopkins Hospital and histologically confirmed. Cases were stratified by either HPV-16-positive HPV-16-negative as determined by in situ hybridization of tumor biopsies	322 cancer-free control subjects referred to the same outpatient otolaryngology clinic, were individually matched. Two controls were matched to each case subject	Interviews using audio computer-assisted self-interview (ACASI) technology.	*HPV-16-positive Cases* Never 67% Former 22% Current 11% *HPV-16-negative Cases* Never 80% Former 13% Current 7% *Controls—HPV-16-positive matched* Never 83% Former 14% Current 3% *Controls - HPV-16-negative matched* Never 86% Former 11% Current 4%	*HPV-16-POSITIVE Adjusted OR* *Smoked marijuana, monthly for ≥1 year* Never Former Current *Number of joints usually smoked per month* ≤1 2–13 14–29 ≥30 *Number of years smoked marijuana* Never 1–10 11–20 ≥21 *Years since quit smoking marijuana* Current smoker 1–10 11–20 >20 Never user *Number of joint-years* 0 1–4 5–14 ≥15	$p = 0.007$ 1 2.3 (0.98–5.4) 4.7 (1.3–17) $p = 0.007$ 1 2.5 (0.89–6.8) 3.2 (1.0–10) 5.4 (1.0–28) $p = 0.011$ 1 1.7 (0.58–5.1) 6.7 (1.8–25) 3.0 (0.58–16) $p = 0.017$ 1 0.49 (0.05–4.7) 0.87 (0.20–3.9) 0.56 (0.13–2.5) 0.27 (0.08–0.93) $p = 0.003$ 1 2.0 (0.76–5.2) 6.0 (1.2–29) 6.4 (1.6–26)	Adjusted for race, tobacco, alcohol, marijuana use, frequency of tooth brushing, number of teeth lost, and number of oral sex partners

Liang et al. (2009). Greater Boston, MA, United States. Cases were identified between Dec. 1999 and Dec. 2003	Head and neck squamous cell carcinoma (HNSCC)	434 cases of incident HNSCCs from 9 medical facilities in the Greater Boston, MA area. Cases and controls were tested serological status for HPV-16 by venous blood samples	547 controls were frequency-matched to cases on age, gender and town of residence, randomly selected from Massachusetts town books	Self-administered questionnaire	*Cases* Never 73.3% Former 8.3% Current 18.4% *Controls* Never 72.9% Former 7.5% Current 20.11%	*Adjusted OR* *Marijuana use status* Never Former Current $p < 0.05$ *Duration of marijuana use in lifetime (years)* None >0– < 10 10– < 20 $p < 0.05$ ≥20 *Average frequency of marijuana use per week (times)* <0.5 0.5–1.5 $p < 0.05$ 1.5 to < 4.5 ≥4.5 $p < 0.05$ *Total numbers of marijuana use in lifetime (times/week/year)* *None* >0 to <5 5 to <15 $p < 0.05$ 15 to <90 $p < 0.05$ ≥90 *Total amount of marijuana use in lifetime (ounces/week/year)* None >0 to <1/16 1/16 to <3 3 to <7.5 ≥7.5 *Average amount of marijuana use per week (ounces)* <3/32 3/32 to <1/4 ¼ to <3/4 ≥3/4 *Years since last marijuana use* None <2 $p < 0.05$ 2 to <12 12 to <22 $p < 0.05$ ≥22 *Age at starting of marijuana use* None 10 to <15 $p < 0.05$ 15 to <20 $p < 0.05$ ≥20 $p < 0.05$	$p_{trend} = 0.00$ 1 0.65 (0.36–1.16) 0.52 (0.34–0.80) $p_{trend} = 0.01$ 1 0.63 (0.38–1.05) 0.38 (0.22–0.67) 0.67 (0.38–1.20) $p_{trend} = 0.01$ 1 0.52 (0.32–0.85) 0.62 (0.34–1.12) 0.55 (0.31–0.99) $p_{trend} = 0.03$ 1 0.63 (0.34–1.17) 0.36 (0.18–0.69) 0.53 (0.30–0.94) 0.78 (0.41–1.47) 1 0.53 (0.26–1.06) 0.57 (0.29–1.12) 0.65 (0.33–1.26) 0.69 (0.36–1.33) 1 0.60 (0.32–1.14) 0.72 (0.36–1.47) 0.76 (0.41–1.41) $p_{trend} = 0.01$ 1 0.57 (0.38–0.86) 0.73 (0.25–2.18) 0.27 (0.09–0.82) 0.68 (0.24–1.94) $p_{trend} = 0.00$ 1 0.62 (0.39–0.96) 0.53 (0.30–0.95) 0.39 (0.17–0.90)	Adjusted for age, gender, race, education, family history of cancer, HPV-16, smoking (pack-year), and average drinks of alcohol per week.

(*Continued*)

TABLE e9.2 Key Characteristics of Eligible Cases-Control Studies Included in the Review (*cont.*)

Study location/ period	Type(s) of cancer	Characteristics of cases	Characteristics of controls	Exposure assessment	Prevalence of cannabis use	Exposure categories (JY = joint years[a])	Hazard ratio (HR)/Odds ratio (OR)/Risk ratio (RR), 95% Confidence Interval (CI)	Adjustment for potential confounders
Daling et al. (2009). Seattle/ Puget Sound region, WA, United States. Subjects were identified between Jan. 1999 and Jan. 2006	Testicular germ cell tumors (TGCTs)	369 cases, men ages 18–44 years, of incident invasive TGCTs. 550 cases identified, participation = 67%. Data from the Adult Testicular Cancer Lifestyle and Blood Specimen (ATLAS) study, a population-based case-control study	979 controls were age-matched men residing in the same counties were used in the analyses. 1875 total potential controls contacted, participation = 52%	Interviewed by trained interviewers	*Cases* Never 27.4% Former 46.3% Current 26.3% *Controls* Never 32.0% Former 48.4% Current 19.6%	*Adjusted OR* *Ever used marijuana* No Yes *Marijuana use as of reference date* Never Former Current *Age at first use among current marijuana users (years)* <18 ≥18 *Length of use among current marijuana users (years)* <10 [a]*95% CI excludes 1* ≥10 *Frequency of current marijuana use* Daily or ≥1 day per week Less than once per week	 1 1.3 (1.0–1.8) 1 1.2 (0.9–1.7) 1.7 (1.1–2.5) 1.8 (1.2–2.8) 1.4 (0.8–2.5) 1.8 (1.0–3.3) 1.6 (1.1–2.5) 2.0 (1.3–3.2) 1.4 (0.9–2.3)	Adjusted for age at reference date, reference year, alcohol use, current smoking, and history of cryptorchidism
Trabert et al. (2011). TX, United States. Patients were diagnosed between Jan. 1990 and Oct. 1996	TGCTs	187 cases with incident primary TGCTs, men ages 18–50 years, enrolled at The University of Texas MD Anderson Cancer Center, and residing in Texas, Arkansas, Louisiana, or Oklahoma	148 controls, men ages 18–50 years, enrolled at the same location, and residing in Texas, Arkansas, Louisiana or Oklahoma	Self-administered questionnaire	*Cases* Never 51.3% Ever 48.7% *Controls* Never 45.2% Ever 54.8%	*Adjusted OR* *Ever use marijuana* No Yes *Frequency of marijuana use* Never <1/day Daily or >1/day *Duration of marijuana use* Never <10 years ≥10 years	 1 0.7 (0.4–1.1) 1 0.5 (0.3–0.9) 2.2 (1.0–5.1) 1 0.6 (0.3–1.0) 1.2 (0.6–2.8)	Adjusted for age, race, cryptorchidism, cigarette smoking, and alcohol intake
Lacson et al. (2012). Los Angeles County, CA, United States. Patients were diagnosed from Dec. 1986 to Apr. 1991	TGCTs	163 cases, with incident primary TGCTs, men ages 18–35 years, participation = 81%	292 controls, men matched to patients on age, race, ethnicity, and neighborhood	Structures, in–person interviews administered by trained interviewers	*Cases* Never 18.7% Former 48.9% Current 32.4% [a]Response rate = 85.3% *Controls* Never 24.0% Former 38.4% Current 37.6% [a]Response rate = 100%	*Adjusted OR* *Marijuana use* *Never* *Ever* *Recency* Former use Current use *Frequency, number/week* <1 ≥1 *Duration, years* <10 ≥10	 1 1.94 (1.02–3.68) 2.28 (1.17–4.43) 1.38 (0.67–2.87) 2.10 (1.09–4.03) 1.53 (0.73–3.24) 2.09 (1.09–3.98) 1.51 (0.66–3.47)	Adjusted for cocaine use, amyl nitrite use, cryptorchidism, religiosity, and education

Feng et al. (2009). Algeria, Morocco, and Tunisia. Patients were diagnosed or recruited in 2001–2004	Nasopharyngeal carcinoma (NPC)	636 cases, with incident and prevalent NPC (about 25%) diagnosed in five hospitals were invited for interview. Participation = more than 90% total for cases and controls	615 controls were hospitalized in 15 noncancer hospital departments (61%) or friends/family of patients diagnosed with other cancers (39%). Controls were frequency-matched by center, age, sex, and childhood household type (urban/rural). Controls were excluded if they had a disease that shared risk factors with NPC	In-person interviews conducted by trained doctors using identical questionnaires	*Cases* Never 88% Ever 12% *Controls* Never 94% Ever 6%	*Adjusted OR* *Marijuana use* Never Ever *Frequency of consumption* Never <30 times per month ≥30 times per month *Lifetime consumption* Never <2000 times ≥2000 times *Mode of consumption* Never Smoking Smoking with tobacco (kiff)	 1 1.54 (0.86–2.76) 1 1.40 (0.58–3.36) 1.85 (0.86–4.02) 1 1.86 (0.79–4.36) 2.62 (1.00–6.86) 1 0.97 (0.37–2.52) 1.94 (0.96–3.92)	Adjusted for age, socioeconomic status (SES) measures, associated dietary factors, and cigarettes smoked per day
Chacko, Heiner, Siu, Macy, and Terris (2006). CA and GA, United States	Transitional cell carcinoma of the bladder	52 cases with incident transitional cell carcinoma of the bladder, men aged 60 years and younger	104 controls, men, were consecutive patients presenting at the urology clinic with other conditions, age-matched	Self-administered questionnaires	*Cases* Never 11.5% Former 57.7% Current 30.8% *Controls* Never 30.8% Former 49.0% Current 20.2%	*Adjusted OR* *Marijuana* Ever smoked Currently smoke Joint years >40 *Combined smoking of tobacco and marijuana* Both Tobacco only Marijuana only	 3.4 1.9 3.5 1.5 0.5 3.3	Marijuana use in joint years, tobacco use in pack years, smoked meat consumption, Agent Orange consumption, radiation exposure, and dye exposure.

(*Continued*)

TABLE e9.2 Key Characteristics of Eligible Cases-Control Studies Included in the Review (*cont.*)

Study location/ period	Type(s) of cancer	Characteristics of cases	Characteristics of controls	Exposure assessment	Prevalence of cannabis use	Exposure categories (JY = joint years[a])	Hazard ratio (HR)/Odds ratio (OR)/Risk ratio (RR), 95% Confidence Interval (CI)	Adjustment for potential confounders
Bluhm et al. (2006). North America. Children were diagnosed between May 1992 and Apr. 1994	Neuroblastoma	538 youth with neuroblastoma aged 19 years or younger. Participation = 73%	504 controls, selected by random-digit dialing, age–matched. Participation = 72%	Maternal interviews were conducted by trained interviewers, using a standardized questionnaire	*Maternal use of marijuana between one month in pregnancy and childbirth* *Cases* None 90.6% Any 9.4% *Controls* None 95% Any 5%	*Maternal use of marijuana between one month in pregnancy and childbirth* None Any	 1 1.37 (0.77–2.49)	Adjusted for the use of other drugs during the 10 month time interval, household income in the year of birth, and age of diagnosis

The table presents the key characteristics of case control studies (n = 17) included in the systematic review.

[a]*This study is a pooled analysis of three studies, one of which is study #2 in this table. The first study that was pooled was already included in the previous review published in 2005, and the third study included was based on brand new, unpublished data.*

[b]*One joint year is the equivalent of 1 joint per day for 1 year.*

TABLE e9.3 Key Characteristics of Eligible Studies Using a Pooled Approach or Re-Analysis of Secondary Data

Study location/ period	Type of cancer	Characteristic of sample	Exposure assessment	Prevalence of marijuana use	Exposure categories	Hazard ratio (HR)/Odds ratio (OR)/Risk ratio (RR), 95% Confidence Interval (CI)	Adjustment for potential confounders
Han et al. (2010) United States. Data was collected in 2005–07	Lung cancer	29,195 respondents to NSDUH, aged 35–49 years, regarding drug-use and health conditions. Household and personal level response rate = 67%	NSDUH administered at personal household visits by Audio computer-assisted self-administered interviewing (ACASI)	Not reported	*Adjusted OR* *Marijuana* Never use ≤1 year 2–10 years ≥11 years	 1 2.68 (0.53–13.47) 2.12 (0.41–10.95) 7.87 (1.28–48.40)	Adjusted for age, gender, race/ ethnicity, education, health insurance, family income, ever smoked cigarettes daily, duration of tobacco use, duration of alcohol use, and durations of nonmedical use of pain-relievers, tranquilizers, stimulants, and sedatives
Berthiller et al. (2009) Pooled data from five case-control studies on head and neck cancer (HNC) that included information on marijuana use. Studies were from various locations in the United States, as well as one study from Brazil. Recruitment periods ranged from 1985 to 2006	HNC	4029 cases including, including 981 oral cavity, 1397 pharynx (1165 oropharynx and 232 hypopharynx), 435 oral cavity or pharynx not otherwise specified, 1159 larynx, and 57 HNC not otherwise specified. Three studies were hospital-based. Case participation rates = 49–95%. 5015 controls, matched by age and sex in four studies. Control participation = rates = 61–90%. Data were collected as part of the International Head and Neck Cancer (INHANCE) consortium	Structured, in-person questionnaires	*Cases* Never 89.9% Ever 10.1% *Controls* Never 85.2% Ever 14.8%	*Adjusted OR* *Marijuana smoking* Never Ever *Frequency of marijuana smoking (times per day)* Never 0–1 >1–3 >3 *Duration of marijuana smoking (in years)* Never >0–5 >5–10 >10–20 >20 *Cumulative exposure (JY)* Never >0–2 >2–5 >5	 1 0.88 (0.67–1.16) 1 0.87 (0.61–1.25) 0.71 (0.35–1.47) 0.87 (0.40–1.89) 1 0.81 (0.53–1.23) 0.87 (0.48–1.57) 0.82 (0.46–1.44) 0.94 (0.53–1.66) 1 0.89 (0.60–1.31) 0.70 (0.31–1.56) 0.86 (0.54–1.37)	Adjusted for age, sex, race, education level, study, pack-years, alcohol duration, duration of smoking pipe, and duration of smoking cigar

(*Continued*)

TABLE e9.3 Key Characteristics of Eligible Studies Using a Pooled Approach or Re-Analysis of Secondary Data (*cont.*)

Study location/ period	Type of cancer	Characteristic of sample	Exposure assessment	Prevalence of marijuana use	Exposure categories	Hazard ratio (HR)/Odds ratio (OR)/Risk ratio (RR), 95% Confidence Interval (CI)		Adjustment for potential confounders
Marks et al. (2014) United States. Pooled data from nine case-control studies on oropharyngeal cancer that included information on marijuana use. Cases were diagnosed during various periods	Oropharyngeal and oral tongue cancer	1921 cases oropharyngeal and 356 cases oral tongue cases. 7639 controls. Data were collected as part of the INHANCE consortium	Self-reported	*Oropharyngeal cases* Never 79.7% Ever 21.3% *Oropharyngeal controls* Never 84.5% Ever 15.5% *Tongue cases* Never 93.0% Ever 7.0% *Tongue controls* Never 89.5% Ever 9.5%	*Adjusted OR* *Marijuana use* Never Ever *Frequency of use (per week)* Never ≤3 >3 *Duration of use* Never ≤10 >10 *Cumulative exposure (JY)* Never >0–1 2–10 >10	*Oropharyngeal* 1 1.24 (1.06–1.47) $p_{trend} = 0.046$ 1 1.24 (1.02–1.52) 1.19 (0.94–1.52) $p_{trend} = 0.031$ 1 1.11 (0.91–1.36) 1.28 (1.02–1.61) 1 1.12 (0.87–1.45) 1.34 (1.04–1.71) 1.14 (0.85–1.52)	*Oral* 1 0.47 (0.29–0.75) $p_{trend} = 0.005$ 1 0.47 (0.25–0.89) 0.47 (0.23–0.95) $p_{trend} = 0.002$ 1 0.43 (0.23–0.77) 0.44 (0.21–0.94) $p_{trend} = 0.004$ 1 0.39 (0.18–0.88) 0.64 (0.31–1.29) 0.31 (0.11–0.89)	Adjusted for age, sex, race, education level, ever use of tobacco, ever use of cigarettes/pipes, pack-year of tobacco smoking, and alcohol-years
Chen et al. (2008). California, United States. Data from a prospective cohort study by Sidney et al. (1997) was obtained between 1979 and 1985. Participants were then followed-up until the appearance of a cancer diagnosis, membership termination, diagnosis of HIV/AIDS, death, or the end date 31 Dec. 1993	All cancer	64,855 Kaiser Permanente Medical Care Program (KPMCP) members aged 15 to 49 years. 1421 incident cases of cancer	Self-administered questionnaires	*Total sample* Never 58.8% Ever 41.2% *Incident cancer cases* Never 60.9% Ever 29.1%	*Effects* *Interaction effects of marijuana use alone and tobacco use alone, men* Tobacco alone Marijuana alone *Main effects of marijuana use collapsing across tobacco use, men* Never Ever *Interaction effects of marijuana use alone and tobacco use alone, women* Tobacco alone Marijuana alone *Main effects of marijuana use collapsing across tobacco, women* Never Ever	$r = 0.36$ $r = 0.09$ $X^2 = 10.35$, $p = 0.001$ $r = 0.65$ $r = 0.35$ $X^2 = 31$, $p \leq 0.001$ $r = 0.34$ $r = 0.08$ $X^2 = 34.60$, $p \leq 0.001$ $r = 0.27$ $r = 0.73$ $X^2 = 36.09$, $p \leq 0.001$		

The table presents the key characteristics of studies which used a pooled approach or re-analyzed secondary data ($n = 4$) included in the systematic review.

the highest tertile of marijuana use (>10.5 joint-years) showed significantly higher risk (RR = 5.7; 95% CI = 1.5–21.6), even after adjustment for age, sex, ethnicity, pack-years of cigarette smoking, and a family history of lung cancer. The study also found an increase in lung cancer risk of 8% for each joint-year of marijuana smoked, after adjustment for confounders, such as cigarette smoking. This finding appears to be consistent with previous research showing that smoking a few joints a day causes histological changes in the tracheobronchial epithelium, similar to those caused by smoking 20–30 tobacco cigarettes a day (Roth et al., 1998). The possibility of residual confounding by tobacco use, however, is highly possible in the study, as cases and controls differed dramatically on tobacco pack-years smoked (eg, ≥23.9 tobacco pack-years smoked: cases = 56.9%; controls = 12%).

Critical Analysis of North African (Maghreb) Studies: Voirin et al. (2006); Berthiller et al. (2008)

Using data from the Maghrebian region of North Africa, a pooled study (Berthiller et al., 2008) was conducted, which included the Voirin et al. (2006) Tunisian study, as well as a previously published study completed in Morocco by the same group (Sasco et al., 2002), and additional new data from Algeria. The pooled analysis included a total of 430 cases and 755 controls from all three studies. The studies employed a similar research approach, all were hospital-based, and all used face-to-face interviews administered by trained physicians. However, one of the key issues present in all three studies is the very low number of current marijuana smokers (a total of only 4, all from the Algerian data), likely due to biases in self-reports resulting from the strict marijuana-prohibition laws in the area. Overall, the pooled analysis found an increase in the risk of lung cancer in the "ever" group versus the "never" group (OR = 2.4, 95% CI = 1.5–3.7), after adjusting for age, occupational exposure, country (within the pooled analysis), and tobacco use. Cases were much more likely to have not only a higher prevalence of current or former smoking status (controls: 95%; cases: 76%), but also a substantially greater proportion of individuals who reported tobacco smoking of more than 35 pack-years (cases: 35–45%; controls: 10–17%). As a result, there is a strong possibility of residual confounding of the results by tobacco use. Also, studies based in North African settings encounter the frequently intractable problem of identifying the independent effect of marijuana from tobacco, in regions where smoking mixed tobacco and marijuana products (kiff) is common practice (Hashibe et al., 2005).

Critical Analysis of Han, Gfroerer, & Colliver (2010)

Using cross-sectional data from individuals aged 35–49 in the 2005–07 US National Surveys on Drug Use and Health (NSDUH, a large, population-based survey), Han et al. (2010) estimated the relations between duration of illicit drug use (including marijuana use) on a set of 20 self-reported lifetime health conditions, adjusting for potential confounding factors. Logistic-regression models were adjusted for a range of covariates. The researchers found that duration of marijuana use of ≥11 years was significantly associated with increased likelihood of lung cancer (OR = 7.87, 95% CI = 1.28–48.40). It is surprising that the researchers limited their sample to individuals aged 35–49 in the survey, especially as the median age of lung cancer diagnosis is approximately 70 years of age in the United States (Hayat, Howlader, Reichman, & Edwards, 2007), and relatively few individuals develop lung cancer before the age of 50 (Howlader et al., 2014). The possibility of Type 1 error also must be considered in the interpretation of the significant results, as the authors conducted 300 multivariate tests in the paper.

Critical Analysis of Zhang et al. (2015)

Presenting one of the strongest papers in the field, researchers from the International Lung Cancer Consortium conducted a pooled case-control study based on data from six studies in the United States, United Kingdom, and New Zealand (Zhang et al., 2015). This study has a critical advantage over all previous studies in the marijuana-use and lung cancer field: the project allowed for the analysis of the relation between marijuana use and lung cancer in a large group of nontobacco smokers. Given the strong possibility that tobacco smoking confounds estimates throughout this literature, this is a key development in the field.

Compared with nonhabitual or never marijuana users, the pooled OR for habitual marijuana smokers was 0.96 (95% CI = 0.66–1.38). For those habitual smokers with 1–9.9 joint-years of marijuana usage, the pooled OR was 0.69 (95% CI = 0.41–1.17), and for those with ≥10 joint-years, the pooled OR was 0.94 (95% CI = 0.67–1.32). For marijuana users with 20 years or more duration of use, the pooled OR was 1.03 (95% CI = 0.54–1.98). In the subgroup of marijuana users who did not use tobacco, results were similar. Nonetheless, the researchers did find a small monotonic relation between joint-years of marijuana use and lung cancer, with more joint-years of marijuana use being associated with greater likelihood of lung cancer. It is important to note, however, that the small number of cases in the subgroup analyses with nontobacco-using marijuana smokers produced relatively imprecise estimates with large confidence intervals,

especially for estimates associated with higher exposure levels.

HEAD AND NECK CANCERS

Critical Analysis of Aldington et al. (2008b)

The association between marijuana use and head and neck cancers was examined in a case-control study with 75 incident cases and 319 controls, 55 years of age or younger (Aldington et al., 2008b). Cases were selected from hospital databases and the New Zealand Cancer Registry, and controls were randomly selected from the electoral roll. Both groups completed interviewer-administered questionnaires. Overall, increasing risk of head and neck cancers was associated with increasing alcohol and tobacco use, and decreasing income—but not associated with increasing marijuana consumption. It may have been that the duration of marijuana smoking in cases and controls was too short for long-term effects of marijuana on head and neck cancer to appear (the highest tertile of >8.3 joint-years of cumulative exposure only included 10 cases and 8 controls). Additionally, the small sample size in this study likely undermined statistical power to detect an effect, especially vis-à-vis site-specific head and neck cancers.

Critical Analysis of Gillison et al. (2008)

In light of the possibility that there may be two distinct pathways for the carcinogenesis of head and neck cancers—one facilitated by the mutagenic effects of tobacco and alcohol, and the other driven by HPV-mediated changes—Gillison et al. (2008) undertook two case-control studies, and assessed risk factors associated separately with HPV-positive (cases, $n = 92$; controls, $n = 184$) and HPV-negative (cases, $n = 148$; controls, $n = 296$) head and neck squamous cell carcinomas. The relation between marijuana-use indicators and HPV-16-positive head and neck cancers increased in strength with increasing marijuana-use intensity (joints per month), duration (in years), and cumulative joint-years of exposure, even after adjustment for potential confounders. Marijuana-use categories were not significantly related to HPV-16-negative head and neck cancers in the adjusted models. From these results, the authors suggest that cannabis use may promote the progression of HPV-16-positive head and neck cancers by increasing the risk of infection upon exposure, promoting persistence of an infection, and inhibiting antitumor immunity. Given that HPV-16-positive head and neck cancer cases reported much greater prevalence of sexual behavior and risky sexual behavior (one of the primary mechanisms for the acquisition of HPV-16 virus) and that marijuana use is related to riskier sexual behavior (Andrade, Carroll, & Petry, 2013), it is possible that the significant relations between marijuana-use categories and HPV-16-positive head and neck cancers may be due to residual confounding by sexual behavior.

Critical Analysis of Liang et al. (2009)

Liang et al. (2009) conducted a case-control study in Boston (434 cases, 547 controls), using cases drawn from cancer clinics and controls (matched on age, gender, and town of residence) from population-based registers. After adjustment for several potential confounders, the models demonstrated that current marijuana usage, increasing frequency of weekly marijuana use, and duration of marijuana smoking between 10 and 20 years were all significantly associated with reduced risk of head and neck cancers. The administration of the questionnaires to controls and cases differed, however, and this research-design difference may have affected the results. Cases were administered questionnaires at the initial clinic visit, and their responses were reviewed by study personnel at the clinic. Researchers mailed the questionnaires to controls, and if controls were more likely to report cannabis use because of this data-collection procedure, then this design factor might have led to the significantly reduced risk associated with marijuana use in the cases.

Critical Analysis of Berthiller et al. (2009)

Drawing upon a pooled analysis of five case-control studies from the United States, Cuba, and Argentina, INHANCE Consortium researchers assessed the potential relation between marijuana use and head and neck cancers, in a large group of cases and controls (cases, $n = 4085$; controls, $n = 5125$) (Berthiller et al., 2009). Given the large sample size, the investigators could undertake a critically important analysis for the field: an assessment of marijuana use in these cases of the head and neck cancers in "never" tobacco users (cases, $n = 353$; controls, $n = 1017$). The findings from these analyses showed no evidence of significant positive relations between lifetime marijuana use (ever versus never), daily frequency of marijuana use, duration of marijuana smoking, or cumulative joint-years of exposure. Low numbers of heavy and chronic marijuana users undermined the statistical power to detect even a moderate increased risk associated with these marijuana-use categories. Thus, as the researchers acknowledge, the study lacked the precision to rule out a moderately increased risk of head and neck cancers among marijuana users, especially in those with chronic or heavy usage.

Critical Analysis of Feng et al. (2009)

Employing a case-control study of participants (cases, $n = 636$; controls, $n = 615$) selected primarily from hospital-based settings in Algeria, Morocco, and Tunisia,

Feng et al. (2009) assessed the potential relation between marijuana use and the development of nasopharyngeal cancers. In the models based on male cases and controls (401 controls, 439 cases), and adjusted for age, socioeconomic status, dietary factors, and cigarettes smoked per day, the authors found no evidence that cannabis-use indicators, such as lifetime usage (never versus ever) or frequency of consumption, were significantly related to nasopharyngeal cancers. However, total frequency of lifetime cannabis consumption ≥2000 times was significantly related to increased risk of nasopharyngeal cancers (OR 2.62; 95% CI = 1.00–6.86).

Critical Analysis of Marks et al. (2014)

Recently, researchers from the INHANCE Consortium also conducted a pooled analysis of the possible relation between marijuana use and oropharyngeal cancers, as well as oral tongue cancers (Marks et al., 2014). The pooled analysis involved individuals from nine case-control studies in the United States and Latin America, including 2395 cases (2002 oropharyngeal and 393 oral tongue) and 7798 controls. Tobacco use and alcohol consumption are powerful risk factors for the development of head and neck cancers (Lubin et al., 2010), and a critical advantage of the current study was that it was able to stratify analyses according to these risk factors. The study found that, compared with never marijuana smokers, ever marijuana smokers manifested no evidence of increased risk of oropharyngeal cancer (OR = 0.99; 95% CI = 0.71–1.25), after adjusting for potential confounding variables, including HPV-16 status.

Among never tobacco smokers and never drinkers, there was some evidence of a trend between cumulative marijuana-use exposure (in joint-years) and oropharyngeal cancers—with greater cumulative exposure being more strongly related to oropharyngeal cancer. However, these analyses (shown in Table e9.3 in the Marks et al., 2014 paper) did not account for HPV-16 status—a critically important potential confounder of the outcome. Also, it is important to acknowledge that the stratified analyses including never tobacco smokers and never alcohol drinkers were likely compromised by low statistical power to detect a moderate effect, especially for higher levels of frequency, duration, and cumulative joint-year exposure categories.

In the Marks et al. (2014) paper, the researchers also assessed the possible link between marijuana use and tongue cancers. The adjusted-model results showed that the risk of oral tongue cancer was consistently reduced across the all levels of marijuana-use categories: "ever" (OR, 0.47; 95% CI = 0.29–0.75), higher frequency of usage (eg, >3 times per week: OR = 0.64; 95% CI = 0.23–0.95), duration of use (eg, >10 years: OR = 0.44; 95% CI = 0.21–0.94), and cumulative joint-years of exposure (>10 years: OR = 0.31; 95% CI = 0.11–0.89). The inverse relation between marijuana use and development of oral tongue cancer might be due to the antineoplastic effects of cannabinoids—active ingredients of marijuana (not to be confused with its psychoactive ingredient, tetrahydrocannabinol)—which appear to support antitumor activity (Cridge & Rosengren, 2013).

TESTICULAR GERM CELL TUMORS

Critical Analysis of Daling et al. (2009); Trabert, Sigurdson, Sweeney, Strom, and McGlynn (2011); Lacson et al. (2012)

Recently, researchers have assessed the relation between marijuana use and the development of testicular germ cell tumors (TGCT). This category of cancer is traditionally divided into two broad groups: pure seminoma (which usually accounts for 60% of incident cases), and nonseminoma. Daling et al. (2009) found that current marijuana use (versus "never" as referent), longer duration of cannabis use among current users (≥10 years), high frequency of current marijuana use (daily or ≥1 day per week), and an earlier age of first-use of marijuana (<18 years) were all significantly associated with *only* nonseminoma TGCT—but not seminoma cases. Similar to the Daling et al. (2009) paper, Trabert et al. (2011) found that higher marijuana-use frequency (>1 per day) and longer marijuana-use duration (≥10 years) were significantly associated with nonseminoma cancer, but not seminoma. In the most recent cannabis-testicular cancer study, Lacson et al. (2012) did not find evidence of any significant relations between marijuana use and seminoma, but they did show that marijuana use was significantly associated with nonseminoma/mixed germ cell tumors—a pattern similar to the prior two studies in the field (ever versus never marijuana use: OR = 2.42; 95% CI = 1.08–5.42). At this time, the field lacks a persuasive description of the biological plausibility of marijuana use as a mechanism specific to nonseminoma tumors.

MISCELLANEOUS: CRITICAL ANALYSIS OF CHACKO ET AL. (2006); BLUHM, DANIELS, POLLOCK, AND OLSHAN (2006); CHEN ET AL. (2008)

In our review, we also identified studies assessing the relation between marijuana use and transitional cell carcinoma, and mothers' marijuana use and cancer in their offspring, as well as a reanalysis of secondary data. Using a small, pilot case-control study (52 cases, 168 age-matched controls), Chacko et al. (2006) found that increasing

joint-years of marijuana use was significantly related, in a univariate analysis, to transitional cell carcinoma. However, even though the authors state that "multivariate adjustment" was undertaken in their supplementary logistic regression model (Chacko et al., 2006, p. 102), the important details of the modeling strategy were not provided and, as a result, it is not possible to evaluate this finding. In addition, Bluhm et al. (2006) found that "any use" of marijuana in the first trimester was significantly related to increased risk of neuroblastoma (OR = 4.75; 95% CI = 1.55–16.48). It is surprising, however, that this model was not adjusted for illicit drug use and, as a result, other kinds of illicit drug use (other than marijuana) may have confounded the estimate. Also, Chen et al. (2008) reanalyzed data from a prior study (Sidney, Quesenberry, Friedman, & Tekawa, 1997) and found that marijuana smokers were "at significantly lower risk of cancer relative to those who smoked tobacco and not marijuana" (Chen et al., 2008, p. 271). The Chen et al. (2008) study, however, used log-linear analyses—an extension of chi-square to multi-factorial contingency tables. The key limitation of the log-linear approach is that it cannot account for potentially confounding variables omitted from the contingency table—in this case, age and alcohol consumption.

CONCLUSIONS

In relation to our primary research question, the findings from the lung cancer area do not yield a definitive response. Of the seven eligible studies, five found that marijuana use significantly increased the risk of lung cancer, but the two best-designed studies—which stratified analyses by tobacco-use status—found no evidence of a relation between marijuana exposure and the outcome. The two critical limitations of these studies published between 2004 and 2014 are: the strong possibility of residual confounding by tobacco use, especially in the studies finding elevated risk associated with marijuana use; and the inclusion of only small numbers of individuals with heavy or chronic marijuana consumption (eg, >10 joint-years of usage). Prior systematic reviews of earlier literature (Hashibe et al., 2005; Mehra et al., 2006) also identified these issues as key limitations undermining clear interpretation of the results in this area. An important, recent advance in the field has been the pooling of estimates across case-control studies (eg, Zhang et al., 2015). This approach has allowed for the stratification of key marijuana-use analyses according to tobacco-use status, and it addresses the threat of residual confounding by tobacco use. Future work should include stratification by tobacco-use into the research design or, at least, match cases and controls according to tobacco consumption. Given the strong biological plausibility of an association between marijuana use and lung cancer (Mehra et al., 2006), the mixed results of the seven studies included in the current review, and the inconsistent findings in prior systematic reviews on this topic (Hashibe et al., 2005; Mehra et al., 2006), it seems reasonable to advise caution regarding lung cancer risk associated with marijuana use until further, more definitive evidence arises in the field.

In the last major review of epidemiological studies assessing the potential link between marijuana use and head and neck cancers, Hashibe et al. (2005) found inconsistent results in the field. In the 10-year interim since the publication of that review, the available literature continues to yield mixed results. Of the six studies reviewed, three showed no evidence of a significant relation between marijuana use and head and neck cancer (or subcategories of head and neck cancer) (Aldington et al., 2008b; Berthiller et al., 2009; Marks et al., 2014); two demonstrated a significantly reduced risk of head and neck cancer (Liang et al., 2009) or oral tongue cancer (Marks et al., 2014); and two studies found that marijuana use indicators were associated with significantly increased risk of head and neck cancers (Feng et al., 2009; Gillison et al., 2008). Given that two studies (Feng et al., 2009; Gillison et al., 2008) found that chronic, heavy marijuana use (ie, use ≥2000 times in lifetime; ≥5 joint-years of use) was significantly related to head and neck cancers, and that the critical limitation in this field is the lack of inclusion of individuals with substantial marijuana-use histories (eg, >10 joint-years of exposure), it is important to stress that a true relation between chronic, heavy marijuana use and head and neck cancer cannot be ruled out.

In light of the three available studies assessing marijuana use and the development of testicular germ cell cancers (Daling et al., 2009; Trabert et al., 2011; Lacson et al., 2012), it is possible to suggest some emerging themes, as well as limitations. All three studies have found an association between marijuana use and nonseminoma/mixed germ cell tumors, but not seminoma cancers. This relation may be true, but a number of caveats need to be considered. First, some of the analyses assessing the potential relation between marijuana use and seminoma lacked sufficient statistical power to detect a meaningful effect (primarily because of relatively small sample sizes of seminoma cases), especially in adjusted multivariate models with numerous covariates. In addition, the field has yet to provide a persuasive description of how marijuana use might specifically affect nonseminoma/mixed germ cell tumors, but not seminoma tumors. In the absence of a description of biological plausibility, readers must consider the strong possibility that the relation may be spurious and a result of confounding by factors associated with marijuana use, but not marijuana use per se.

Since the decade-ago publication of Hashibe et al. (2005), systematic review on the topic of marijuana use and cancer, the scientific field has progressed by producing 19 relevant papers. However, many of these studies have substantial limitations that undermine confident interpretation of their results. For the field to continue to advance, researchers need to make strong efforts to include the following: detailed marijuana exposure assessments; information about primary route of marijuana administration; strict account of tobacco smoking, the best being through stratification across tobacco-use history; sufficiently large samples of chronic or heavy marijuana users (eg, individuals with more than 10 joint-years of exposure); and, where possible, pooled analyses or meta-analytic approaches to identify risk patterns across combined samples or studies.

MINI-DICTIONARY

Germ cells In humans, germ cells are reproductive cells: egg cells in females, and sperm cells in males.
Germ cell tumors A mixed grouping of neoplasms usually occurring at the gonads: ovaries in females, or testes in males.
Gliomas A category of primary brain tumors originating in glial cells, common cells serving a supportive function to neurons.
In vitro Refers to an experiment taking place outside of a living organism in a laboratory or experimental environment—for example, in a test tube or culture dish.
In vivo Refers to an experiment or process taking place inside of a living organism.
Joint-years (JYs) A joint-year is equivalent to smoking 1 marijuana "joint" per day, every day, for an entire year.

References

Alberg, A. J., Ford, J. G., & Samet, J. M. American College of Chest Physicians. (2007). Epidemiology of lung cancer: ACCP evidence-based clinical practice guidelines (2nd edition). *Chest*, *132*(Suppl. 3), 29S–55S.

Aldington, S., Harwood, M., Cox, B., Weatherall, M., Beckert, L., Hansell, A., et al. (2008a). Cannabis use and risk of lung cancer: a case-control study. *European Respiratory Journal*, *31*(2), 280–286.

Aldington, S., Harwood, M., Cox, B., Weatherall, M., Beckert, L., Hansell, A., et al. (2008b). Cannabis use and cancer of the head and neck: case-control study. *Otolaryngology, Head and Neck Surgery*, *138*(3), 374–380.

Andrade, L. F., Carroll, K. M., & Petry, N. M. (2013). Marijuana use is associated with risky sexual behaviors in treatment-seeking poly-substance abusers. *American Journal of Drug and Alcohol Abuse*, *39*(4), 266–271.

Berg, C. J., Stratton, E., Schauer, G. L., Lewis, M., Wang, Y., Windle, M., et al. (2015). Perceived harm, addictiveness, and social acceptability of tobacco products and marijuana among young adults: marijuana, hookah, and electronic cigarettes win. *Substance Use and Misuse*, *50*(1), 79–89.

Berthiller, J., Straif, K., Boniol, M., Voirin, N., Benhaim-Luzon, V., Ayoub, W. B., et al. (2008). Cannabis smoking and risk of lung cancer in men: a pooled analysis of three studies in Maghreb. *Journal of Thoracic Oncology*, *3*(12), 1398–1403.

Berthiller, J., Lee, Y. C., Boffetta, P., Wei, Q., Sturgis, E. M., Greenland, S., et al. (2009). Marijuana smoking and the risk of head and neck cancer: pooled analysis in the INHANCE consortium. *Cancer Epidemiology, Biomarkers and Prevention*, *18*(5), 1544–1551.

Bluhm, E. C., Daniels, J., Pollock, B. H., & Olshan, A. F. Children's Oncology Group (United States). (2006). Maternal use of recreational drugs and neuroblastoma in offspring: a report from the children's oncology group (United States). *Cancer Causes and Control*, *17*(5), 663–669.

Callaghan, R. C., Allebeck, P., & Sidorchuk, A. (2013). Marijuana use and risk of lung cancer: a 40-year cohort study. *Cancer Causes and Control*, *24*(10), 1811–1820.

Centers for Disease Control and Prevention. (2013). *Smoking-attributable mortality, morbidity, and economic costs (SAMMEC)*. Atlanta, GA: CDCP, Available from https://apps.nccd.cdc.gov/sammec/index.asp.

Chacko, J. A., Heiner, J. G., Siu, W., Macy, M., & Terris, M. K. (2006). Association between marijuana use and transitional cell carcinoma. *Urology*, *67*(1), 100–104.

Chen, A. L., Chen, T. J., Braverman, E. R., Acuri, V., Kemer, M., Varshavskiy, M., et al. (2008). Hypothesizing that marijuana smokers are at a significantly lower risk of carcinogenicity relative to tobacco-non-marijuana smokers: evidenced based on statistical reevaluation of current literature. *Journal of Psychoactive Drugs*, *40*(3), 263–272.

Cridge, B. J., & Rosengren, R. J. (2013). Critical appraisal of the potential use of cannabinoids in cancer management. *Cancer Management and Research*, *5*, 301–313.

Daling, J. R., Doody, D. R., Sun, X., Trabert, B. L., Weiss, N. S., Chen, C., et al. (2009). Association of marijuana use and the incidence of testicular germ cell tumours. *Cancer*, *115*(6), 1215–1223.

Feng, B. J., Khyatti, M., Ben-Ayoub, W., Dahmoul, S., Ayad, M., Maachi, F., et al. (2009). Cannabis, tobacco and domestic fumes intake are associated with nasopharyngeal carcinoma in North Africa. *British Journal of Cancer*, *101*(7), 1207–1212.

Gillison, M. L., D'Souza, G., Westra, W., Sugar, E., Xiao, W., Begum, S., et al. (2008). Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. *Journal of the National Cancer Institute*, *100*(6), 407–420.

Han, B., Gfroerer, J. C., & Colliver, J. D. (2010). Associations between duration of illicit drug use and health conditions: results from the 2005–2007 national surveys on drug use and health. *Annals of Epidemiology*, *20*(4), 289–297.

Hashibe, M., Straif, K., Tashkin, D. P., Morgenstern, H., Greenland, S., & Zhang, Z. F. (2005). Epidemiologic review of marijuana use and cancer risk. *Alcohol*, *35*(3), 265–275.

Hashibe, M., Morgenstern, H., Cui, Y., Tashkin, D. P., Zhang, Z. F., Cozen, W., et al. (2006). Marijuana use and the risk of lung and upper aerodigestive tract cancers: results of a population-based case-control study. *Cancer Epidemiology, Biomarkers and Prevention*, *15*(10), 1829–1834.

Hayat, M. J., Howlader, N., Reichman, M. E., & Edwards, B. K. (2007). Cancer statistics, trends, and multiple primary cancer analyses from the surveillance, epidemiology, and end results (SEER) program. *Oncologist*, *12*(1), 20–37.

Howlader, N., Noone, A. M., Krapcho, M., Garshell, J., Miller, D., Altekruse, S. F., et al. (2014). *SEER cancer statistics review, 1975–2011*. Bethesda, MD: National Cancer Institute. Available from http://seer.cancer.gov/csr/1975_2011/

Lacson, J. C., Carroll, J. D., Tuazon, E., Castelao, E. J., Bernstein, L., & Cortessis, V. K. (2012). Population-based case-control study of recreational drug use and testis cancer risk confirms an association between marijuana use and nonseminoma risk. *Cancer*, *118*(21), 5374–5383.

Liang, C., McClean, M. D., Marsit, C., Christensen, B., Peters, E., Nelson, H. H., et al. (2009). A population-based case-control study of marijuana use and head and neck squamous cell carcinoma. *Cancer Prevention Research*, *2*(8), 759–768.

Lubin, J. H., Gaudet, M. M., Olshan, A. F., Kelsey, K., Boffetta, P., Brennan, P., et al. (2010). Body mass index, cigarette smoking, and alcohol consumption and cancers of the oral cavity, pharynx, and larynx: modeling odds ratios in pooled case-control data. *American Journal of Epidemiology*, *171*(12), 1250–1261.

Maertens, R. M., White, P. A., Rickert, W., Levasseur, G., Douglas, G. R., Bellier, P. V., et al. (2009). The genotoxicity of mainstream and sidestream marijuana and tobacco smoke condensates. *Chemical Research in Toxicology*, *22*(8), 1406–1414.

Marks, M. A., Chaturvedi, A. K., Kelsey, K., Straif, K., Berthiller, J., Schwartz, S. M., et al. (2014). Association of marijuana smoking with oropharyngeal and oral tongue cancers: pooled analysis from the INHANCE consortium. *Cancer Epidemiology, Biomarkers and Prevention*, *23*(1), 160–171.

Mehra, R., Moore, B. A., & Crothers, K. (2006). The association between marijuana smoking and lung cancer: a systematic review. *Archives of Internal Medicine*, *166*(13), 1359–1367.

National Institute on Drug Abuse (2014). DrugFacts: Marijuana. Available from: http://www.drugabuse.gov/publications/drugfacts/marijuana

Room, R., Fischer, B., Hall, W., Lenton, S., & Reuter, P. (2010). *Cannabis policy: moving beyond stalemate*. Oxford, UK: Oxford University Press.

Roth, M. D., Arora, A., Barsky, S. H., Kleerup, E. C., Simmons, M., & Tashkin, D. P. (1998). Airway inflammation in young marijuana and tobacco smokers. *American Journal of Respiratory and Critical Care Medicine*, *157*(3 Pt 1), 928–937.

Sasco, A. J., Merrill, R. M., Dari, I., Benhaim-Luzon, V., Carriot, F., Can, C. I., et al. (2002). A case–control study of lung cancer in Casablanca, Morocco. *Cancer Causes and Control*, *13*(7), 609–617.

Sidney, S., Quesenberry, C. P., Jr., Friedman, G. D., & Tekawa, I. S. (1997). Marijuana use and cancer incidence (California, United States). *Cancer Causes and Control*, *8*(5), 722–728.

Tashkin, D. P., Baldwin, G. C., Sarafian, T., Dubinett, S., & Roth, M. D. (2002). Respiratory and immunologic consequences of marijuana smoking. *Journal of Clinical Pharmacology*, *42*(11S), 71S–81S.

Trabert, B., Sigurdson, A. J., Sweeney, A. M., Strom, S. S., & McGlynn, K. A. (2011). Marijuana use and testicular germ cell tumors. *Cancer*, *117*(4), 848–853.

United Nations Office on Drugs and Crime. (2014). *World Drug Report*. New York, NY: United Nations.

Voirin, N., Berthiller, J., Benhain-Luzon, V., Boniol, M., Straif, K., & Ben Ayoub, W. (2006). Risk of lung cancer and past use of cannabis in Tunisia. *Journal of Thoracic Oncology*, *1*(6), 577–579.

Zhang, L. R., Morgenstern, H., Greenland, S., Chang, S. C., Lazarus, P., Teare, M. D., et al. (2015). Cannabis smoking and lung cancer risk: pooled analysis in the international lung cancer consortium. *International Journal of Cancer*, *136*(4), 894–903.

CHAPTER

e10

Cannabis and the Use of Amphetamine-Like Substances

A. Porcu, M.P. Castelli

Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, Cittadella Universitaria, Monserrato, CA, Italy

SUMMARY POINTS

- This chapter focuses on coabuse of cannabis and amphetamine-like substances (ALS).
- Cannabis (main psychoactive compound Δ^9-Tetrahydrocannabinol, Δ^9-THC) is consumed by 2.9–4.3% of the global population aged 16–64 in the world.
- Cannabis consumption is frequently associated with simultaneous use of other psychoactive compounds such as alcohol, cocaine, and ALS (ie, amphetamine, methamphetamine, and 3,4-methylenedioxymethamphetamine).
- New psychoactive substances (NPS), such as cathinones and its derivatives, are frequently coabused with cannabis.
- Cannabis + ASL coabuse enhances the subjective effects of the drugs in humans, counteracts the dysphoric symptoms of "coming down" from 3,4-methylenedioxymethamphetamine or methamphetamine (MDMA and METH), and/or improves and mellows the drug experience.
- Both pharmacological and genotype animal studies support the role of the cannabinoid type 1 receptor (CB1R) in the rewarding and addictive properties of MDMA.
- Preclinical and clinical studies investigating the effects of cannabis + ASL and their interaction in METH/MDMA-induced neurotoxicity have reported conflicting findings: cannabis might exacerbate or attenuate METH/MDMA induced neurotoxicity.

KEY FACTS ON THE MONOAMINERGIC TARGET SYSTEM

- Monoamines refer to the particular neurotransmitters dopamine, norepinephrine and serotonin.
- These neurotransmitters are involved in mediating a wide range of physiological and homeostatic functions such as cognition, memory, learning, mood, and behavior.
- These neurotransmitters are synthesized within particular neurons, and exert an effect when they are released into the synapses, where they are able to act on specific receptors.
- The dopamine transporter (DAT), serotonin transporter (SERT), and the norepinephrine transporter (NET) transport monoamines in or out of a cell.
- ALS may increase the activity of dopamine, norepinephrine, or serotonin by either increasing release, blocking reuptake, inhibiting metabolism, or acting directly on a receptor.

KEY FACTS ON NEW PSYCHOACTIVE SUBSTANCES (NPS)

- NPS are known in the drug market under the names of "designer drugs," "legal highs," "herbal highs," "bath salts," or "research chemicals."
- NPS have become a global phenomenon, and they have affected all regions of the world; 70 (out of 80) countries and territories (88%) examined by UNODC (2014) have reported the emergence of NPS.
- The number of NPS on the global market more than doubled over the period 2009–13. Legislation has not

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00066-1
Copyright © 2017 Elsevier Inc. All rights reserved.

been established, making their sale easy and officially legal in almost all countries.
- The most popular of these drugs are based on the substance cathinone (2-amino-1-phenylpropanone) and its synthetic analogs.
- The chemical structure of NPS is similar to that of amphetamine, as well as their physiological and psychological actions.

KEY FACTS ON POLYDRUG USE

- Research on polydrugs use is typically done on relatively small convenient samples (eg, street-based, emergency rooms, and club patrons), where multiple drugs are frequently used consecutively or simultaneously, often for their perceived counteracting or complementary effects.
- It is associated with a higher risk of developing psychiatric and health problems.

LIST OF ABBREVIATIONS

Δ^9-THC	Δ^9-Tetrahydrocannabinol
5HT	Serotonin
ADHD	Attention-deficit/hyperactivity disorder
AMPH	Amphetamine
ALS	Amphetamine-like substances
BDNF	Brain-derived neurotrophic factor
CB1-KO	Cannabinoid type 1 receptor knockout mice
CB1R	Cannabinoid type 1 receptor
CB2-KO	Cannabinoid type 2 receptors knockout mice
CB2R	Cannabinoid type 2 receptor
CPP	Conditioned place preference
DA	Dopamine
DAT	Dopamine transporter
DEA	Drug Enforcement Administration
dUTP	2′-deoxyuridine 5′-triphosphate
ECS	Endocannabinoid system
FDA	Food and Drug Administration
fMRI	Functional magnetic resonance technique
GFAP	Glial fibrillary acidic protein
MAO	Monoamine oxidase
MDMA	3,4-Methylenedioxymethamphetamine
METH	Methamphetamine
MJ	Marijuana
NE	Norepinephrine
NET	Norepinephrine transporter
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NPS	New psychoactive substances
pCREB	Phosphorylated cAMP response element–binding protein
PKA	Protein kinase A
PKC	Protein kinase C
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SERT	Serotonin transporter
TH	Tyrosine hydroxylase
TTAR1	Activate trace amine associated receptor 1
TUNEL	Terminal deoxynucleotidyl transferase
VMAT-2	Vesicular monoamine transporter-2

INTRODUCTION

Cannabis is the most widely consumed illicit drug worldwide, with an estimated global use by 2.9–4.3% of the population aged 16–64 (UNODC, 2010) (Fig. e10.1).

While the prevalence of illicit drugs such as cocaine, amphetamine-like substances (ALS) [ie, amphetamine (AMPH), methamphetamine (METH), and 3,4-methylenedioxymethamphetamine (MDMA)] appeared to remain stable between 2009 and 2011, since 2009 the extent of cannabis and opioids use has increased. Cannabis consumption is frequently associated with simultaneous and/or concurrent use of other psychoactive compounds such as alcohol, cocaine, and ALS (Gouzoulis-Mayfrank & Daumann, 2006).

A growing body of clinical and preclinical studies has highlighted the simultaneous use of MDMA, also called ecstasy, and cannabis (Parrott, Milani, Gouzoulis-Mayfrank, & Daumann, 2007). Strote, Lee, & Wechsler (2002), surveying a sample of over 10,000 US college students, reported a prevalence of ecstasy use of 4.7%, with 92% of consumers also consuming cannabis. Wish, Fitzelle, O'Grady, & Hsu (2006) confirmed that 98% of recreational MDMA users in a sample of East Coast College students also consume cannabis. Moreover, the presence of a metabolite of the main psychoactive compound Δ^9-tetrahydrocannabinol (Δ^9-THC), 11-nor-9 carboxy-THC, was found in urine samples collected from 198 ecstasy users between 2005 and 2008 (Black, Cawthon, Robert, & Moser, 2009).

In Europe, the prevalence of couse of MDMA + cannabis ranges from 73% to 100% in different countries (Sala & Braida, 2005), and is higher in males and in regular cannabis users than in women and occasional

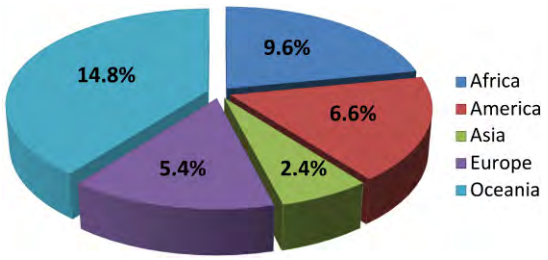


FIGURE e10.1 Estimated number of people who used cannabis at least once in the past year, and its prevalence among the population aged 15–64, by region. *Source: UNODC (2010).*

cannabis consumers (ESPAD, 2000). Due to its low cost, wide availability, and high potential for abuse, METH is the second most frequently abused recreational drug among adolescents, and is often coabused with cannabis (Gonzalez, Rippeth, Carey, & Heaton, 2004).

Several psychosocial and functional reasons can account for the high percentage of concurrent use of Δ^9-THC and METH or MDMA. Cannabis is often considered a "gateway drug," and its consumption might predict a significantly higher risk for subsequent use of heavy illicit drugs such as ALS. However, numerous reports have failed to support this theory, while other studies point to social, environmental, and genetic factors to explain the couse of cannabis and other drugs (Parrott et al., 2007).

MDMA and METH are psychostimulant drugs that induce euphoria, increased sociability, and empathy (Parrott, 2013), while cannabis produces relaxation and feelings of happiness (Green, Kavanagh, & Young, 2003). One of the main reasons for using cannabis in association with MDMA or METH is that the combination of the two drugs enhances the subjective drug effects, and counteracts the dysphoric symptoms of the ecstasy or METH "coming down" (Schulz, 2011). Indeed, MDMA abusers often consume cannabis in the immediate post-ecstasy period as a symptomatic relief against the negative physiological and emotional states (eg, anhedonia, depression) that follow ecstasy's "high." Many ecstasy users also report taking cannabis in the initial acute phase of MDMA to "improve" and "mellow" the experience (Parrott et al., 2007).

In the last few years, many recreational drugs have been synthesized as legal alternatives to scheduled cannabis and AMPH/METH stimulants. New psychoactive substances (NPS) have been found mostly in Europe and North America, but also in Oceania, South America, and Africa. Because they are new, legislation has not been established yet, making their sale easy and officially legal. Their global market has increased dramatically in recent years: by December 2013, the number of NPS reported to UNODC had reached 348, up from 251 as of July 2012, and 166 in 2009 (UNODC, 2014).

The most popular of these drugs are based on the substance cathinone (2-amino-1-phenylpropanone), an analogue of amphetamine, a natural compound of the khat plant (Paillet-Loilier, Cesbron, Le Boisselier, Bourgine, & Debruyne, 2014), which acts on the central dopamine system. Dopaminergic stimulation of the reward system could explain the development of dependence after frequent consumption of cathinone derivatives.

Due to the recent discovery of the NPS, only a few survey studies are currently available on their simultaneous use with cannabis, but no preclinical or clinical studies. On the other hand, despite the high prevalence of cannabis use among MDMA and METH users, the neurobiological interactions between these two drugs are complex, and data regarding their coadministration in human and animal studies are contradictory (Mohamed, Ben Hamida, Cassel, & de Vasconcelos, 2011; Schulz, 2011).

Δ^9-THC and other cannabis derivatives act via cannabinoid type 1 receptors (CB1R) on the endocannabinoid system (ECS), while the ALS, including MDMA, METH and the cathinones, act by increasing synaptic levels of the monoamines dopamine (DA), serotonin (5HT), and norepinephrine (NE).

For a detailed description of the ECS and the mechanism of action of Δ^9-THC and other cannabinoids, see designated Chapters of this book.

In the following paragraphs, we will briefly describe the ALS, including cathinones, their molecular pharmacology, and the pharmacological/neurobiological effects induced by ALS + cannabis.

AMPHETAMINE-LIKE SUBSTANCES: AMPH, METH, MDMA, AND CATHINONES

Amphetamine (AMPH) is the parent compound of a class of molecules with similar chemical structures and biological properties, referred to as amphetamines (Fig. e10.2) (Fleckenstein, Volz, Riddle, & Gibb, 2007).

In 1935, AMPH was introduced to the market by the pharmaceutical company Smith, Kline and French, with the brand name of "Benzedrine." for the treatment of narcolepsy, mild depression, postencephalitic Parkinsonism, and other disorders. Since 1971, AMPH and METH, a substituted amphetamine, due to their addictive potential, have been inserted in Schedule II of the Controlled Substances Act, and are Food and Drug Administration (FDA)-approved for treatment of attention-deficit/hyperactivity disorder (ADHD), narcolepsy, and obesity (DEA Congressional Testimony by Terrance Woodworth; 2000, May 10).

METH is one of the most common ALS; also known as "crystal," "chalk," or "ice," it is taken orally, smoked,

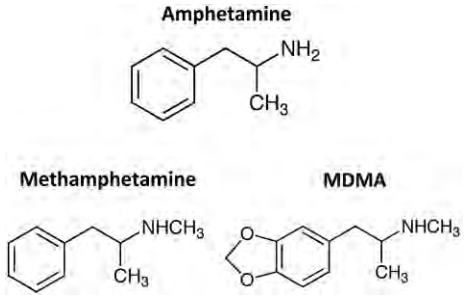


FIGURE e10.2 Chemical structures of amphetamines. Amphetamine, methamphetamine, and MDMA are the amphetamines most frequently coabused with cannabis.

snorted, or dissolved in water or alcohol, and injected. The annual prevalence of METH in the United States was 0.5% of the population aged 15–64 in 2012, similar to that estimated between 2009 and 2011 (SAMHSA, 2013).

MDMA, developed and patented in 1918 by Merck for use in synthesis of styptic drugs, was used in the United States by psychotherapists from 1978 to 1985, when, due to its high abuse potential, the DEA (Drug Enforcement Administration) included it in Schedule I. After cannabis, the second most widely used illegal drugs worldwide are ALS, particularly MDMA (Parrott, 2013). Also known as "ecstasy," "Molly," "lover's speed," or "the love drug," MDMA is used by 10.5–25.8 million people worldwide, corresponding to 0.2–0.6% of the adult population (16–64 years) (UNODC, 2010). MDMA is mostly consumed at late-night parties ("raves," "dance clubs," "techno parties"), where the percentage of users can be up to 95%. The 2009 National Survey on Drug Use and Health reported that the percent consuming MDMA was 1.4% among adolescents (12–17 years old), 3.9% among young adults (18–25 years old) and 0.3% for adults (older than 26 years) (NSDUH, 2010).

Among the NPS, the most abused are derivatives of cathinone, which is a β-cheto analogue of AMPH used as a structural template for the discovery of compounds with a wide range of pharmacological activities (Carroll, Blough, Mascarella, & Navarro, 2010).

NPS are usually marketed as tablets of various colors and shapes, or capsules, powders, and crystals, and sold as "bath salts" or "plant fertilizers." In recent years, several synthetic analogs of cathinone have emerged on the market, and circulate on the Internet or are sold in smart shops with the misleading indication that they are "not for human consumption" (Fig. e10.3).

The general structure of these substituted compounds shows substitution patterns at four locations on the cathinone molecule, for example, on the carbon atom linked to the carbon in the alpha position (R1), on the nitrogen atom (R2 and R3), and on the phenyl group (R4) (Fig. e10.4).

FIGURE e10.3 **Representative packaging of NPS products.** The indication that this substance is "not for human consumption" makes it automatically legal.

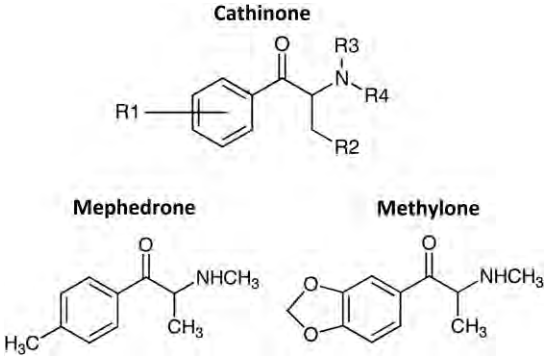


FIGURE e10.4 **Chemical structures of cathinones.** R1, R2, R3, R4 are the various groups that can be substituted to obtain other synthetic derivatives.

ALS Pharmacology

The psychostimulant and rewarding properties of AMPH, METH, and MDMA are due to their ability to enhance neuronal excitability in the brain and, in particular, to increase dopaminergic neuronal activity in the mesolimbic pathway.

Indeed, ALS are historically considered potent indirect monoaminergic agonists, raising the synaptic levels of DA, NE, and, to a lesser extent, 5HT through different mechanisms such as inhibition of: (1) the transporters of DA (DAT), NE (NET), and 5HT (SERT); (2) the vesicular monoamine transporter-2 (VMAT-2); and (3) monoamine oxidase (MAO) activity. Specifically, although with different affinities, AMPH and its derivatives are substrates of plasmalemma DAT, NET, and SERT, with METH and AMPH having greater affinity for DAT, and MDMA having greater affinity for SERT (Sulzer, Sonders, Poulsen, & Galli, 2005).

According to the "exchange-diffusion model," the lipophilic AMPH, METH, and MDMA at high concentrations can diffuse directly across the plasmalemma membrane, while at low concentrations they are transported into the cell by competing with synaptic DA or 5HT at the extracellular site of DAT or SERT. Then, AMPH can disrupt the proton gradient between the inside and outside of the storage vesicle, resulting in an increased release of DA or 5HT from vesicular compartments. At physiological concentrations, AMPH binds to VMAT2, thus inhibiting vesicular monoamine uptake and enhancing cytoplasmic DA levels. As cytoplasmic DA levels rise, DA exits the neuron via reverse transport and/or channel like transport (Fleckenstein et al., 2007).

AMPH can also interact indirectly with DAT. Once entering the neuron, it can bind to and activate trace amine associated receptor 1 (TTAR1), a G-protein

coupled receptor that is important for the regulation of monoamine transporter function and METH's action on DAT activity. In particular, AMPH-induced TTAR1 activation via protein kinase A (PKA) and protein kinase C (PKC) signaling determines DAT phosphorylation and internalization, thereby decreasing DAT DA uptake. METH and MDMA also increase glutamate release in the striatum and hippocampus, respectively (Panenka, Procyshyn, Lecomte, & MacEwan, 2013).

Besides its acute actions, moderate to high doses of ALS administered in a "binge pattern" (repeated administrations at short time intervals) induce long-term effects such as dopaminergic and serotoninergic deficits, including reductions in expression of the enzymes tyrosine and tryptophan hydroxylase and DAT and SERT density, as well as decreases in DA and 5HT tissue content (Marshall & O'Dell, 2012). In addition to damage to DA and 5HT terminals, METH and MDMA cause activation of microglia, and a significant increase in glial fibrillary acidic protein (GFAP), an index of gliosis and central nervous system (CNS) toxicity and cell death. METH increases terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), a marker of apoptotic cell death and DNA damage, and alters expression of the Bcl2 gene and causes apoptosis by increasing caspase-3 activity, and the Fas/Fasl cell death pathways. MDMA exposure in vitro causes an increase in reactive nitrogen species (RNS) and caspase-3 and, in vivo, an enhancement in protein levels associated with apoptosis (eg, BAX, cytochrome c, caspase-3) (Capela, Carmo, Remião, & Bastos, 2009, Krasnova & Cadet, 2009).

Moreover, the increased extracellular glutamate levels induced by METH-binging activate N-methyl-D-aspartate (NMDA) receptors, promoting an increase in intracellular Ca^{2+} that leads to activation of neuronal nitric oxide synthase (nNOS) and subsequent production of nitric oxide (NO), and activation of apoptotic pathways (Halpin, Collins, & Yamamoto, 2014).

Increasing evidence now suggests the involvement of several interacting mechanisms underlying ALS-induced neurotoxicity, such as hyperthermia, increased production of reactive oxygen (ROS) and RNS, induction of inflammatory responses, apoptotic pathways, DNA damage, and excitotoxic injury. Indeed, ALS-neurotoxicity is associated with long-lasting impairments in mood and cognitive functions in humans and animals (Marshall & O'Dell, 2012).

As concerns synthetic cathinones, data concerning their pharmacological and toxicological properties, mechanisms of action and toxic effects are still inconclusive.

Just as AMPH and cocaine, cathinones in the CNS increase synaptic concentrations of catecholamines by inhibiting their uptake transporters. The meth-cathinone analogs mephedrone and methylone function as substrates for NET, DAT, and SERT at nanomolar concentrations, but are nonselective substrates for monoamine transporters in vitro, exhibiting a potency and selectivity comparable to those of MDMA. Mephedrone and methylone produce concurrent elevations in extracellular DA and 5HT in vivo, and high-dose administration produces hyperthermia and motor stimulation, but no lasting changes in brain tissue monoamines (Baumann, Ayestas, Partilla, & Sink, 2012).

ALS + CANNABIS

Animal Studies

Pharmacological and genotype studies in animals support the existence of interactions between cannabinoid and MDMA/METH related to their rewarding effects, and the role played by CB1Rs in these effects (Sala & Braida, 2005). The reinforcing properties elicited by the simultaneous use of MDMA and Δ^9-THC have been investigated by means of several animal paradigms, such as conditioned place preference (CPP) and intracranial and intravenous self-administration. In particular, in rats trained to self-administer MDMA intracerebroventricularly, the CB1R agonist CP55940 reduced the amount of lever-pressing to obtain MDMA, while the CB1R antagonist SR141617A (SR) increased MDMA-associated lever-pressing, suggesting an increase and a decrease in MDMA's reinforcing effects, respectively. SR decreases MDMA-induced CPP in rats, further supporting the role of the CB1R in reward processes (Sala & Braida, 2005). Accordingly, CB1-KO mice fail to self-administer MDMA, while MDMA induces CPP and enhances extracellular DA levels in the nucleus accumbens of both wild type and CB1-KO mice (Touriño et al., 2008). This discrepancy might be attributed to the different animal species (rats in the pharmacological studies, and mice in the genetic studies), or to the different behavioral paradigms utilized (self-administration vs CPP). Indeed, several brain circuits are involved in the self-administration paradigm, such as those regulating motivation, consciousness, arousal, and learning, while CPP is mostly based on conditioning processes. Intriguingly, subthreshold doses of Δ^9-THC coadministered with a subthreshold dose of MDMA produced CPP in mice, while administration of the same dose of Δ^9-THC with a rewarding dose of MDMA decreased CPP (Robledo et al., 2007). At low doses, the CB1R agonist WIN55212-2 (WIN) increased the rewarding properties of low doses of MDMA, while MDMA reinstated WIN-induced CPP, although WIN was not able to reinstate MDMA-induced CPP in animals with no prior exposure to cannabinoids (Manzanedo, Rodríguez-Arias, Daza-Losada, & Maldonado, 2010).

Accumulating evidence supports the role of the ECS in a crucial phase of the addiction cycle, that is, relapse to drug-seeking after a period of abstinence. Animal studies have shown that a priming with CB1R agonists reinstates not only the extinguished cannabinoid-seeking, but also cocaine-, heroin-, and alcohol-seeking, while reinstatement of cannabinoid-, heroin-, nicotine-, and methamphetamine-seeking is prevented by blockade of CB1Rs (Fattore, Fadda, & Fratta, 2007). Notably, a recent study reported that SR, either alone or coadministered with a priming dose of any of the MDMA, reinstated MDMA-induced CPP. Conversely, WIN had no effect on CPP reinstatement, but potentiated the effects of a subthreshold priming dose of MDMA (Daza-Losada, Miñarro, Aguilar, & Valverde, 2011). Collectively, all these findings supported interactions between the ECS and MDMA regulating either drug-taking or drug-seeking behaviors.

The rewarding effects of Δ^9-THC and MDMA/METH are likely mediated through a common neurochemical system, that is, the mesocorticolimbic pathway. In fact, MDMA raises DA levels by different mechanisms, while cannabinoids influence the synthesis, release, and turnover of DA. In addition, cannabinoid and dopamine receptor expression overlaps in some brain areas, including the nucleus accumbens. Finally, the ECS modulates DAergic transmission by controlling excitatory and inhibitory inputs on dopaminergic neurons. Both cannabinoids and MDMA also modify the glutamatergic system in areas involved in reward and addiction (Maldonado, Valverde, & Berrendero, 2006).

A plethora of data obtained from animal models has shown that administration of high or repeated doses of MDMA and METH results in neurotoxicity to the serotoninergic or dopaminergic system. MDMA neurotoxicity is species-dependent, as in rats and monkeys MDMA mostly affects the serotoninergic pathway, while in mice it targets the dopaminergic one. As revealed by biochemical, histological and immunohistochemical analyses (see the Section "ALS Pharmacology"), administration of high or repeated doses of MDMA or METH causes long-lasting depletion of 5HT and DA in several brain regions.

In association with these neurotoxic effects on the DAergic and 5HTergic systems, binge METH regimens also cause cognitive and behavioral alterations similar to those observed in METH abusers (Marshall, Belcher, Feinstein, & O'Dell, 2007). Recently, our group demonstrated long-term memory deficits and startle responsiveness (index of pathological anxiety) in adult rats, as well as loss of DAergic and 5HTergic brain terminals, following a METH binge neurotoxic regimen (Bortolato, Frau, Piras, & Luesu, 2009). Moreover, the same neurotoxic METH treatment upregulated CB1R expression in brain regions that play an important role in the regulation of emotional and cognitive responses, including the medial prefrontal cortex, striatum, basolateral amygdala, and hippocampal formation (Bortolato, Frau, Bini, & Luesu, 2010).

MDMA-induced neurotoxicity is associated with functional deficits, such as the loss of the ability to thermoregulate, and alterations in locomotor activity in rats (Capela et al., 2009).

The ECS exerts either protective or toxic properties in several in vitro and in vivo models of neuronal injury and neurodegenerative pathologies, including glutamate excitotoxicity, hypoxia, ischemic stroke, brain trauma, oxidative stress, Alzheimer's disease, Parkinson's disease, and Huntington's disease (Fernández-Ruiz, García, Sagredo, & Gómez-Ruiz, 2010).

Given the frequent coabuse of Δ^9-THC and MDMA in humans (see Section "Introduction"), their interaction in MDMA-induced neurotoxicity has also been investigated in terms of neurotoxicity.

Coadministration of Δ^9-THC and CP55940 prevented MDMA-induced hyperthermia, hyperlocomotion, and long-term increase in anxiety measured in the emergence test, but not in the social interaction test in rats (Morley, Li, Hunt, & Mallet, 2004). Accordingly, pretreatment with 3 mg/kg of Δ^9-THC, considered a dose equivalent to that consumed by moderate cannabis users, prevented MDMA-induced hyperthermia in mice receiving a neurotoxic regimen of MDMA (Touriño et al., 2010). This hyperthermic effect was blocked by the CB1R antagonist AM251 and absent in CB1-KO mice, while neither a CB2R antagonist nor deletion of the CB2R gene abolished Δ^9-THC's preventive effect on hyperthermia. Moreover, MDMA-induced microglial and astrocyte activation was abolished by Δ^9-THC in wild-type mice, and partially attenuated in CB2-KO mice, while Δ^9-THC had no effect in CB1-KO and double CB1/CB2-KO mice. Recently, it was demonstrated that an ultra-low dose of Δ^9-THC protects mice from various insults, including ecstasy-induced neurotoxicity and cognitive deficits. This protective effect was long-lasting, and accompanied by increased levels of pCREB (phosphorylated cAMP response element-binding protein) and BDNF (brain-derived neurotrophic factor) in some brain areas (Fishbein, Gov, Assaf, & Gafni, 2012). Altogether, these findings suggest that Δ^9-THC protects an animal from MDMA-neurotoxicity by lowering body temperature and neuroinflammation through both CB1 and CB2Rs.

Chronic administration of Δ^9-THC during adolescence attenuated MDMA-induced hyperthermia, and blunted some behavioral MDMA-induced effects (Shen, Ali, & Meyer, 2011). Additionally, Δ^9-THC prevented MDMA-induced decreases in exploratory behavior, and reductions in 5HT and SERT levels in the striatum, frontal, and parietal cortex, suggesting that chronic coadministration during adolescence might provide some protection

against MDMA-neurotoxicity. Indeed, chronic treatment with Δ^9-THC and MDMA during adolescence produced sex-dependent long-lasting molecular, behavioral, and endocrine-effects, along with modifications of astrocytes, microglia reactive markers, CB1R, and SERT expression. Specifically, coadministration of Δ^9-THC and MDMA impaired working memory in female rats, and attentional capabilities in both sexes, more severely than when administered alone. Moreover, both drugs—either separately or in combination—induced increases in reactive microglia cells, suggesting that chronic administration of these drugs might account for persistent modifications of microglia reactivity.

As regards the METH + cannabis association, only two groups, including ours, have investigated the ability of the ECS to prevent METH-induced neurotoxicity in rats and mice (Castelli et al., 2014; Nader, Rapino, Gennequin, & Chavant, 2014). Specifically, pre- and posttreatment with Δ^9-THC reduced overexpression of nNOS and astrogliosis in the caudate-putamen and prefrontal cortex, respectively, in rats exposed to a neurotoxic regimen of METH. SR reversed the neuroprotective effects of Δ^9-THC on METH-induced nNOS expression, while it failed to counteract the effect of Δ^9-THC on decreasing METH-induced astrogliosis. Our findings indicate that Δ^9-THC prevents METH-induced neurotoxicity through inhibition of nNOS and astrogliosis via both CB1 receptor-dependent and -independent mechanisms (Castelli et al., 2014). According to this, it has been reported that Δ^9-THC, URB597, and JZL184 (inhibitors of degradation of anandamide and 2-arachidonoyl glycerol, respectively) attenuated the decrease in TH levels in the striatum, demonstrating that, in mice, stimulation of the ECS reduced METH neurotoxicity to dopaminergic terminals (Nader et al., 2014).

As concerns cathinone derivatives, no animal studies are currently available on the concomitant use of cannabis.

Human Studies

In contrast to basic research, human studies investigating acute and long-term effects of the consumption of cannabis + MDMA are limited and often contradictory. When consumed acutely, cannabis can enhance MDMA-induced subjective effects and counteract adverse MDMA effects, such as fatigue, insomnia, reduced appetite, irritability, panic attacks, visual hallucinations, and paranoid delusions that appear when "coming off" MDMA. As previously demonstrated in animals (Morley et al., 2004), cannabis can also acutely attenuate body temperature in dance club recreational ecstasy users (Parrott et al., 2007).

Both drugs taken alone may have detrimental effects on memory and cognition (Parrott, 2013; Solowij, Stephens, Roffman, & Babor, 2002), and when taken together can determine neurocognitive deficits in several domains. Noteworthy, while in animal studies MDMA increases oxidative stress, cannabinoids have antioxidative and antiinflammatory properties, supporting the idea that cannabinoids might also have neuroprotective effects against MDMA-neurotoxicity in humans.

The literature on the long-term effects of each substance and/or their combination on cognitive functions, memory, mood, and impulsivity is inconsistent. Indeed, several reports demonstrated that deficits in memory, learning, or verbal fluency, as well as self-reported psychopathological problems such as depression, anxiety, paranoid ideation, and obsessive-compulsive behavior are mainly associated with cannabis use, rather than with ecstasy (Gouzoulis-Mayfrank & Daumann, 2006).

However, other studies revealed a clear relationship between neuropsychobiological deficits and MDMA use, rather than cannabis use, that is, memory/cognitive deficits were related to ecstasy, but not to cannabis use. Indeed, higher self-rated psychiatric symptoms were reported in both light and heavy ecstasy polydrug users than in the control group that included cannabis users (Parrott, 2006). Several factors might explain these conflicting findings, including the relative use of cannabis and ecstasy, the amount of each drug used (heavy versus light users), and the psychobiological function examined. MDMA and cannabis may also influence different cognitive aspects, that is, cannabis use has been associated with everyday memory problems, while MDMA use accentuates prospective memory problems (Parrott, 2006).

As regards the potential neuroprotective effect of cannabis on ecstasy users, cannabis + MDMA users were found to have fewer psychobiological problems (ie, lower rates of anger, hostility, depression, and negative symptoms) than noncannabis users (Gouzoulis-Mayfrank & Daumann, 2006). Moreover, altered brain activation and poor working performance were detected in MDMA users, and the concomitant use of cannabis was suggested to improve the MDMA-induced alterations (Parrott, 2006). Notably, coadministration of these two drugs did not worsen cognitive impairments induced by either drug (Dumont, van Hasselt, de Kam, & Van Gerven, 2011).

Despite the wide consumption of METH + cannabis, only a few clinical studies have investigated the interactions between these two drugs, and their acute and long-lasting effects.

A positron emission tomography study showed that the combined use of these two drugs caused reduced regional cerebral glucose metabolism in the frontal, temporal, and striatal brain areas of METH + marijuana (MJ) users during completion of a continuous performance task, in comparison to individuals that abused METH

alone, but no differences were observed in continuous performance task accuracy between the two groups (Gouzoulis-MayFrank & Daumann, 2006). Accordingly, cognitive functioning in several ability areas (ie, verbal fluency, abstraction/executive functioning, attention/working memory, learning, and delayed recall/retention) in METH + MJ were found not to differ significantly from the control group, or METH users without a history of MJ dependence (Gonzalez et al., 2004). Recently, decreased frontal N-acetylaspartyl glutamate levels, an indicator of intact neuronal integrity, were found in adolescents using METH + MJ, compared to healthy controls and METH groups, supporting the idea that concomitant heavy cannabis and METH use may induce neurotoxicity in the adolescent brain (Sung, Carey, Stein, & Ferrett, 2013).

Further clinical and preclinical studies are needed to clarify if concurrent use of cannabis and MDMA/METH worsens or improves neurocognitive functions.

Finally, a paucity of clinical data are available on the effects of cathinone derivatives given alone or in combination with cannabis. In humans, cathinones induce primarily stimulant effects such as euphoria, elevated mood, mental stimulation, intensification of sensory sensations, increased energy and sociability, empathy connection, and decreased inhibition (Prosser & Nelson, 2012). However, high doses of cathinones lead to hallucinations, paranoia, agitation, self-mutilation, psychosis, tachycardia, increased blood pressure, and hemorrhage.

Chronic khat use is associated with impaired inhibitory control, and long-term use of cathinone is associated with dysfunctions in the prefrontal cortex, and reduced DA levels in the striatum (Colzato, Ruiz, van den Wildenberg, & Bajo, 2011). Even before clubbing (and after recent drug use), mephedrone users performed worse than nonusers on cognitive tasks and, contrary to controls, showed decreased performance in verbal learning and fluency (Herzig, Brooks, & Mohr, 2013). Yet, when subjects were analyzed for their use of other drugs or preexisting factors, it turned out that prior polydrug use (cannabis + alcohol in particular, but also AMPH) and related psychological traits (depression) were likely to affect cognitive functioning in the sample studied.

The presence of some of these substances was detected through surveys/questionnaires, studies on drug samples and biological fluids, and case reports. In one instance, a driver stopped by officers of the Massachusetts State Police and subjected to blood sample analysis revealed the presence of clonazepam, 7-aminoclonazepam, carboxy-THC, diphenhydramine, and MDMA (Elian & Hackett, 2014). The analysis of fluid body samples obtained from suspected NPS intoxication cases detected synthetic cannabinoids of the JHW series and cathinones (methylone, butylone, mephedrone, flephedrone, and methcathinone), often present in combinations of two or more drugs (Papaseit, Farrè, Schifano, & Torrens, 2014). Most worrisome, the recent trend seems to be to mix different types of designer drugs like cathinones (stimulants) or tryptamines (hallucinogens) with synthetic cannabinoids.

MINI-DICTIONARY

Amphetamine-like substances Although there are a variety of amphetamines, in this chapter, amphetamine-like substances (ALS) stands for amphetamine (AMPH), methamphetamine (METH), 3,4-methylenedioxymethamphetamine (MDMA), and cathinones. The first three compounds are frequently coabused with cannabis, while cathinones are the most popular new psychoactive substances (NPS).

Chatinone 2-amino-1-phenylpropanone, a natural compound of the khat plant.

Drug addiction A chronic, relapsing brain disease that causes compulsive drug-seeking and use, despite harmful consequences to the drug addict and those around them.

Dopamine A monoamine neurotransmitter. In particular, the mesolimbic dopamine system that projects from the ventral tegmental area to the nucleus accumbens has been implicated in the rewarding effects of drugs of abuse.

Ecstasy or MDMA This drug is a phenylethylamine, a molecule that belongs to the group of synthetic derivatives of methamphetamine. It is an illegal drug, synthesized for the first time in 1912, with a prevailing action on the serotonergic system.

NPS A new psychoactive substance is defined as a new narcotic or psychotropic drug, in pure form or in preparation.

Polydrug use A common phenomenon defined as the consumption of more than one drug during a specific time period.

Reward system The brain circuit that controls an individual's response to natural rewarding stimuli, such as food, sex, and social interaction.

References

Baumann, M. H., Ayestas, M. A., Jr., Partilla, J. S., Sink, J. R., et al. (2012). The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. *Neuropsychopharmacology*, *37*, 1192–1203.

Black, D. L., Cawthon, B., Robert, T., Moser, F., et al. (2009). Multiple drug ingestion by ecstasy abusers in the United States. *Journal of Analytical Toxicology*, *33*, 143–147.

Bortolato, M., Frau, R., Piras, A. P., Luesu, W., et al. (2009). Methamphetamine induces long-term alterations in reactivity to environmental stimuli: correlation with dopaminergic and serotonergic toxicity. *Neurotoxicity Research*, *15*, 232–245.

Bortolato, M., Frau, R., Bini, V., Luesu, W., et al. (2010). Methamphetamine neurotoxicity increases brain expression and alters behavioral functions of CB_1 cannabinoid receptors. *Journal of Psychiatric Research*, *44*, 944–955.

Capela, J. P., Carmo, H., Remião, F., Bastos, M. L., et al. (2009). Molecular and cellular mechanisms of ecstasy-induced neurotoxicity: an overview. *Molecular Neurobiology*, *39*, 210–271.

Carroll, F. I., Blough, B. E., Mascarella, S. W., Navarro, H. A., et al. (2010). Synthesis and biological evaluation of bupropion analogues as potential pharmacotherapies for smoking cessation. *Journal of Medicinal Chemistry*, *53*, 2204–2214.

Castelli, M.P., Madeddu, C., Casti, A., Casu, A., et al. (2014) Δ9-tetrahydrocannabinol prevents methamphetamine-induced neurotoxicity. *PLoS One*. 20, 9(5):e98079.

Colzato, S. L., Ruiz, M. J., van den Wildenberg, W. P. M., Bajo, M. T., et al. (2011). Long-term effects of chronic khat use: impaired inhibitory control. *Frontiers in Psychology*, *219*, 1–5.

Daza-Losada, M., Miñarro, J., Aguilar, M. A., Valverde, O., et al. (2011). Acute blockade of CB1 receptor leads to reinstatement of MDMA-induced conditioned place preference. *Pharmacology Biochemistry and Behavior*, *100*, 33–39.

Dumont, G. J., van Hasselt, J. G., de Kam, M., Van Gerven, J. M., et al. (2011). Acute psychomotor, memory and subjective effects of MDMA and THC co-administration over time in healthy volunteers. *Journal of Psychopharmacology*, *25*, 478–489.

Elian, A. A., & Hackett, J. (2014). A polydrug intoxication involving methoxetamine in a drugs and driving case. *Journal of Forensic Sciences*, *59*, 854–858.

ESPAD (2000). The 1999 alcohol and other drug use among students in 30 Eruropean countries, Swedish council for information on alcohol and other drugs (CAN). The Pompidou group of the Council of Europe.

Fattore, L., Fadda, P., & Fratta, W. (2007). Endocannabinoid regulation of relapse mechanisms. *Pharmacological Research*, *5*, 418–427.

Fernández-Ruiz, J., García, C., Sagredo, O., Gómez-Ruiz, M., et al. (2010). The endocannabinoid system as a target for the treatment of neuronal damage. *Expert Opinion in Therapeutic Targets*, *14*, 387–404.

Fishbein, M., Gov, S., Assaf, F., Gafni, M., et al. (2012). Long-term behavioral and biochemical effects of an ultra-low dose of Δ9-tetrahydrocannabinol (THC): neuroprotection and ERK signaling. *Experimental Brain Research*, *221*, 437–448.

Fleckenstein, A. E., Volz, T. J., Riddle, E. L., Gibb, J. W., et al. (2007). New insights into the mechanism of action of amphetamines. *Annual Reviews of Pharmacology and Toxicology*, *47*, 681–698.

Gonzalez, R., Rippeth, J. D., Carey, C. L., Heaton, R. K., et al. (2004). Neurocognitive performance of methamphetamine users discordant for history of marijuana exposure. *Drug and Alcohol Dependence*, *76*, 181–190.

Gouzoulis-Mayfrank, E., & Daumann, J. (2006). The confounding problem of polydrug use in recreational ecstasy/MDMA users: a brief overview. *Journal of Psychopharmacology*, *20*, 188–193.

Green, B., Kavanagh, D., & Young, R. (2003). Being stoned: a review of self-reported cannabis effects. *Drug and Alcohol Reviews*, *22*, 453–460.

Halpin, L. E., Collins, S. A., & Yamamoto, B. K. (2014). Neurotoxicity of methamphetamine and 3,4-methylenedioxymethamphetamine. *Life Sciences*, *97*, 37–44.

Herzig, D. A., Brooks, R., & Mohr, C. (2013). Inferring about individual drug and schizotypy effects on cognitive functioning in polydrug using mephedrone users before and after clubbing. *Human Psychopharmacology*, *28*, 168–182.

Krasnova, I. N., & Cadet, J. L. (2009). Methamphetamine toxicity and messengers of death. *Brain Research Reviews*, *60*, 379–407.

Maldonado, R., Valverde, O., & Berrendero, F. (2006). Involvement of the endocannabinoid system in drug addiction. *Trends in Neuroscience*, *29*, 225–232.

Manzanedo, C., Rodríguez-Arias, M., Daza-Losada, M., Maldonado, C., et al. (2010). Effect of the CB1 cannabinoid agonist WIN 55212-2 on the acquisition and reinstatement of MDMA-induced conditioned place preference in mice. *Behavior and Brain Function*, *22*, 6–19.

Marshall, J. F., & O'Dell, S. J. (2012). Methamphetamine influences on brain and behavior: unsafe at any speed? *Trends in Neuroscience*, *35*, 536–545.

Marshall, J. F., Belcher, A. M., Feinstein, E. M., & O'Dell, S. J. (2007). Methamphetamine-induced neural and cognitive changes in rodents. *Addiction*, *102*, 61–69.

Mohamed, W. M., Ben Hamida, S., Cassel, J. C., de Vasconcelos, A. P., et al. (2011). MDMA: interactions with other psychoactive drugs. *Pharmacology Biochemistry and Behavior*, *99*, 759–774.

Morley, K. C., Li, K. M., Hunt, G. E., Mallet, P. E., et al. (2004). Cannabinoids prevent the acute hyperthermia and partially protect against the 5HT depleting effects of MDMA("Ecstasy") in rats. *Neuropharmacology*, *46*, 954–965.

Nader, J., Rapino, C., Gennequin, B., Chavant, F., et al. (2014). Prior stimulation of the endocannabinoid system prevents methamphetamine-induced dopaminergic neurotoxicity in the striatum through activation of CB(2) receptors. *Neuropharmacology*, *5*, pii: S0028-3908.

NSDUH (2010). Results from the 2009 National Survey on Drug use and Health. Summary of National Findings, volume 1, n2010 (september 2010). http://www.oas.samhsa.gov/NSDUH

Paillet-Loilier, M., Cesbron, A., Le Boisselier, R., Bourgine, et al. (2014). Emerging drugs of abuse: current perspectives on substituted cathinones. *Substance Abuse and Rehabilitation*, *5*, 37–52.

Panenka, W. J., Procyshyn, R. M., Lecomte, T., MacEwan, G. W., et al. (2013). Methamphetamine use: a comprehensive review of molecular, preclinical and clinical findings. *Drug and Alcohol Dependence*, *129*, 167–179.

Papaseit, E., Farrè, M., Schifano, F., & Torrens, M. (2014). Emerging drugs in Europe. *Current Opinion in Psychiatry*, *27*, 243–250.

Parrott, A. C. (2006). MDMA in humans: factors which affect the neuropsychobiological profiles of recreational ecstasy users, the integrative role of bioenergetic stress. *Journal of Psychopharmacology*, *20*, 147–163.

Parrott, A. C. (2013). Human psychobiology of MDMA or 'Ecstasy': an overview of 25 years of empirical research. *Human Psychopharmacology, Clinical and Experimental*, *28*, 289–307.

Parrott, A. C., Milani, R. M., Gouzoulis-Mayfrank, E., & Daumann, J. (2007). Cannabis and Ecstasy/MDMA (3,4-methylenedioxymethamphetamine): an analysis of their neuropsychobiological interactions in recreational users. *Journal of Neural Transmission*, *114*, 959–968.

Prosser, J. M., & Nelson, L. S. (2012). The toxicology of bath salts: a review of synthetic cathinones. *Journal of Medical Toxicology*, *8*, 33–42.

Robledo, P., Trigo, J. M., Panayi, F., de la Torre, R., et al. (2007). Behavioural and neurochemical effects of combined MDMA and THC administration in mice. *Psychopharmacology*, *195*, 255–264.

Sala, M., & Braida, D. (2005). Endocannabinoids and 3,4-methylenedioxymethamphetamine (MDMA) interaction. *Pharmacology Biochemistry and Behavior*, *81*, 407–416.

SAMHSA. (2013). *Substance Abuse and Mental Health Services Administration, Results from the 2012 National Survey on Drug Use and Health: Summary of National Findings, NSDUH Series H-46, HHS Publication No. (SMA) 13-4795*. Rockville, MD: Substance Abuse and Mental Health Services Administration.

Schulz, S. (2011). MDMA & cannabis: a mini-review of cognitive, behavioral, and neurobiological effects of co-consumption. *Current Drug Abuse Reviews*, *4*, 81–86.

Shen, E. Y., Ali, S. F., & Meyer, J. S. (2011). Chronic administration of THC prevents the behavioral effects of intermittent adolescent MDMA administration and attenuates MDMA-induced hyperthermia and neurotoxicity in rats. *Neuropharmacology*, *61*, 1183–1192.

Solowij, N., Stephens, R., Roffman, R. A., & Babor, T. (2002). Does marijuana use cause long-term cognitive deficits? *JAMA*, *287*, 2653–2654.

Strote, J., Lee, J. E., & Wechsler, H. (2002). Increasing MDMA use among college students: results of a national survey. *Journal of Adolescent Health*, *30*, 64–72.

Sulzer, D., Sonders, M. S., Poulsen, N. W., & Galli, A. (2005). Mechanisms of neurotransmitter release by amphetamines: a review. *Progress in Neurobiology*, *75*, 406–433.

Sung, Y. H., Carey, P. D., Stein, D. J., Ferrett, H. L., et al. (2013). Decreased frontal N-acetylaspartate levels in adolescents concurrently using both methamphetamine and marijuana. *Behavior and Brain Research*, *246*, 154–161.

Touriño, C., Ledent, C., Maldonado, R., & Valverde, O. (2008). CB1 cannabinoid receptor modulates 3,4-methylenedioxymethamphetamine acute responses and reinforcement. *Biological Psychiatry*, *63*, 1030–1038.

Touriño, C., Zimmer, A., & Valverde, O. (2010). THC Prevents MDMA neurotoxicity in mice. *PLoS One*, *5*, 9143.

United Nations Office on Drugs and Crime, UNODC (2010). World Drug Report. United Nations Publication.

United Nations Office on Drugs and Crime, UNODC (2014). World Drug Report. United Nations Publication.

Wish, E. D., Fitzelle, D. B., O'Grady, K. E., Hsu, M. H., et al. (2006). Evidence for significant polydrug use among ecstasy-using college students. *Journal of the American College Health*, *55*, 99–104.

PART V

PHARMACOLOGY AND CELLULAR ACTIVITIES OF CANNABINOIDS AND ENDOCANNABINOIDS

58 *Cannabinoids and the cannabinoid receptors: an overview* 553
59 *Signaling and regulation of the cannabinoid CB_1 receptor* 564
60 *Allosteric modulation of the cannabinoid CB1 receptor* 573
61 *Polymorphisms of the CB2 cannabinoid receptor* 584
62 *Chemistry of cannabinoid receptor agonists* 592
63 *The endocannabinoid system as a target for new antiseizure drugs* 606
64 *Pharmacological aspects of anandamide and 2-arachidonoyglycerol as bioactive lipids* 616
65 *Pharmacological aspects of NMDA receptors, mGluR5, and endocannabinoids* 630
66 *Peripheral CB1 receptors and ghrelin in feeding regulation: Pharmacological implications* 639
67 *Pharmacological aspects of novel antiobesity agents related to cannabinoids* 649
68 *Cannabinoid reward and dependence: focus on the main psychoactive ingredients of marijuana in preclinical studies* 659
69 *Peroxisome proliferator activated receptors and cannabinoids* 671
70 *The protein-protein interactions of cannabinoid receptor interacting protein 1a (CRIP1a) and cannabinoid 1 receptor: the molecular mechanism study through an integrated molecular modeling approach* 680
71 *Synthetic cannabinoids: a summary of selected phenomena with respect to behavioral pharmacology and abuse liability* 691

Page left intentionally blank

CHAPTER

58

Cannabinoids and the Cannabinoid Receptors: An Overview

D. Lu, D.E. Potter

Department of Pharmaceutical Sciences, Rangel College of Pharmacy, Texas A&M University, Kingsville, TX, United States

SUMMARY POINTS

- Cannabinoids are a group of biologically active ingredients isolated from cannabis. The representative member of cannabinoids is (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC), which binds to cannabinoid receptors to exert its biological effects, including psychoactive effects.
- So far, at least two types of cannabinoid receptors have been identified and defined respectively as cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2), which are G-protein coupled receptors specifically responding to phytocannabinoids, endocannabinoids and synthetic cannabimimetic compounds.
- The cannabinoid receptors are widely distributed. The CB1 receptors are primarily found in the central nervous system, whereas the CB2 receptors are mainly found in the cells of immune and hematopoietic systems.
- Activations of the cannabinoid CB1 and CB2 receptors generally lead to the inhibition of adenylyl cyclase, and the activation of mitogen-activated protein kinases (MAPKs).
- The cannabinoid receptors are involved in a large number of physiological processes, such as neuronal development, food intake and energy balance, perception process, and immune modulation.

KEY FACTS

- According to world health organization, about 147 million people worldwide (2.5% of the world population) consume cannabis mainly for recreational purposes.
- Both short-term and long-term uses of cannabis have been linked to certain health hazards such as altered senses, impaired body movement, memory, and learning functions.
- Some synthetic cannabis-mimetics are more potent than natural cannabis ingredients. They may affect the body functions much more powerfully than cannabis. Their actual effects are unpredictable. In some cases, they can be even life-threatening.
- While cannabis is an illicit drug, it has been demonstrated with a number of potential medical usefulness including treatment of asthma and glaucoma, as an antidepressant, appetite stimulant, anticonvulsant, antispasmodic, antiemetic, and analgesic.
- By far, three cannabis-based medications have been developed, which include Dronabinol®, Cesamet®, and Sativex®. The first two were proved by FDA for the treatment of nausea and vomiting in the advanced stages of cancer and AIDS, whilst Sativex® was approved in some European countries for the treatment of spasticity from multiple sclerosis.
- By far in the United Sates, 25 states and Washington D.C. have legalized cannabis for medical uses.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00068-5
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

Δ^9-THC	(−)-Δ^9-Tetrahydrocannabinol
AP-3	Adaptor protein complex-3
BC	Before Christ
cAMP	Cyclic adenosine monophosphate
CB1	Cannabinoid receptor type 1
CB2	Cannabinoid receptor type 2
cDNA	Complementary DNA
CHO	Chinese hamster ovary
CNS	Central nervous system
DNA	Deoxyribonucleic acid
FAAH	Fatty acid amide hydrolase
FAN	Factor associated with neutral sphingomyelinase activation
G-protein	Guanine nucleotide-binding protein
GABA	γ-Aminobutyric acid
GASP1	GPCR-associated sorting protein 1
GPCR	G-protein-coupled receptor
JNK	C-Jun N-terminal kinase
MAGL	Monoacylglycerol lipase
PI3K	Phosphatidylinositide 3-kinase
TM	Transmembrane

INTRODUCTION

Cannabis has a long history for medical application, and is a psychoactive substance for recreational use. Isolation of its major active components (cannabinoids) and the synthesis of potent cannabimimetics led to the discoveries of two G-protein coupled receptors that specifically respond to cannabinoids, and are designated as cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2). Subsequently, endogenous ligands for the cannabinoid receptors (endocannabinoids) were identified. The cannabinoid receptors and the endocannabinoids are involved in modulation of many physiological functions, not only in the central and peripheral nervous systems, but also in a number of peripheral sites within the human body: endocrine system, immune system, the gastrointestinal tract, cardiovascular system, and the reproductive system. The two described cannabinoid receptors are encoded by different genes, and exhibit a 44% homology in their primary structures. Functionally, they belong to the super family of G-protein coupled receptors, and transduce signals of ligand binding into various biological responses, through both G-protein-mediated and G-protein-independent pathways.

Generally, the major role of the CB1 receptor is to modulate the release of neurotransmitters, whereas the major function of the CB2 receptor is to mediate the immune modulatory effects of cannabinoids, among others. The functions of cannabinoid receptors can be modulated by: (1) a variety of ligands (agonists, partial agonists, neutral antagonists, and inverse agonists) that target the orthosteric binding sites on the receptors, or (2) allosteric modulators that bind to the allosteric binding sites of the receptors. With the development of a variety of new ligands of cannabinoid receptors, intensive research has been carried out in the last two decades to clarify the roles of cannabinoid receptors in physiological and pathological conditions, as well as to determine how the disease states involving the cannabinoid receptors can be treated more effectively and safely. In this chapter, some historical and fundamental aspects about cannabinoids and cannabinoid receptors are reviewed.

CANNABIS AND CANNABINOIDS

The plant *Cannabis sativa* Linne is one of the oldest plants used by humans as folklore medicine. The earliest record of medical use of cannabis appeared around 2737 BC in a Chinese pharmaceutical compendium, *Shennong Bencao Jing* (The Divine Farmer's Materia Medica), in which *Cannabis* was documented as a magic mood- and memory-booster, an antiinflammatory herb, and as a therapeutic for many other conditions, including disorders of the female reproductive tract, constipation, gout, rheumatism, stroke, and absent-mindedness (Yang, 1998). During the long history of agricultural migration of cannabis from China and India to the Middle East and Europe, various medical usefulness of cannabis have been noted (Mack & Joy, 2000). In 1850, cannabis was included in the US pharmacopeia for many ailments, including neuralgia, tetanus, typhus, cholera, rabies, dysentery, alcoholism, opiate addiction, anthrax, leprosy, incontinence, gout, convulsive disorders, tonsillitis, insanity, excessive menstrual bleeding, and uterine bleeding (Boire & Feeney, 2006). The broad pharmacological effects of cannabis stimulated the search for its bioactive ingredients, and led to the isolation and structural characterization of (−)-Δ^9-trans-(6a*R*,10a*R*)-tetrahydrocannabinol (Δ^9-THC) in 1964 (Gaoni & Mechoulam, 1964). Δ^9-THC is the most psychoactive substance present in cannabis. To date, 489 constituents have been isolated from cannabis (Elsohly & Slade, 2005). These natural compounds can be classified into 18 distinct chemical groups such as cannabinoids (~70), terpenoids (~103), hydrocarbons (~50), amino acids (~18), steroids (~11), and flavaniidglycosides (~19), among others (Elsohly & Slade, 2005; Turner, Elsohly, & Boeren, 1980). Cannabinoids is a group of 70 terpenophenolic compounds that generally contain a monoterpene moiety connected to a phenol ring, having a 3- to 5-carbon alkyl chain located in the position meta to the phenolic hydroxyl group. There are 12 major

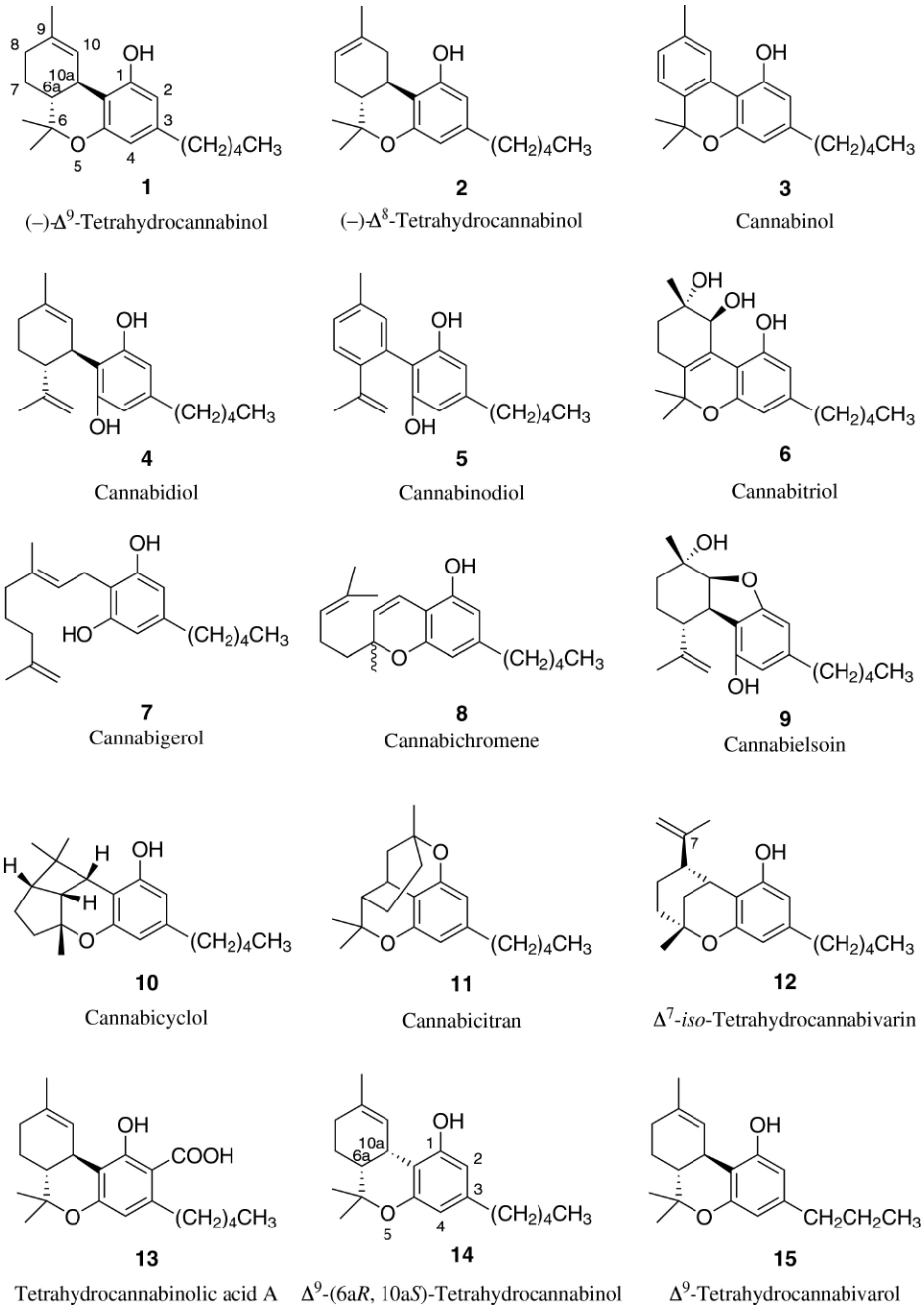


FIGURE 58.1 **Chemical structures of main phytocannabinoids isolated from *Cannabis sativa L.***

types of cannabinoids, all of which are derived from cannabigerol type precursors, and differ mainly in the way how the precursors are chemically cyclized within the structure. The representative members of cannabinoids are illustrated in Fig. 58.1.

The discoveries of endogenous cannabinoids (endocannabinoids) and the development of synthetic cannabimimetic compounds have prompted the use of "phytocannabinoids" to describe plant-derived cannabinoids. Among these phytocannabinoids, (−)-Δ^9-THC (1) and (−)-Δ^8-THC (2) are two psychologically active constituents. Besides the psychoactive cannabinoids, other cannabinoids such as cannabinol (3), cannabidiol (4), and cannabigerol (7) also exhibit a variety of pharmacological effects (Izzo, Borrelli, Capasso, Di Marzo, & Mechoulam, 2009). Notably, cannabidiol (4) and cannabigerol (7) are not psychotropic because of their inability to interact with cannabinoid CB1 receptors in the brain (Izzo et al., 2009).

Consumption of *Cannabis* can lead to a variety of behavioral effects such as sedation, impairment

of short-term memory, motor incoordination; mood alternations that include euphoria, calmness, anxiety, or paranoia. Because of the highly lipophilic properties of phytocannabinoids, early scientific inquires on how cannabis works led to a hypothesis that cannabinoids may elicit their biological effects through nonspecific perturbing the membrane lipids (Lawrence & Gill, 1975). However, this concept was diminished by the discovery of the first cannabinoid receptor in the late 1980s.

CANNABINOID RECEPTORS: WHAT THEY ARE

Discovery of Cannabinoid Receptors

Although nonspecific action of cannabis was thought to be possible, a series of pharmacological studies in late 1980s indicated a receptor-mediated mechanism of action (Howlett & Fleming, 1984; Howlett, Qualy, & Khachatrian, 1986; Little, Compton, Johnson, Melvin, & Martin, 1988). The first evidence for the existence of a brain type cannabinoid receptor came from demonstration of the specific binding sites of synthetic cannabimimetics in rat brain P2 membranes and synaptosomes (Devane, Dysarz, Johnson, Melvin, & Howlett, 1988). This landmark finding was followed by the confirmation of cannabinoid-specific binding sites in the brains of several other mammalian species, including human (Herkenham et al., 1990). The conclusive proof for the existence of a cannabinoid receptor was provided by cloning and expression of a complementary DNA (cDNA), SKR6, from a rat cerebral cortex cDNA library (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990). The protein product of SKR6 differed significantly from any other known receptor in amino acid sequence, and shows high selectivity for binding to phytocannabinoids and cannabimimetic agents. Because the distribution of its mRNA matched well with the binding sites of cannabimimetics in the brain, this receptor was named as cannabinoid receptor, formalized later as cannabinoid receptor type 1 (CB1). Soon after the identification of CB1 receptor, homology cloning disclosed another form of cannabinoid receptor from the human promyelocytic cell line HL60 (Munro, Thomas, & Abu-Shaar, 1993). Because its mRNA could not be detected in the central nervous system during the initial characterizations, this receptor was firstly recognized as a peripheral cannabinoid receptor, and later was formally designated as cannabinoid receptor type 2 (CB2). Following the cloning of human CB2, the rat (Griffin, Tao, & Abood, 2000) and mouse (Shire et al., 1996), CB2 receptors were disclosed. Unlike the CB1 receptor which exhibits high amino acid identity across human, rat, and mouse (>97%) (Abood, Ditto, Noel, Showalter, & Tao, 1997; Gerard, Mollereau, Vassart, & Parmentier, 1991), the CB2 receptor displays only 81% amino acid identity between human and rat, and 82% identity between human and mouse, respectively. Notably, the sequences of human, rat, and mouse CB2 receptors markedly differ at the C-terminus. The mouse CB2 is shorter by 13 amino acids; the rat CB2 is 50 amino acids longer than the human CB2 (Console-Bram, Marcu, & Abood, 2012).

Endogenous Ligands of Cannabinoid Receptors

The cloning of CB1 and CB2 receptors motivated numerous efforts to pursue the endogenous ligands that can activate the cannabinoid receptors. It was then demonstrated that mammalian tissues do synthesize and release lipid-derived molecules that can activate cannabinoid receptors and generate pharmacological effects similar to those from Δ^9-THC (Di Marzo, De Petrocellis, & Bisogno, 2005; Hanuš, 2009; Martin, Mechoulam, & Razdan, 1999). The first identified endogenous cannabinoid, *N*-arachidonoylethanolamine (anandamide), was isolated from porcine brain in 1992 (Hanuš, 2009). Its identity as an endogenous cannabinoid was proven by many in vitro and in vivo studies (Felder et al., 1996; Martin et al., 1999). Later, another lipid derivative, 2-arachidonoylglycerol (2-AG) isolated from canine gut was shown to be a potent endogenous cannabinoid, and binds to the CB1 and CB2 receptors more devotedly than anandamide (Martin et al., 1999). In the brain, the concentration of 2-AG is about 50–500-fold higher than the concentration of anandamide. Additionally, 2-AG behaves as a full agonist for the CB1 and CB2 receptors, whereas anandamide is a partial agonist at both receptors (Di Marzo et al., 2005). The degradation of anandamide is primarily catalyzed by fatty acid amide hydrolase (FAAH), while 2-AG is mainly metabolized by monoacylglycerol lipase (MAGL) (Di Marzo et al., 2005). Other compounds that have been shown as endocannabinoids include noladin ether, *N*-arachidonoyldopamine, *N*-docasatetraenoylethanolamide, dihomo-γ-linolenoylethanolamide, O-arachidonoyl-ethanolamine (virodhamine), and meadethanolamide (Di Marzo et al., 2005; Hanuš, 2009; Martin et al., 1999). The structures of these endocannabinoids are presented in Fig. 58.2. The identification of endogenous ligands completed the discovery of cannabinoid CB1 and CB2 receptors.

The Structural Characteristics of CB1 and CB2 Receptors

The CB1 receptor is a protein encoded by CNR1 gene located on human chromosome 6, whereas the CB2 receptor is encoded by CNR2 gene located on chromosome 1 (Yao & Mackie, 2009). Expressions of the human CNR1 and CNR2 genes, respectively, provide a protein with 472 amino acids (CB1), and a protein with 360 amino acids (CB2). The primary structures of human CB1 and CB2 receptors are illustrated in Fig. 58.3.

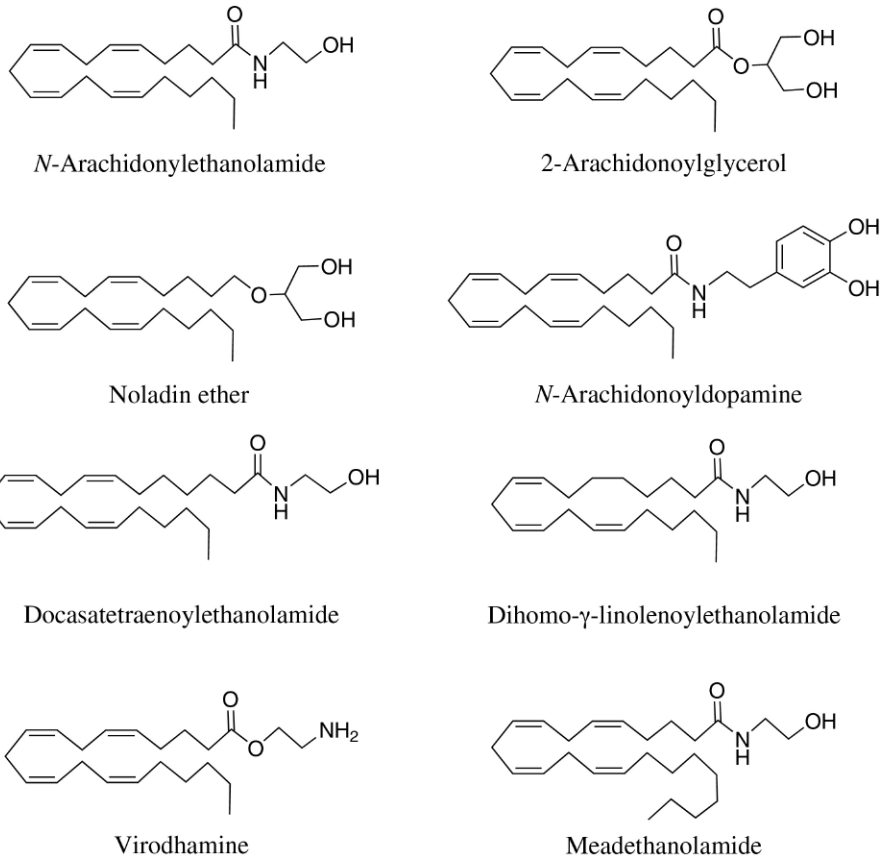


FIGURE 58.2 **Chemical structures of known endocannabinoids.**

```
CB1
MKSILDGLAD TTFRTITTDL LYVGSNDIQY EDIKGDMASK LGYFPQKFPL TSFRGSPFQE
KMTAGDNPQL VPADQVNITE FYNKSLSSFK ENEENIQCGE NFMDIECFMV LNPSQQLAIA
VLSLTLGTFT VLENLLVLCV ILHSRSLRCR PSYHFIGSLA VADLLGSVIF VYSFIDFHVF
HRKDSRNVFL FKLGGVTASF TASVGSLFLT AIDRYISIHR PLAYKRIVTR PKAVVAFCLM
WTIAIVIAVL PLLGWNCEKL QSVCSDIFPH IDETYLMFWI GVTSVLLLFI VYAYMYILWK
AHSHAVRMIQ RGTQKSIIIH TSEDGKVQVT RPDQARMDIR LAKTLVLILV VLIICWGPLL
AIMVYDVFGK MNKLIKTVFA FCSMLCLLNS TVNPIIYALR SKDLRHAFRS MFPSCEGTAQ
PLDNSMGDSD CLHKHANNAA SVHRAAESCI KSTVKIAKVT MSVSTDTSAE AL

CB2
MEECWVTEIA NGSKDGLDSN PMKDYMILSG PQKTAVAVLC TLLGLLSALE NVAVLYLILS
SHQLRRKPSY LFIGSLAGAD FLASVVFACS FVNFHVFHGV DSKAVFLLKI GSVTMTFTAS
VGSLLLTAID RYLCLRYPPS YKALLTRGRA LVTLGIMWVL SALVSYLPLM GWTCCPRPCS
ELFPLIPNDY LLSWLLFIAF LFSGIIYTYG HVLWKAHQHV ASLSGHQDRQ VPGMARMRLD
VRLAKTLGLV LAVLLICWFP VLALMAHSLA TTLSDQVKKA FAFCSMLCLI NSMVNPVIYA
LRSGEIRSSA HHCLAHWKKC VRGLGSEAKE EAPRSSVTET EADGKITPWP DSRDLDLSDC
```

FIGURE 58.3 **Primary structures of CB1 and CB2 receptors.** The amino acid sequence of the human CB1 (top panel) and CB2 (lower panel) receptors. The human CB1 receptor comprises of 472 amino acids, whereas the human CB2 receptor contains 360 amino acids. The individual amino acid is presented by one letter abbreviation. The regions shown with *red letters* are transmembrane domains. The length and sequence homology of the third intracellular loop (regions represented with *blue characters*) and the C-terminals (regions represented with *green characters*) of the two receptor subtypes are notably different. The protein sequences and domain assignments were retrieved from the freely accessible protein-sequence database Uniprot (http://www.uniprot.org).

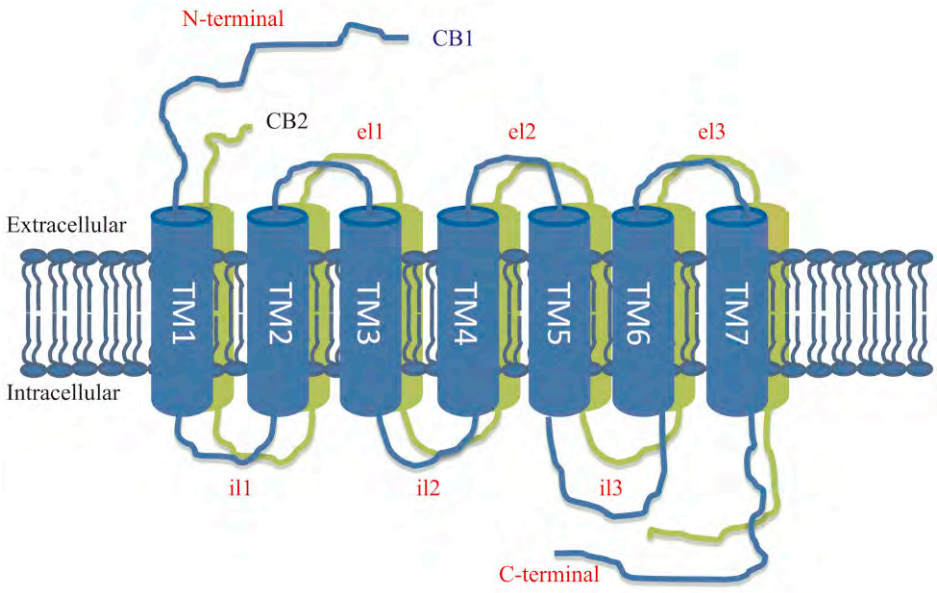


FIGURE 58.4 **Schematic representation of the cannabinoid CB1 and CB2 receptors.** The *blue* model represents CB1 receptor and the *green* model represents the CB2 receptor. *TM*, transmembrane; *el*, extracellular loop; *il*, intracellular loop. The CB1 receptor is a larger protein, and has additional 72 amino acid residues in the N-terminal segment, 13 additional residues in the third intracellular loop, and 14 additional residues in the C-terminal region.

The two cannabinoid receptors belong to the largest family of receptors, which couple to guanine-nucleotide-binding proteins (G-proteins) at their intracellular sites. They share the structural characteristics of all G-protein-coupled receptors (GPCRs), possessing seven hydrophobic domains that transpass the cell membrane seven times, forming helix bundles when embedded in cell membrane. The seven transmembrane domains are linked by three extracellular and three intracellular loops, an extracellular N-terminal, and an intracellular C-terminal (Fig. 58.3). Structurally, the CB1 and CB2 receptors distinguish themselves from many other GPCRs in two aspects (Matsuda, 1997). Unlike many other GPCRs, both CB1 and CB2 receptors do not contain conserved cysteine residues within the second extracellular domain (el1), whereas the third extracellular domain (el2) contains two or more cysteines. The conserved cysteine residues in the second and third extracellular domains of GPCRs appear to stabilize the tertiary structure of the receptor through the formation of intramolecular disulfide linkage with the neighboring transmembrane domains (TMs). Additionally, both CB1 and CB2 lack a highly conserved proline residue in the fifth transmembrane (TM5) domain, by which a structural kink could be formed usually in the helix (Matsuda, 1997). In comparison with the CB2 receptor, the CB1 receptor has an additional 72 amino acid residues in the N-terminal segment, 15 additional residues in the third intracellular loop, and 13 additional residues in the C-terminal region (Figs. 58.3 and 58.4). The sequence homology between the two cannabinoid receptors is 44% in the full-length protein, and ranges from 33% to 82% within the seven transmembrane regions (Fig. 58.4). The highest homology between CB1 and CB2 is found in the transmembrane TM2, TM3, TM6, and TM7 domains (TM1: 50%; TM2: 66%; TM3: 80%; TM4: 33%; TM5: 40%; TM6: 60%; and TM7: 82%) (Matsuda, 1997). The primary structures of CB1 and CB2 receptors show that the length and sequence homology of the third intracellular loops and the C-terminals of the two receptors are considerably different. These variations might affect their cellular response to a ligand, because these regions are known to mediate G-protein signal transduction (Howlett & Shim, 2004), as well as receptor desensitization and internalization (Hsieh, Brown, Derleth, & Mackie, 1999).

At present, X-ray crystallographic structures of cannabinoid receptors remain unknown. In the absence of crystallographic structures for CB1 and CB2 receptors, computational homology models of human CB1 receptor (Shim, Welsh, & Howlett, 2003) and human CB2 receptor (Raitio, Salo, Nevalainen, Poso, & Jarvinen, 2005; Xie, Chen, & Billings, 2003) have been respectively constructed using bovine rhodopsin as the prototype of GPCRs. Site-directed mutation and computer-modeling have been employed to identify the key amino acids (or motif) involved in ligand binding and receptor activation/deactivation. Notable residues identified include Lys192 of CB1 receptor (Song & Bonner, 1996) and Ser112/T116 of CB2 receptor (Tao et al., 1999), which are instrumental for the binding of most cannabinoid ligands. The functional residues/motif involved in ligand binding to the CB1/CB2 receptor has been thoroughly reviewed (Ortega-Gutierrez & Lopez-Rodriguez, 2005).

THE CANNABINOID RECEPTORS: WHERE THEY ARE

The cannabinoid receptors are widely distributed in the human body. CB1 is expressed primarily in the central nervous system (CNS), and is the most abundant G-protein-coupled receptor in the brain (Mackie, 2005). Quantitative autoradiography revealed that the concentration of CB1 receptors in brain is comparable to those of cortical benzodiazepine receptor, striatal dopamine receptor, and whole-brain glutamate receptor (Herkenham et al., 1990). The highest density of CB1 receptors is found in the basal ganglia, particularly in putamen, which is involved in motor control. High levels of CB1 receptors are also detected in cerebellum, hippocampus, and cerebral cortex. These brain regions are involved in coordinating body movements, learning and memory, and higher cognitive functions, respectively (Mackie, 2005; Pertwee, 1997). Moderate levels of CB1 receptors were also found in the brainstem, spinal cord, hypothalamus, amygdala, central gray, and nucleus of the solitary tract (Pertwee, 1997). Besides their presence in CNS, CB1 receptors have been found in periphery, but at much lower level compared to its brain level. Some immunocytochemical analyses of CB1 mRNA indicated the presence of CB1 receptor in a variety of peripheral tissues/organs, including liver, pancreas, testis, fat, skeletal muscle, retina, sperms, peripheral neurons, adrenal gland, heart, lung, prostate, uterus, ovary, bone marrow, spleen, thymus, and tonsils (Bouaboula et al., 1993; Galiegue et al., 1995; Pertwee, 1997; Rice, Shannon, Burton, & Fiedeldey, 1997).

Early investigations of the mRNA of CB2 receptor in the cerebral tissues led to the inference that CB2 receptor is absent in the central nervous system, and predominantly expressed in the tissues/organs of the immune system, such as spleen, tonsil, thymus, and lymphoid tissues (Galiegue et al., 1995; Munro et al., 1993; Schatz, Lee, Condie, Pulaski, & Kaminski, 1997). Of these peripheral sites, tonsils exhibit the highest CB2 mRNA level, which is even higher than the total concentration of CB1 mRNA in the brain (Galiegue et al., 1995). Quantitative analysis of CB2 mRNA reveals that moderate to low levels of CB2 receptors can be found in pancreas, bone marrow, uterus, and lung. Likewise, high concentrations of CB2 receptors can be identified in human leukocytes such as B-cells, NK-cells, monocytes polymorphonuclear neutrophils, $CD8^+$-T-lymphocytes and $CD4^+$-T-lymphocytes (Bouaboula et al., 1993; Galiegue et al., 1995). Although early investigations of CB2 receptor did not detect this receptor in the brain, Van Sickle et al. (2005) have demonstrated that functional CB2 receptors do exist in the central nervous system, albeit in much lower concentration than in the periphery. Several recent reports also disclosed the CB2 immunoreactivity in many brain areas, such as the hippocampal formation, olfactory bulb, cortex, midbrain, and thalamus (Gong et al., 2006; Onaivi et al., 2006, 2008a,b). These results support the conclusion that CB2 receptor is not an exclusively peripheral cannabinoid receptor. Additionally, CB2 receptors show an inducible nature under certain disease conditions. For example, microglial cells in healthy brain do not appear to express CB2. However, in neurodegenerative disorders associated with the activation of brain microglial cells, such as Alzheimer and Huntington diseases, the expression of CB2 receptors in the brain can be significantly elevated (Benito et al., 2003; Palazuelos et al., 2009). The multifocal expressions of CB2 receptors in the brain suggest that CB2 receptors may play broader roles in the central nervous system than previously postulated.

CANNABINOID RECEPTORS: WHAT THEY DO

The cannabinoid receptors as part of the endocannabinoid system have been shown to play important roles in a variety of physiological conditions, including neuronal development, neuronal plasticity, food intake and energy balance, perception process, immune modulation, cell apoptosis, cardiovascular and reproductive functions (Marzo, Bifulco, & De Petrocellis, 2004; Rodriguez de Fonseca et al., 2005). The multifaceted functions of cannabinoid receptors are rooted by their cellular physiology and complex signaling pathways in transducing the binding of cannabinoid ligands into biological responses.

Cell Physiology of Cannabinoid Receptors

Many studies have demonstrated that endocannabinoids function as retrograde messengers in the CNS. They are released from postsynaptic neurons in a calcium-dependent manner, and traverse retrogradely onto presynaptic cannabinoid CB1 receptors. Subsequently, they produce a transient or prolonged reduction in the release of neurotransmitters such as the inhibitory γ-aminobutyric acid (GABA), excitatory glutamate, acetylcholine, and noradrenaline (Piomelli, 2003; Rodriguez de Fonseca et al., 2005). Unlike classical peptide neurotransmitters, endocannabinoids are not presynthesized and stored in vesicles before release. In contrast, they are synthesized "on demand," via enzymatic pathways triggered by a rapid increase of intracellular calcium through cell depolarization or receptor activation. At present, the knowledge concerning the physiology of CB2 receptors is relatively less than that of CB1 receptors. Given the high level of CB2 receptors on the cells of immune and hematopoietic systems, CB2 receptors are most likely involved in inflammation-associated pathologies. In general, CB2 receptors are involved in regulation of immune cells in several ways, including: (1) inducing immune cell apoptosis, (2) suppressing proliferation of immune cells and the

production of proinflammatory cytokines/chemokines, (3) increasing secretion of antiinflammatory cytokines, (4) inducing regulatory T cells, and (5) promoting the trafficking and migration of immune cells (Klein & Cabral, 2006; Rom & Persidsky, 2013).

Signal Transductions of CB1 and CB2 Receptors

Cannabinoid receptors transduce signals through various pathways: G-protein-dependent processes (Demuth & Molleman, 2006; Pertwee, 1997) and G-protein-independent processes (Turu & Hunyady, 2010; Velasco et al., 2005). The major signal transductions of both CB1 and CB2 receptors are primarily mediated through the inhibitory G_i and G_o proteins. However, under certain conditions, signal transductions of CB1 receptor through $G_{q/11}$ and stimulatory G_s are also possible (Turu & Hunyady, 2010). In contrast to CB1 receptors, CB2 receptors do not transduce signals through stimulatory G_s (Demuth & Molleman, 2006). Upon stimulation by agonists, cannabinoid receptors modulate adenylyl cyclase and, consequently, alter the cellular production of cAMP. Although activation of cannabinoid receptors typically suppresses adenylyl cyclase and downregulates signaling responses mediated by second messenger cAMP, evidence suggests that the isoforms of adenylyl cyclase expressed in cells determine the outcome of cAMP production. For instance, in COS-7 cells that coexpress CB1/CB2 with nine isoforms of adenylyl cyclase 1 to 9, activation of the cannabinoid receptors CB1 and CB2 inhibited the activity of adenylyl cyclase isoforms 1, 3, 5, 6, and 8. In contrast, adenylyl cyclase isozymes 2, 4, and 7 were stimulated (Rhee, Bayewitch, Avidor-Reiss, Levy, & Vogel, 1998). In addition, the CB1 and CB2 receptors can carry signal transductions through the activation of phosphatidylinositide 3-kinases (PI3K), but with different mechanisms (Demuth & Molleman, 2006; Howlett & Shim, 2004). Both the CB1 and CB2 cannabinoid receptors regulate the phosphorylation and activation of different members of all three families of mitogen-activated protein kinases (MAPKs), including p44/42 MAP kinase, p38 kinase, and JUN-terminal kinase (Atwood & Mackie, 2010; Turu & Hunyady, 2010). Unlike the activation of CB2 receptor, which generally has no effect on ion channels, activation of CB1 receptor inhibits N- and P/Q-type voltage-gated Ca^{2+} channels and activates A-type and G-protein-coupled inwardly-rectifying K^+ channels (GIRK) (Howlett, 2005). Along with the G-protein mediated signal transductions, the CB1 receptor can interact with a variety of non-G protein partners (eg, β-arrestins, adaptor protein AP-3, GPCR-associated sorting protein 1 (GASP1), and the adaptor protein FAN) to control receptor signaling or trafficking (Howlett, Blume, & Dalton, 2010; Smith, Sim-Selley, & Selley, 2010). The CB1 and CB2 cannabinoid receptors can also transduce signals through G-protein independent pathways that employ ceramide as the second messenger (Velasco et al., 2005). Activation of cannabinoid receptors impacts both the short-term and long-term ceramide accumulation, which is respectively resulted from sphingomylin hydrolysis and ceramide de novo synthesis. Ceramide, in turn, mediates cannabinoid-induced metabolic regulation and cell apoptosis (Herrera et al., 2006; Velasco et al., 2005). The major signaling pathways of the CB1 and CB2 receptors are summarized in Fig. 58.5.

The signaling events of CB1 and CB2 receptors can be attenuated by desensitization of the receptor, followed by an internalization process (Atwood, Straiker, & Mackie, 2012). Desensitization involves G-protein-receptor-kinase-mediated phosphorylation of multiple serine/threonine residues of the receptors, followed by binding of the β-arrestins. The internalized receptors can be either transported to endosomes for dephosphorylation, and then returned to the cell membrane for the next signaling event, or transported to lysosomes for degradation. Apart from the agonist-initiated signal transductions, both CB1 and CB2 receptors can induce cellular responses through constitutive activities (so called "constitutive tone"). In the absence of agonists, the two cannabinoid receptors have been shown to exhibit a spontaneous activation of $G_{i/o}$-proteins, and increased constitutive MAPK-activating properties (Yao & Mackie, 2009). These constitutive activities can be respectively attenuated by inverse agonists that can selectively interact with the CB1 and CB2 receptors.

CONCLUDING REMARKS

The isolation of Δ^9-THC from cannabis, the identification and cloning of the two cannabinoid receptors, and the discovery of endogenous CB ligands, revealed the existence of the endocannabinoid system. In the past three decades, investigation of the molecular biology and pharmacology of the endocannabinoid system has implicated its importance in a large number of physiological functions in human. Modulating the functions of the cannabinoid receptors, either directly or indirectly, holds therapeutic promises for ameliorating a number of pathological conditions, ranging from the diseases of nervous systems, such as psychiatric disorders, Parkinson and Huntington disease, multiple sclerosis and spinal cord and brain injuries, to pain, inflammation, and cancer, as well as some cardiovascular and metabolic disorders including atherosclerosis, myocardial infarction, hypertension, stroke, obesity/metabolic syndrome, and osteoporosis, among others (Pacher, Bátkai, & Kunos, 2006). Further understanding the molecular biology and pharmacology of the cannabinoid receptors will create more opportunities for the discovery of novel and safer medications by targeting of cannabinoid receptors more selectively.

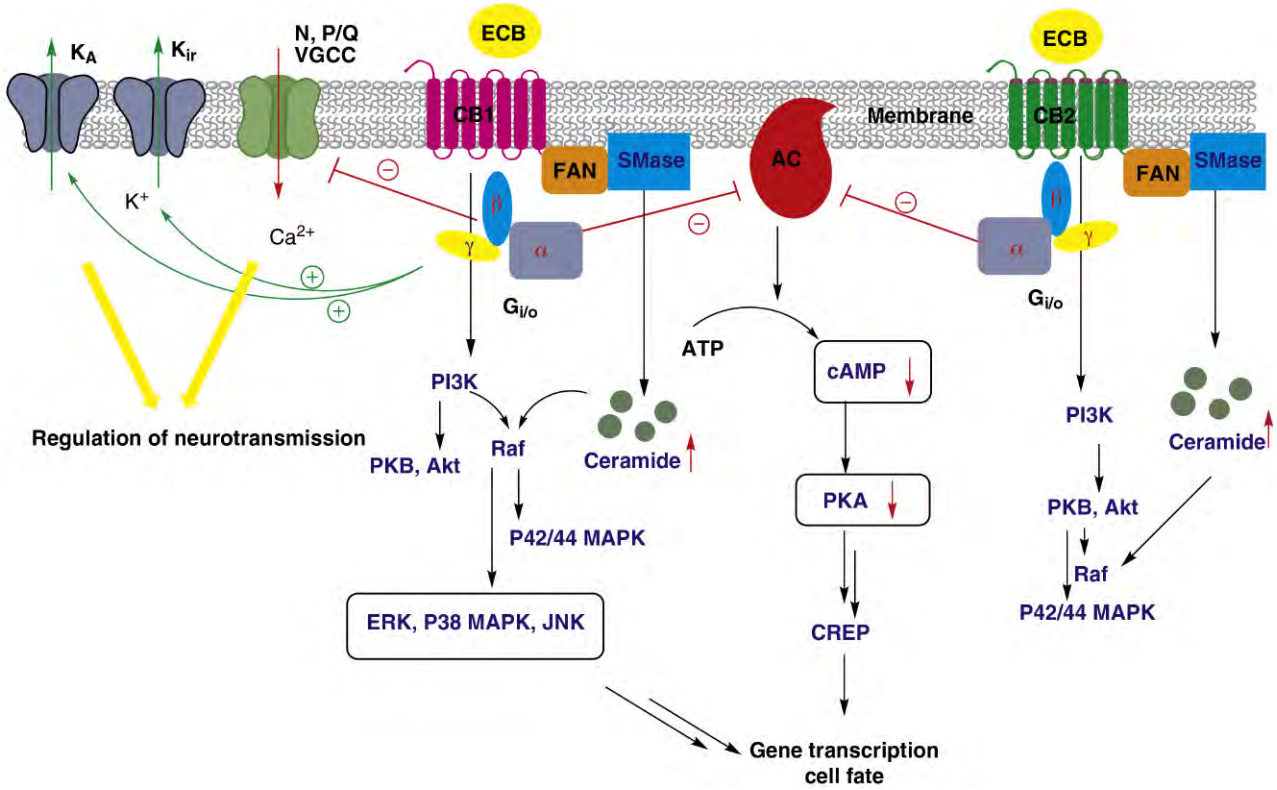


FIGURE 58.5 **Major signaling pathways initiated from activation of the cannabinoid CB1 and CB2 receptors.** The major signaling pathways initiated by the binding of agonists (ECB) to cannabinoid receptors involve the activation and dissociation of the coupled $G_{i/o}$-protein heterotrimers (ie, α and βγ subunits). The released G_α subunits interact with adenylyl cyclase (AC) and inhibit its activity in synthesis of cAMP. This decreases the activation of protein kinase A (PKA), which in turn downregulates PKA-mediated signaling events. The βγ subunits can evoke the phosphatidylinositide 3-kinase (PI3K) and protein kinase B (PKB) pathways, which in turn induce the phosphorylation of P42/44 MAPK and other members of MAPK such as JNK and p38 MAPK. The CB1 and CB2 receptors can also couple to non-G-protein partners such as FAN, which activates neutral sphingomyelinase (SMase) that mediates the generation of ceramide from sphingomyelin. While ceramide is involved in other functions, it can function as a second messenger to activate several downstream effectors, including ERK, JNK, and p38 that are further involved in the control of gene transcriptions and cell fate. Activation of CB1 receptor inhibits N and P/Q types of voltage gated calcium channels (VGCC), while positively regulates A-type K^+ (K_A), and inwardly rectifying K^+ (K_{ir}) channels. The modulations of ion channels by CB1 are independent of the cAMP pathway, and are involved in the regulation of neurotransmitter release. In contrast, activation of CB2 receptors generally does not regulate ion channels.

MINI-DICTIONARY

Cannabinoid receptors Cannabinoid receptors are the G-protein-coupled receptors that mediate the biological effects of phytocannabinoids, endocannabinoids, and synthetic cannabimimetic compounds.

Cannabinoids Cannabinoids are a group of biologically active compounds isolated from the plant cannabis, and are often referred to as phytocannabinoids, some of which are responsible for the psychoactive effects of cannabis.

Endocannabinoids Endocannabinoids are the molecules produced by human and animals which exhibit similar biological activities as phytocannabinoids.

G-protein-coupled receptors G-protein-coupled receptors (GPCRs) are membrane-bound signal transduction proteins that associate with guanosine nucleotide-binding proteins (G-proteins) on their intracellular side. Upon binding to certain type of chemical compounds, the GPCRs change shapes, and cause the disassembly of the associated G-proteins into subunits, which further transduce the signal to the downstream proteins and eventually lead to cellular responses.

Synthetic cannabimimetics Chemical compounds synthesized in laboratories that exhibit similar biological activities as phytocannabinoids.

References

Abood, M. E., Ditto, K. E., Noel, M. A., Showalter, V. M., & Tao, Q. (1997). Isolation and expression of a mouse CB1 cannabinoid receptor gene. Comparison of binding properties with those of native CB1 receptors in mouse brain and N18TG2 neuroblastoma cells. *Biochemical Pharmacology*, *53*(2), 207–214.

Atwood, B. K., & Mackie, K. (2010). CB2: a cannabinoid receptor with an identity crisis. *British Journal of Pharmacology*, *160*(3), 467–479.

Atwood, B. K., Straiker, A., & Mackie, K. (2012). CB(2): therapeutic target-in-waiting. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *38*(1), 16–20.

Benito, C., Nunez, E., Tolon, R. M., Carrier, E. J., Rabano, A., Hillard, C. J., & Romero, J. (2003). Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *Journal of Neuroscience*, *23*(35), 11136–11141.

Boire, R. G., & Feeney, K (2006). *Medical marijuana law*. Oakland, CA: Ronin Publishing.

Bouaboula, M., Rinaldi, M., Carayon, P., Carillon, C., Delpech, B., Shire, D., . . ., Casellas, P. (1993). Cannabinoid-receptor expression in human leukocytes. *European Journal of Biochemistry*, *214*(1), 173–180.

Console-Bram, L., Marcu, J., & Abood, M. E. (2012). Cannabinoid receptors: nomenclature and pharmacological principles. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *38*(1), 4–15.

Demuth, D. G., & Molleman, A. (2006). Cannabinoid signalling. *Life Sciences*, *78*(6), 549–563.

Devane, W. A., Dysarz, F. A., III, Johnson, M. R., Melvin, L. S., & Howlett, A. C. (1988). Determination and characterization of a cannabinoid receptor in rat brain. *Molecular Pharmacology*, *34*(5), 605–613.

Di Marzo, V., Bifulco, M., & De Petrocellis, L. (2004). The endocannabinoid system and its therapeutic exploitation. *Nature Reviews Drug Discovery*, *3*(9), 771–784.

Di Marzo, V., De Petrocellis, L., & Bisogno, T. (2005). The biosynthesis, fate and pharmacological properties of endocannabinoids. *Handbook of Experimental Pharmacology*, *168*, 147–185.

Elsohly, M. A., & Slade, D. (2005). Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life Sciences*, *78*(5), 539–548.

Felder, C. C., Nielsen, A., Briley, E. M., Palkovits, M., Priller, J., Axelrod, J., Becker, G. W., et al. (1996). Isolation and measurement of the endogenous cannabinoid receptor agonist, anandamide, in brain and peripheral tissues of human and rat. *FEBS Letters*, *393*(2–3), 231–235.

Galiegue, S., Mary, S., Marchand, J., Dussossoy, D., Carriere, D., Carayon, P., . . ., Casellas, P. (1995). Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *European Journal of Biochemistry*, *232*(1), 54–61.

Gaoni, Y., & Mechoulam, R. (1964). Isolation, structure and partial synthesis of an active constituent of hashish. *Journal of the American Chemistry Society*, *86*, 1646–1647.

Gerard, C. M., Mollereau, C., Vassart, G., & Parmentier, M. (1991). Molecular cloning of a human cannabinoid receptor which is also expressed in testis. *Biochemical Journal*, *279*(Pt 1), 129–134.

Gong, J. P., Onaivi, E. S., Ishiguro, H., Liu, Q. R., Tagliaferro, P. A., Brusco, A., & Uhl, G. R. (2006). Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Research*, *1071*(1), 10–23.

Griffin, G., Tao, Q., & Abood, M. E. (2000). Cloning and pharmacological characterization of the rat CB(2) cannabinoid receptor. *Journal of Pharmacology and Experimental Therapeutics*, *292*(3), 886–894.

Hanuš, L. O. (2009). Pharmacological and therapeutic secrets of plant and brain (endo) cannabinoids. *Medicinal Research Reviews*, *29*(2), 213–271.

Herkenham, M., Lynn, A. B., Little, M. D., Johnson, M. R., Melvin, L. S., De Costa, B. R., & Rice, K. C. (1990). Cannabinoid receptor localization in brain. *Proceedings of the National Academy of Sciences of the United States of America*, *87*(5), 1932–1936.

Herrera, B., Carracedo, A., Diez-Zaera, M., Gomez del Pulgar, T., Guzman, M., & Velasco, G. (2006). The CB2 cannabinoid receptor signals apoptosis via ceramide-dependent activation of the mitochondrial intrinsic pathway. *Experimental Cell Research*, *312*(11), 2121–2131.

Howlett, A. C. (2005). Cannabinoid receptor signaling. *Handbook of Experimental Pharmacology*, *168*, 53–79.

Howlett, A. C., Blume, L. C., & Dalton, G. D. (2010). CB(1) cannabinoid receptors and their associated proteins. *Current Medicinal Chemistry*, *17*(14), 1382–1393.

Howlett, A. C., & Fleming, R. M. (1984). Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. *Molecular Pharmacology*, *26*(3), 532–538.

Howlett, A. C., Qualy, J. M., & Khachatrian, L. L. (1986). Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. *Molecular Pharmacology*, *29*(3), 307–313.

Howlett, A. C., & Shim, J. Y. (2004). Cannabinoid receptors and signal transduction. In V. Di Marzo (Ed.), *Cannabinoids* (pp. 84–97). New York, NY/Georgetown, TX: Kluwer Academic/Plenum Publishers–Landes Bioscience.

Hsieh, C., Brown, S., Derleth, C., & Mackie, K. (1999). Internalization and recycling of the CB1 cannabinoid receptor. *Journal of Neurochemistry*, *73*(2), 493–501.

Izzo, A. A., Borrelli, F., Capasso, R., Di Marzo, V., & Mechoulam, R. (2009). Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends in Pharmacological Sciences*, *30*(10), 515–527.

Klein, T. W., & Cabral, G. A. (2006). Cannabinoid-induced immune suppression and modulation of antigen-presenting cells. *Journal of Neuroimmune Pharmacology*, *1*(1), 50–64.

Lawrence, D., & Gill, E. (1975). The effects of Δ1-tetrahydrocannabinol and other cannabinoids on spin-labeled liposomes and their relationship to mechanisms of general anesthesia. *Molecular Pharmacology*, *11*(5), 595–602.

Little, P. J., Compton, D. R., Johnson, M. R., Melvin, L. S., & Martin, B. R. (1988). Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *Journal of Pharmacology and Experimental Therapeutics*, *247*(3), 1046–1051.

Mack, A., & Joy, J. (2000). A brief history of medical marijuana. In *Marijuana as medicine? The science beyond the controversy* (pp. 13–19). Washington, DC: National Academies Press.

Mackie, K. (2005). Distribution of cannabinoid receptors in the central and peripheral nervous system cannabinoids. *Handbook of Experimental Pharmacology*, *168*, 299–325.

Martin, B. R., Mechoulam, R., & Razdan, R. K. (1999). Discovery and characterization of endogenous cannabinoids. *Life Sciences*, *65*(6–7), 573–595.

Matsuda, L. A. (1997). Molecular aspects of cannabinoid receptors. *Critical Reviews in Neurobiology*, *11*(2–3), 143–166.

Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., & Bonner, T. I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature*, *346*(6284), 561–564.

Munro, S., Thomas, K. L., & Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, *365*(6441), 61–65.

Onaivi, E. S., Ishiguro, H., Gong, J. P., Patel, S., Meozzi, P. A., Myers, L., . . ., Uhl, G. R. (2008). Brain neuronal CB2 cannabinoid receptors in drug abuse and depression: from mice to human subjects. *PLoS One*, *3*(2), e1640.

Onaivi, E. S., Ishiguro, H., Gong, J. P., Patel, S., Meozzi, P. A., Myers, L., . . ., Uhl, G. R. (2008). Functional expression of brain neuronal CB2 cannabinoid receptors are involved in the effects of drugs of abuse and in depression. *Annals of the New York Academy of Sciences*, *1139*, 434–449.

Onaivi, E. S., Ishiguro, H., Gong, J. P., Patel, S., Perchuk, A., Meozzi, P. A., . . ., Uhl, G. R. (2006). Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. *Annals of the New York Academy of Sciences*, *1074*, 514–536.

Ortega-Gutierrez, S., & Lopez-Rodriguez, M. L. (2005). CB1 and CB2 cannabinoid receptor binding studies based on modeling and mutagenesis approaches. *Mini Reviews in Medicinal Chemistry*, *5*(7), 651–658.

Pacher, P., Bátkai, S., & Kunos, G. (2006). The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacological Reviews*, *58*(3), 389–462.

Palazuelos, J., Aguado, T., Pazos, M. R., Julien, B., Carrasco, C., Resel, E., . . ., Galve-Roperh, I. (2009). Microglial CB2 cannabinoid receptors are neuroprotective in Huntington's disease excitotoxicity. *Brain*, *132*(Pt 11), 3152–3164.

Pertwee, R. G. (1997). Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacology and Therapeutics*, *74*(2), 129–180.

Piomelli, D. (2003). The molecular logic of endocannabinoid signalling. *Nature Reviews Neuroscience*, *4*(11), 873–884.

Raitio, K., Salo, O., Nevalainen, T., Poso, A., & Jarvinen, T. (2005). Targeting the cannabinoid CB2 receptor: mutations, modeling and development of CB2 selective ligands. *Current Medicinal Chemistry*, *12*(10), 1217–1237.

Rhee, M. H., Bayewitch, M., Avidor-Reiss, T., Levy, R., & Vogel, Z. (1998). Cannabinoid receptor activation differentially regulates the various adenylyl cyclase isozymes. *Journal of Neurochemistry*, *71*(4), 1525–1534.

Rice, W., Shannon, J. M., Burton, F., & Fiedeldey, D. (1997). Expression of a brain-type cannabinoid receptor (CB1) in alveolar Type II cells in the lung: regulation by hydrocortisone. *European Journal of Pharmacology*, *327*(2–3), 227–232.

Rodriguez de Fonseca, F., Del Arco, I., Bermudez-Silva, F. J., Bilbao, A., Cippitelli, A., & Navarro, M. (2005). The endocannabinoid system: physiology and pharmacology. *Alcohol and Alcoholism*, *40*(1), 2–14.

Rom, S., & Persidsky, Y. (2013). Cannabinoid receptor 2: potential role in immunomodulation and neuroinflammation. *Journal of Neuroimmune Pharmacology*, *8*(3), 608–620.

Schatz, A. R., Lee, M., Condie, R. B., Pulaski, J. T., & Kaminski, N. E. (1997). Cannabinoid receptors CB1 and CB2: a characterization of expression and adenylate cyclase modulation within the immune system. *Toxicology and Applied Pharmacology*, *142*(2), 278–287.

Shim, J. Y., Welsh, W. J., & Howlett, A. C. (2003). Homology model of the CB1 cannabinoid receptor: sites critical for nonclassical cannabinoid agonist interaction. *Biopolymers*, *71*(2), 169–189.

Shire, D., Calandra, B., Rinaldi-Carmona, M., Oustric, D., Pessegue, B., Bonnin-Cabanne, O., . . ., Ferrara, P. (1996). Molecular cloning, expression and function of the murine CB2 peripheral cannabinoid receptor. *Biochimica et Biophysica Acta*, *1307*(2), 132–136.

Smith, T. H., Sim-Selley, L. J., & Selley, D. E. (2010). Cannabinoid CB1 receptor-interacting proteins: novel targets for central nervous system drug discovery? *British Journal of Pharmacology*, *160*(3), 454–466.

Song, Z. H., & Bonner, T. I. (1996). A lysine residue of the cannabinoid receptor is critical for receptor recognition by several agonists but not WIN55212-2. *Molecular Pharmacology*, *49*(5), 891–896.

Tao, Q., McAllister, S. D., Andreassi, J., Nowell, K. W., Cabral, G. A., Hurst, D. P., . . ., Abood, M. E. (1999). Role of a conserved lysine residue in the peripheral cannabinoid receptor (CB2): evidence for subtype specificity. *Molecular Pharmacology*, *55*(3), 605–613.

Turner, C. E., Elsohly, M. A., & Boeren, E. G. (1980). Constituents of Cannabis sativa L. XVII. A review of the natural constituents. *Journal of Natural Products*, *43*(2), 169–234.

Turu, G., & Hunyady, L. (2010). Signal transduction of the CB1 cannabinoid receptor. *Journal of Molecular Endocrinology*, *44*(2), 75–85.

Van Sickle, M. D., Duncan, M., Kingsley, P. J., Mouihate, A., Urbani, P., Mackie, K., . . ., Sharkey, K. A. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science*, *310*(5746), 329–332.

Velasco, G., Galve-Roperh, I., Sanchez, C., Blazquez, C., Haro, A., & Guzman, M. (2005). Cannabinoids and ceramide: two lipids acting hand-by-hand. *Life Sciences*, *77*(14), 1723–1731.

Xie, X. Q., Chen, J. Z., & Billings, E. M. (2003). 3D structural model of the G-protein-coupled cannabinoid CB2 receptor. *Proteins*, *53*(2), 307–319.

Yang, S. (1998). *The divine farmer's materia medica: a translation of the Shen nong ben cao jing*. Boulder, CO: Blue Poppy Enterprises, Inc.

Yao, B., & Mackie, K. (2009). Endocannabinoid receptor pharmacology. In D. Kendall, & S. Alexander (Eds.), *Behavioral neurobiology of the endocannabinoid system* (pp. 37–63). Berlin Heidelberg, Germany: Springer.

CHAPTER

59

Signaling and Regulation of the Cannabinoid CB_1 Receptor

M.R. Hunter, D.B. Finlay, M. Glass

Department of Pharmacology and Clinical Pharmacology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

SUMMARY POINTS

- CB_1 primarily signals through $G\alpha_{i/o}$ proteins, resulting in decreased adenylate cyclase activity and phosphorylation of ERK and other mitogen kinases.
- CB_1 can also signal through $G\alpha_s$, $G\alpha_q$, $G\alpha_{12/13}$, and $G\alpha_z$ pathways, depending on the cellular conditions and the agonist applied.
- A wide range of ion channels are modulated in response to CB_1 signaling.
- CB_1 is a constitutively active receptor.
- CB_1 signaling is attenuated by the processes of desensitization and internalization, which ultimately result in receptors being degraded. The cell must repopulate the cell surface with newly-synthesized receptors to regain CB_1-mediated signaling responses.

KEY FACTS ABOUT GPCRs

- Also called seven transmembrane or heptahelical receptors.
- Located in the cell membrane, and transduce signals across the cell membrane.
- Ligands (such as neurotransmitters, hormones or drugs) bind to the extracellular region resulting in a conformational change in the receptor which activates G proteins inside the cell.
- Activation of the G protein triggers a series of intracellular signaling reactions.
- G protein coupled receptors are thought to be desensitized by phosphorylation and internalization.

KEY FACTS ABOUT GPCR PHARMACOLOGY

- A drug which binds to a receptor and activates it is called an agonist.
- A drug which binds to a receptor, but does not activate it, or inactivate it, is called an antagonist. Antagonists produce their effects by preventing agonists from binding to the receptor.
- If a receptor has high basal activity in the absence of agonist (constitutive activity), then inverse agonism can be seen. An inverse agonist is a drug which binds to a receptor and inactivates it, resulting in decreased signaling.

LIST OF ABBREVIATIONS

Δ^9-THC	Δ^9-Tetrahydrocannabinol
2-AG	2-arachidonoylglycerol
cAMP	Cyclic adenosine monophosphate
CB_1	Cannabinoid receptor 1
CB_2	Cannabinoid receptor 2
ERK/pERK	Extracellular signal-regulated kinase/ phosophorylated extracellular signal-regulated kinase
GDP/GTP	Guanosine diphosphate/guanosine triphosphate
GIRK	G protein coupled inwardly rectifying K^+ channels

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00069-7
Copyright © 2017 Elsevier Inc. All rights reserved.

GPCR G protein coupled receptor
GRK G protein coupled receptor kinases
PI3K Phosphoinositide 3-kinase
PLC Phospholipase C
PTX Pertussis toxin

INTRODUCTION

Cannabis-like (cannabinoid) agonists such as Δ^9-tetrahydrocannabinol (Δ^9-THC) have long been reported to exert inhibitory effects on the activity of the enzyme adenylate cyclase, which regulates intracellular levels of the second messenger cyclic adenosine monophosphate (cAMP) (Dill & Howlett, 1988). The receptors found to be responsible for this functional effect are G protein coupled receptors (GPCRs). There are currently two confirmed GPCRs which primarily respond to cannabinoids, named the type 1 cannabinoid receptor (CB_1) and type 2 cannabinoid receptor (CB_2). While there is some evidence that a third, or possibly fourth, cannabinoid receptor may exist (Pertwee et al., 2010), this chapter will focus on CB_1 as the receptor mediating the psychoactive effects of Δ^9-THC.

GPCRs signal predominantly by coupling to a family of heterotrimeric G proteins, anchored on the cytoplasmic face of the plasma membrane. Activation of the receptor enables a recruited G protein to exchange a bound GDP molecule for GTP, causing the trimer to dissociate into α and βγ subunits, which proceed to affect a variety of cellular functions. GPCRs in general are capable of activating a large number of intracellular signaling cascades, depending on factors such as the cell type in which they are expressed, the type of G protein bound by the active receptor, and coexpression of the receptor with accessory proteins which may modify the effector coupling. The "coupling" of a GPCR describes the type of G protein α subunit that it activates, as different subunits exert inhibitory ($G\alpha_{i/o}$) or stimulatory ($G\alpha_s$) effects on adenylate cyclase function; cause activation of phospholipase C ($G\alpha_q$); or alter Rho-GTPase signaling pathways ($G\alpha_{12/13}$) (Neves, Ram, & Iyengar, 2002). Both CB_1 and CB_2 are $G\alpha_{i/o}$-coupling receptors (Felder et al., 1995), although this classification is not absolute, and is discussed in detail later in the chapter. The activity of Gβγ subunits is less well characterized, but the various subtypes are able to activate a wide range of effectors, including mitogen-activated protein kinases, ion channels, other receptors, as well as affecting adenylate cyclase function (Dupré, Robitaille, Rebois, & Hébert, 2009). GPCRs also interact with arrestins, and these interactions have intrinsic effects on cell signaling pathways, as well as modifying the activity state of the receptor itself.

CB_1 Agonists

Once the primary psychoactive constituent in marijuana, Δ^9-THC, was elucidated in 1964 (Gaoni & Mechoulam, 1964), a raft of synthetic cannabinoid ligands (such as CP55,940 and WIN55,212-2) were developed. The identification of the endogenous ligands anandamide and 2-arachidonoylglycerol (2-AG) has led to a greater appreciation for the function of the endocannabinoid system. However, it should be noted that cannabinoid receptor pharmacology is largely understood in the context of synthetic agonists, due to their greater potency. Significantly, despite exhibiting high affinity for CB_1 (low nanomolar concentrations), Δ^9-THC is characterized as a partial agonist at this receptor; that is, compared to ligands like HU210, CP55,940, and 2-AG, maximal Δ^9-THC-induced CB_1 signaling is of lower magnitude (eg, Pertwee, 2008; see Fig. 59.1 for structures).

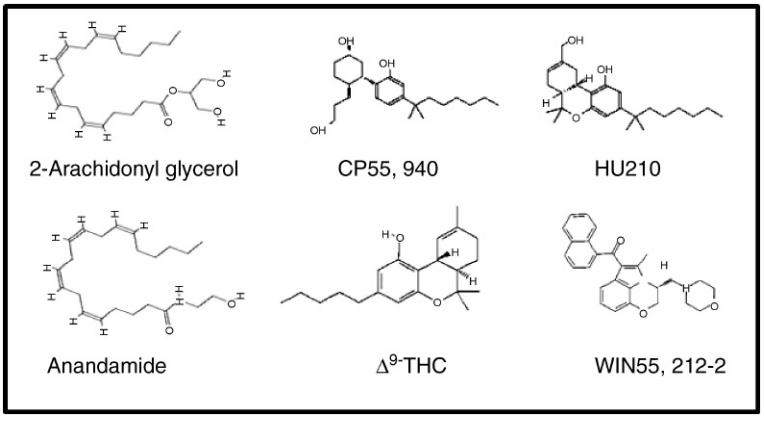


FIGURE 59.1 **Structures of Δ^9-THC; the endocannabinoids 2-arachidonylglycerol (2-AG) and anandamide; and commonly utilized synthetic cannabinoids CP55,940, WIN55,212-2, and HU210.**

CB_1 $G\alpha_I$ PROTEIN COUPLING AND CANONICAL SIGNALING

G Protein Coupling and Modulation of Adenylate Cyclase

Characterization of CB_1 signaling was initiated by Howlett (1984), prior to identification of the cannabinoid receptors. Howlett began investigating the modulatory effects on adenylate cyclase of arachidonic acid derivatives and phytocannabinoids, including Δ^9-THC. This was based on the understanding that cAMP is central to cellular metabolism by, for example, modulating the activity of cAMP-dependent protein kinase A, phosphodiesterases, and ion channels (Antoni, 2012). Δ^9-THC was found to inhibit adenylate cyclase, mediating a cAMP reduction in neuroblastoma cells (Howlett, 1984; Howlett & Fleming, 1984).

Howlett, Qualy, and Khachatrian (1986) also observed that this inhibition of cAMP was sensitive to pertussis toxin (PTX). PTX prevents $G\alpha_i$ protein-receptor interactions by locking the G protein in the GDP-bound state, so this sensitivity implicated $G\alpha_i$-coupling as important in cannabinoid receptor signaling, and suggested that Δ^9-THC was acting upon a GPCR. Indeed, $G\alpha_i$-coupling is today the best-characterized CB_1 signaling phenotype, and is generally considered the canonical signaling pathway for receptor activity (see Fig. 59.2).

The first characterization of brain cannabinoid binding sites was reported by Devane, Dysarz, Johnson, Melvin, and Howlett (1988). However, the molecular identity of CB_1 was only revealed in 1990, upon ligand screening of a novel 473 amino acid protein whose gene was first isolated from a rat cortex cDNA library (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990). In a Chinese hamster ovary K1 (CHO K1) cell line stably expressing this novel GPCR, treatment with the cannabinoid ligands Δ^9-THC and CP55,940 was found to inhibit adenylate cyclase activity. It was further determined that this modulation of adenylate cyclase activity was sensitive to PTX and, therefore, a consequence of $G\alpha_i$ protein coupling.

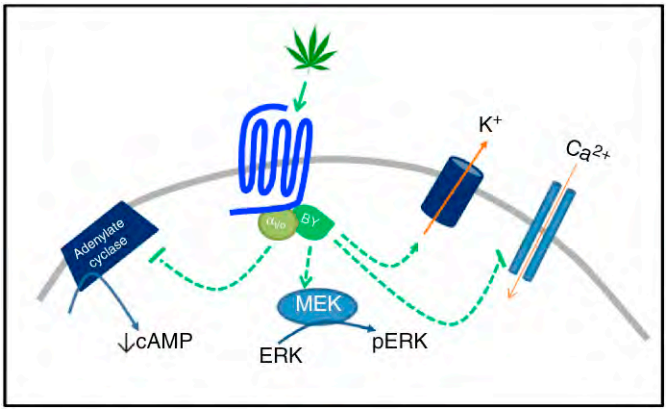


FIGURE 59.2 **Canonical $G\alpha_{i/o}$ mediated CB_1 signaling.** Following activation of CB_1 by an agonist, such as Δ^9-THC (indicated by the cannabis leaf), $G\alpha_{i/o}$ signaling is activated. This results in the activation of inwardly rectifying potassium channels; inhibition of N-, P-, and Q-type calcium channels; activation of MAP-kinase kinase (MEK), leading to phosphorylation of ERK; and inhibition of adenylate cyclase leading to decreased intracellular levels of cAMP.

Although GPCR signaling has traditionally centered around Gα subunit-modulated signal transduction pathways, the role of the Gβγ dimer has become increasingly important in the field of GPCR signaling. Gβγ effectors are now described independently of Gα-mediated signaling, and include modulation of (among others) Gα-independent adenylate cyclase, phosphatidylinositol-3-kinase (PI3K), extracellular signal-regulated kinase (ERK) phosphorylation, and GPCR kinase (GRK) activity (Dupré et al., 2009). Free Gβγ can also selectively activate inwardly rectifying potassium channels and inhibit N-, P/Q-, and R-type voltage-gated Ca^{2+} channels (Currie, 2010; Huang, Slesinger, Casey, Jan, & Jan, 1995).

Phosphorylation of ERK by CB_1

The extracellular signal regulated kinases ERK1/2 are mitogen-activated kinases activated in response to a variety of extracellular signals, and are involved in a wide range of processes, such as cellular proliferation, differentiation, and cell cycle progression. Bouaboula et al. (1995) reported cannabinoid-mediated ERK phosphorylation in CHO cells heterologously expressing CB_1. This pERK stimulation was sensitive to PTX (meaning that the activation pathway was $G\alpha_i$-protein-mediated), but was not Gα or cAMP-mediated, suggestive of a Gβγ-mediated signal. Different researchers have empirically demonstrated CB_1-mediated Gβγ signaling through ERK: nonpigmented ciliary epithelial cells (which endogenously express CB_1) treated with the CB_1 agonist WIN55212-2 induced an outwardly rectifying Cl^- current (Shi et al., 2003). When Gβγ was sequestered, treatment with WIN55212-2 failed to activate this Cl^- current. Interestingly, through pharmacological methods, it was established this effect on Cl^- currents was the consequence of CB_1 signaling, via Gβγ, downstream of phosphorylation of ERK (Shi et al., 2003).

Phosphorylation of ERK has also been reported in hippocampal brain slices on treatment with endocannabinoid ligands (anandamide, 2-AG), in addition to Δ^9-THC. Although CB_1 signaling through pERK has been reported as being PI3K-dependent in other biological systems (Bouaboula et al., 1997), in hippocampal slices the endocannabinoid-induced pERK response was not blocked when PI3K was inhibited with LY294002. Rather, ERK phosphorylation was found to be downstream of cAMP inhibition (Derkinderen et al., 2003). The different pathways for ERK signaling illustrate the complexity

of cannabinoid receptor signaling; receptor activation results in signal transduction through different pathways, depending on the physiology of the host cell type.

Some reports also suggest that CB_1 induces ERK phosphorylation through a pathway involving activation of receptor tyrosine kinases. CB_1 transactivation of vascular endothelial growth factor receptor was shown to induce pERK in a $G\alpha_i$ protein-mediated manner (Rubovitch, Gafni, & Sarne, 2004), and similar effects were induced on cannabinoid-mediated epidermal growth factor transactivation, although the signaling pathway involved was not described (Hart, Fischer, & Ullrich, 2004). Fyn, a member of the Src family of tyrosine kinases, also appears to be involved in the CB_1 pERK response, as Fyn knockout mice demonstrated a loss of cannabinoid-mediated pERK induction (Derkinderen et al., 2003).

CB_1 Associated Ion Channel Modulation

CB_1 mediated modulation of ion channels is well-reported in the current literature, but the large variety of channels influenced by receptor activity makes their characterization complex because, as noted, not all are regulated through the canonical $G\alpha_i$ protein-mediated signaling pathway. In particular, calcium ion channel modulation by CB_1 signaling has layers of complexity. Different pathways are involved in regulation of N-type (Pan, Ikeda, & Lewis, 1996; Mackie & Hille, 1992), L-type (Gebremedhin, Lange, Campbell, Hillard, & Harder, 1999), P/Q-type (Twitchell, Brown, & Mackie, 1997), and T-type (Chemin, Monteil, Perez-Reyes, Nargeot, & Lory, 2001) channels.

Calcium Channels

Cannabinoid inhibition of N-type (neural) Ca^{2+} channels is the most widely established Ca^{2+} ion channel target of CB_1 activity. Mackie et al. discovered that cannabinoid agonists reversibly reduced Ca^{2+} current in differentiated NG108-15 (mouse neuroblastoma) cells. This inhibition was concentration-dependent and $G\alpha_i$ protein-mediated (Mackie & Hille, 1992). The channel responsible for the conduction of these Ca^{2+} currents was determined as N-type, because pretreatment of cells with the selective N-type channel blocker ω-conotoxin-GVIA attenuated cannabinoid-mediated current inhibition (Mackie & Hille, 1992). Similar cannabinoid-mediated inhibition of N-type channels was also reported in superior cervical ganglion neurons (Pan et al., 1996).

Channels conducting Ca^{2+} currents detected in cat arterial smooth muscle cells were identified as L-type (long-lasting), due to their high voltage threshold for channel activation, and blockade by nifedipine and cadmium ions. The effects of cannabinoid agonists on these channels were investigated to probe a possible role in cannabinoid-mediated cerebral vasodilation. Treating cells with cannabinoid agonists resulted in a significant and concentration-dependent inhibition of peak Ca^{2+} current (Gebremedhin et al., 1999). It was also noted that cannabinoid-mediated inhibition of L-type Ca^{2+} currents was unexpected, as other publications have ruled out L-type channels as contributing appreciably to cannabinoid-driven Ca^{2+} currents (Mackie & Hille, 1992), and GPCRs were only seldom associated with Ca^{2+} current modulation via L-type channels. Cannabinoid-mediated L-type Ca^{2+} current inhibition was abolished by PTX pretreatment (Gebremedhin et al., 1999), demonstrating that these effects are $G\alpha_i$ protein-dependent, although the downstream pathways (and whether the effect was Gα- or Gβγ-mediated) were not delineated. L-type Ca^{2+} channel modulation by cannabinoids has been reported more recently, when the Gα subunit of the G protein was found to be responsible for channel conductance in nucleus tractus solitarius neurons, as antibodies against $G\alpha_i$ subunits, but not Gβγ subunits, attenuated WIN55212-2-induced Ca^{2+} current inhibition (Endoh, 2006).

P-(Purkinje) and Q-type Ca^{2+} channels are also inhibited by CB_1 signaling. WIN55212-2 inhibited Ca^{2+} currents in transfected AtT20 cells in a PTX-sensitive manner, but these effects were attenuated when the cells were pretreated with ω-conotoxin-MVIIC (a selective blocker of P/Q Ca^{2+} channels). It was also noted that the extent of cannabinoid-mediated Ca^{2+} current inhibition through P/Q-type channels was subordinate to the influence of cannabinoids on N-type channels: approximately 70% of WIN55212-2-induced current inhibition was carried by N-type channels (Twitchell et al., 1997). Similar P-type channel-directed Ca^{2+} inhibition has been reported for anandamide in Purkinje neurons (Fisyunov, Tsintsadze, Min, Burnashev, & Lozovaya, 2006).

Given the number of Ca^{2+} channels involved in cannabinoid-mediated Ca^{2+} ion conductance, it is challenging to determine the signaling pathways through which these effects are elicited. In general, however, all channels discussed are sensitive to PTX.

Potassium Channels

Cannabinoid-mediated regulation of potassium ion (K^+) channels has also been detailed. In some systems, K^+ conductance is known to be both Gβγ subunit-dependent, and sensitive to changes in intracellular cAMP levels [reviewed by (Yamada, Inanobe, & Kurachi, 1998)], implicating both the α and βγ subunits of G_i in the signaling. For example, CB_1's modulation of inwardly rectifying potassium currents (through G protein coupled inwardly rectifying K^+ channels, GIRKs) were found to be sensitive to PTX, and may be Gβγ-mediated (Ho, Uezono, Takada, Takase, & Izumi, 1999; Mackie, Lai, Westenbroek, & Mitchell, 1995). Specifically, GIRK1 and GIRK4 are implicated in cannabinoid-mediated K^+

conductance, as both increase inward K^+ flux downstream of CB_1 stimulation with WIN55212-2 (Mcallister, Griffin, Satin, & Abood, 1999).

CB_1 CONSTITUTIVE SIGNALING

Traditionally, drug action was interpreted on the basis that receptor systems have no activity unless stimulated by agonist. This notion has been dispelled, as constitutive (ligand-independent) activities have been reported for many GPCRs [reviewed in (Seifert & Wenzel-Seifert, 2002)]. This is supported by the key observation that drugs which had been classified as antagonists (drugs that displace agonists but do not influence receptor activity), are often seen acting as inverse agonists; they are capable of reducing the constitutive activity of their target. In the case of the CB_1 constitutive activity has been detected through a variety of endpoints.

Experiments first demonstrated a constitutive phenotype for CB_1 by revealing inverse agonist action of the drug SR141716A (also known as rimonabant). Firstly, CB_1 mediated G protein turnover in the absence of agonist was demonstrated using the GTPγS assay, which measures G protein cycling through accumulation of radioisotope-labeled GTPγS (a poorly-hydrolysable GTP analog). In this experiment, SR141716A induced a reduction in the extent of GTPγS accumulation (Landsman, Burkey, Consroe, Roeske, & Yamamura, 1997). It has also been observed that in human CB_1-expressing CHO cells, basal levels of ERK phosphorylation were higher than those observed in matched wild-type controls. This effect could be potently attenuated upon treatment with SR141716A (Bouaboula et al., 1997). Similarly, Bouaboula et al. (1997) showed that CB_1 exert basal inhibitory effects on cAMP levels in a manner sensitive to SR141716A. Notably, the potencies with which SR141716A induced ERK inhibition and cAMP increases in unstimulated cells were similar to its binding properties, arguing that the response was directly receptor-mediated, and not due to endogenous agonists present in the culture, or off-target effects on other components of the cell background (Bouaboula et al., 1997).

Furthermore, CB_1 constitutive activity has also been demonstrated in G protein-mediated ion channel conductance, and receptor trafficking, as SR141716A potently blocked agonist-mediated inhibition of Ca^{2+} currents through N-type Ca^{2+} channels. SR141716A also increased Ca^{2+} conductance above basal levels in otherwise unstimulated cells which were microinjected with CB_1 cRNA (Pan, Ikeda, & Lewis, 1998). Recent publications have also reported unstimulated CB_1 trafficking activity, quantifiable in the absence of any drug, and abatable on treatment with multiple CB_1 inverse agonists, including SR141716A (Leterrier, Bonnard, Carrel, Rossier, & Lenkei, 2004; Grimsey, Graham, Dragunow, & Glass, 2010).

CB_1 G PROTEIN COUPLING PROMISCUITY

Alongside the canonical $G\alpha_i$ protein-associated functional consequences of CB_1 signaling, potential for CB_1-mediated activation of other pathways has also been reported in response to specific environmental or pharmacological conditions. In particular, receptor coupling to three non-$G\alpha_i$-type G proteins has been reported, leading CB_1 to be described as a "promiscuous G protein coupler" (see Fig. 59.3).

The most-reported and possibly dominant "secondary" signaling pathway for CB_1 is the coupling of stimulatory $G\alpha_s$ proteins, first reported by Glass and Felder (1997). In cells where CB_1 is coexpressed with D_2 dopamine receptors, costimulation with a CB_1 agonist (HU210) and a D_2 agonist (quinpirole) resulted in an increase in forskolin-induced intracellular cAMP levels (Glass & Felder, 1997)—a finding which challenged the understanding that both receptors couple $G\alpha_i$ proteins. More recent research has revealed that this coupling switch is coexpression-dependent (Jarrahian, Watts, & Barker, 2004), and possibly mediated by a CB_1/D_2 receptor heteromer (Kearn, Blake-Palmer, Daniel, Mackie, & Glass, 2005). PTX pretreatment is sufficient to unmask

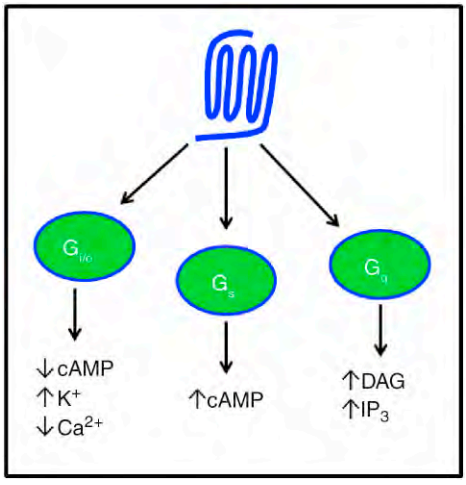


FIGURE 59.3 G protein coupling of CB_1. Activation of CB_1 has been suggested to activate multiple G proteins under particular circumstances, resulting in a diverse range of signaling pathway activation. The predominant pathway is thought to be activation of $G\alpha_{i/o}$ proteins, resulting in decreased cAMP; stimulation of inwardly rectifying potassium channels; and blockade of N-, P-, and Q-type calcium channels. Under conditions of limited G_i, G_s signaling has been described, resulting in increased levels of cAMP. Following activation with some ligands (notably WIN55,212-2), G_q mediated signaling has been reported, consisting of activation of phospholipase C, leading to increased levels of inositol triphosphate (IP3), and diacyl glycerol (DAG).

a CB_1-mediated $G\alpha_s$ response, in both primary striatal neurons (where D2 is coexpressed) and transfected CB_1-CHO cells (Glass & Felder, 1997).

$G\alpha_q$ coupling by CB_1 has also been reported. Unlike $G\alpha_i$ and $G\alpha_s$ signaling, $G\alpha_q$ signaling does not influence adenylate cyclase activity. Rather, $G\alpha_q$ activates phospholipase C (PLC), and its downstream signaling cascade, resulting in Ca^{2+} release from the endoplasmic reticulum into the cytoplasm. Stimulation of CB_1 in hippocampal neurons or transfected HEK293 cells with a high concentration (5 μM) of WIN55,212-2 caused a large, SR141716A-sensitive increase of cytosolic Ca^{2+} (Lauckner, Hille, & Mackie, 2005). This response was PTX-sensitive, and was specific to WIN55,212-2. However, the effect was markedly reduced in cells treated with a PLC inhibitor or expressing a $G\alpha_q$ dominant negative, therefore identifying $G\alpha_q$ as the effector (Lauckner et al., 2005).

$G\alpha_{12/13}$ protein signaling is perhaps the least characterized G protein pathway, and is mainly involved in regulation of the actin cytoskeleton; that is, maintenance and remodeling of cell morphology. The small GTPase RhoA is known to be activated downstream of $G\alpha_{12/13}$ signaling, and is also involved in actin dynamics (Suzuki, Nakamura, Mano, & Kozasa, 2003). Furthering the characterization of CB_1 as a promiscuous coupler of G proteins, anandamide was shown to induce rounding of B103 rat neuroblastoma cells, in a manner consistent with $G\alpha_{12/13}$ protein coupling [insensitive to PTX-treatment, but sensitive to treatment with a Rho inhibitor (Ishii & Chun, 2002)]. Finally, in vivo studies have suggested coupling of CB_1 to the $G\alpha_{i/o}$-related G protein $G\alpha_z$, as mice with reduced levels of $G\alpha_z$ proteins, unlike WT mice, demonstrate attenuated cannabinoid-mediated tolerance to analgesia (Garzon, De La Torre-Madrid, Rodriguez-Munoz, Vicente-Sanchez, & Sanchez-Blazquez, 2009).

CB_1 REGULATION

Desensitization of CB_1

CB_1 receptors lose their responsiveness to ligand (desensitize) within minutes of application of agonist (Jin et al., 1999). As for most GPCRs, this is believed to occur as the result of receptor phosphorylation, followed by removal from the cell surface; this process is illustrated in Fig. 59.4 (Hsieh, Brown, Derleth, & Mackie, 1999; Zastrow, 2003).

GRK proteins act to phosphorylate the intracellular faces of GPCRs, such that G proteins can no longer be activated. Putative phosphorylation sites are found in the C-terminal tail, with mutations of these residues resulting in reduced desensitization in both cell line models and transgenic mice, although phosphorylation of these sites may not be necessary for subsequent receptor internalization (Jin et al., 1999; Straiker, Wager-Miller, & Mackie, 2012; Morgan et al., 2014). Subsequent recruitment of β-arrestins abrogates G protein coupling, and internalization occurs via clathrin-coated pits (Marchese, Paing, Temple, & Trejo, 2008; Hsieh et al., 1999). In cultured rat neurons, CB_1 desensitization is GRK2-dependent (Kouznetsova, Kelley, Shen, & Thayer, 2002), and may also interact with GRK3 (Jin et al., 1999). However,

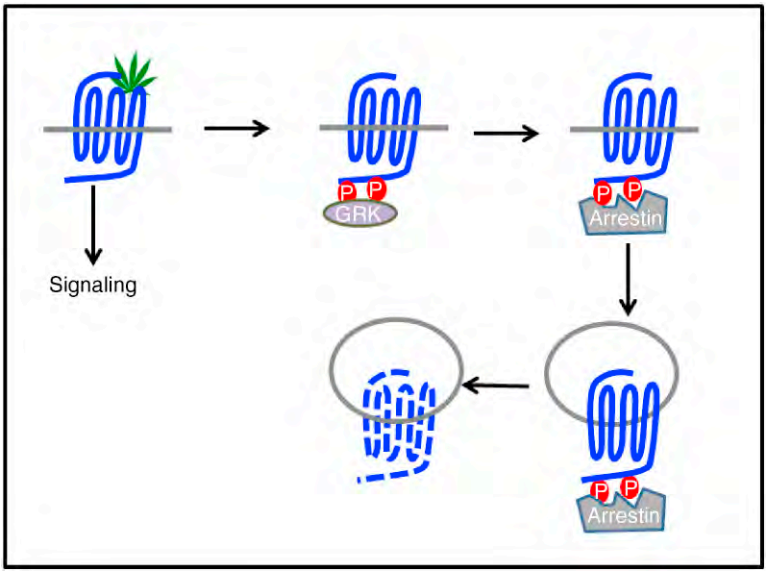


FIGURE 59.4 Regulation of CB_1. Following activation by an agonist, CB_1 (like most G protein coupled receptors) are believed to be phosphorylated and internalized by a conserved pathway. Thus, following activation, the intracellular face of the receptor is phosphorylated by G protein receptor kinase (GRK), leading to arrestin recruitment, and subsequent internalization into a vesicle. Following internalization, CB_1 is thought to undergo lysosomal degradation.

in cultured rabbit smooth muscle cells, GRK5 was found to control phosphorylation (Mahavadi, Sriwai, Huang, Grider, & Murthy, 2014). In the mouse brain, Δ^9-THC treatment results in receptor mediated GRK2 recruitment, and chronic Δ^9-THC treatment results in upregulation of both GRK2 and GRK4 (Rubino et al., 2006). However, it is likely that the exact mechanism of phosphorylation and desensitization may vary between cell types, including standard cell line models, depending on their particular complement of accessory proteins (Atwood, Lopez, Wager-Miller, Mackie, & Straiker, 2011).

Arrestin Recruitment and Signaling

As for other GPCRs, CB_1 is able to interact with β-arrestins, which control aspects of receptor regulation and non-G protein-mediated signaling (Magalhaes, Dunn, & Ferguson, 2012). In general terms, arrestin-mediated signaling can include activation of multiple kinase pathways including Src, ERK, and JNK pathways (Luttrell & Lefkowitz, 2002; Zhan, Kook, Gurevich, & Gurevich, 2014). This provides a second complement of GPCR signaling cascades beyond canonical G protein effects. However, arrestin coupling by CB_1 (and any downstream signaling cascades) is still relatively uncharacterized. In cultured cells, CB_1 is generally observed to interact weakly with β-arrestin2 through a phosphorylation site in the C-terminal tail (Gyombolai, Boros, Hunyady, & Turu, 2013; Daigle, Kwok, & Mackie, 2008), and potentially very weakly with β-arrestin1 (Jin et al., 1999). Furthermore, it has been reported that CB_1 couples to both β-arrestin1 and β-arrestin2 in a ligand-dependent manner (Ahn, Mahmoud, Shim, & Kendall, 2013). Under some conditions, activation of ERK and Src pathways may be solely arrestin-dependent (Mahavadi et al., 2014), although this is not universally observed.

Despite the conflicting data from cell culture experiments, there is some evidence from animal studies that CB_1-arrestin interactions are physiologically important. The behavioral effects of acute doses of Δ^9-THC (eg, antinociception) are increased in β-arrestin2 knockout mice (Breivogel, Lambert, Gerfin, Huffman, & Razdan, 2008; Nguyen et al., 2012), indicating that this arrestin contributes to the desensitization process. However, β-arrestin2 knockout only changes the development of tolerance to chronic Δ^9-THC dosing in some brain regions. For example, receptors in the globus pallidus (which mediate catalepsy) show increased tolerance to stimulation with Δ^9-THC, while receptors in the spinal cord (which mediate nociception) showed reduced tolerance, with behavioral responses reflecting this (Nguyen et al., 2012). Similarly, chronic Δ^9-THC increases β-arrestin1 protein specifically in some (striatum, cerebellum, and hippocampus), but not all, regions of the mouse brain (Rubino et al., 2006). This may reflect the different desensitization-tolerance pathways available in different cell types, which depend on the complement of CB_1-interacting proteins available. It is interesting to note, however, that only Δ^9-THC responses appear to be affected in these β-arrestin2 knockout animals, while responses to synthetic cannabinoids (including synthetic partial agonists) and analogs of the endocannabinoids are unchanged (Breivogel et al., 2008), suggesting a level of complexity yet to be understood.

Internalization and Degradation of CB_1

CB_1 is removed from the cell surface and internalized quickly (Mikasova, Groc, Choquet, & Manzoni, 2008; Grimsey et al., 2010), a process which appears to be controlled by the distal end of the CB_1 C-terminus (independent of the suggested GRK phosphorylation sites) (Straiker et al., 2012). Following internalization into an early endosome, CB_1 is sorted into a degradative pathway (Grimsey et al., 2010). CB_1's helix 8 contains an interaction site for G protein receptor-associated sorting protein 1 (GASP1), a protein which directs receptors to degradative lysosomes (Martini et al., 2007). Indeed, in mice, GASP1 has been observed to be required for the downregulation of CB_1 in response to chronic agonist treatment (Tappe-Theodor et al., 2007). The degradative phenotype of internalized CB_1 affects the cellular regulation of CB_1 signaling, as the state of reduced responsiveness to agonists will persist until new receptors have been synthesized and trafficked to the cell surface.

CONCLUSIONS

CB_1 can signal through a wide range of G protein mediated and independent pathways. Exactly which signaling pathway is activated in a particular cell will depend on the signaling components of the cells. To date, most of our knowledge is based on signaling observed in either transfected cell lines, or primary rodent cells, with a small number of signaling studies in vivo. Further studies will hopefully focus on signaling of CB_1 in primary human cells or tissues, as the culturing of these cells becomes more advanced. Such knowledge should greatly aid in our understanding of how receptors mediate the actions of Δ^9-THC, the endocannabinoids, and the synthetic cannabinoids in the human brain.

MINI-DICTIONARY

Adenylate cyclase/adenylyl cyclase A family of enzymes which convert ATP into cAMP.
Constitutive activity (of a GPCR) GPCR activation which occurs in the absence of agonist.
Desensitization The uncoupling of a receptor from its signaling cascade, resulting in reduced responsiveness to agonist.

ERK (extracellular signal-regulated kinase) pathway Intracellular signaling pathway consisting of a cascade of enzymes which add phosphate groups to the next protein in the pathway. Ultimately, it ends in changes in transcriptional activity in the nucleus.

G proteins A family of GTPases which, when activated by a GPCR, transmit information from the GPCR to downstream effectors (also known as guanosine nucleotide-binding proteins).

G protein coupled receptor A family of seven transmembrane cell surface proteins which respond to external stimuli by coupling (primarily) to intracellular G proteins.

Internalization The cellular process of removing a GPCR from the surface of the cell, thereby decreasing the number of receptors available to extracellular agonist.

Partial agonist A ligand which is able to activate a receptor, but does not elicit the maximum functional response (ie, partial efficacy, even at full receptor occupancy).

References

Ahn, K. H., Mahmoud, M. M., Shim, J. -Y., & Kendall, D. A. (2013). Distinct roles of β-arrestin 1 and β-arrestin 2 in ORG27569-induced biased signaling and internalization of the cannabinoid receptor 1 (CB1). *Journal of Biological Chemistry*, *288*, 9790–9800.

Antoni, F. A. (2012). New paradigms in cAMP signalling. *Molecular and Cellular Endocrinology*, *353*, 3–9.

Atwood, B. K., Lopez, J., Wager-Miller, J., Mackie, K., & Straiker, A. (2011). Expression of G protein-coupled receptors and related proteins in HEK293, AtT20, BV2, and N18 cell lines as revealed by microarray analysis. *BMC Genomics*, *12*, 14.

Bouaboula, M., Perrachon, S., Milligan, L., Canat, X., Rinaldi-Carmona, M., Portier, M., Barth, F., Calandra, B., Pecceu, F., Lupker, J., Maffrand, J. P., Le Fur, G., & Casellas, P. (1997). A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. *Journal of Biological Chemistry*, *272*, 22330–22339.

Bouaboula, M., Poinot-Chazel, C., Bourrie, B., Canat, X., Calandra, B., Rinaldi-Carmona, M., Le Fur, G., & Casellas, P. (1995). Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochemical Journal*, *312*(Pt 2), 637–641.

Breivogel, C. S., Lambert, J. M., Gerfin, S., Huffman, J. W., & Razdan, R. K. (2008). Sensitivity to delta9-tetrahydrocannabinol is selectively enhanced in beta-arrestin2 -/- mice. *Behavioral Pharmacology*, *19*, 298–307.

Chemin, J., Monteil, A., Perez-Reyes, E., Nargeot, J., & Lory, P. (2001). Direct inhibition of T-type calcium channels by the endogenous cannabinoid anandamide. *EMBO Journal*, *20*, 7033–7040.

Currie, K. P. (2010). G protein modulation of CaV2 voltage-gated calcium channels. *Channels (Austin)*, *4*, 497–509.

Daigle, T. L., Kwok, M. L., & Mackie, K. (2008). Regulation of CB1 cannabinoid receptor internalization by a promiscuous phosphorylation-dependent mechanism. *Journal of Neurochemistry*, *106*, 70–82.

Derkinderen, P., Valjent, E., Toutant, M., Corvol, J. C., Enslen, H., Ledent, C., Trzaskos, J., Caboche, J., & Girault, J. A. (2003). Regulation of extracellular signal-regulated kinase by cannabinoids in hippocampus. *Journal of Neuroscience*, *23*, 2371–2382.

Devane, W. A., Dysarz, F. A., III, Johnson, M. R., Melvin, L. S., & Howlett, A. C. (1988). Determination and characterization of a cannabinoid receptor in rat brain. *Molecular Pharmacology*, *34*, 605–613.

Dill, J. A., & Howlett, A. C. (1988). Regulation of adenylate cyclase by chronic exposure to cannabimimetic drugs. *Journal of Pharmacology and Experimental Therapeutics*, *244*, 1157–1163.

Dupré, D. J., Robitaille, M., Rebois, R. V., & Hébert, T. E. (2009). The role of Gβγ subunits in the organization, assembly, and function of GPCR signaling complexes. *Annual Review of Pharmacology and Toxicology*, *49*, 31–56.

Endoh, T. (2006). Pharmacological characterization of inhibitory effects of postsynaptic opioid and cannabinoid receptors on calcium currents in neonatal rat nucleus tractus solitarius. *British Journal of Pharmacology*, *147*, 391–401.

Felder, C. C., Joyce, K. E., Briley, E. M., Mansouri, J., Mackie, K., Blond, O., Lai, Y., Ma, A. L., & Mitchell, R. L. (1995). Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. *Molecular Pharmacology*, *48*, 443–450.

Fisyunov, A., Tsintsadze, V., Min, R., Burnashev, N., & Lozovaya, N. (2006). Cannabinoids modulate the P-type high-voltage-activated calcium currents in purkinje neurons. *Journal of Neurophysiology*, *96*, 1267–1277.

Gaoni, Y., & Mechoulam, R. (1964). Isolation, structure, and partial synthesis of an active constituent of hashish. *Journal of the American Chemistry Society*, *86*, 1646–1647.

Garzon, J., De La Torre-Madrid, E., Rodriguez-Munoz, M., Vicente-Sanchez, A., & Sanchez-Blazquez, P. (2009). Gz mediates the long-lasting desensitization of brain CB1 receptors and is essential for cross-tolerance with morphine. *Molecular Pain*, *5*, 11.

Gebremedhin, D., Lange, A. R., Campbell, W. B., Hillard, C. J., & Harder, D. R. (1999). Cannabinoid CB1 receptor of cat cerebral arterial muscle functions to inhibit L-type Ca^{2+} channel current. *American Journal of Physiology*, *276*, H2085–H2093.

Glass, M., & Felder, C. C. (1997). Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. *Journal of Neuroscience*, *17*, 5327–5333.

Grimsey, N. L., Graham, E. S., Dragunow, M., & Glass, M. (2010). Cannabinoid receptor 1 trafficking and the role of the intracellular pool: implications for therapeutics. *Biochemical Pharmacology*, *80*, 1050–1062.

Gyombolai, P., Boros, E., Hunyady, L., & Turu, G. (2013). Differential beta-arrestin2 requirements for constitutive and agonist-induced internalization of the CB1 cannabinoid receptor. *Molecular and Cellular Endocrinology*, *372*, 116–127.

Hart, S., Fischer, O. M., & Ullrich, A. (2004). Cannabinoids induce cancer cell proliferation via tumor necrosis factor alpha-converting enzyme (TACE/ADAM17)-mediated transactivation of the epidermal growth factor receptor. *Cancer Research*, *64*, 1943–1950.

Ho, B. Y., Uezono, Y., Takada, S., Takase, I., & Izumi, F. (1999). Coupling of the expressed cannabinoid CB1 and CB2 receptors to phospholipase C and G protein-coupled inwardly rectifying K^+ channels. *Receptors and Channels*, *6*, 363–374.

Howlett, A. C. (1984). Inhibition of neuroblastoma adenylate cyclase by cannabinoid and nantradol compounds. *Life Sciences*, *35*, 1803–1810.

Howlett, A. C., & Fleming, R. M. (1984). Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. *Molecular Pharmacology*, *26*, 532–538.

Howlett, A. C., Qualy, J. M., & Khachatrian, L. L. (1986). Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. *Molecular Pharmacology*, *29*, 307–313.

Hsieh, C., Brown, S., Derleth, C., & Mackie, K. (1999). Internalization and recycling of the CB1 cannabinoid receptor. *Journal of Neurochemistry*, *73*, 493–501.

Huang, C. L., Slesinger, P. A., Casey, P. J., Jan, Y. N., & Jan, L. Y. (1995). Evidence that direct binding of G beta gamma to the GIRK1 G protein-gated inwardly rectifying K^+ channel is important for channel activation. *Neuron*, *15*, 1133–1143.

Ishii, I., & Chun, J. (2002). Anandamide-induced neuroblastoma cell rounding via the CB1 cannabinoid receptors. *Neuroreport*, *13*, 593–596.

Jarrahian, A., Watts, V. J., & Barker, E. L. (2004). D2 dopamine receptors modulate Galpha-subunit coupling of the CB1 cannabinoid receptor. *Journal of Pharmacology and Experimental Therapeutics*, *308*, 880–886.

Jin, W., Brown, S., Roche, J. P., Hsieh, C., Celver, J. P., Kovoor, A., Chavkin, C., & Mackie, K. (1999). Distinct domains of the CB1

cannabinoid receptor mediate desensitization and internalization. *Journal of Neuroscience*, *19*, 3773–3780.
Kearn, C. S., Blake-Palmer, K., Daniel, E., Mackie, K., & Glass, M. (2005). Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? *Molecular Pharmacology*, *67*, 1697–1704.
Kouznetsova, M., Kelley, B., Shen, M., & Thayer, S. A. (2002). Desensitization of cannabinoid-mediated presynaptic inhibition of neurotransmission between rat hippocampal neurons in culture. *Molecular Pharmacology*, *61*, 477–485.
Landsman, R. S., Burkey, T. H., Consroe, P., Roeske, W. R., & Yamamura, H. I. (1997). SR141716A is an inverse agonist at the human cannabinoid CB1 receptor. *European Journal of Pharmacology*, *334*, R1–R2.
Lauckner, J. E., Hille, B., & Mackie, K. (2005). The cannabinoid agonist WIN55,212-2 increases intracellular calcium via CB1 receptor coupling to Gq/11 G proteins. *Proceedings of the National Academy of Sciences of the United States of America*, *102*, 19144–19149.
Leterrier, C., Bonnard, D., Carrel, D., Rossier, J., & Lenkei, Z. (2004). Constitutive endocytic cycle of the CB1 cannabinoid receptor. *Journal of Biological Chemistry*, *279*, 36013–36021.
Luttrell, L. M., & Lefkowitz, R. J. (2002). The role of β-arrestins in the termination and transduction of G-protein-coupled receptor signals. *Journal of Cell Science*, *115*, 455–465.
Mackie, K., & Hille, B. (1992). Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proceedings of the National Academy of Sciences of the United States of America*, *89*, 3825–3829.
Mackie, K., Lai, Y., Westenbroek, R., & Mitchell, R. (1995). Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. *Journal of Neuroscience*, *15*, 6552–6561.
Magalhaes, A. C., Dunn, H., & Ferguson, S. S. (2012). Regulation of GPCR activity, trafficking and localization by GPCR-interacting proteins. *British Journal of Pharmacology*, *165*, 1717–1736.
Mahavadi, S., Sriwai, W., Huang, J., Grider, J. R., & Murthy, K. S. (2014). Inhibitory signaling by CB1 receptors in smooth muscle mediated by GRK5/β-arrestin activation of ERK1/2 and Src kinase. *American Journal of Physiology: Gastrointestinal and Liver Physiology*, *306*(6), G535–G545.
Marchese, A., Paing, M. M., Temple, B. R. S., & Trejo, J. (2008). G protein-coupled receptor sorting to endosomes and lysosomes. *Annual Review of Pharmacology and Toxicology*, *48*, 601–629.
Martini, L., Waldhoer, M., Pusch, M., Kharazia, V., Fong, J., Lee, J. H., Freissmuth, C., & Whistler, J. L. (2007). Ligand-induced down-regulation of the cannabinoid 1 receptor is mediated by the G-protein-coupled receptor-associated sorting protein GASP1. *The FASEB Journal*, *21*, 802–811.
Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., & Bonner, T. I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature*, *346*, 561–564.
Mcallister, S. D., Griffin, G., Satin, L. S., & Abood, M. E. (1999). Cannabinoid receptors can activate and inhibit G protein-coupled inwardly rectifying potassium channels in a xenopus oocyte expression system. *Journal of Pharmacology and Experimental Therapeutics*, *291*, 618–626.
Mikasova, L., Groc, L., Choquet, D., & Manzoni, O. J. (2008). Altered surface trafficking of presynaptic cannabinoid type 1 receptor in and out synaptic terminals parallels receptor desensitization. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 18596–18601.
Morgan, D. J., Davis, B. J., Kearn, C. S., Marcus, D., Cook, A. J., Wager-Miller, J., Straiker, A., Myoga, M. H., Karduck, J., Leishman, E., Sim-Selley, L. J., Czyzyk, T. A., Bradshaw, H. B., Selley, D. E., & Mackie, K. (2014). Mutation of putative GRK phosphorylation sites in the cannabinoid receptor 1 (CB1R) confers resistance to cannabinoid tolerance and hypersensitivity to cannabinoids in mice. *The Journal of Neuroscience*, *34*, 5152–5163.
Neves, S. R., Ram, P. T., & Iyengar, R. (2002). G protein pathways. *Science*, *296*, 1636–1639.
Nguyen, P. T., Schmid, C. L., Raehal, K. M., Selley, D. E., Bohn, L. M., & Sim-Selley, L. J. (2012). β-arrestin2 regulates cannabinoid CB1 receptor signaling and adaptation in a central nervous system region-dependent manner. *Biological Psychiatry*, *71*, 714–724.
Pan, X., Ikeda, S. R., & Lewis, D. L. (1996). Rat brain cannabinoid receptor modulates N-type Ca^{2+} channels in a neuronal expression system. *Molecular Pharmacology*, *49*, 707–714.
Pan, X., Ikeda, S. R., & Lewis, D. L. (1998). SR141716A acts as an inverse agonist to increase neuronal voltage-dependent Ca^{2+} currents by reversal of tonic CB1 cannabinoid receptor activity. *Molecular Pharmacology*, *54*, 1064–1072.
Pertwee, R. G. (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. *British Journal of Pharmacology*, *153*, 199–215.
Pertwee, R. G., Howlett, A. C., Abood, M. E., Alexander, S. P. H., Di Marzo, V., Elphick, M. R., Greasley, P. J., Hansen, H. S., Kunos, G., Mackie, K., Mechoulam, R., & Ross, R. A. (2010). International union of basic and clinical pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. *Pharmacological Reviews*, *62*, 588–631.
Rubino, T., Viganò, D., Premoli, F., Castiglioni, C., Bianchessi, S., Zippel, R., & Parolaro, D. (2006). Changes in the expression of G protein-coupled receptor kinases and β-arrestins in mouse brain during cannabinoid tolerance. *Molecular Neurobiology*, *33*, 199–213.
Rubovitch, V., Gafni, M., & Sarne, Y. (2004). The involvement of VEGF receptors and MAPK in the cannabinoid potentiation of Ca^{2+} flux into N18TG2 neuroblastoma cells. *Brain Research. Molecular Brain Research*, *120*, 138–144.
Seifert, R., & Wenzel-Seifert, K. (2002). Constitutive activity of G-protein-coupled receptors: cause of disease and common property of wild-type receptors. *Naunyn Schmiedebergs Archives of Pharmacology*, *366*, 381–416.
Shi, C., Szczesniak, A., Mao, L., Jollimore, C., Coca-Prados, M., Hung, O., & Kelly, M. E. (2003). A3 adenosine and CB1 receptors activate a PKC-sensitive Cl^- current in human nonpigmented ciliary epithelial cells via a G beta gamma-coupled MAPK signaling pathway. *British Journal of Pharmacology*, *139*, 475–486.
Straiker, A., Wager-Miller, J., & Mackie, K. (2012). The CB1 cannabinoid receptor C-terminus regulates receptor desensitization in autaptic hippocampal neurones. *British Journal of Pharmacology*, *165*, 2652–2659.
Suzuki, N., Nakamura, S., Mano, H., & Kozasa, T. (2003). Galpha 12 activates Rho GTPase through tyrosine-phosphorylated leukemia-associated RhoGEF. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 733–738.
Tappe-Theodor, A., Agarwal, N., Katona, I., Rubino, T., Martini, L., Swiercz, J., Mackie, K., Monyer, H., Parolaro, D., Whistler, J., Kuner, T., & Kuner, R. (2007). A molecular basis of analgesic tolerance to cannabinoids. *Journal of Neuroscience*, *27*, 4165–4177.
Twitchell, W., Brown, S., & Mackie, K. (1997). Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. *Journal of Neurophysiology*, *78*, 43–50.
Yamada, M., Inanobe, A., & Kurachi, Y. (1998). G protein regulation of potassium ion channels. *Pharmacology Reviews*, *50*, 723–760.
Zastrow, M. V. (2003). Mechanisms regulating membrane trafficking of G protein-coupled receptors in the endocytic pathway. *Life Sciences*, *74*, 217–224.
Zhan, X., Kook, S., Gurevich, E., & Gurevich, V. (2014). Arrestin-dependent activation of JNK family kinases. In V. V. Gurevich (Ed.), *Arrestins—pharmacology and therapeutic potential*. Berlin Heidelberg, Germany: Springer.

CHAPTER

60

Allosteric Modulation of the Cannabinoid CB1 Receptor

E.E. Cawston, M.R. Hunter, M. Glass

Department of Pharmacology and Clinical Pharmacology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

SUMMARY POINTS

- Several allosteric modulators of CB1 cannabinoid receptors have been described.
- The most studied of these are ORG27569 and PSNCBAM-1.
- These compounds have paradoxical action, increasing affinity but decreasing efficacy of orthosteric ligands in most studied signaling pathways.
- These ligands may also show some probe dependence, having greater effect on CP55,940 than on WIN55,212-2 activity.
- Several other ligands have been suggested to act allosterically at CB1 including lipoxin A4, pregnenolone, and pepcans.
- Receptor heteromerization is another mechanism by which receptor signaling can be altered allosterically.
- CB1 forms heteromers with a number of other GPCRs to alter signaling.
- CB1 heteromers may, therefore, provide a means to selectively target only a subset of CB1 receptors.

KEY FACTS OF ALLOSTERIC MODULATION

- Allosteric regulation is regulation of a receptor by binding of a molecule at a site other than the proteins active (orthosteric) site.
- Allosteric modulators bind to the receptor leading to a conformational change in the protein.
- This might enhance or inhibit the activity of the receptor in the absence of an orthosteric ligand, now termed allo-agonism or allo-antagonism.
- Allosteric modulators can alter the response of the receptor to orthosteric ligand, either by enhancing the ligand's affinity and/or efficacy (positive allosteric modulation) or inhibiting it (negative allosteric modulation).

KEY FACTS ABOUT THE THERAPEUTIC POTENTIAL OF ALLOSTERIC MODULATORS OF GPCRS

- If the allosteric modulator has no intrinsic activity at the receptor, then it will only exert its effects when the endogenous ligand is present, thereby maintaining the anatomical and temporal aspects of endogenous signaling.
- Higher sequence divergence in the allosteric site should enable design of subtype selective modulators.
- Allosteric modulation with limited cooperativity produce a ceiling above which there is no further on-target effect, for example, a negative allosteric modulator could decrease signaling without completely preventing it.

LIST OF ABBREVIATIONS

cAMP	Cyclic adenosine monophosphate
CB1	Cannabinoid receptor 1
CP55,940	(−)-*cis*-3-[2-Hydroxy-4-(1,1-dimethylheptyl) phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00070-3
Copyright © 2017 Elsevier Inc. All rights reserved.

ERK1/2	Extracellular signal-regulated kinase 1/2
GPCR	G protein-coupled receptor
[^{35}S]GTPγS	Guanosine 5′-O-(3-[^{35}S]thio) triphosphate binding
MAP kinases	Mitogen-activated protein kinases
mIPSC	Miniature inhibitory postsynaptic currents
ORG27569	5-Chloro-3-ethyl-*N*-[2-[4-(1-piperidinyl)phenyl]ethyl-1H-indole-2-carboxamide
PSNCBAM-1	1-(4-Chlorophenyl)-3-[3-(6-pyrrolidin-1-ylpyridin-2-yl)phenyl]urea
SR141716A	*N*-(piperidin-1-yl)-5-(4-cholrophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide
WIN55, 212-2	*R*(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo [1,2,3,-de]-1,4-benzoxacin-6-yl]-1-naphtalenylmethanone mesylate

INTRODUCTION

To date, most efforts in G protein-coupled receptor (GPCR) drug design have focused on targeting drugs to the same binding site on the receptor as the endogenous ligand binds to, the orthosteric site. Orthosteric ligands can activate (agonists), inactivate (inverse agonists), or prevent the action of the endogenous ligand (antagonists). Developing specific drugs in this way can be challenging, especially when the target receptor belongs to a larger family that shares high sequence homology at the orthosteric site, as is usually required to bind the endogenous ligand across a family of receptors. Moreover, most GPCR therapies are based on chronic exposure of the receptor to orthosteric ligands, activating regulatory processes, and potentially altering clinical efficacy due to desensitization and up/down regulation of the target receptor. An alternative approach is, therefore, desirable, and targeting of allosteric sites on GPCRs has become an area of intense research (Wenthur, Gentry, Mathews, & Lindsley, 2014).

ALLOSTERIC MODULATION OF GPCRS

The term "allostery" comes from the Greek *allos* meaning "else" or "different" (Kenakin & Miller, 2010). The concept of allosterism was first described over 50 years ago with regard to activity of bacterial enzymes (Umbarger, 1956), with the use of the term "allosteric" being formalized a few years later (Monod, Changeux, & Jacob, 1963); it has expanded to encompass modulatory sites on receptors, including GPCRs (Christopoulos et al., 2014; Kenakin & Miller, 2010).

A GPCR allosteric modulator is a molecule that binds at a topographically distinct site to the orthosteric ligand, and results in a change in receptor conformation, thus creating a receptor with novel binding and/or functional properties (Kenakin & Miller, 2010; Conn, Christopoulos, & Lindsley, 2009). Allosteric modulators have three main modes of action, as illustrated graphically in Fig. 60.1A. First, binding of an allosteric modulator may result in a conformational change that can impact the affinity of the orthosteric binding pocket, such that either the association or dissociation rate (or both) of an orthosteric ligand is modified (affinity modulation). Second, allosteric modulators may modulate the signaling response produced by the orthosteric ligand through altering the efficacy of the receptor conformation induced by the orthosteric ligand (efficacy modulation). Thus, positive allosteric modulators enhance the affinity and/or efficacy of an orthosteric ligand, while negative allosteric modulators decrease the affinity and/or efficacy of the orthosteric ligand (Fig. 60.1B). Finally, the allosteric modulator may have effects on receptor signaling independent of the orthosteric ligand—increasing (allosteric agonism) or decreasing (allosteric antagonism/inverse agonism) signaling (Kenakin & Miller, 2010).

Allosteric modulation of GPCRs is an emerging therapeutic strategy that can potentially provide improved selectivity and safety (Conn et al., 2009). Improved selectivity is predicted as allosteric sites are under less evolutionary pressure to be conserved than would be required to maintain an orthosteric binding pocket capable of accommodating an endogenous ligand across a family of receptors. Furthermore, allosteric modulators that lack intrinsic activity, and only modulate the action of the orthosteric ligand, may overcome some of the receptor regulation produced by orthosteric activation, as well as providing the opportunity to selectively tune up or down the actions of endogenous ligand. Such selective modulation would provide spatial and temporal control not currently possible with orthosteric ligands. Furthermore, allosteric modulation is saturable; thus, once allosteric sites are occupied, no additional on-target effects are observed.

GPCRs have the additional ability to form multireceptor complexes called "heterodimers" or "heteromers," in which two or more GPCRs interact to form a functional unit with modified ligand binding and signaling, compared to monomeric receptors (Fig. 60.4). Therefore, heteromerization can also be considered as a form of allosteric modulation, which is available for pharmacological manipulation, and may be particularly useful for restricting a drug's site of action to a cell type containing a specific heteromer. For the purpose of this review, we will be addressing GPCR allosteric modulation with regard to allosteric ligands and heteromerization.

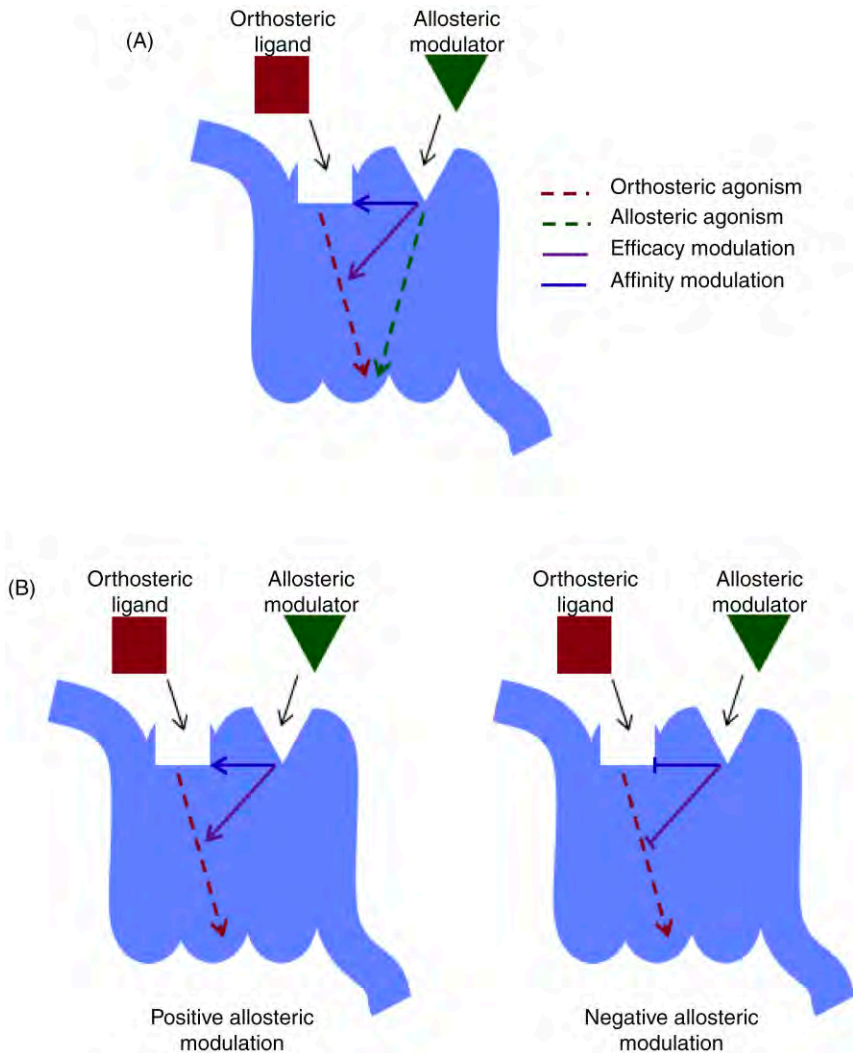


FIGURE 60.1 Different actions of allosteric modulators and how they influence orthosteric function. (A) An allosteric ligand *(green triangle)* binds to topographically distinct sites on the G protein-coupled receptor relative to the orthosteric ligand *(red square)*. An allosteric modulator bound to the allosteric site on a receptor can modulate orthosteric ligand affinity *(blue line)* and/or efficacy *(purple line)*. Additionally, the allosteric modulator may directly modulate signaling in their own right in the absence of orthosteric ligand *(dashed green line)*. (B) Positive allosteric modulators (left) enhance the affinity and/or efficacy of an orthosteric ligand. Negative allosteric modulators (right) decrease the affinity and/or efficacy of the orthosteric ligand.

POTENTIAL ADVANTAGES OF ALLOSTERIC MODULATORS FOR CB1

Cannabinoid receptor 1 (CB1) is of considerable therapeutic interest for the treatment of a range of disorders, including neurodegenerative disorders (Gowran, Noonan, & Campbell, 2011; Scotter, Goodfellow, Graham, Dragunow, & Glass, 2010; Sagar et al., 2009). The widespread distribution of cannabinoid receptors leads to wide-ranging physiological responses to cannabinoid-based therapies, therefore interest is now turning to improved drug therapies for these receptors through development of allosteric ligands, and drugs targeting receptor heteromers.

ALLOSTERIC LIGANDS OF CB1

In 2005, the first description of allosteric modulators for CB1 was reported (Price et al., 2005). Three novel compounds from Organon (ORG27569, ORG27759, and ORG29647) were shown to significantly increase the binding of the orthosteric CB1 agonist [^{3}H]CP55,940, with ORG27569 having the greatest potency (Fig. 60.2A). In 2007, a second CB1 allosteric modulator compound, structurally similar to the Organon compounds, was described through screening of a small molecule library of compounds from Prosidion Limited (PSN) (Horswill et al., 2007). The compound PSNCBAM-1 (Fig. 60.2B) was identified, and was found to produce a similar

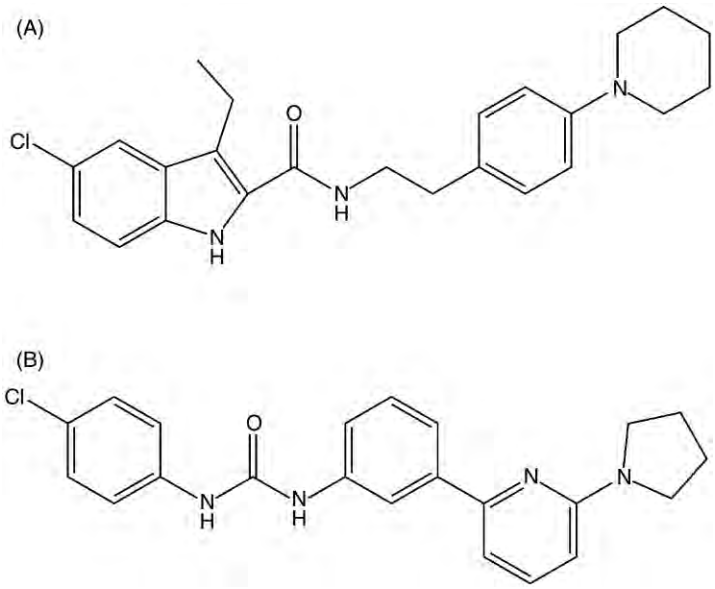


FIGURE 60.2 Structures of CB1 allosteric modulators ORG27569 and PSNCBAM-1. (A) ORG27569: 5-chloro-3-ethyl-*N*-[2-[4-(1-piperidinyl) phenyl]ethyl-1H-indole-2-carboxamide (Price et al., 2005) and (B) PSNCBAM-1: 1-(4-chlorophenyl)-3-[3-(6-pyrrolidin-1-ylpyridin-2-yl)phenyl] urea (Horswill et al., 2007).

increase in binding of [^{3}H]CP55,940 as seen with the Organon compounds (Horswill et al., 2007). The majority of the subsequent studies have focused on the effects of ORG27569 and PSNCBAM-1 on CB1 activity, therefore these will be the main focus of this review.

ALLOSTERIC MODULATORS ALTER THE BINDING OF ORTHOSTERIC LIGANDS TO CB1

Initial studies demonstrated that both ORG27569 and PSNCBAM-1 appeared to increase the binding of the orthosteric agonist [^{3}H]CP55,940 to the receptor (Price et al., 2005; Horswill et al., 2007), as illustrated in Fig. 60.3. Increased radioligand binding suggests positive cooperativity, mediated by an increase in agonist affinity in the presence of the allosteric modulator. This has been further investigated in kinetic studies which have investigated the dissociation rate of [^{3}H]CP55,940 in the presence of ORG27569 or PSNCBAM-1. [^{3}H]CP55,940 has been reported to show biphasic dissociation (Devlin & Christopoulos, 2002), thought to reflect rapid dissociation from low affinity uncoupled receptors, and slow dissociation from high affinity activated receptors. Initial studies with ORG27569 suggested a reduction in both the slow and fast dissociation rate constants, without changing the proportion of receptor in either state (Price et al., 2005). This finding suggests that ORG27569 reduces the orthosteric ligand dissociation without affecting G protein-coupling status (Price et al., 2005). In contrast, more recent kinetic binding assays with PSNCBAM-1 found no alteration in dissociation rates, but rather an increased proportion of receptors undergoing slow dissociation, suggesting that the increased binding observed is not due to a change in affinity, but rather a greater proportion of receptors in a high affinity state (Baillie et al., 2013). This was supported by saturation binding assays, which found no change in affinity (K_d) for [^{3}H]CP55,940 in the presence of either ORG27569 or PSNCBAM, but rather found an increase in total receptor binding (B_{max}), consistent with the suggestion that the ligands increase the available binding sites for the orthosteric ligand, rather than acting directly on ligand affinity (Baillie et al., 2013).

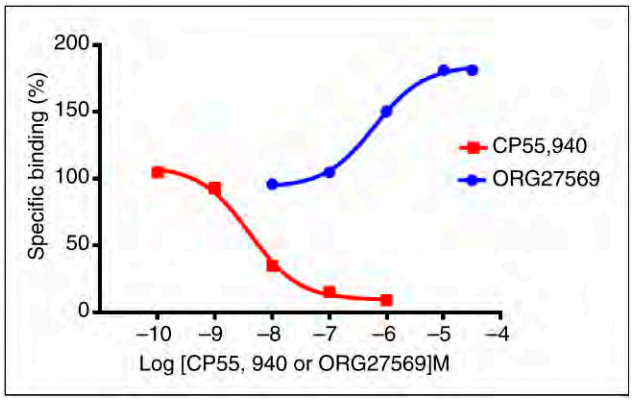


FIGURE 60.3 Influence of ORG27569 on binding of [^{3}H]CP55,940. Classical orthosteric ligands such as CP55,940 cause displacement of the radioligand [^{3}H]CP55,940. In contrast, the allosteric ligand ORG27569 produces a concentration-dependent increase in binding of the radioligand.

An increased proportion of receptors in a high affinity state is also consistent with the finding of Price et al. (2005) that ORG27569 decreased the binding of the inverse agonist SR141716A to rat brain membranes, as inverse agonist is thought to bind preferentially to low affinity receptor conformations. Ahn, Mahmoud, and Kendall (2012) further investigated this with receptor mutations they had previously demonstrated to display enhanced (T201I) or reduced (T210A) constitutive activity (D'Antona, Ahn, & Kendall, 2006). Competition binding assays showed the greatest enhancement of [^{3}H] CP55,940 binding with the inactive state CB1 receptor T210A, while the wild-type receptor showed a slightly lesser increase, and the constitutively active receptor showed no change in [^{3}H] CP55,940 binding, with increasing concentrations of ORG27569 (Ahn et al., 2012). Thus, these findings were again consistent with the concept that the allosteric modulator is driving the receptor into a high affinity agonist binding conformation.

ALLOSTERIC MODULATION OF CB1 RECEPTOR SIGNALING

Despite binding studies demonstrating increased orthosteric ligand binding, as described earlier, a paradoxical decrease in orthosteric ligand efficacy has generally been observed in most signaling pathways. The guanosine 5′-O-(3-[^{35}S]thio)triphosphate binding ([^{35}S]GTPγS) assay measures very early signaling events—the activation of the G protein, in response to agonist binding. Initial functional studies of the effect of the Organon compounds on CB1 receptor showed they acted as insurmountable antagonists of the CB1 receptor in the [^{35}S]GTPγS assay, eliciting a significant reduction in the maximum stimulation achieved by the CB1 agonists (Price et al., 2005). Similarly, PSNCBAM-1 displayed a concentration-dependent blockade of [^{35}S]GTPγS binding stimulated with either CP55,940 or the endocannabinoid anandamide (Horswill et al., 2007). These findings were subsequently confirmed by Baillie et al. (2013), who further extended the signaling assays to include inhibition of adenylate cyclase, a pathway downstream of $G\alpha_i$ activation. This study demonstrated a concentration dependent blockade of CB1-mediated inhibition of adenylate cyclase by ORG27569, a finding confirmed by Cawston et al. (2013). Intriguingly the extent of G protein-coupled inwardly rectifying potassium channel activation induced by CB1 was not inhibited by high concentrations of ORG27569 (Cawston et al., 2013), suggesting biased signaling modulation.

In addition to coupling to $G\alpha_i$, CB1 can also couple to $G\alpha_s$ to stimulate the activation of adenylate cyclase, and this $G\alpha_s$ linkage can be observed in cells following pertussis toxin treatment (Glass & Felder, 1997). Consistent with its effect on $G\alpha_i$, ORG27569 has also been shown to antagonize CB1's $G\alpha_s$-like coupling (Baillie et al., 2013; Cawston et al., 2013).

More complex interactions with signaling pathways have been suggested by studies investigating the effects of the allosteric modulators on MAP kinase signaling pathways (Bouaboula et al., 1995; Derkinderen, Ledent, Parmentier, & Girault, 2001). In contrast to the inhibition of orthosteric agonist signaling observed in other assays, ORG27569 has been suggested to enhance CP55,940-induced pERK stimulation (Baillie et al., 2013) (although see the findings of Ahn et al., 2012 discussed later).

ALLOSTERIC MODULATION OF CB1 RECEPTOR REGULATION

Studies examining the kinetics of blockade of adenylate cyclase inhibition by ORG27569 and PSNCBAM-1 have suggested that rather than purely antagonizing signaling, the blockade of signaling described earlier may be due to enhanced receptor regulation. Thus, Cawston et al. (2013) found that early inhibition of adenylate cyclase by CB1 was not prevented by the allosteric modulators, but that the modulators produced a rapid desensitization of this pathway. Likewise, CB1 mediated recovery of membrane potential following agonist-mediated hyperpolarization was more rapid in the presence of ORG275689 and PSNCBAM-1 (Cawston et al., 2013).

Current receptor theory would suggest that enhanced receptor desensitization is likely to be mediated by enhanced G protein-coupled receptor kinase phosphorylation of the receptor, followed by increased recruitment of β-arrestin and receptor internalization; however, this is not the case with the CB1 allosteric modulators studied to date. Baillie et al. (2013) demonstrated that ORG27569 inhibited β-arrestin recruitment, while Cawston et al. (2013) have shown that ORG27569 and PSNCBAM-1 produce a concentration-dependent decrease in agonist-induced receptor internalization. Interestingly, ORG27569 treatment of the constitutively inactive CB1 variant (T210A) resulted in a robust acceleration in receptor internalization, as compared to CP55,940 alone (Ahn et al., 2012) a finding that is hard to reconcile with the previously mentioned experiments on wild-type receptors, but that may be due to enhanced β-arrestin recruitment by these mutant receptors (Ahn, Mahmoud, Shim, & Kendall, 2013).

ALLOSTERIC AGONISM

There have also been some suggestions in the literature that ORG27569 can act as an allosteric agonist, producing efficacy in signaling pathways in the absence of

orthosteric ligand, although these are by no means consistent. The initial report by Price et al. (2005), suggested no activity of the compounds alone, in a mouse vas deference contractility assay. Similarly, in mouse brain samples, ORG27569 produced no effect on [^{35}S]GTPγS binding; however, in cell lines expressing human CB1, ORG27569 produced a small but significant decrease in basal [^{35}S]GTPγS binding (Baillie et al., 2013), a finding confirmed by Ahn et al. (2012), and consistent with an inverse agonist effect of ORG27569. ORG27569 alone was also found to produce a small stimulation of adenylate cyclase in the presence of forskolin (Baillie et al., 2013), an effect that was confirmed by Cawston et al. (2013). However, this increase was found to be pertussis toxin insensitive in the initial study—suggestive of $G\alpha_s$ coupling (Baillie et al., 2013), but was pertussis toxin-sensitive in the subsequent study (Cawston et al., 2013)—suggestive of inverse agonism. This discrepancy is yet to be resolved.

Ahn et al. (2012) utilized their mutant CB1 receptors with both enhanced and reduced constitutive activity to investigate how ORG27569 influenced additional downstream signaling cascades, including the MAP kinase signaling pathway. Interestingly, ORG27569 alone at high concentrations (10 μM) induced robust ERK1/2 phosphorylation (higher than that of the orthosteric agonist CP55,940). This effect was shown to be pertussis toxin-insensitive, suggesting it was $G\alpha_i$ independent (Ahn et al., 2012), and was subsequently found to be β-arrestin-1 dependent (Ahn et al., 2013). In contrast, Baillie et al. (2013) observed only a small increase in ERK1/2 phosphorylation in response to ORG27569 alone, which was pertussis toxin-sensitive. The cause of these differences between studies is not immediately obvious, particularly as most studies have used transfected HEK293 cells, but could possibly suggest that HEK293 cells in different labs have substantively different β-arrestin expression.

PROBE DEPENDENCE

Probe dependence is when different allosteric effects may be observed for the same modulator, acting at the same receptor, but with different orthosteric ligands (Kenakin, 2005). In the case of CB1, PSNCBAM-1 has been shown to cause noncompetitive antagonism in [^{35}S]GTPγS binding studies, with a higher potency against CB1 receptor agonist CP55,940 than WIN55,212-2 (Wang, Horswill, Whalley, & Stephens, 2011). The electrophysiology studies carried out by Wang et al. (2011), using both WIN55,212-2 and CP55,940, showed a reduction in miniature inhibitory postsynaptic currents (mIPSCs) frequency, but not amplitude. PSNCBAM-1 pretreatment revealed agonist-dependent functional antagonism, abolishing CP55,940-induced reductions in mIPSC frequency, but having no clear effect on WIN55,212-2 actions, suggestive of ligand-dependent effects. In line with these findings, Baillie et al. (2013) showed that both ORG27569 and PSNCBAM-1 displayed a marked ligand selectivity in [^{35}S]GTPγS, β-arrestin and cAMP assays, with both allosteric modulators being significantly less potent as inhibitors of WIN55,212-2 signaling, as compared with CP55,940. This was confirmed for ORG27569 by Cawston et al. (2013) in the cAMP signaling assay; however, they found that PSNCBAM-1 was equipotent for CP55,940 and WIN212-55. However, differences were observed, as when WIN55,212-2 was the orthosteric ligand, ORG27569 was unable to produce the same extent of inverse agonism as CP55,940, suggesting that the presence of WIN55,212-2 prevented the receptor from adopting an inactive conformation (Cawston et al., 2013). Thus, there are suggestions that the allosteric modulators may differentiate between orthosteric ligands, but this still has not been fully investigated.

ELUCIDATION OF ORG27569 BINDING SITE IN CB1

Site-directed fluorescence labeling has been used to gain insight into why the presence of ORG27569 results in increased agonist binding, but also in blockade of agonist mediated signaling (Fay & Farrens, 2012). To do this, a fluorescent label was attached to the cytoplasmic end of CB1's transmembrane 6 (a helix shown to move during activation in other GPCRs by the same method). It was shown that ORG27569 blocks conformational changes in CB1 that accompany G protein binding and/or activation and, thus, inhibits formation of a fully active CB1 structure. It was determined that the reason for this is that the simultaneous binding of ORG27569 and agonist produces a unique conformation resembling an intermediate state (Fay & Farrens, 2012). Furthermore, the binding site of ORG27569 in CB1 has recently been identified via computational, synthesis, mutation, and functional studies as being present in transmembrane 3-6-7 (Shore et al., 2014). The ORG27569 binding site was shown to overlap with the previously determined SR141716A binding site, and extends extracellularly, with an interaction between the ORG27569 piperidine ring nitrogen, and the lysine at position 192 of CB1, enabling ORG27569 to act as an inverse agonist.

IN VIVO STUDIES OF ORG27569

Initial characterization studies with PSNCBAM-1 by Horswill et al. (2007) showed that, in vivo in an acute rat-feeding model, PSNCBAM-1 decreased food intake and body weight. Subsequently, ORG27569 was evaluated

in vivo in the well-established mouse behavioral assays sensitive to CB1 orthosteric agonists and inverse agonist (Gamage et al., 2014). Similar to the orthosteric CB1 antagonist/inverse agonist SR141716A, ORG27569 reduced food intake, however this occurred independently of the CB1 receptor. ORG27569 did not elicit CB1-mediated effects alone, and lacked efficacy in altering antinociceptive, cataleptic, and hypothermic actions of anandamide, CP55,940, or Δ^9-tetrahydrocannabidiol (Gamage et al., 2014). While pharmacokinetic issues cannot be ruled out, this lack of efficacy in common behavioral pathways is surprising, given the complex effects on signaling described earlier, and requires further investigation.

PROPOSED ALLOSTERIC MODULATORS OF CB1

There have been additional reports of potential allosteric modulators for CB1 that have not yet been as extensively investigated as the two compounds described earlier. The endogenous antiinflammatory lipid Lipoxin A4 has been shown as a potential allosteric enhancer of CB1 (Pamplona et al., 2012). Lipoxin A4 enhanced the affinity of anandamide in mouse brain membranes, thus potentiating its effects both in vivo and in vitro. In HEK cells transfected with mouse CB1, Lipoxin A4 increased the potency of anandamide in reducing forskolin-induced cAMP levels ($G\alpha_i$), but had no effect in the [^{35}S]GTPγS assay (Pamplona et al., 2012).

The α-hemoglobin-derived dodecapeptide RVD-hemopressin has been shown to interact orthosterically with CB1 (Heimann et al., 2007). Bauer et al. (2012) investigated this further by raising monoclonal antibodies to the C-terminal part of hemopressin, and screening them on rodent brain extracts, and human and mouse plasma. From this screen, a family of N-terminally extended peptides in addition to hemopressin were identified. The peptides were designated Pepcan-12 and Pepcan-23 (referring to the peptide length). Pepcan-12 was shown to be the most efficacious Pepcan in displacing [^{3}H]CP55,940 and [^{3}H]WIN55,212-2, but only partial displacement occurred, consistent with an allosteric effect. The data was fitted to the allosteric ternary complex model, and revealed a negative cooperativity factor, thus identifying Pepcans as negative allosteric modulators of CB1. Pepcan-12 was shown to be a potent negative allosteric modulator of orthosteric agonist-induced cAMP accumulation, [^{35}S]GTPγS binding, and CB1 internalization (Bauer et al., 2012).

Recently, the inactive precursor of all steroid hormones, pregnenolone has been described as an endogenous negative allosteric modulator of CB1 (Vallee et al., 2014). In rats, Δ^9-tetrahydrocannabidiol CB1 activation was shown to increase the level of brain pregnenolone, which in turn, exerted a negative feedback on the CB1 receptor activity. Binding studies in cell lines expressing human CB1 showed that pregnenolone (up to 100 μM) did not modify equilibrium binding of [^{3}H]CP55,940 and [^{3}H]WIN55,212-2 to CB1 and, thus, the authors have suggested that pregnenolone is acting as a negative allosteric modulator (Vallee et al., 2014).

Finally, several dopamine transporter inhibitor compounds (RTI-370, RTI-371, JWH007, and GBR12909) have been suggested, via a cell-based calcium mobilization assay, to be positive allosteric modulators of human CB1 (Navarro, Howard, Pollard, & Carroll, 2009). The positive allosteric effect on calcium signaling of RTI-370, RTI-371, and JWH007 occurred with very little increase in potency, whereas GBR12909 showed a decrease in CP55,940 potency (Navarro et al., 2009).

CB1 DIMERIZATION AS A FORM OF ALLOSTERIC MODULATION

It has become well-established that GPCRs can interact together to form two- or multireceptor assemblies called "heterodimers" or "heteromers" (Pin et al., 2007). Heterodimerization can be considered as a form of allosteric modulation, which can be exploited pharmacologically for therapeutic advantage (Milligan & Smith, 2007), as illustrated in Fig. 60.4. This could mean designing ligands which disrupt or encourage heterodimer formation, or utilize functional crosstalk within the dimer, therefore targeting tissues specifically where that dimer partnership occurs.

CB1 has been demonstrated to dimerize with many other GPCRs, with functional consequences for CB1's signaling pathways. So far, CB1 has been suggested to dimerize with adenosine A2A (Carriba et al., 2007, 2008); dopamine D2 (Carriba et al., 2008; Glass & Felder, 1997; Marcellino et al., 2008); orexin 1 (Ellis, Pediani, Canals, Milasta, & Milligan, 2006); β2-adenergic (Hudson, Hébert, & Kelly, 2010); and μ-, κ-, and δ-opioid receptors (Rios, Gomes, & Devi, 2006). Here, we will just discuss some better-characterized examples.

The CB1–D2 heterodimer is probably the most well-described cannabinoid receptor heterodimer. These two receptors are coexpressed in neurons, particularly striatal medium spiny neurons (Glass, Dragunow, & Faull, 1997), and within the nucleus accumbens (Pickel, Chan, Kearn, & Mackie, 2006). When coexpressed, these receptors change from their standard $G\alpha_i$ signaling, to one which has been variously reported to either switch to stimulation of cAMP accumulation (Kearn, Blake-Palmer, Daniel, Mackie, & Glass, 2005; Glass & Felder, 1997; Jarrahian, Watts, & Barker, 2004; Maneuf & Brotchie, 1997), or at least nonsynergistic adenylate cyclase inhibition (Meschler & Howlett, 2001; Khan &

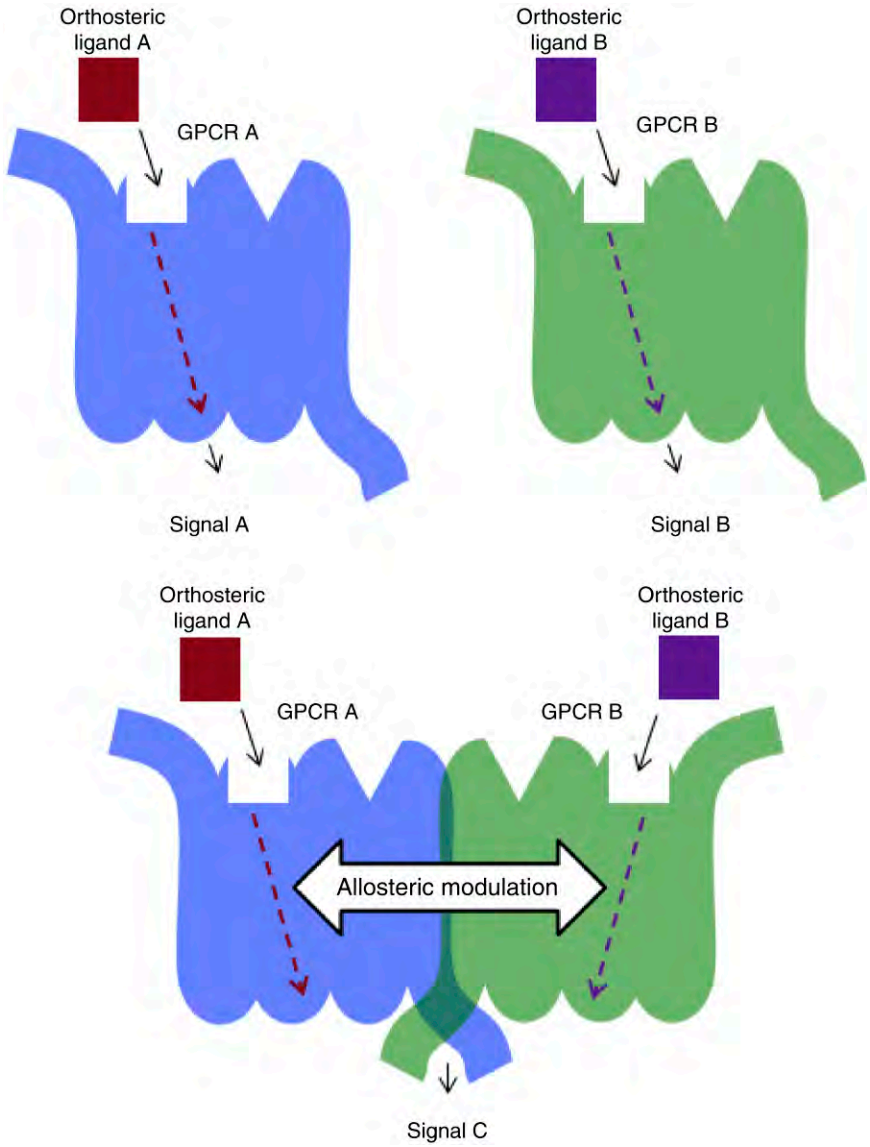


FIGURE 60.4 **Heteromerization as a form of allosteric modulation.** Heterodimerization can be considered as a form of allosteric modulation and, thus, two G protein-coupled receptors A and B can interact together to form a heterodimer, and this results in a change in receptor conformation, thus creating a GPCR heterodimer with novel binding and/or functional properties.

Lee, 2014). Fluorescence resonance energy transfer data (Marcellino et al., 2008; Carriba et al., 2008) and coimmunoprecipitation data (Kearn et al., 2005) strongly suggest that this receptor interaction is due to physical contact between CB1 and D2.

Electron microscopy and FRET data suggest that a CB1–orexin 1 receptor heterodimer can also occur (Ellis et al., 2006; Hilairet, Bouaboula, Carrière, Le Fur, & Casellas, 2003). CB1's dimerization with the orexin 1 receptor affects its constitutive trafficking phenotype (Ellis et al., 2006), and also both receptors' ability to signal through pERK1/2 (Ellis et al., 2006; Hilairet et al., 2003). However, this interaction may not be entirely within the structure of a heterodimer, as orexin receptor stimulation causes 2AG release, which can act at CB1 receptors in an autocrine manner (Jäntti et al., 2013).

The CB1 and μ-opioid receptors are coexpressed in the nucleus accumbens (Pickel, Chan, Kash, Rodríguez, & MacKie, 2004; Schoffelmeer, Hogenboom, Wardeh, & De Vries, 2006), caudate putamen nucleus (Rodríguez, Mackie, & Pickel, 2001), and spinal cord dorsal horn (Salio et al., 2001), allowing for molecular interactions of these receptors. The CB1–μ opioid dimer creates a mutually inhibitory effect on signaling (ie, both receptors signal less), as measured by G protein turnover, pERK1/2, and Src activation pathways (Rios et al., 2006; Canals & Milligan, 2008), and coactivation of both receptors affects neurotransmitter release in nucleus accumbens slices (Schoffelmeer et al., 2006).

The importance of GPCR heteromerization in vivo remains to be elucidated and exploited, largely because of a lack of selective pharmacological tools. Among various efforts to modulate GPCR heteromers, bivalent ligands, are defined as two pharmacophores linked by spacers, represent a promising approach, and may provide such a tool (Berque-Bestel, Lezoualc'h, & Jockers, 2008; Liu, Zhang, & Zhang, 2009). Bivalent ligands are expected to bind with greatly enhanced affinity to ligand recognition sites on heteromers because of the small containment volume for the second pharmacophore after the binding of the first, and the formation of more thermodynamically stable complexes. At the same time, bivalent ligands may display unique properties, since they interact with more than one receptor simultaneously. Indeed, bivalent ligands have been developed for a variety of G protein-coupled receptor targets, and exhibit unique pharmacological properties, compared to their monomeric subunits (Hiller, Kuhhorn, & Gmeiner, 2013).

CONCLUSIONS

In conclusion, CB1 receptor signaling can be modified by allosteric modulation, whether it be by direct binding to allosteric binding sites, or through modulation of the receptor conformation by heteromer partners. Considering the therapeutic potential of cannabinoid receptors, allosteric modulation provides an attractive alternative to orthosteric modulation.

MINI-DICTIONARY

Adenylate cyclase/adenylyl cyclase A family of enzymes which make cAMP, the activity of which can be controlled by GPCRs.
Affinity The strength of interaction between a ligand and its binding site.
Allosteric modulator A molecule that binds at a topographically distinct site to the orthosteric ligand, and results in a change in receptor conformation, thus creating a GPCR with novel binding and/or functional properties.
Efficacy The maximum signaling effect which a ligand can produce.
Heterodimers/heteromers A functional unit formed by the physical interaction of two or more GPCRs, often with modified ligand binding and signaling, compared to monomeric receptors.
MAP kinase signaling A signaling cascade which can be triggered by GPCRs. ERK1/2 phosphorylation is a common experimental endpoint.
Negative allosteric modulator An allosteric modulator that decreases the affinity and/or efficacy of the orthosteric ligand.
Orthosteric agonist An agonist which binds in the same site as the endogenous ligand.
Orthosteric site The binding site on the receptor where the endogenous ligand binds.
Positive allosteric modulators An allosteric modulator that enhances the affinity and/or efficacy of an orthosteric ligand.
Probe dependence When different allosteric effects may be observed for the same modulator, acting at the same receptor, but with different orthosteric ligands.

References

Ahn, K. H., Mahmoud, M. M., & Kendall, D. A. (2012). Allosteric modulator ORG27569 induces CB1 cannabinoid receptor high affinity agonist binding state, receptor internalization, and Gi protein-independent ERK1/2 kinase activation. *Journal of Biological Chemistry*, *287*, 12070–12082.

Ahn, K. H., Mahmoud, M. M., Shim, J. Y., & Kendall, D. A. (2013). Distinct roles of beta-arrestin 1 and beta-arrestin 2 in ORG27569-induced biased signaling and internalization of the cannabinoid receptor 1 (CB1). *Journal of Biological Chemistry*, *288*, 9790–9800.

Baillie, G. L., Horswill, J. G., Anavi-Goffer, S., Reggio, P. H., Bolognini, D., Abood, M. E., McAllister, S., Strange, P. G., Stephens, G. J., Pertwee, R. G., & Ross, R. A. (2013). CB(1) receptor allosteric modulators display both agonist and signaling pathway specificity. *Molecular Pharmacology*, *83*, 322–338.

Bauer, M., Chicca, A., Tamborrini, M., Eisen, D., Lerner, R., Lutz, B., Poetz, O., Pluschke, G., & Gertsch, J. (2012). Identification and quantification of a new family of peptide endocannabinoids (Pepcans) showing negative allosteric modulation at CB1 receptors. *Journal of Biological Chemistry*, *287*, 36944–36967.

Berque-Bestel, I., Lezoualc'h, F., & Jockers, R. (2008). Bivalent ligands as specific pharmacological tools for G protein-coupled receptor dimers. *Current Drug Discovery and Technology*, *5*, 312–318.

Bouaboula, M., Poinot-Chazel, C., Bourrie, B., Canat, X., Calandra, B., Rinaldi-Carmona, M., Le Fur, G., & Casellas, P. (1995). Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochemistry Journal*, *312*(Pt. 2), 637–641.

Canals, M., & Milligan, G. (2008). Constitutive activity of the cannabinoid CB1 receptor regulates the function of co-expressed mu opioid receptors. *Journal of Biological Chemistry*, *283*, 11424–11434.

Carriba, P., Navarro, G., Ciruela, F., Ferré, S., Casadó, V., Agnati, L., Cortés, A., Mallol, J., Fuxe, K., & Canela, E. I. (2008). Detection of heteromerization of more than two proteins by sequential BRET-FRET. *Nature Methods*, *5*, 727–733.

Carriba, P., Ortiz, O., Patkar, K., Justinova, Z., Stroik, J., Themann, A., Muller, C., Woods, A. S., Hope, B. T., Ciruela, F., Casado, V., Canela, E. I., Lluis, C., Goldberg, S. R., Moratalla, R., Franco, R., & Ferre, S. (2007). Striatal adenosine A2A and cannabinoid CB1 receptors form functional heteromeric complexes that mediate the motor effects of cannabinoids. *Neuropsychopharmacology*, *32*, 2249–2259.

Cawston, E. E., Redmond, W. J., Breen, C. M., Grimsey, N. L., Connor, M., & Glass, M. (2013). Real-time characterization of cannabinoid receptor 1 (CB1) allosteric modulators reveals novel mechanism of action. *British Journal of Pharmacology*, *170*, 893–907.

Christopoulos, A., Changeux, J. P., Catterall, W. A., Fabbro, D., Burris, T. P., Cidlowski, J. A., Olsen, R. W., Peters, J. A., Neubig, R. R., Pin, J. P., Sexton, P. M., Kenakin, T. P., Ehlert, F. J., Spedding, M., & Langmead, C. J. (2014). International Union of Basic and Clinical Pharmacology. XC. Multisite pharmacology: recommendations for the nomenclature of receptor allosterism and allosteric ligands. *Pharmacological Reviews*, *66*, 918–947.

Conn, P. J., Christopoulos, A., & Lindsley, C. W. (2009). Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. *Nature Reviews Drug Discovery*, *8*, 41–54.

D'Antona, A. M., Ahn, K. H., & Kendall, D. A. (2006). Mutations of CB1 T210 produce active and inactive receptor forms correlations with ligand affinity, receptor stability, and cellular localization. *Biochemistry*, *45*, 5606–5617.

Derkinderen, P., Ledent, C., Parmentier, M., & Girault, J. A. (2001). Cannabinoids activate p38 mitogen-activated protein kinases through CB1 receptors in hippocampus. *Journal of Neurochemistry*, *77*, 957–960.

Devlin, M. G., & Christopoulos, A. (2002). Modulation of cannabinoid agonist binding by 5-HT in the rat cerebellum. *Journal of Neurochemistry*, *80*, 1095–1102.

Ellis, J., Pediani, J. D., Canals, M., Milasta, S., & Milligan, G. (2006). Orexin-1 receptor-cannabinoid CB1 receptor heterodimerization results in both ligand-dependent and -independent coordinated alterations of receptor localization and function. *Journal of Biological Chemistry*, *281*, 38812–38824.

Fay, J. F., & Farrens, D. L. (2012). A key agonist-induced conformational change in the cannabinoid receptor CB1 is blocked by the allosteric ligand Org 27569. *Journal of Biological Chemistry*, *287*, 33873–33882.

Gamage, T. F., Ignatowska-Jankowska, B. M., Wiley, J. L., Abdelrahman, M., Trembleau, L., Greig, I. R., Thakur, G. A., Tichkule, R., Poklis, J., Ross, R. A., Pertwee, R. G., & Lichtman, A. H. (2014). In-vivo pharmacological evaluation of the CB1-receptor allosteric modulator Org-27569. *Behavioural Pharmacology*, *25*, 182–185.

Glass, M., Dragunow, M., & Faull, R. L. (1997). Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience*, *77*, 299–318.

Glass, M., & Felder, C. C. (1997). Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. *Journal of Neuroscience*, *17*, 5327–5333.

Gowran, A., Noonan, J., & Campbell, V. A. (2011). The multiplicity of action of cannabinoids: implications for treating neurodegeneration. *CNS Neuroscience and Therapeutics*, *17*, 637–644.

Heimann, A. S., Gomes, I., Dale, C. S., Pagano, R. L., Gupta, A., de Souza, L. L., Luchessi, A. D., Castro, L. M., Giorgi, R., Rioli, V., Ferro, E. S., & Devi, L. A. (2007). Hemopressin is an inverse agonist of CB1 cannabinoid receptors. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 20588–20593.

Hilairet, S., Bouaboula, M., Carrière, D., Le Fur, G., & Casellas, P. (2003). Hypersensitization of the orexin 1 receptor by the CB1 receptor: evidence for cross-talk blocked by the specific cb1 antagonist, sr141716. *Journal of Biological Chemistry*, *278*, 23731–23737.

Hiller, C., Kuhhorn, J., & Gmeiner, P. (2013). Class A G-protein-coupled receptor (GPCR) dimers and bivalent ligands. *Journal of Medical Chemistry*, *56*, 6542–6559.

Horswill, J. G., Bali, U., Shaaban, S., Keily, J. F., Jeevaratnam, P., Babbs, A. J., Reynet, C., & Wong Kai In, P. (2007). PSNCBAM-1, a novel allosteric antagonist at cannabinoid CB1 receptors with hypophagic effects in rats. *British Journal of Pharmacology*, *152*, 805–814.

Hudson, B. D., Hébert, T. E., & Kelly, M. E. M. (2010). Physical and functional interaction between CB1 cannabinoid receptors and β2-adrenoceptors. *British Journal of Pharmacology*, *160*, 627–642.

Jäntti, M. H., Putula, J., Turunen, P. M., Näsman, J., Reijonen, S., Lindqvist, C., & Kukkonen, J. P. (2013). Autocrine endocannabinoid signaling through CB1 receptors potentiates OX1 orexin receptor signaling. *Molecular Pharmacology*, *83*, 621–632.

Jarrahian, A., Watts, V. J., & Barker, E. L. (2004). D2 dopamine receptors modulate Gα-subunit coupling of the CB1 cannabinoid receptor. *Journal of Pharmacology and Experimental Therapeutics*, *308*, 880–886.

Kearn, C. S., Blake-Palmer, K., Daniel, E., Mackie, K., & Glass, M. (2005). Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? *Molecular Pharmacology*, *67*, 1697–1704.

Kenakin, T. (2005). New concepts in drug discovery: collateral efficacy and permissive antagonism. *Nature Reviews Drug Discovery*, *4*, 919–927.

Kenakin, T., & Miller, L. J. (2010). Seven transmembrane receptors as shapeshifting proteins: the impact of allosteric modulation and functional selectivity on new drug discovery. *Pharmacological Reviews*, *62*, 265–304.

Khan, S., & Lee, F. S. (2014). Delineation of domains within the cannabinoid CB1 and dopamine D2 receptors that mediate the formation of the heterodimer complex. *Journal of Molecular Neuroscience*, *53*, 10–21.

Liu, Z., Zhang, J., & Zhang, A. (2009). Design of multivalent ligand targeting G-protein-coupled receptors. *Current Pharmaceutical Design*, *15*, 682–718.

Maneuf, Y. P., & Brotchie, J. M. (1997). Paradoxical action of the cannabinoid WIN 55, 212-2 in stimulated and basal cyclic AMP accumulation in rat globus pallidus slices. *British Journal of Pharmacology*, *120*, 1397–1398.

Marcellino, D., Carriba, P., Filip, M., Borgkvist, A., Frankowska, M., Bellido, I., Tanganelli, S., Müller, C. E., Fisone, G., Lluis, C., Agnati, L. F., Franco, R., & Fuxe, K. (2008). Antagonistic cannabinoid CB1/dopamine D2 receptor interactions in striatal CB1/D2 heteromers. A combined neurochemical and behavioral analysis. *Neuropharmacology*, *54*, 815–823.

Meschler, J. P., & Howlett, A. C. (2001). Signal transduction interactions between CB1 cannabinoid and dopamine receptors in the rat and monkey striatum. *Neuropharmacology*, *40*, 918–926.

Milligan, G., & Smith, N. J. (2007). Allosteric modulation of heterodimeric G-protein-coupled receptors. *Trends in Pharmacological Sciences*, *28*, 615–620.

Monod, J., Changeux, J. P., & Jacob, F. (1963). Allosteric proteins and cellular control systems. *Journal of Molecular Biology*, *6*, 306–329.

Navarro, H. A., Howard, J. L., Pollard, G. T., & Carroll, F. I. (2009). Positive allosteric modulation of the human cannabinoid (CB) receptor by RTI-371, a selective inhibitor of the dopamine transporter. *British Journal of Pharmacology*, *156*, 1178–1184.

Pamplona, F. A., Ferreira, J., Menezes de Lima, O., Jr., Duarte, F. S., Bento, A. F., Forner, S., Villarinho, J. G., Bellocchio, L., Wotjak, C. T., Lerner, R., Monory, K., Lutz, B., Canetti, C., Matias, I., Calixto, J. B., Marsicano, G., Guimaraes, M. Z., & Takahashi, R. N. (2012). Anti-inflammatory lipoxin A4 is an endogenous allosteric enhancer of CB1 cannabinoid receptor. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 21134–21139.

Pickel, V. M., Chan, J., Kash, T. L., Rodríguez, J. J., & MacKie, K. (2004). Compartment-specific localization of cannabinoid 1 (CB1) and μ-opioid receptors in rat nucleus accumbens. *Neuroscience*, *127*, 101–112.

Pickel, V. M., Chan, J., Kearn, C. S., & Mackie, K. (2006). Targeting dopamine D2 and cannabinoid-1 (CB1) receptors in rat nucleus accumbens. *Journal of Comparative Neurology*, *495*, 299–313.

Pin, J. -P., Neubig, R., Bouvier, M., Devi, L., Filizola, M., Javitch, J. A., Lohse, M. J., Milligan, G., Palczewski, K., Parmentier, M., & Spedding, M. (2007). International Union of Basic and Clinical Pharmacology. LXVII. Recommendations for the recognition and nomenclature of G protein-coupled receptor heteromultimers. *Pharmacological Reviews*, *59*, 5–13.

Price, M. R., Baillie, G. L., Thomas, A., Stevenson, L. A., Easson, M., Goodwin, R., McLean, A., McIntosh, L., Goodwin, G., Walker, G., Westwood, P., Marrs, J., Thomson, F., Cowley, P., Christopoulos, A., Pertwee, R. G., & Ross, R. A. (2005). Allosteric modulation of the cannabinoid CB1 receptor. *Molecular Pharmacology*, *68*, 1484–1495.

Rios, C., Gomes, I., & Devi, L. A. (2006). μ Opioid and CB1 cannabinoid receptor interactions: reciprocal inhibition of receptor signaling and neuritogenesis. *British Journal of Pharmacology*, *148*, 387–395.

Rodríguez, J. J., Mackie, K., & Pickel, V. M. (2001). Ultrastructural localization of the CB1 cannabinoid receptor in μ-opioid receptor patches of the rat caudate putamen nucleus. *Journal of Neuroscience*, *21*, 823–833.

Sagar, D. R., Gaw, A. G., Okine, B. N., Woodhams, S. G., Wong, A., Kendall, D. A., & Chapman, V. (2009). Dynamic regulation of the endocannabinoid system: implications for analgesia. *Molecular Pain*, *5*, 59.

Salio, C., Fischer, J., Franzoni, M. F., Mackie, K., Kaneko, T., & Conrath, M. (2001). CB1-cannabinoid and μ-opioid receptor co-localization on postsynaptic target in the rat dorsal horn. *NeuroReport*, *12*, 3689–3692.

Schoffelmeer, A. N. M., Hogenboom, F., Wardeh, G., & De Vries, T. J. (2006). Interactions between CB1 cannabinoid and μ opioid receptors mediating inhibition of neurotransmitter release in rat nucleus accumbens core. *Neuropharmacology*, *51*, 773–781.

Scotter, E. L., Goodfellow, C. E., Graham, E. S., Dragunow, M., & Glass, M. (2010). Neuroprotective potential of CB1 receptor agonists in an in vitro model of Huntington's disease. *British Journal of Pharmacology*, *160*, 747–761.

Shore, D. M., Baillie, G. L., Hurst, D. H., Navas, F., III, Seltzman, H. H., Marcu, J. P., Abood, M. E., Ross, R. A., & Reggio, P. H. (2014). Allosteric modulation of a cannabinoid G protein-coupled receptor: binding site elucidation and relationship to G protein signaling. *Journal of Biological Chemistry*, *289*, 5828–5845.

Umbarger, H. E. (1956). Evidence for a negative-feedback mechanism in the biosynthesis of isoleucine. *Science*, *123*, 848.

Vallee, M., Vitiello, S., Bellocchio, L., Hebert-Chatelain, E., Monlezun, S., Martin-Garcia, E., Kasanetz, F., Baillie, G. L., Panin, F., Cathala, A., Roullot-Lacarriere, V., Fabre, S., Hurst, D. P., Lynch, D. L., Shore, D. M., Deroche-Gamonet, V., Spampinato, U., Revest, J. M., Maldonado, R., Reggio, P. H., Ross, R. A., Marsicano, G., & Piazza, P. V. (2014). Pregnenolone can protect the brain from cannabis intoxication. *Science*, *343*, 94–98.

Wang, X., Horswill, J. G., Whalley, B. J., & Stephens, G. J. (2011). Effects of the allosteric antagonist 1-(4-chlorophenyl)-3-[3-(6-pyrrolidin-1-ylpyridin-2-yl)phenyl]urea (PSNCBAM-1) on CB1 receptor modulation in the cerebellum. *Molecular Pharmacology*, *79*, 758–767.

Wenthur, C. J., Gentry, P. R., Mathews, T. P., & Lindsley, C. W. (2014). Drugs for allosteric sites on receptors. *Annual Review of Pharmacology and Toxicology*, *54*, 165–184.

CHAPTER

61

Polymorphisms of the CB2 Cannabinoid Receptor

P. Kumar, Z.-H. Song

Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY, United States

SUMMARY POINTS

- This chapter focuses on the single nucleotide polymorphisms (SNPs) of the human CB2 cannabinoid receptor (*CNR2*).
- Functional and genetic association studies on SNPs of *CNR2* are reviewed.
- Nonsynonymous SNPs in *CNR2* have been reported to cause changes of the responses of the CB2 to various cannabinoid ligands.
- Nonsynonymous SNPs, synonymous SNPs, as well as SNPs in the noncoding region of *CNR2* have been shown to be associated with a variety of diseases.
- The disorders associated with *CNR2* include autoimmune diseases, liver diseases, osteoporosis, and disorders of central nervous system.

KEY FACTS OF SNPs

- SNPs are single-nucleotide base substitutions that occur in greater than 1% of the population.
- SNPs can be used to predict an individual's susceptibility to environmental factors.
- SNPs play a central role in identifying possible risk factors for diseases.
- SNPs can help predict how an individual will respond a drug treatment.
- SNPs can serve as diagnostic markers to help make informed decisions regarding specific medical treatments.

LIST OF ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
BBRI	Breaking bending resistance index
BMD	Bone mineral density
CB2	Cannabinoid receptor 2
CD	Celiac disease
CHC	Chronic hepatitis C
CNS	Central nervous system
dbSNP	The single nucleotide polymorphism database
GWAS	Genome-wide association studies
ITP	Immune thrombocytopenic purpura
MI	Myocardial infarction
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
PBC	Primary biliary cirrhosis
SNP	Single nucleotide polymorphism

CB2 CANNABINOID RECEPTOR: DISTRIBUTION AND FUNCTION

The CB2 cannabinoid receptor, cloned in 1993 (Munro, Thomas, & Abu-Shaar, 1993), is a G-protein coupled receptor which is coupled to $G_{i/o}$ protein (Howlett, 2005; Pertwee, 2005; Pertwee & Ross, 2002). Activation of CB2 results in an inhibition of adenylate cyclase through the activation of a pertussis toxin-sensitive G protein (Howlett, 2005; Pertwee, 2005; Pertwee & Ross, 2002).

CB2 has been shown to be expressed on the cells and tissues of the immune system, cardiovascular system, gastrointestinal system, and skeletal system, with recent evidence implicating its expression in the central nervous system (CNS) (Howlett, 2005; Pertwee, 2005; Pertwee & Ross, 2002).

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00071-5
Copyright © 2017 Elsevier Inc. All rights reserved.

In the immune system, CB2 has been shown to mediate immunosuppressive and antiinflammatory effects of cannabinoids (Malfitano, Basu, Maresz, Bifulco, & Dittel, 2014). In the cardiovascular system, CB2 has been shown to play a role in modulating the inflammatory response during myocardial ischemia/reperfusion injury (Steffens & Pacher, 2012). In the gastrointestinal system, the CB2 receptor has been implicated in regulating inflammation in the liver, and playing a hepatoprotective role (Mallat, Teixeira-Clerc, Deveaux, Manin, & Lotersztajn, 2011). In the skeletal system, CB2 receptors have been shown to play a role in bone remodeling (Tam et al., 2005). In the CNS, CB2 may play a role in modulating a variety of diseases such as substance abuse and depression (Onaivi, Ishiguro, Gu, & Liu, 2012).

GENETIC POLYMORPHISMS

Genetic polymorphisms, which are DNA sequence variations in a population, are often the result of mutations. A polymorphism refers to a mutation that displays an allele frequency greater than or equal to 1%. Single nucleotide polymorphisms (SNPs) are the simplest type of polymorphism, and result from a single base mutation which substitutes one nucleotide for another. SNPs are estimated to occur at 1 out of every 1000 base pairs, consist of 80% of all sequence variants, and represent the most common type of polymorphisms and genetic variation among individuals (Gray, Campbell, & Spurr, 2000; Syvanen, 2001).

In a gene, SNPs are typically located in (1) coding sequences, (2) noncoding regions, or (3) intergenic regions between genes (Gray et al., 2000; Syvanen, 2001). SNPs in the coding sequence fall within two categories: synonymous (do not affect protein sequence) and nonsynonymous (affect protein sequence). While synonymous SNPs are typically referred to as silent changes (unable to result in function changes), recently there have been several reports according to which these mutations can result in functional changes. Synonymous SNPs may have altered translation kinetics of a define mRNA which may cause the in vivo folding of the same polypeptide chain into different conformations (Komar, 2007; Sauna, Kimchi-Sarfaty, Ambudkar, & Gottesman, 2007). SNPs that are not in the coding region may still be significant for gene splicing, binding of transcription factors, or the sequence of noncoding RNA (Gray et al., 2000; Syvanen, 2001).

GENOTYPING METHODS

The detection of SNPs can be categorized into two areas: (1) scanning DNA sequences for previously unidentified SNPs and (2) genotyping individuals for known SNPs. SNP genotyping is commonly defined as the measurement of genetic variations of SNPs between members of a species. The most common SNP genotyping methods include hybridization-based (eg, SNP microarrays), enzyme-based (eg, primer extension), oligonucleotide ligation, endonuclease cleavage, or a variation of the aforementioned techniques (Gray et al., 2000; Syvanen, 2001).

TYPES OF INVESTIGATIONS

Association Studies

Genetic association is commonly defined as the relationship between a phenotype and a genetic polymorphism (eg, single nucleotide polymorphism) or between two genetic polymorphisms. Typically, the purpose of genetic association studies is to investigate whether single-locus alleles or genotype frequencies differ between two groups of subjects (patients with disease versus healthy control subjects) (Cargill & Daley, 2000; Farrall & Morris, 2005; Schork, Fallin, & Lanchbury, 2000).

Case–Control Studies

Case–control studies are the most widely employed genetic association studies which compare two groups of subjects (a healthy control group versus a case group displaying the disease). Briefly, all subjects are genotyped (DNA sample analysis) for the known SNPs, and the allele frequency is calculated to determine if it is significantly different between the control and case group. Most commonly, an odds ratio is reported which is the proportion of individuals in the case group exhibiting a specific allele, and the proportion of individuals in the control group exhibiting the same specific allele. An odds ratio significantly greater than one demonstrates that the genetic variant is associated with the disease (Cargill & Daley, 2000; Farrall & Morris, 2005; Schork et al., 2000).

Genome-Wide Association Studies

Genome-wide association studies (GWAS) represent an emerging method to identify important genes which modulate human disease. GWAS primarily focus on relationships between SNPs and disease characteristics (traits). Briefly, this method scans the genome for SNPs, and can analyze hundreds or even thousands of SNPs simultaneously. GWAS typically compare the DNA of two groups of patients: (1) patients who exhibit the disease (cases) and (2) subjects who do no exhibit the disease (controls). This can potentially result in the determination of genes that may contribute to an individual's risk of developing a certain disease (Farrall & Morris, 2005).

THE SINGLE NUCLEOTIDE POLYMORPHISM DATABASE (DBSNP) AND "RS" NUMBERS

One of the most commonly used databases is the dbSNP database, established by the National Center for Biotechnology Information (NCBI), in collaboration with the National Human Genome Research Institute, in 1998. This database serves as the central public source for information regarding genetic variation. The dbSNP is a free public archive for cataloging genetic variation across different species. Reference SNP identification numbers (eg, rs251432) is a unique identification label assigned by NCBI to catalogue a group of SNPs that map to an identical location. The "rs" designation is typically assigned postsubmission, and is always in lower case. The "rs" number does not contain any information regarding position, as it is just an identifier, and is used for all types of variants. It is possible for two different "rs" numbers to refer to the same SNP, if they have not yet been merged (Sherry et al., 2001).

CNR2 GENE LOCATION AND STRUCTURE

The human CB2 cannabinoid receptor gene (*CNR2*) is located on chromosome 1 (1p36.11), and consists of two exons and one intron. The first exon (consists part of the promoter sequence) does not encode for protein, but regulates *CNR2* transcription (Sherwood et al., 2009). In addition, the human *CNR2* has been reported to have two splice variants (Liu et al., 2009).

NONSYNONYMOUS SNPs IN *CNR2*

The following nonsynonymous SNPs in *CNR2* have been shown to cause functional changes of CB2 and/or linked to various diseases:

1. rs2501432, rs35761398: arginine for glutamine at position 63(Q63R) at the beginning of the first intracellular loop.
2. rs4131193: leucine for isoleucine at position 133 (L133I) in the second intracellular loop.
3. rs2501431: alanine for glycine at position 155 (A155G) in the third transmembrane domain.
4. rs2229579: tyrosine for histidine at position 316 (H316Y) at the C-terminal tail.

FUNCTIONAL CONSEQUENCES OF NONSYNONYMOUS SNPs IN *CNR2*

To date, three studies have investigated the functional effects of nonsynonymous SNPs at the *CNR2* gene (Carrasquer, Nebane, Williams, & Song, 2010; Ishiguro et al., 2010b; Sipe, Arbour, Gerber, & Beutler, 2005).

The first study, conducted by Sipe and coworkers, investigated the relationship between the CB2 receptor polymorphism and immune cell regulation and function. They studied a missense polymorphism at the CB2 cDNA position 188-189 which results in the amino acid substitution of glutamine with arginine at position 63 (Q63R). Sipe and coworkers demonstrated that, in T lymphocytes from CB2 188-189 GG/GG homozygotes, there was a twofold reduction of endocannabinoid-induced inhibition of proliferation, compared with cells from CB2 188-189 AA/AA homozygotes. Overall, Sipe et al. (2005) has provided evidence that this missense polymorphism leads to a disruption of the endocannabinoid inhibition of the immune system function, and suggests that the CB2 188-189 variant may be a genetic risk factor for autoimmune diseases.

In 2010, Ishiguro and coworkers investigated the effect of the cannabinoid agonists 2-arachidonoylglycerol (2-AG) and JWH015 on cAMP levels in Q63- or R63-allele *CNR2*-transfected CHO cells. Their results showed that, in cells transfected with the R63-allele, the cAMP response to both 2-AG and JWH-015 was reduced, comparing to cells transfected with Q63 allele. Ishiguro et al. (2010b) concluded that CB2 carrying R63 had a reduced function when activated by cannabinoid agonists.

Carrasquer et al. (2010) investigated if two nonsynonymous SNPs at the *CNR2* gene result in functional consequences at the CB2 receptor. Q63R, H316Y, and Q63R/H316 mutations were generated in recombinant human CB2 and stably transfected in HEK293 cells. Carrasquer and coworkers reported that the CB2 polymorphic receptors were able to bind cannabinoid ligands, and modulate signal transduction. However, in ligand-induced cAMP accumulation assays, the cannabinoid agonists WIN55212-2 and 2-AG exhibited reduced efficacy in cells expressing the polymorphic receptors compared to the CB2 wild-type receptor. Furthermore, in constitutive activity assays, the H316Y and Q63R/H316Y polymorphic receptors displayed higher constitutive activity, compared with the CB2 wild-type receptor. Carrasquer et al. (2010) concluded that the *CNR2* Q63R and H316Y polymorphisms resulted in alterations of receptor function.

CB2 POLYMORPHISM GENETIC ASSOCIATION STUDIES

Association Between CB2 Polymorphisms and Immune System Disorders

This section will review the three studies in which the *CNR2* Q63R variant has been linked to immune system disorders.

Immune thrombocytopenic purpura (ITP) is an acquired autoimmune disorder, and is the most common cause of thrombocytopenia in children. In 2011, Rossi and coworkers genotyped 190 children with ITP and 600 healthy subjects for the *CNR2* rs35761398 variant which results in the missense Q63R. They demonstrated, for the first time, an association between the functional variant of the *CNR2* gene and ITP. The relative odds ratio indicated that the risk of acquiring childhood chronic ITP was more than double in children who were homozygous for the variant. The results from this study indicated that the *CNR2* variant Q63R is significantly linked to childhood chronic ITP. The results from their study confirm the role that CB2 plays in autoimmunity susceptibility, and divulged a previously unknown association between this receptor and childhood chronic ITP (Rossi et al., 2011).

In 2013, Gouda and coworkers investigated the link between the *CNR2* Q63R polymorphism and childhood ITP in a case–control association study consisting of 100 patients. This is the first study to describe this association in a population of Egyptian children. The relative odds ratio indicated that the risk of acquiring childhood chronic ITP was more than double in children who were homozygous for the variant. In contrast, there was no significant difference between the acute ITP patient group and the control group, regarding the Q63R genotype. Therefore, it was concluded that the *CNR2* Q63R polymorphism is a predictor of chronic, but not acute ITP (Mahmoud Gouda & Mohamed Kamel, 2013). The results from this study are consistent with the findings from Rossi et al. (2011).

Celiac disease (CD) is a digestive and autoimmune disorder often characterized by chronic inflammation that damages the lining of the small intestine. CD can be triggered by the consumption of gluten, a protein found in several grain products. In 2012, Rossi and coworkers investigated the association of rs35761398 (CAA/CGG), the Q63R *CNR2* variant, with CD in a cohort of 327 southern Italian children. The finding of this study demonstrated that the Q63R variant was significantly associated with CD. Specifically, the Q63R variant conferred a greater than sixfold risk for developing CD (Rossi et al., 2012a).

Overall, the three studies discussed in this section provide evidence that the Q63R polymorphism of *CNR2* is associated with disorders of the immune system (ie, ITP and CD).

Association Between CB2 Polymorphisms and Cardiovascular Disorders

Reinhard et al. hypothesized that variations within *CNR2* may be linked to myocardial infarction (MI) or cardiovascular risk factors. They conducted a large case–control study involving 1968 individuals from German MI families. Specifically, the association between 13 SNPs (within and near the *CNR2* gene) with MI or cardiovascular risk factors (eg, arterial hypertension, obesity, hypercholesterolemia, and diabetes mellitus) was investigated. Reinhard et al. concluded that, in this large German population, common SNPs within the *CNR2* gene confer no risk to MI or to cardiovascular risk factors. However, it should be pointed out that Reinhard et al. (2008) did not investigate the association between Q63R and MI or cardiovascular factors.

Association Between CB2 Polymorphisms and Liver Disorders

This section will review the three studies in which the Q63R variant has been investigated for its contribution to liver disorders.

Primary biliary cirrhosis (PBC) is a liver disorder involving cholestasis, fibrosis, and biliary cirrhosis. PBC is thought to be modulated by autoimmune processes. In 2010, Floreani and coworkers investigated if the CB2 receptor is modulated in the livers of PBC patients, and if genetic variations in *CNR2* may be associated with PBC. They found that there was no significant increase in the mRNA expression of CB2 in patients with PBC. In addition, since the CB2 polymorphism Q63R (188-189 AA/GG) was observed in 24.4% of Italian patients, 30.4% of US patients, and 28.0% of Italian control samples (p = n.s.), they concluded that the association between CB2 polymorphism and PBC was not significant (Floreani et al., 2010).

Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) consist of a broad spectrum of conditions, including steatosis and inflammatory steatohepatitis, which can be associated with differing degrees of fibrosis, and can potentially progress to cirrhosis. The endocannabinoid system has recently been implicated in liver diseases. In 2012, Rossi and coworkers investigated the association of the rs35761398 *CNR2* Q63R variant and liver disease in a population of 118 Italian children with NAFLD, as confirmed by histopathology. They reported that the Q63R variant was not linked to steatosis or fibrosis, but was associated with the severity of the inflammation and appearance of NASH. However, it should be mentioned that the lack of association of Q63R with fibrosis can possibly be due to the young age of the subjects in this study, as the disease simply may have not progressed. Overall, Rossi et al. (2012b) concluded that the Q63R polymorphism may play a role in modulating the inflammatory state in obese children and can potentially predispose these children to liver damage.

In patients with chronic hepatitis C (CHC), CB2 seems to display hepatoprotective properties. Recently,

Coppola et al. conducted the first study to investigate the association between rs35761398 Q63R CB2 polymorphism and CHC, in a cohort of 169 Italian patients with anti-HCV/HCV RNA-positive asymptomatic chronic hepatitis. Coppola et al. (2014) concluded that the Q63R variant of the *CNR2* gene is associated with severe inflammation and hepatocellular necrosis.

Overall, it seems that, whether or not there is an association with *CNR2* Q63R polymorphism, depends on the type of liver disease studied.

Association Between CB2 Polymorphisms and Bone Disorders

Osteoporosis is a skeletal disorder characterized by a decrease in bone mineral density (BMD), reduction in bone mass, and a weakening of the microarchitecture of the bone which results in an increased liability to bone fractures. To date, four studies have investigated the association between SNPs in *CNR2* and bone disorders (Huang, Li, & Kung, 2009; Karsak et al., 2005, 2009; Yamada, Ando, & Shimokata, 2007).

Karsak et al. (2005) were the first to show that the CB2 receptor maybe involved in the etiology of osteoporosis in humans by pursuing a systematic genetic case-controlled association study. The subjects consisted of 388 women of Caucasian and French origin (168 patients with postmenopausal osteoporosis and 220 controls); 26 SNPs in the *CNR2* gene were genotyped and 13 of the SNPs were located in the coding exon. Of the 26 SNPs, 18 are significant for allele and genotype distributions. All 13 SNPs in the coding exon were significant for allele and genotype distributions. Of the 13 SNPs in the coding exon, rs4649124 (synonymous) and rs2501431 (A155G-nonsynmous) showed the most significant *P*-values for allele and genotype distributions. Among the 13 SNPs in the coding exon, 3 were nonsynonymous (rs2501432 Q63R, rs2501431 A155G, rs2229579 H316Y). Karsak et al. (2005) concluded that the CNR2 gene is associated with human osteoporosis.

Yamada et al. (2007) genotyped the rs2501431 (A155G) SNP of *CNR2* in a Japanese cohort of 1110 women and 1128 men, in a population-based prospective cohort study, and the results demonstrated that polymorphisms of *CNR2* were associated with BMD in premenopausal and postmenopausal women.

In 2009, Huang and coworkers conducted an association study of four candidate genes, including *CNR2*, in 1243 case–control Chinese subjects. Six SNPs in the *CNR2* gene were analyzed. Of the six SNPs that were analyzed, only A155G was significant for allelic associations with both trochanter BMD and hip BMD. Of the six SNPs that were analyzed, four are located within exon 2. Among the SNPs in exon 2, only one was nonsynonymous (rs2501431-A155G). Their conclusion was that rs2501431 (A155G) has significant allelic association with both trochanter BMD and hip BMD. Huang et al. (2009) also investigated the nonsynonymous *CNR2* SNP rs2229579 (H316Y), and found it was not significantly associated with trochanter BMD or hip BMD.

In 2009, Karsak et al. investigated the association of polymorphisms in *CNR2* with hand bone strength in a family-based study of 574 subjects (290 men, 277 women) from an ethnically homogeneous healthy family sample of European origin (Chuvashians) living in Russia. Specifically, Karsak et al. genotyped 16 SNPs spanning the extended *CNR2* locus. Of the 16 SNPs, 8 were significantly associated with BMD and breaking bending resistance index (BBRI). Furthermore, of the 16 SNPs, 6 were located in the coding exon and contained 3 nonsynonymous SNPs (rs2501432 Q63R, rs2229579 H316Y, and rs2501431 A155G). Of the SNPs in the coding exon, five were significantly associated with BMD and BBRI which included the two nonsynonymous SNPs (Q63R, A155G), but not H316Y. Overall, there were five SNPs in the coding exon of *CNR2* that are significantly related to both BMD and BBRI. The results from this study confirmed and extended previous studies regarding the association of *CNR2* and bone phenotypes (Karsak et al., 2009).

While the studies described earlier provide evidence that implicates *CNR2* as a candidate gene for osteoporosis, the *CNR2* locus was not reported as loci for BMD or osteoporosis in recent GWAS (Richards et al., 2008; Styrkarsdottir et al., 2008). However, it is worth mentioning that these two GWAS used platforms which showed a gap in SNP coverage of about 200 kb which included *CNR2* and neighboring genes. In other words, the *CNR2* locus was not sufficiently covered in these GWAS, and future studies using novel arrays (covering this locus) are needed to determine the contribution of *CNR2* variants in BMD and osteoporosis (Richards et al., 2008; Styrkarsdottir et al., 2008).

Association Between CB2 Polymorphisms and CNS Disorders

There have been eight published studies that investigated the association between the SNPs in *CNR2* and CNS disorders, including alcoholism, eating disorders, depression, bipolar disorders, and schizophrenia.

Association Between CB2 Q63 Polymorphism and Alcoholism

In 2007, Ishiguro and coworkers published data demonstrating that there is an association (odds ratio = 1.25) between the rs2501432 Q63R polymorphism of the *CNR2* gene and alcoholism, in a Japanese population (667 male

and 118 female and 487 age- and gender-matched controls). Ishiguro et al. (2007) concluded that the Q63R *CNR2* polymorphism may influence the susceptibility for developing alcoholism.

Association Between CB2 Polymorphisms and Eating Disorders

In 2010, Ishiguro and coworkers tested the hypothesis that *CNR2* genetic variants may be important for food consumption by examining the association between the *CNR2* gene and eating disorders (anorexia nervosa and bulimia nervosa) in 204 subjects and 1876 healthy volunteers in a Japanese population. Ishiguro et al. (2010a) demonstrated a link between the R63Q polymorphism of the *CNR2* gene and eating disorders (odds ratio 1.24).

In 2014, Ketterer and coworkers investigated the association between the *CNR2* polymorphisms and body weight in a cross-sectional cohort study of 2006 subjects who were at an increased risk for type 2 diabetes. In this study, five SNPs were analyzed. Of the five SNPs, two were located in exon 2 [rs2229579 (H316Y) and rs3123554]. Of the SNPs in exon 2, only the minor allele of rs3123554 was significant. Ketterer et al. demonstrated that the minor of allele of rs3123554 was associated with reduced body weight. In addition, interestingly, carriers of this allele lose less body weight during lifestyle intervention, which is associated to the lower cerebral insulin sensitivity of these individuals. The authors of this study noted that the reduced body mass index (BMI) in female carriers of this allele may be due to peripheral metabolic mechanisms (Ketterer et al., 2014).

Association Between CB2 Q63R Polymorphism and Depression

In 2008, Onaivi and coworkers hypothesized that genetic variants of the *CNR2* gene may be associated with depression in a Japanese population. They demonstrated, in depressed Japanese subjects, there is a high occurrence of the Q63R, but not H316Y polymorphism in the *CNR2* gene. This study provided the first evidence for a role of CB2 in depression (Onaivi et al., 2008a,b).

In 2012, Mitjans and coworkers conducted a longitudinal study that analyzed the genetic variability in the *CNR2* gene in response to a 12-week treatment to citalopram. They demonstrated that AA allele carriers (rs2501431–A155G) exhibited higher Hamilton Depression Rating Scale, as compared to the G allele carriers. This scale is used by clinicians to determine the level of depression in a patient before, during, and after treatment. Mitjans, Gasto, Catalan, Fananas, and Arias (2012) concluded that the *CNR2* gene is associated more with severity of disease outcome versus treatment response.

Association Between CB2 Polymorphisms and Bipolar Disorder

In 2011, Minocci et al. tested the hypothesis that *CNR2* genetic variants might be associated with BD. Specifically, three *CNR2* missense SNPs, rs2501432 (315ANG; Q63R), rs41311993 (L133I), and rs2229579 (H316Y) were investigated in a case–control study that included 80 patients and 160 healthy subjects. A significant correlation was found between BD and *CNR2* 524C > A (L133I polymorphism). In contrast, no association was observed for the other two SNPs. Overall, Minocci et al. (2011) showed for the first time a link between bipolar disorder and the I133L polymorphism in the *CNR2* gene in a population of patients of Italian descent.

Association Between CB2 Polymorphisms and Schizophrenia

Previously, there have been several reports describing the link between marijuana use and schizophrenia.

In 2010, Ishiguro and coworkers examined genetic associations between *CNR2* gene polymorphisms and schizophrenia in two independent case–control populations. The first group consisted of 1152 East Japanese subjects and 1194 control subjects. The second group consisted of 768 West Japanese subjects and 726 control subjects. Differences in the alleles of associated SNPs were analyzed using postmortem (human) brain tissues, and in cultured cells. They analyzed five SNPs. Three of the SNPs are located in the intron region of *CNR2*, and two SNPs [rs2501432 (R63Q) and rs2229579 (H316Y)] are located in exon 2 of *CNR2*. Among the SNPs analyzed, Ishiguro et al. identified two SNPs in *CNR2* gene that are associated with schizophrenia in Japanese populations: (1) rs2501432, which is the missense polymorphism R63Q; and (2) rs12744386, which is located in the intron region, and was found to be associated with low CB2 mRNA and protein expression levels. Ishiguro et al. (2010b) concluded that individuals who exhibit low CB2 receptor expression or low CB2 function may have an increased risk to schizophrenia.

In 2013, Tong and coworkers investigated the association between 3 *CNR2* gene polymorphisms (rs2501432 Q63R, rs2229579 H316Y, rs2501401 G/A) and schizophrenia in 316 patients and 334 healthy control subjects. The *CNR2* polymorphisms Q63R and H316Y are located on the second exon, while rs2501401 is found in the intron. Tong et al. (2013) demonstrated that the TT genotype and T allele frequencies of Q63R were lower, as compared to controls, indicating a protective effect. In addition, they concluded that the T allele of H316Y may increase the risk for developing schizophrenia. Furthermore, there was no significant difference in the genotype and rs2501401 G/A allele frequencies between the case and control groups, demonstrating that the rs2501401 G/A polymorphism may not be related to the risk of developing schizophrenia (Tong et al., 2013).

POSSIBLE ROLE OF *CNR2* IN PERSONALIZED MEDICINE?

Personalized medicine refers to the determination of an individual's genetic makeup which can result in a more fine-tuned diagnosis and treatment. In addition, by doing so, a more specific treatment can be selected, which may increase the likelihood of a successful outcome and reduce adverse drug reactions (Howland, 2012a,b).

Diagnosis

Recent genetic studies (described earlier) provide evidence that *CNR2* variants are associated with a broad range of conditions (eg, immune disorders, liver disorders, osteoporosis, and CNS diseases), suggesting that high-risk individuals could possibly be identified through genetic testing. For example, Sipe et al. (2005) demonstrated that there is a link between the *CNR2* variant Q63R and immune cell regulation and function in humans. This can potentially be used as a diagnostic marker for individuals who are at higher risk for certain autoimmune diseases.

Treatment

The CB2 receptor represents a novel pharmacological target. Drugs acting specifically on CB2 would not have the psychoactive effects of CB1 agonists. CB2 receptor agonists could be potentially useful for the treatment of a variety of diseases, including autoimmune diseases, liver diseases, osteoporosis, and CNS disorders.

As a potential therapeutic target, CB2 receptor can be genotyped and, based on the presence of a particular SNPs in CB2, health professionals can potentially determine the specific cannabinoid drug and proper dosages of cannabinoids to be administered to the specific patient. For example, Sipe et al. (2005) demonstrated that in T lymphocytes from individuals who are homozygous (GG) for the CB2 188-189 variant, the inhibition produced by the endogenous cannabinoid 2-AG was consistently less, compared with cells from the CB2 188-189 AA/AA homozygotes. Therefore, in individuals who are homozygous for the CB2 188-189 variant, a higher concentration of exogenous cannabinoid will need to be administered to achieve therapeutic response.

MINI-DICTIONARY

Allele A variant form of a gene defined as one of a number of alternative forms of the same gene or same genetic locus.
Case–control studies A term commonly used in genetics which refers to comparing the frequency of alleles or genotypes between cases and control.
Genetic polymorphisms A polymorphism refers to a mutation that displays an allele frequency greater than or equal to 1%.
Genotyping A commonly used method to determine differences in the genetic make-up of an individual by investigating an individual's DNA sequence, and comparing it to another individual's sequence or a reference sequence.
Genome-wide association study (GWAS) A method that involves the rapid scanning of markers across complete sets of DNA or genomes of individuals to determine genetic variations associated with a particular disease. These studies are most commonly performed by comparing cases (affected individuals) and controls (unaffected individuals) unrelated to each other.
Nonsynonymous SNP A coding SNP that alters the protein sequence. Two types of nonsynonymous SNPs are missense (results in a different amino acid) or nonsense (results in a premature stop codon).
Odds ratio A measurement of association typically used in case–control studies. It is defined as the odds of exposure to the susceptible genetic variant in cases compared with the odds of exposure in controls. If the odds ratio is significantly greater than one, the genetic variant is associated with the disease.
Reference single nucleotide polymorphism identification number ("rs" ID) An identification number assigned by NCBI which refers to a cluster of SNPs that map to an identical location.
Single nucleotide polymorphism (SNP) A SNP is a change in a single base pair in a specific position along the genome that may or may not be functionally relevant.
Synonymous SNP A coding SNP that usually does not alter the protein sequence.

References

Cargill, M., & Daley, G. Q. (2000). Mining for SNPs: putting the common variants—common disease hypothesis to the test. *Pharmacogenomics*, *1*(1), 27–37.

Carrasquer, A., Nebane, N. M., Williams, W. M., & Song, Z. H. (2010). Functional consequences of nonsynonymous single nucleotide polymorphisms in the CB2 cannabinoid receptor. *Pharmacogenetics and Genomics*, *20*(3), 157–166.

Coppola, N., Zampino, R., Bellini, G., Macera, M., Marrone, A., Pisaturo, M., …, Rossi, F. (2014). Association between a polymorphism in cannabinoid receptor 2 and severe necroinflammation in patients with chronic hepatitis C. *Clinical Gastroenterology and Hepatology*, *12*(2), 334–340.

Farrall, M., & Morris, A. P. (2005). Gearing up for genome-wide gene-association studies. *Human Molecular Genetics*, *14*(Spec No. 2), R157–R162.

Floreani, A., Lazzari, R., Macchi, V., Porzionato, A., Variola, A., Colavito, D., …, Bergasa, N. V. (2010). Hepatic expression of endocannabinoid receptors and their novel polymorphisms in primary biliary cirrhosis. *Journal of Gastroenterology*, *45*(1), 68–76.

Gray, I. C., Campbell, D. A., & Spurr, N. K. (2000). Single nucleotide polymorphisms as tools in human genetics. *Human Molecular Genetics*, *9*(16), 2403–2408.

Howland, R. H. (2012a). Future prospects for pharmacogenetics in the quest for personalized medicine. *Journal of Psychosocial Nursing and Mental Health Services*, *50*(12), 13–16.

Howland, R. H. (2012b). Where are we today with personalized medicine? *Journal of Psychosocial Nursing and Mental Health Services*, *50*(11), 11–13.

Howlett, A. C. (2005). Cannabinoid receptor signaling. *Handbook of Experimental Pharmacology*, *168*, 53–79.

Huang, Q. Y., Li, G. H., & Kung, A. W. (2009). Multiple osteoporosis susceptibility genes on chromosome 1p36 in Chinese. *Bone*, *44*(5), 984–988.

Ishiguro, H., Carpio, O., Horiuchi, Y., Shu, A., Higuchi, S., Schanz, N., …, Onaivi, E. S. (2010a). A nonsynonymous polymorphism in cannabinoid CB2 receptor gene is associated with eating disorders in humans and food intake is modified in mice by its ligands. *Synapse*, *64*(1), 92–96.

Ishiguro, H., Horiuchi, Y., Ishikawa, M., Koga, M., Imai, K., Suzuki, Y., …, Arinami, T. (2010b). Brain cannabinoid CB2 receptor in schizophrenia. *Biological Psychiatry*, *67*(10), 974–982.

Ishiguro, H., Iwasaki, S., Teasenfitz, L., Higuchi, S., Horiuchi, Y., Saito, T., …, Onaivi, E. S. (2007). Involvement of cannabinoid CB2 receptor in alcohol preference in mice and alcoholism in humans. *Pharmacogenomics Journal*, *7*(6), 380–385.

Karsak, M., Cohen-Solal, M., Freudenberg, J., Ostertag, A., Morieux, C., Kornak, U., …, Zimmer, A. (2005). Cannabinoid receptor type 2 gene is associated with human osteoporosis. *Human Molecular Genetics*, *14*(22), 3389–3396.

Karsak, M., Malkin, I., Toliat, M. R., Kubisch, C., Nurnberg, P., Zimmer, A., & Livshits, G. (2009). The cannabinoid receptor type 2 (CNR2) gene is associated with hand bone strength phenotypes in an ethnically homogeneous family sample. *Human Genetics*, *126*(5), 629–636.

Ketterer, C., Heni, M., Stingl, K., Tschritter, O., Linder, K., Wagner, R., …, Fritsche, A. (2014). Polymorphism rs3123554 in CNR2 reveals gender-specific effects on body weight and affects loss of body weight and cerebral insulin action. *Obesity*, *22*(3), 925–931.

Komar, A. A. (2007). Genetics. SNPs, silent but not invisible. *Science*, *315*(5811), 466–467.

Liu, Q. R., Pan, C. H., Hishimoto, A., Li, C. Y., Xi, Z. X., Llorente-Berzal, A., …, Uhl, G. R. (2009). Species differences in cannabinoid receptor 2 (CNR2 gene): identification of novel human and rodent CB2 isoforms, differential tissue expression and regulation by cannabinoid receptor ligands. *Genes Brain and Behavior*, *8*(5), 519–530.

Mahmoud Gouda, H., & Mohamed Kamel, N. R. (2013). Cannabinoid CB2 receptor gene (CNR2) polymorphism is associated with chronic childhood immune thrombocytopenia in Egypt. *Blood Coagulation and Fibrinolysis*, *24*(3), 247–251.

Malfitano, A. M., Basu, S., Maresz, K., Bifulco, M., & Dittel, B. N. (2014). What we know and do not know about the cannabinoid receptor 2 (CB2). *Seminars in Immunology*, *26*, 369–379.

Mallat, A., Teixeira-Clerc, F., Deveaux, V., Manin, S., & Lotersztajn, S. (2011). The endocannabinoid system as a key mediator during liver diseases: new insights and therapeutic openings. *British Journal of Pharmacology*, *163*(7), 1432–1440.

Minocci, D., Massei, J., Martino, A., Milianti, M., Piz, L., Di Bello, D., …, Nieri, P. (2011). Genetic association between bipolar disorder and 524A > C (Leu133Ile) polymorphism of CNR2 gene, encoding for CB2 cannabinoid receptor. *Journal of Affective Disorders*, *134*(1–3), 427–430.

Mitjans, M., Gasto, C., Catalan, R., Fananas, L., & Arias, B. (2012). Genetic variability in the endocannabinoid system and 12-week clinical response to citalopram treatment: the role of the CNR1, CNR2 and FAAH genes. *Journal of Psychopharmacology*, *26*(10), 1391–1398.

Munro, S., Thomas, K. L., & Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, *365*(6441), 61–65.

Onaivi, E. S., Ishiguro, H., Gong, J. P., Patel, S., Meozzi, P. A., Myers, L., …, Uhl, G. R. (2008a). Brain neuronal CB2 cannabinoid receptors in drug abuse and depression: from mice to human subjects. *PLoS One*, *3*(2), e1640.

Onaivi, E. S., Ishiguro, H., Gong, J. P., Patel, S., Meozzi, P. A., Myers, L., …, Uhl, G. R. (2008b). Functional expression of brain neuronal CB2 cannabinoid receptors are involved in the effects of drugs of abuse and in depression. *Annals of the New York Academy of Sciences*, *1139*, 434–449.

Onaivi, E. S., Ishiguro, H., Gu, S., & Liu, Q. R. (2012). CNS effects of CB2 cannabinoid receptors: beyond neuro-immuno-cannabinoid activity. *Journal of Psychopharmacology*, *26*(1), 92–103.

Pertwee, R. G. (2005). Pharmacological actions of cannabinoids. *Handbook of Experimental Pharmacology*, *168*, 1–51.

Pertwee, R. G., & Ross, R. A. (2002). Cannabinoid receptors and their ligands. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, *66*(2–3), 101–121.

Reinhard, W., Stark, K., Neureuther, K., Sedlacek, K., Fischer, M., Baessler, A., …, Hengstenberg, C. (2008). Common polymorphisms in the cannabinoid CB2 receptor gene (CNR2) are not associated with myocardial infarction and cardiovascular risk factors. *International Journal of Molecular Medicine*, *22*(2), 165–174.

Richards, J. B., Rivadeneira, F., Inouye, M., Pastinen, T. M., Soranzo, N., Wilson, S. G., …, Spector, T. D. (2008). Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. *Lancet*, *371*(9623), 1505–1512.

Rossi, F., Bellini, G., Alisi, A., Alterio, A., Maione, S., Perrone, L., …, Nobili, V. (2012a). Cannabinoid receptor type 2 functional variant influences liver damage in children with non-alcoholic fatty liver disease. *PLoS One*, *7*(8), e42259.

Rossi, F., Bellini, G., Tolone, C., Luongo, L., Mancusi, S., Papparella, A., …, del Giudice, E. M. (2012b). The cannabinoid receptor type 2 Q63R variant increases the risk of celiac disease: implication for a novel molecular biomarker and future therapeutic intervention. *Pharmacological Research*, *66*(1), 88–94.

Rossi, F., Mancusi, S., Bellini, G., Roberti, D., Punzo, F., Vetrella, S., …, Perrotta, S. (2011). CNR2 functional variant (Q63R) influences childhood immune thrombocytopenic purpura. *Haematologica*, *96*(12), 1883–1885.

Sauna, Z. E., Kimchi-Sarfaty, C., Ambudkar, S. V., & Gottesman, M. M. (2007). Silent polymorphisms speak: how they affect pharmacogenomics and the treatment of cancer. *Cancer Research*, *67*(20), 9609–9612.

Schork, N. J., Fallin, D., & Lanchbury, J. S. (2000). Single nucleotide polymorphisms and the future of genetic epidemiology. *Clinical Genetics*, *58*(4), 250–264.

Sherry, S. T., Ward, M. H., Kholodov, M., Baker, J., Phan, L., Smigielski, E. M., & Sirotkin, K. (2001). dbSNP: the NCBI database of genetic variation. *Nucleic Acids Research*, *29*(1), 308–311.

Sherwood, T. A., Nong, L., Agudelo, M., Newton, C., Widen, R., & Klein, T. W. (2009). Identification of transcription start sites and preferential expression of select CB2 transcripts in mouse and human B lymphocytes. *Journal of Neuroimmune Pharmacology*, *4*(4), 476–488.

Sipe, J. C., Arbour, N., Gerber, A., & Beutler, E. (2005). Reduced endocannabinoid immune modulation by a common cannabinoid 2 (CB2) receptor gene polymorphism: possible risk for autoimmune disorders. *Journal of Leukocyte Biology*, *78*(1), 231–238.

Steffens, S., & Pacher, P. (2012). Targeting cannabinoid receptor CB2 in cardiovascular disorders: promises and controversies. *British Journal of Pharmacology*, *167*(2), 313–323.

Styrkarsdottir, U., Halldorsson, B. V., Gretarsdottir, S., Gudbjartsson, D. F., Walters, G. B., Ingvarsson, T., …, Stefansson, K. (2008). Multiple genetic loci for bone mineral density and fractures. *New England Journal of Medicine*, *358*(22), 2355–2365.

Syvanen, A. C. (2001). Accessing genetic variation: genotyping single nucleotide polymorphisms. *Nature Reviews Genetics*, *2*(12), 930–942.

Tam, J., Ofek, O., Alexandrovich, A., Yirmiya, R., Mackie, K., Mechoulam, R., …, Bab, I. (2005). Traumatic brain injury-induced stimulation of peripheral bone formation: role of neuronal cannabinoid receptor. *Journal of Neurotrauma*, *22*(10), 1182.

Tong, D., He, S., Wang, L., Jin, L., Si, P., & Cheng, X. (2013). Association of single-nucleotide polymorphisms in the cannabinoid receptor 2 gene with schizophrenia in the Han Chinese population. *Journal of Molecular Neuroscience*, *51*(2), 454–460.

Yamada, Y., Ando, F., & Shimokata, H. (2007). Association of candidate gene polymorphisms with bone mineral density in community-dwelling Japanese women and men. *International Journal of Molecular Medicine*, *19*(5), 791–801.

CHAPTER

62

Chemistry of Cannabinoid Receptor Agonists

M. Aghazadeh Tabrizi, P.G. Baraldi

Department of Chemistry and Pharmaceutical Science, University of Ferrara, Ferrara, Italy

SUMMARY POINTS

- Cannabinoid ligands are characterized by a wide chemical diversity. This diversity has different consequences. First, it has been fairly complicated to supply a common pharmacophore for all cannabinoid agonists. Second, the structure–activity relationships are not the same in the whole family.
- Conspicuous antinociceptive effects of CB agonists in different models of inflammatory or neuropathic pain have been demonstrated.
- The CB_1 selective agonists include anandamide analogs (ACEA, ACPA) and O-1812, cyano analog of methandamide.
- The CB_2 selective agonists include nonendogenous cannabinoids, traditional cannabinoids such as L-759,633, JWH-133, HU-308, aminoalkylindoles (AM-1241), and several different chemical structures are the purpose of the discussion in this chapter.
- Several cannabinoid receptor agonists show distinct stereoselectivity in pharmacological assays, reflecting the presence of chiral centers in these molecules.

KEY FACTS OF CANNABINOID RECEPTORS

- Cannabinoid receptors are responsible for the mediation of the psychoactive effects of cannabis they are also implicated in a variety of physiological processes.
- The two types of cannabinoid receptors named CB_1 and CB_2 are G protein coupled receptors.
- Activation of the cannabinoid receptors causes inhibition of adenylate cyclase, and a subsequent decrease in the concentration of cyclic adenosine monophosphate in the cell, resulting in the inhibition of neurotransmission.
- Δ^9-Tetrahydrocannabinol (Δ^9-THC), representing the psychoactive principle of *Cannabis*, was identified in 1964.
- The CB_1 receptor is expressed mainly in brain, lungs, liver, and kidneys; the CB_2 receptor is expressed principally in the immune system.
- Cannabinoid receptors are activated by phytocannabinoids (found in *Cannabis*), endocannabinoids (produced naturally in the body by humans and animals), and synthetic cannabinoids (produced chemically by humans).

LIST OF ABBREVIATIONS

2-AG	2 Arachidonoylglycerol
ACEA	Arachidonyl-2′-chloroethylamide
ACPA	Arachidonylcyclopropylamide
AEA	Anandamide
cAMP	Cyclic adenosine monophosphate
CBD	Cannabidiol
CNS	Central nervous system
EC_{50}	Median effective concentration
FAAH	Fatty acid amide hydrolase
K_i	Inhibition constant
SAR	Structure–activity relationship
THC	Tetrahydrocannabinol

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00072-7
Copyright © 2017 Elsevier Inc. All rights reserved.

INTRODUCTION

Cannabinoids, the active components of *Cannabis sativa* and their derivatives, are known to mediate some of their actions through the cannabinoid receptors. Two distinct cannabinoid receptors, named CB_1 and CB_2, have been cloned and characterized from mammalian tissues. The CB_1 receptor is abundantly expressed in the central nervous system (CNS) and is responsible for the psychotropic side effects. The CB_2 receptor is mainly found in cells of the immune system, though it may be upregulated in the CNS under pathological conditions. The main signal transduction pathway triggered is through G_i proteins, resulting in an inhibition of adenylate cyclase activity, and a decrease in cyclic adenosine monophosphate (cAMP) levels (Pertwee et al., 2010). Several studies have demonstrated noticeable antinociceptive effects of CB agonists in different models of inflammatory or neuropathic pain (Guindon & Hohmann, 2008). These models could be useful for the preclinical evaluation and validation of the therapeutic efficacy of novel putative analgesics (Ashton & Milligan, 2008). However, it was mainly drugs interacting with CB_1 receptors that showed marked central side effects that have disallowed their prevalent acceptance and therapeutic application (Kreitzer & Stella, 2009).

The first part of this chapter describes cannabinoid ligands commonly classified into the following chemical groups: tricyclic and bicyclic analogs of tetrahydrocannabinol (THC); endogenous cannabinoids, and the aminoalkylindoles.

In the second part, we describe the chemical structures of the recent entry of several different cores as cannabinoid ligands.

TRICYCLIC AND BICYCLIC ANALOGS OF TETRAHYDROCANNABINOL

Chemical structures are shown in Fig. 62.1; biological data are shown in Table 62.1.

The tricyclic natural compounds fall into the classical cannabinoids. The prototypical (6aR,10aR)-Δ^9-THC (**1**) is the mainly well-known phytocannabinoid, and it is a nonselective agonist that binds to both CB_1 and CB_2 with a similar affinity in the order of 40 nM (Howlett et al., 2002). This natural product readily isomerizes to thermodynamically more stable isomer (**2**, Δ^8-THC) under acidic conditions, which maintains a similar level of affinity. Classical and nonclassical cannabinoids include the tricyclic and bicyclic derivatives of Δ^9-THC and, in particular, derived from more stable isomer Δ^8-THC. For example, (6aR,10aR)-9-(hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,10,10a-tetrahydrobenzo[*c*]chromen-1-ol **3** (HU-210**)** is the (+)-1,1-dimethylheptyl analog of 11-hydroxy-Δ^8-THC which is more potent than natural THC for both CB_1 and CB_2 receptors. The (−)enantiomer (HU-211) being inactive at cannabinoid receptors highlighted the preference for the 6aR, 10aR enantiomer.

The 1-deoxy-Δ^8-THC derivatives were synthesized by replacing the phenolic hydroxyl function leading to **4** (JWH-051), that exhibited significantly enhanced affinity for both the CB_2 and the CB_1 receptors. Other 1-deoxy-Δ^8-THC compounds such as **5** (JWH-056), and **6 (**JWH-133) showed a total loss of CB_1 affinity. In this series, JWH-133 has very high affinity and selectivity for the CB_2 receptor (K_i 3.4 nM). Alternatively, methylation of the phenolic hydroxyl group also reduced affinity for the CB_1 receptor such as that found in compound **7** (L759,633, K_i CB_1 1043 nM) (Huffman, 2000). The bicyclic compounds fall into the nonclassical cannabinoids and derived from opening of the pyran ring of Δ^9-THC. Cannabidiol (CBD) (**8**) is a bicyclic cannabinoid devoid of any psychoactive properties produced by Δ^8-THC or Δ^9-THC. Interestingly, a 3-phenylcyclohexanol derivative **9** (CP-47,497) which has been described as producing many of the pharmacological effects produced by Δ^9-THC, acts as full receptor agonists for both the CB_1 and CB_2 cannabinoid receptors. Another related bicyclic analog 2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-5-(2-methyloctan-2-yl) phenol **10** (CP-55,940) has been used to identify a cannabinoid binding site (Showalter, Compton, Martin, & Abood, 1996). The bicyclic ligand **11** (HU-308) which has the opposite absolute configuration from all other analogs, is a CB_2 specific agonist. A slight modification of dimethylbicycloheptene leading to the **12** (HU-910), enhanced the affinity of the molecule for CB_2 receptor (Horvath et al., 2012).

ENDOGENOUS CANNABINOIDS AND SYNTHETIC ANALOGS

Chemical structures are shown in Fig. 62.2; biological data are shown in Table 62.1.

Generally, the endocannabinoids show better affinity for the CB_1 than CB_2 receptor. In 1992, the first endogenous ligand cannabinoid receptors was isolated and named anandamide (arachidonoylethanolamide, AEA, **13**) (Howlett et al., 2002). Anandamide exerts its biological effects through both cannabinoid receptors as partial agonist. The endogenous agonist of the CB_1 receptor, 2-arachidonoylglycerol (**14**, 2-AG), is the arachidonic acid ester of glycerol and has higher affinity and efficacy to CB_1 and CB_2 receptors than AEA. 2-Arachidonoylglyceryl ether (**15**, 2-AGE or noladin ether) is an ether-linked analog of 2-AG, binds to CB_1 and very weakly to CB_2 receptor.

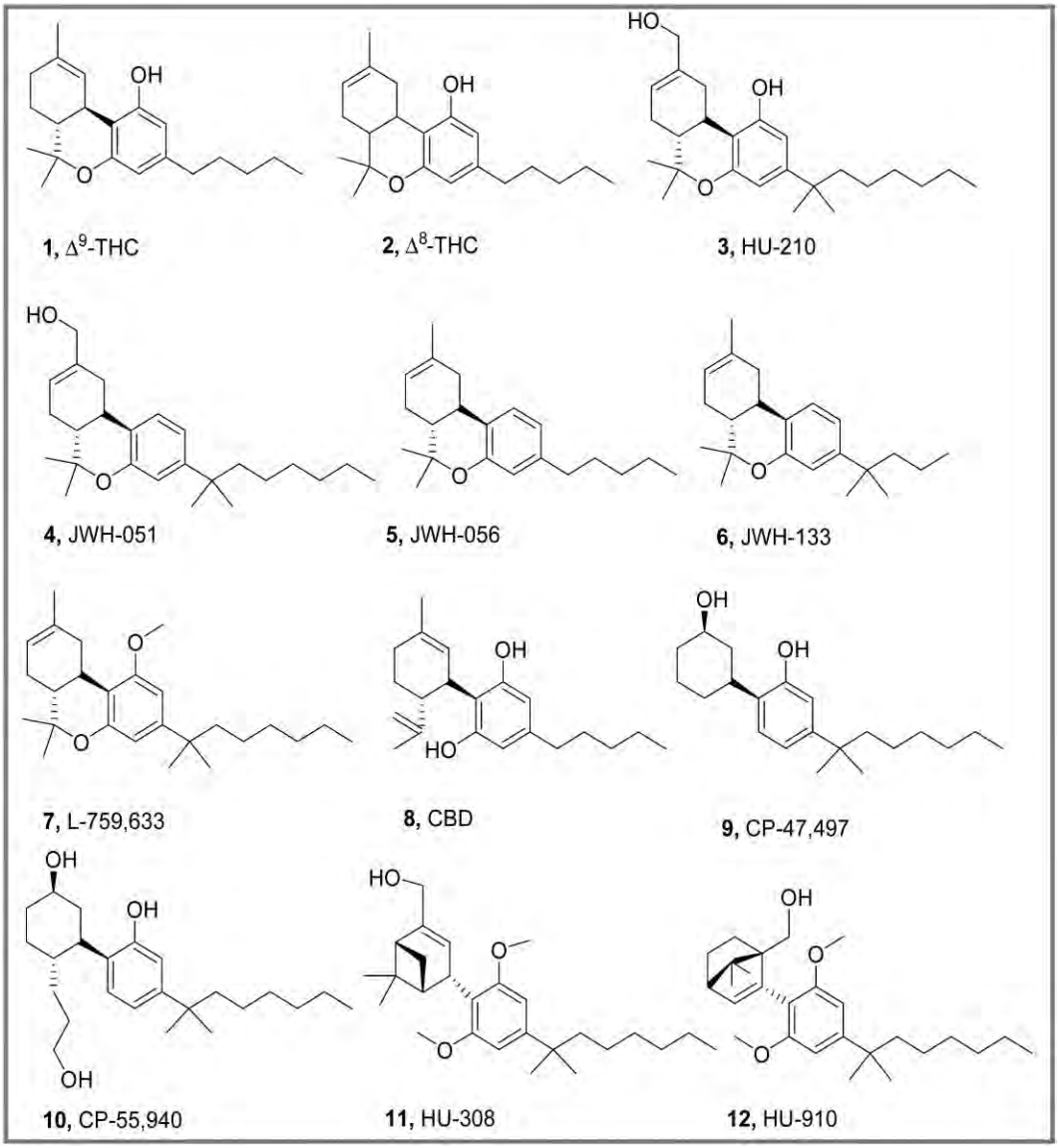


FIGURE 62.1 **Chemical structures of tricyclic and bicyclic analogs of THC.**

To improve metabolic stability and to increase the CB_1 selectivity, the methanandamide series was synthesized. Compound **16** (R-(+)-methanandamide), named also AM356, is a stable chiral analog of anandamide and the CB_1 selectivity of R-(+)-methanandamide derived from the introduction of a methyl group on the 1 carbon of AEA, a structural change that also confers better resistance to the hydrolytic inactivation by fatty acid amide hydrolase (FAAH). O-1812 (compound **17**) is a cyano analog of methanandamide, behaves as potent CB_1 receptor agonist, and acts as a potent and highly selective agonist for the CB_1 receptor (K_i of 3.4 nM at CB_1 and 3870 nM at CB_2 receptors). Unlike most related compounds, O-1812 is metabolically stable against rapid breakdown by FAAH. Arachidonyl-2′-chloroethylamide (**18**, ACEA) and arachidonylcyclopropylamide **19** (ACPA) were prepared by replacement of ethanolamide chain with lipophilic moieties. These analogs are considered to be selective cannabinoid agonists, as they bind primarily to the CB_1 receptor (Pertwee, 2005).

INDOLES

Chemical structures are shown in Fig. 62.3; biological data are shown in Table 62.1.

These ligands consisted of an indole core substituted by the lipophilic aroyl group at C3 and an aminoalkyl side chain at N1 positions (Huffman, 1999). The prototypical **20** (R-(+)-WIN-55,212), is one of the best characterized synthetic cannabinoids from the aminoalkylindole series that has been employed extensively in a number of investigations into the pharmacology of this group of compounds. The R-enantiomer is a potent, full

TABLE 62.1 CB_1 and CB_2 Binding Affinities of Traditional Cannabinoid Ligands and Indoles

Compound	CB_1 K_i(nM)[a]	CB_2 K_i(nM)[a]	References
Agonists with similar CB_1 and CB_2 Affinities			
1, Δ^9-THC	53.3	75.5	Pertwee (2005)
2, Δ^8-THC	47.6 (rat)	39.3 (mouse)	Pertwee (2005)
3, HU-210	0.06	0.52	Pertwee (2005)
8, CBD	4350 (rat)	2860 (rat)	Showalter et al. (1996)
10, CP-55,940	5.0	1.8	Howlett et al. (2002)
12, HU-910	1.37	6.0	Horvath et al. (2012)
14, 2-AG	472[b]	1400[b]	Pertwee (2005)
20, R(+)-WIN55212	9.9 (rat)	16.2 (rat)	Pertwee (2005)
21, JWH-018	9.5	2.9	Pertwee (2005)
Agonists with higher CB_1 than CB_2 affinity			
9, CP-47,497	2.2	ND	Weissman, Milne, and Melvin (1982)
13, AEA	61 (rat)	1930 (rat)	Howlett et al. (2002)
15, 2-AGE	21.2 (rat)	>3000[b]	Pertwee (2005)
16, AM-356	17.9 (mouse)	868 (rat)	Pertwee (2005)
17, O-1812	3.4 (rat)	3870 (mouse)	Pertwee (2005)
18, ACEA	1.4 (rat)	>2000 (rat)	Pertwee (2005)
19, ACPA	2.2 (rat)	715 (rat)	Pertwee (2005)
Agonists with higher CB_2 than CB_1 affinity			
4, JWH-051	1.2 (rat)	0.03	Howlett et al. (2002)
5, JWH-056	>10000 (rat)	32	Han, Thatte, Buzard, and Jones (2013)
6, JWH-133	677 (rat)	3.4	Pertwee (2005)
7, L-759,633	1043	6.4	Huffman (2000)
11, HU-308	>10000 (rat)	22.7	Pertwee (2005)
22, JWH-015	383	14	Howlett et al. (2002)
23, AM-1241	580	7.1	Yao et al. (2009)
24, UR-12	245	11	Han et al. (2013)
25	16	1.0	Han et al. (2013)
26, A-796,260	945	4.6	Frost et al. (2010)
27, L-768242	2043	14	Gallant et al. (1996)

The structures of compounds listed in this table are shown in Figs. 72.1–72.3.
[a] *Human cannabinoid receptors, unless otherwise noted: nM, nanomolar.*
[b] *Species unspecified.*

agonist of both CB_1 and CB_2 receptors. In contrast, the S-(-)-WIN 55,212 was found to behave as a competitive neutral antagonist of the human CB_2 receptor, and acts as a low potency partial inverse agonist at the human CB_1 receptor (Savinainen et al., 2005).

A summary of SAR described by the research groups indicated that key structural features for potent cannabinoid binding activity of aminoalkylindoles are a bicyclic (particularly l-naphthyl) substituent at the 3-position of the indole, a small substituent, or better, no substituent, at

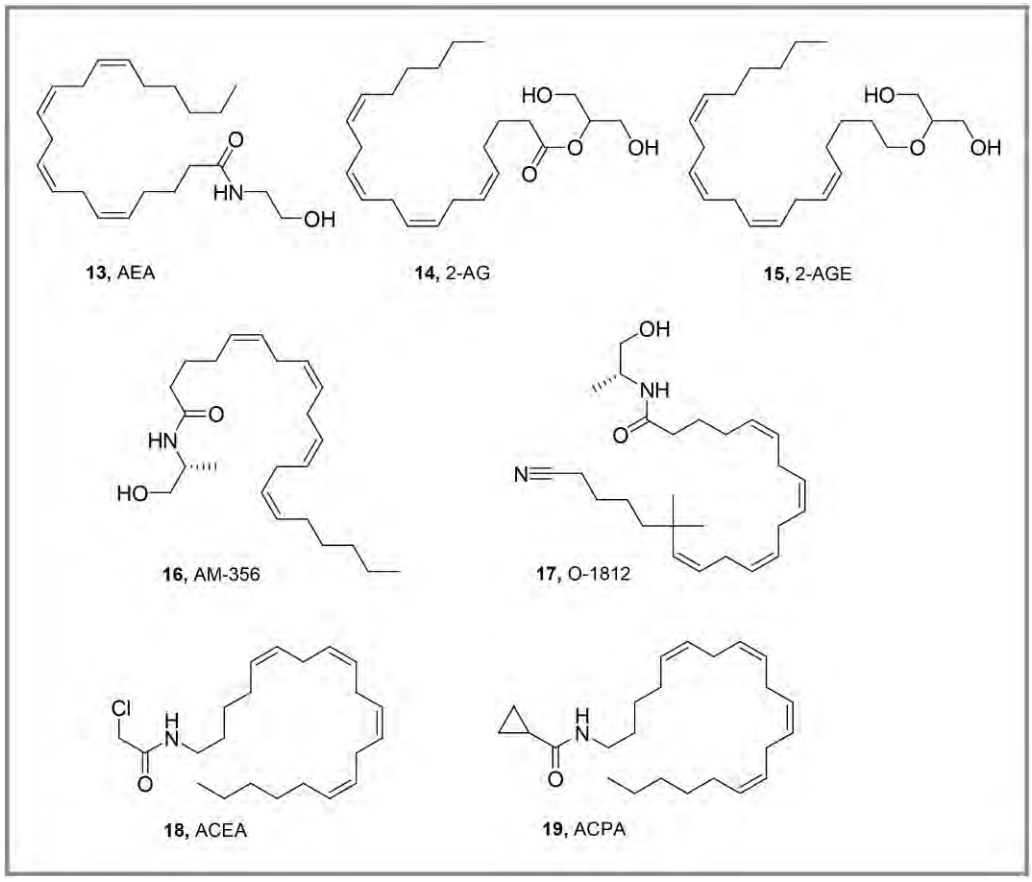


FIGURE 62.2 **Chemical structures of endogenous cannabinoids and synthetic analogs.**

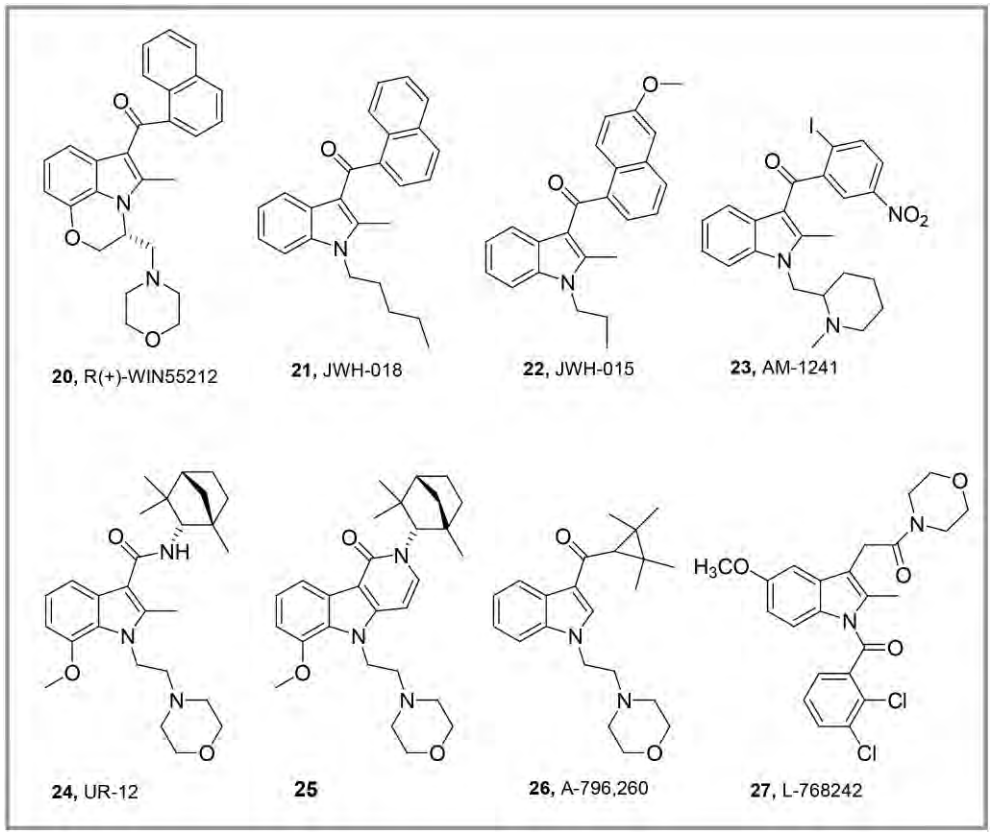


FIGURE 62.3 **Chemical structures of indoles.**

the 2-position of the indole, and a morpholinoethyl or other cyclic aminoethyl substituent at the 1-position. Several studies have been established that the aminoalkyl segment of the molecule could be replaced by an alkyl group. In particular, 1-pentyl-2-methyl-3-(1-naphtoyl)indole **21** (JWH-018) demonstrates modest preference for CB_2, it has high affinity for the brain receptor and exhibits typical cannabinoid pharmacology in vivo. The 1-propyl analog **22** (JWH-015) has selective affinity for the peripheral cannabinoid spleen receptor. In the family of aminoalkylindoles, the 3-benzoyl derivative **23 (**AM-1241, 1-(methylpiperidin-2-ylmethyl)-3-(2-iodo-5-nitrobenzoyl)indole) was prepared as a hydrochloride salt, and showed high selectivity for the human CB_2 receptor versus human CB_1 in recombinant binding assays (Eissenstat et al., 1995). The C3 amido indole and its cyclized and conformationally constrained indolopyridone were found to bind selectively to the CB_2 receptor. Representative compounds are *N*-(S)-fenchyl-1-(2 morpholinoethyl)-7-methoxyindole-3-carboxamide **24** (UR-12**)** and compound **25** (Han et al., 2013). Replacing the aromatic 3-benzoyl or 3-naphthoyl group of indole derivatives with the 3-tetramethylcyclopropylmethanone, enhances selectivity for CB_2 receptors. Compound **26 (**A-796,260) is an example which showed high affinity and selectivity for CB_2 receptor having K_i values of 4.6 and 945 nM in a competition binding assay for human CB_2 and CB_1, respectively (Frost et al., 2010). CB_2 selective cannabimimetic indoles include 1-(2,3-dichlorobenzoyl)-2-methyl-3-(2-[1-morpholine]ethyl)-5-methoxyindole **27** (L-768242) obtained by introduction of benzoyl group on the N1 position (K_i values are 3.92 and 4772 nM for human CB_2 and CB_1 receptors, respectively). The morpholine moiety may be linked to the indole core through an ethylene or acetyl spacer with minimal change in potency on the human CB_2 receptor. In this series, for compounds bearing a morpholine unit at C3, the 2,3-dichlorobenzoyl indole derivative was more active than the another chloro derivatives (30-fold) (Gallant et al., 1996).

The second part of this chapter is focused on advances that have risen with the recent entry of several diverse chemical cores as cannabinoid ligands. Generally, these molecules are characterized by an aromatic heterocyclic core linked to a lipophilic segment, and commonly possess CB_2 receptor affinity.

MONOCYCLIC CORES

Chemical structures are shown in Figs. 62.4 and 62.5; biological data are shown in Table 62.2.

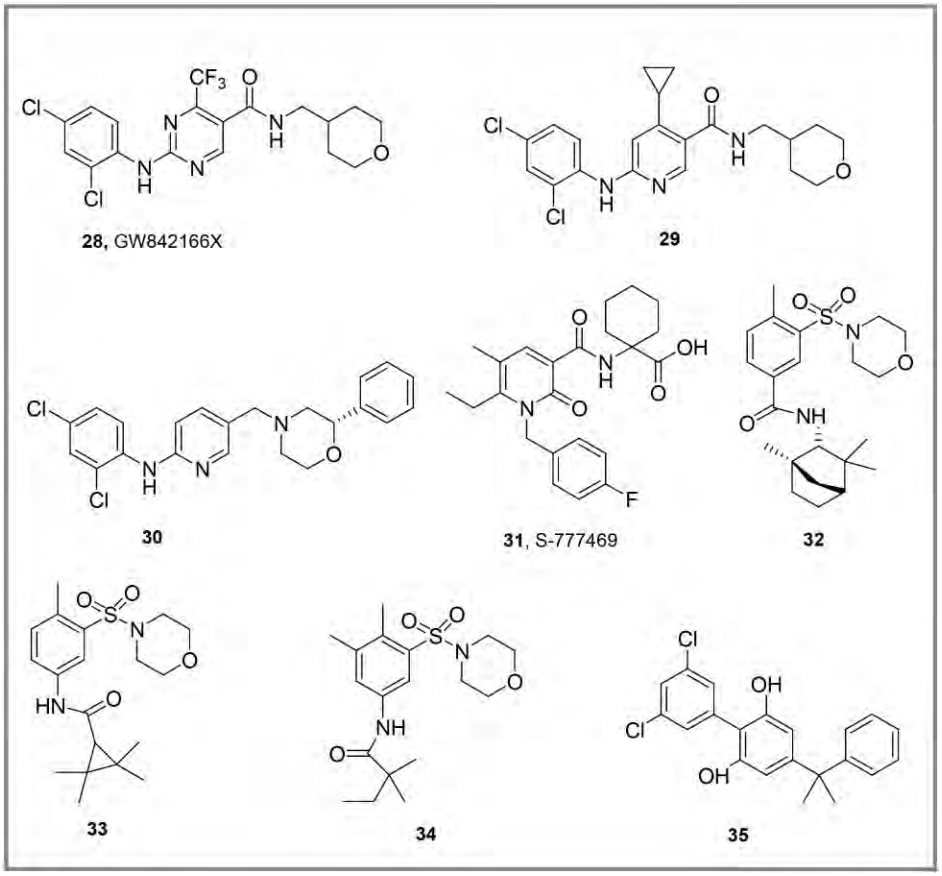


FIGURE 62.4 **Chemical structures of monocyclic cores.**

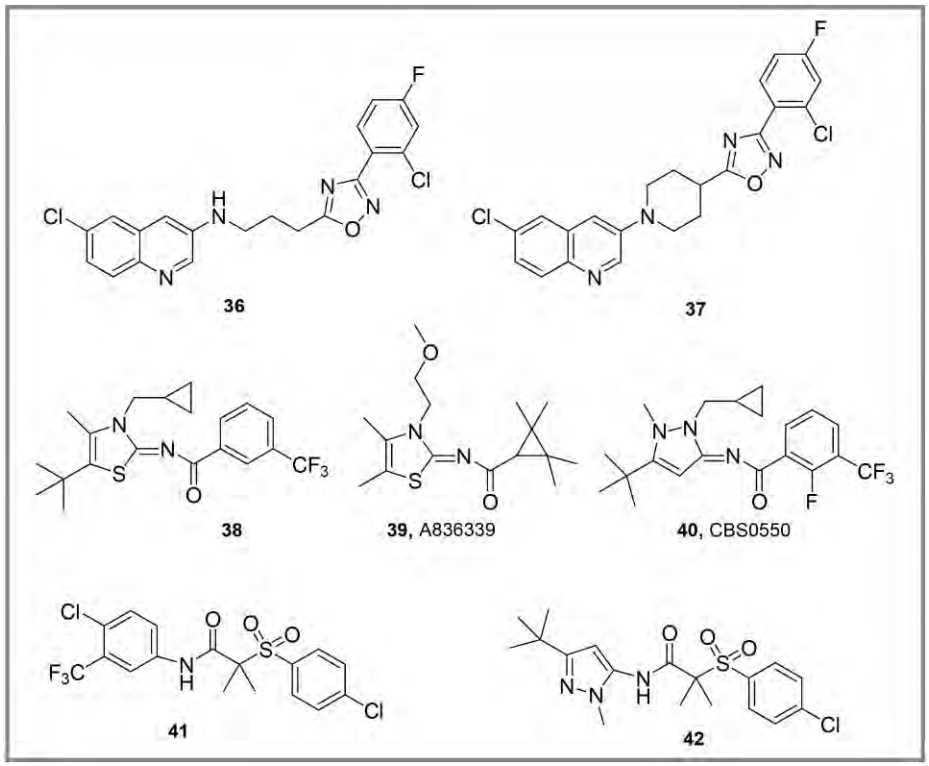


FIGURE 62.5 **Chemical structures of monocyclic cores.**

Pyrimidines and Pyridines

Focused screen identified the pyrimidine ester as a partial agonist at the CB_2 receptor with micromolar potency. Subsequent lead optimization identified **28** (GW842166X) as the optimal compound in the series. GW842166X is a potent and selective full agonist at the CB_2 receptor. Replacement of the pyrimidine core of compound GW842166X with pyridine was investigated to improve the aqueous solubility over GW842166X (Mitchell et al., 2009). An extended SAR of the new pyridine-3-carboxamides template was evaluated, and was concluded in the identification of analog 6-(2,4-dichlorophenylamino)-4-cyclopropyl-*N*-((tetrahydro-2H-pyran-4-yl)methyl)pyridine-3-carboxamide **29** which demonstrated efficacy in an in vivo model of inflammatory pain, despite its low aqueous solubility. Next studies reported a conformationally restricted morpholinyl motif (compound **30**) which retained activity and selectivity (Zindell et al., 2009). A novel CB_2 ligand based on the 3-carbamoyl-2-pyridone derivatives by adjusting the size of side chain at 1-, 5-, and 6-position was discovered. The structure–activity relationship around this template led to the identification of **31** (S-777469) as a selective CB_2 receptor agonist, which exhibited moderate potency for CB_2 receptor (hCB_2 K_i 36 nM) and good selectivity (>120) (Odan et al., 2012).

Sulfamoyl Benzamides

Chemical structures are shown in Fig. 62.4; biological data are shown in Table 62.2.

Several sufamoyl benzamides have been described (Worm et al., 2008), large lipophilic substituents such as S-fenchyl residue, led to improved receptor binding and selectivity for the CB_2 receptor such as compound **32**, with 120-fold functional selectivity for the CB_2 receptor. Small changes in the sulfonamide part of the molecule produced a change from full agonist to inverse agonist. Replacement of the amide moiety with various heterocycles did not significantly improve affinity or selectivity for the CB_2 receptor, in comparison to the lead compound **32**; while reversal of the amide linkage led to the sulfamoyl benzamides with improved affinity and selectivity for the CB_2 receptor. The best compound of this study, the tetramethylcyclopropy derivative **33**, displayed poor metabolic stability in rat pain model (Goodman et al., 2009). Further SAR investigation in the sulfonamide series led to the identification of several compounds, showing potent affinity for the CB_2 receptor (K_i < 10 nM) and improved CB_2 receptor selectivity (>200-fold). Compound **34**, *N*-[3,4-dimethyl-5-(morpholin-4-ylsulfonyl)phenyl]-2,2-dimethyl butanamide, displayed the best overall in vitro profile, and demonstrated robust efficacy in an animal model of postoperative pain (Sellitto et al., 2010).

TABLE 62.2 CB_1 and CB_2 Activities of Mono-, Bi-, and Tricyclic Cannabinoids

Compound	CB_1 (nM)[a]	CB_2 (nM)[a]	References
28, GW842166X	EC_{50} > 30000	EC_{50} 63	Mitchell et al. (2009)
29	EC_{50} > 30000	EC_{50} 79	Zindell et al. (2009)
30	EC_{50} > 20000	EC_{50} 10	Zindell et al. (2009)
31	K_i 4.6	K_i 36	Odan et al. (2012)
32	EC_{50} 550000	EC_{50} 4.6	Worm et al. (2008)
33	K_i 3400	K_i 23	Goodman et al. (2009)
34	K_i 2500	K_i 17	Sellitto et al. (2010)
35	K_i 93.6	K_i 1.0	Bhattacharjee, Gurley, and Moore (2009)
36	EC_{50} 220	EC_{50} 1.7	Cheng et al. (2008)
37	EC_{50} 1430	EC_{50} 7.0	DiMauro et al. (2008)
38	EC_{50} 3500	EC_{50} 13	Ohta et al. (2007)
39, A836339	K_i 270	K_i 0.64	Dart et al. (2007)
40, CBS0550	EC_{50} 4000	EC_{50} 2.9	Ohta et al. (2008)
41	EC_{50} > 2000	EC_{50} 5.0	Marx et al. (2009)
42	EC_{50} > 2000	EC_{50} 25	Marx et al. (2009)
43	K_i > 4600	K_i 38	Page et al. (2008)
44	ND	EC_{50} 0.15	Ryckmans et al. (2009)
45	EC_{50} > 25000	EC_{50} 30	Ando and Iwata (2010)
46	EC_{50} > 10000	EC_{50} 2.7	Ryckmans et al. (2009)
47	EC_{50} 1422	EC_{50} 0.3	Verbist et al. (2008)
48	EC_{50} 17000	EC_{50} 33	Trotter et al. (2011)
49	EC_{50} 2600	EC_{50} 5.0	Trotter et al. (2011)
50	ND	EC_{50} 17	Wu, Green, and Hartnett (2009)
51, GRC-10693	K_i 985	K_i 11.8	Muthuppalanippan, Balasubramanian, Gullapalli, Joshi, and Narayanan (2006)
52	K_i > 10000	K_i 0.6	Manera et al. (2007)
53	K_i 560	K_i 11	Pasquini et al. (2011)
54	K_i 4568	K_i 11.2	Aghazadeh Tabrizi et al. (2012)
55	K_i > 10000	K_i 2.5	Aghazadeh Tabrizi et al. (2013)
56	K_i 363 (mouse)	K_i 0.03 (mouse)	Murineddu et al. (2006)
57, Sch35966	K_i 2633	K_i 6.8 (rat)	Gonsiorek et al. (2007)
58	K_i 6183	K_i 17.6 (rat)	Page et al. (2007)
59	K_i 310	K_i 0.81	Baraldi et al. (2012)
60	K_i > 10000	K_i 8.12	Vincenzi et al. (2013)

[a] *Human cannabinoid receptors unless otherwise noted: nM, nanomolar; ND, no data.*

Triaryl Derivatives

Chemical structures are shown in Fig. 62.4; biological data are shown in Table 62.2.

A series of cannabinoid ligands with a structurally triaryl core has been designed. This study was based on the previously observations, wherein an enhancement of CB_2 receptor affinity was observed with a dichlorophenyl substituted ring in the biaryl series. The presence of a gem dimethyl group as a linker (compound **35**) results in a CB_2 selective compound with a K_i value of 1.07 nM at the CB_2 receptor, compared to 93.6 nM at the CB_1 receptor subtype (Bhattacharjee et al., 2009).

N-Arylamide Oxadiazoles

Chemical structures are shown in Fig. 62.5; biological data are shown in Table 62.2.

Targeted library screening and subsequent hit assessment have identified the five-membered oxadiazole core as a novel class of potent and selective CB_2 agonists. SAR investigation into this group of compounds showed that the oxadiazole core was fundamental and substitutions at the 2- and 4-position of the phenyl ring joined to the oxadiazole were important for desired CB_2 activity. The amino quinoline derivative **36** is a highly potent, selective CB_2 agonist that displayed an excellent pharmacokinetic profile, with oral bioavailability in rats (Cheng et al., 2008). Structural modifications in the central portion of the *N*-arylamide oxadiazole scaffold led to the identification of *N*-arylpiperidine oxadiazoles as conformationally constrained analogs that offered improved stability, comparable potency, and selectivity (compound **37**) (DiMauro et al., 2008).

Thiazolylidene, Pyrazolylidene, Amidosulfone

Chemical structures are shown in Fig. 62.5; biological data are shown in Table 62.2.

The five-membered heterocyclic cores such as thiazole ring are reported as CB_2 selective agonists. The SAR study showed that the functional groups at 3- and 5-position in the thiazole ring and the aromatic group in the amide deeply influence the affinity and selectivity for the CB_2 receptor. Thiazolylidene compounds with a bulky functional group like trifluoromethyl, exhibited much high CB_2 affinities (compound **38**, EC_{50} hCB_2 13 nM, 270-fold selectivity) (Ohta et al., 2007). Another thiazolylidene, compound **39** (A-836339), bearing a bulky cycloalkylcarboxamide group, exhibited subnanomolar potency in competition binding assays for human and rat CB_2, with good selectivity against the human and rat CB_1 receptors (425- and 189-fold, respectively) (Dart et al., 2007).

Pyrazole-based CB_2 agonists have also been explored, the SAR study highlighted that heterocycle nucleus operated as a linker, and that the functional groups at the 2-position in the pyrazole ring, and the aromatic group in the amide significantly affected affinity and selectivity for the CB_2 receptor Pyrazolylidene **40** (CBS-0550) has good solubility in water, and in animal studies it was found to produce analgesic and antihyperalgesic effects. It acts as a potent and selective cannabinoid CB_2 receptor agonist (EC_{50} human CB_2 2.9 nM) (Ohta et al., 2008). The strictly correlated α-amidosulfones were found to be potent and selective agonists of CB_2. The effect of the amide substituent on CB_2 activity and selectivity was explored. 3,4-Disubstituted phenyl ring enhanced the potency and selectivity (compound **41**), while disubstituted phenyl rings diminished agonist activity on both CB_1 and CB_2 receptors. For the five-membered heterocycles, it was found that *tert*-butyl group was important for activity, such as in compound 2-(4-chlorophenylsulfonyl)-*N*-(3-*tert*-butyl-1-methyl-1H-pyrazol-5-yl)-2-methylpropanamide **42**. α-Amidosulfones behaved as selective full agonists of CB_2 and showed high functional and cellular activity on CB_2 receptors (Marx et al., 2009).

BICYCLIC CORES

Chemical structures are shown in Fig. 62.6; biological data are shown in Table 62.2.

Benzimidazoles

CB_2 agonists based on benzimidazole template have been reported. One of the first was 2-(4-ethoxybenzyl)-1-(2-(dimethylamino)ethyl)-*N*,*N*-diethyl-1H-benzo[*d*]imidazole-5-carboxamide **43**. This molecule was studied as a starting template to improve the potency of benzoimidazole ligands. SAR studies of the N1 substituent exposed that various alkyl and aromatic groups were tolerated, with a preference for alkyl substituents, and that the binding interactions at the amide position appeared to be essentially hydrophobic (Page et al., 2008). Further investigations around this new class of ligands allowed the discovery of sulfone compounds with CB_2 receptor agonistic activity. Incorporation of neopentyl chain into the 2-position, together with introduction of a sulfonyl group into the 5-position, led to compound 1-(1-(cyclopropylmethyl)-2-neopentyl-1H-benzo[*d*]imidazol-5-ylsulfonyl)cyclopropanecarboxamide (**44**) (Ryckmans et al., 2009), a selective and potent full agonist of CB_2 receptor. In the sulfonyl family, it has been found out that a new class of N-substituted saturated heterocyclic sulfone compounds show CB_2 agonistic activity with little affinity for CB_1, such as 3-(1-(2-(dimethylamino)

ethyl)-2-neopentyl-1H-benzo[*d*]imidazol-5-ylsulfonyl) azetidine-carboxamide **45** (Ando & Iwata, 2010). In this context, 2-*tert*-butyl-substituent benzoimidazole was found to influence both potency and efficacy. Among them, 2-*tert*-butyl-1-(cyclopropylmethyl)-5-(ethylsulfonyl)-1H-benzo[*d*]imidazole **46** was described as a potent and selective CB_2 agonist (>38,000-fold against CB_1) (Ryckmans et al., 2009; Watson et al., 2011). A strictly related class of benzimidazole CB_2 receptor agonists was synthesized, and the SAR was explored. The size of the substituent on the 2-position determined the level of agonism, ranging from inverse to partial or full agonism, which was more pronounced for the rat CB_2 receptor. The benzoimidazole cannabinoid agonists

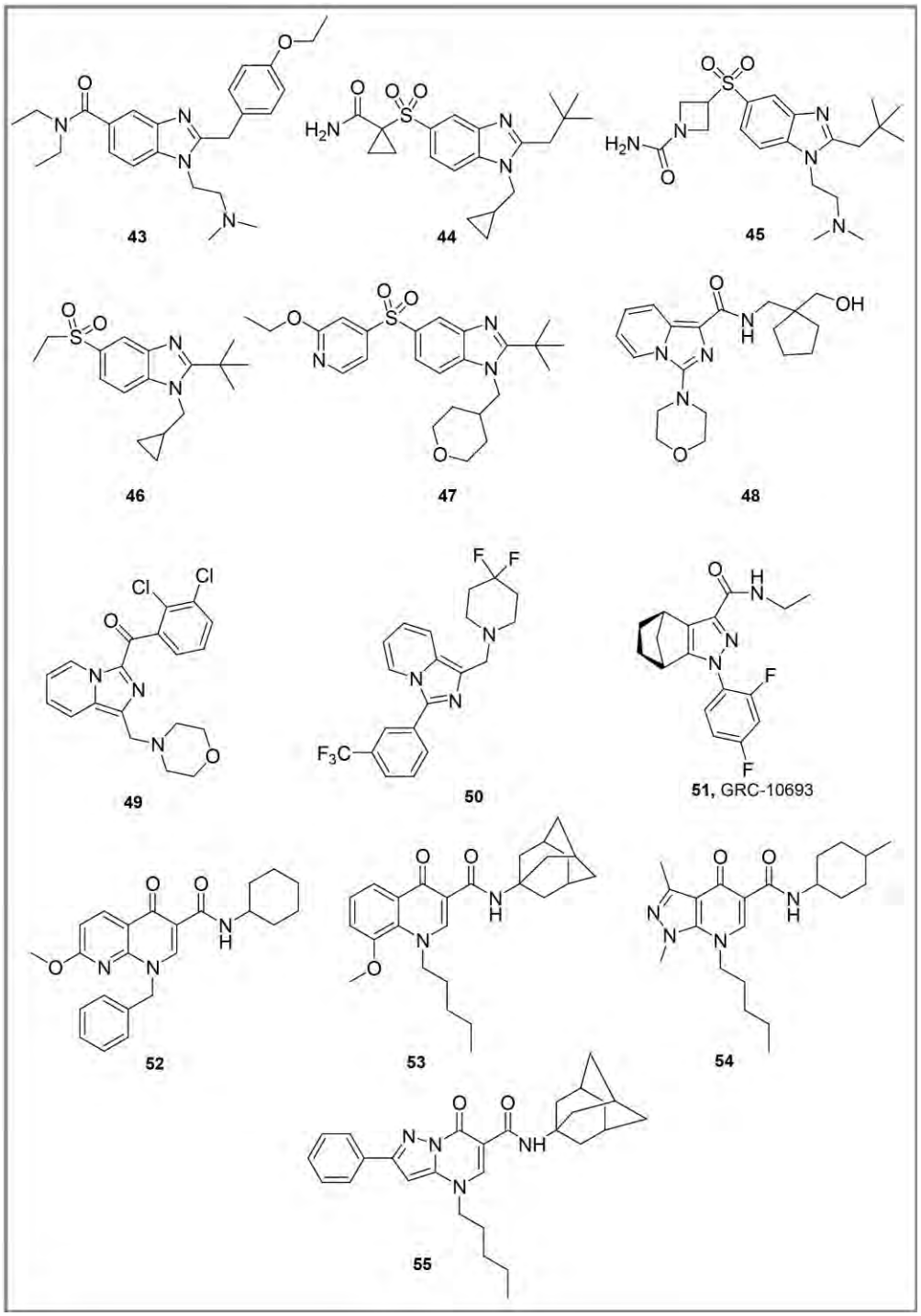


FIGURE 62.6 **Chemical structures of bicyclic cores.**

bearing a substituted aryl group, in particular, the pyridyl sulfone such as compound 2-*tert*-(butyl-5-(2-ethoxypyridin-4-sulfonyl)-1-tetrahydropyran-4-ylmethyl)-1H-benzimidazole. **47** had excellent binding affinity and selectivity for CB_2 (Verbist et al., 2008).

Imidazopyridines

Imidazopyridine-based CB_2 agonists have been described. Exploration of SAR in this chemical series by variation of substituents on both carbon positions of the imidazo ring has produced CB_2 agonists. Compounds *N*-((1-(hydroxymethyl)cyclopentyl)methyl)-3-morpholinoH-imidazo[1,5-*a*]pyridine-1-carboxamide **48** and (2,3-dichlorophenyl)(1-(morpholinomethyl)H-imidazo[1,5-*a*]pyridin-3-yl)methanone **49**, which were completely selective for CB_2 versus CB_1 are representatives of this series. In vivo evaluation of these compounds indicates a significant impact of the degree of selectivity for CB_2 on analgesic effects (Trotter et al., 2011).

A related series of 3-arylimidazopyridine analogs was reported, wherein 3-(3-(trifluoromethyl)phenyl)-1-((4,4-difluoropiperidin-1-yl)methyl)H-imidazo[1,5-*a*]pyridine **50** was the most potent CB_2 agonist disclosed (Wu et al., 2009).

Bicyclic Pyrazoles

The bridged bicyclic pyrazole compounds are reported to be CB_2 agonists. Within this family, (4S,7R)-1-(2,4-difluorophenyl)-*N*-(1,1-dimethylethyl)-4,5,6,7-tetrahydro-4,7-methano-1Hindazole-3-carboxamide (**51**, GRC-10693) has >4700-fold functional selectivity for CB_2 over CB_1, making it one of the most selective CB_2 agonists reported in development (Muthuppalanippan et al., 2006).

Quinolones and Naphthyridines

The CB_2 receptor agonists also include 4-oxo-1,8-naphthyridine-3-carboxamide and 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives, endowed with high affinity and selectivity toward CB_2 (Manera et al., 2007). Some of these analogs were demonstrated to act as agonists or inverse agonists in functional activity assays, depending on the nature of the substituents on the different positions of the heterocyclic scaffold. In the series of 8-substituted quinolones, the best results in terms of both CB_2 affinity and selectivity were obtained with the introduction of electron-donating groups, such as methyl or methoxy group (compound **52**, K_i human CB_1 > 10,000, human CB_2 0.6 nM). In contrast, the double substitution at positions 6 and 8 of the quinolone nucleus was not favorable. The quinolone derivative **53** is reported to be potent and selective for CB_2 receptor (K_i human CB_1 > 10,000 nM, human CB_2 0.6 nM), and possessed antinociceptive effects (Pasquini et al., 2011).

Pyrazolopyridines

Recently, a related series of heteroaryl-4-oxopyridine derivatives has been described (Aghazadeh Tabrizi et al., 2012). In this family, the core structure of molecules defines the type of activity to be seen, while the substituents around the core structure can be used to modulate the potency of that activity. Compound **54** was among the most potent analogs (K_i values of 4.6 μM for human CB_1 and 11.4 nM for human CB_2 receptor). This compound was found to act as partial agonists while the isoxazolopyridine parent compound behaved as full agonist. Further efforts led to discovery of 7-oxopyrazolo[1,5-*a*]pyrimidine-6-carboxamides, structural isomers of the previously reported pyrazolo[3,4-*b*]pyridines. The novel series exemplified by structure such as compound **55** shows stimulatory effects on forskolin-induced cAMP production acting as inverse agonists (Aghazadeh Tabrizi et al., 2013).

TRICYCLIC CORES

Chemical structures are shown in Fig. 62.7; biological data are shown in Table 62.2.

Rigidifying the CB_1 antagonist *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (SR141716A) resulted in 1,4-dihydroindeno[1,2-*c*]pyrazol-based CB_2 agonist ligands. Compound **56** exerted affinity and selectivity for CB_2 receptor (mouse K_iCB_2 0.03 nM) (Murineddu et al., 2006). Tricyclic benzoquinolizinone compound **57** (Sch35966) was found to be a potent CB_2 agonist in four different species; it binds with low nanomolar potency to CB_2 in both primates and rodents. It has >450-fold selectivity for CB_2 over central cannabinoid receptor CB_1 in primates (humans and cynomolgus monkeys) and rodents (rats and mice). Sch35966 is an agonist as it effectively inhibited forskolin-stimulated cAMP synthesis in CHO-human CB_2 cells (Gonsiorek et al., 2007).

Several molecules, based on a 1,2,3,4-tetrahydropyrrolo[3,4-*b*]indole moiety, are described as cannabinoid agonists. These ligands demonstrated good binding affinities and potencies toward the CB_2 receptor, showing moderate to good selectivity over the CB_1 receptor. SAR studies showed that the sulfonamide moiety produced higher CB_2 binding affinities (compound **58**) than the corresponding amide or *N*-alkyl analogs, and the piperidine nucleus enhanced CB_2 binding affinity. Compound **58** is described as a full agonist (EC_{50} human CB_2 17.6 nM), and possessed the best binding selectivity in the series (>350-fold over CB_1) (Page et al., 2007).

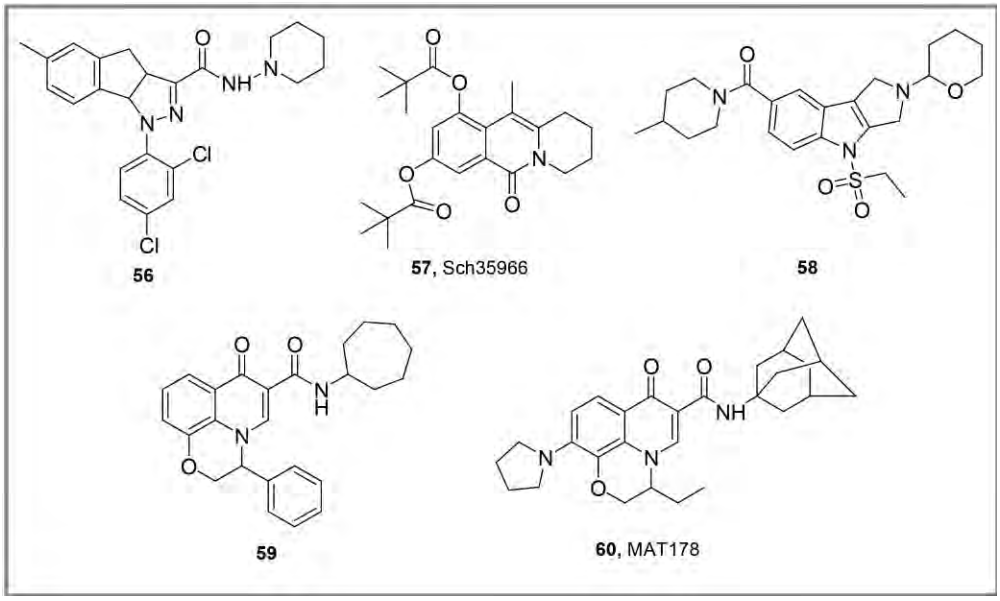


FIGURE 62.7 **Chemical structures of tricyclic cores.**

Very recently, 7-oxo-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamide chemotype is reported as a novel cannabinoid ligand possessing high CB_2 receptor affinity. Structural modifications led to the identification of several compounds as potent and selective cannabinoid receptor agonists (**59**, K_i human CB_2 0.81, human CB_1 310 nM). The effect of a chiral center on the biological activity was also investigated, and it was found that the R-enantiomers exhibited greater affinity at the CB_2 receptor than the S-enantiomers. The novel series behaved as full agonists, exhibiting functional activity at the human CB_2 receptor (Baraldi et al., 2012). Oxazinoqiunolone **60** (MT178) produced a robust analgesia in different pain models via CB_2 receptors, providing an interesting approach to analgesic therapy in inflammatory and chronic pain, without CB_1-mediated central side effects (Vincenzi et al., 2013).

MINI-DICTIONARY

Affinity The affinity of a drug for a receptor is the power with which it binds to the receptor.

Enantiomers Enantiomers are optical isomers that have the same chemical and physical properties, except for their capacity to rotate the plane of polarized light by identical amounts in opposing sense.

Ligand Ligand is a molecule capable to make a complex with a biomolecule to induce a biological function.

Structure–activity relationship (SAR) The relationship between the chemical structure of a molecule and its biological activity is SAR, and allows it to modify the activity of a compound by changing its chemical structure.

The half maximal effective concentration (EC_{50}) The EC_{50} is a measure of the potency of a substance required to produce 50% of the maximal effect.

The inhibitory or affinity constant (K_i) The K_i expresses the binding affinity, and is the direct indicator of affinity between a ligand and a receptor; it could be defined as the concentration of competing ligand that is mandatory to decrease the maximal effect of the reactions by half.

References

Aghazadeh Tabrizi, M., Baraldi, P. G., Saponaro, G., Moorman, A. R., Romagnoli, R., Preti, D., et al. (2012). Design, synthesis, and pharmacological properties of new heteroarylpyridine/heteroarylpyrimidine derivatives as CB(2) cannabinoid receptor partial agonists. *Journal of Medicinal Chemistry*, *56*(3), 1098–1112.

Aghazadeh Tabrizi, M., Baraldi, P. G., Saponaro, G., Moorman, A. R., Romagnoli, R., Preti, D., et al. (2013). Discovery of 7-oxopyrazolo[1,5-a]pyrimidine-6-carboxamides as potent and selective CB(2) cannabinoid receptor inverse agonists. *Journal of Medicinal Chemistry*, *56*(11), 4482–4496.

Ando, K., & Iwata, Y. (2010). N-substituted saturated heterocyclic sulfone compounds with CB2 receptor agonistic activity. Patent No. WO2010084767.

Ashton, J. C., & Milligan, E. D. (2008). Cannabinoids for the treatment of neuropathic pain: clinical evidence. *Current Opinion in Investigational Drugs*, *9*(1), 65–75.

Baraldi, P. G., Saponaro, G., Moorman, A. R., Romagnoli, R., Preti, D., Baraldi, S., et al. (2012). 7-Oxo-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxamides as selective CB(2) cannabinoid receptor ligands: structural investigations around a novel class of full agonists. *Journal of Medicinal Chemistry*, *55*(14), 6608–6623.

Bhattacharjee, H., Gurley, S. N., & Moore, B. M. (2009). Design and synthesis of novel tri-aryl CB2 selective cannabinoid ligands. *Bioorganic and Medicinal Chemistry Letters*, *19*(6), 1691–1693.

Cheng, Y., Albrech, B. K., Brown, J., Buchanan, J. L., Buckner, W. H., DiMauro, E. F., et al. (2008). Discovery and optimization of a novel series of N-arylamide oxadiazoles as potent, highly selective and orally bioavailable cannabinoid receptor 2 (CB(2)) agonists. *Journal of Medicinal Chemistry*, *51*(16), 5019–5034.

Dart, M. J., Carroll, W. A., Florjancic, A. S., Frost, J. M., Gallagher, M. E., Li, T., et al. (2007). Novel compound as cannabinoid receptor ligands and uses thereof. Patent No. WO2007140385.

DiMauro, E. F., Buchanan, J. L., Cheng, A., Emkey, R., Hitchcock, S. A., Huang, L., et al. (2008). Structural modifications of N-arylamide oxadiazoles: identification of N-arylpiperidine oxadiazoles as potent and selective agonists of CB(2). *Bioorganic and Medicinal Chemistry Letters*, *18*(15), 4267–4274.

Eissenstat, M. A., Bell, M. R., D'Ambra, T. E., Alexander, E. J., Daum, S. J., Ackerman, J. H., et al. (1995). Aminoalkylindoles: structure-activity relationships of novel cannabinoid mimetics. *Journal of Medicinal Chemistry*, *38*, 3094–3105.

Frost, J. M., Dart, M. J., Tietje, K. R., Garrison, T. R., Grayson, G. K., Daza, A. V., et al. (2010). Indol-3-ylcycloalkyl ketones: effects of N1 substituted indole side chain variations on CB2 cannabinoid receptor activity. *Journal of Medicinal Chemistry*, *53*(1), 295–315.

Gallant, M., Dufresne, C., Gareau, Y., Guay, D., Leblanc, Y., Prasit, P., et al. (1996). New class of potent ligands for the human peripheral cannabinoid receptor. *Bioorganic and Medicinal Chemistry Letters*, *6*(19), 2263–2268.

Gonsiorek, W., Lunn, C. A., Fan, X., Deno, G., Kozlowski, J., & Hipkin, R. W. (2007). Sch35966 is a potent, selective agonist at the peripheral cannabinoid receptor (CB2) in rodents and primates. *British Journal of Pharmacology*, *151*(8), 1262–1271.

Goodman, A. J., Ajello, C. W., Worm, K., Le Bourdonnec, B., Savolainen, M. A., O'Hare, H., et al. (2009). CB(2) selective sulfamoyl benzamides: optimization of the amide functionality. *Bioorganic and Medicinal Chemistry Letters*, *19*(2), 309–313.

Guindon, J., & Hohmann, A. G. (2008). Cannabinoid CB2 receptors: a therapeutic target for the treatment of inflammatory and neuropathic pain. *British Journal of Pharmacology*, *153*(2), 319–334(Huffman, 1999)..

Han, S., Thatte, J., Buzard, D. J., & Jones, R. M. (2013). Therapeutic utility of cannabinoid receptor type 2 (CB(2)) selective agonists. *Journal of Medicinal Chemistry*, *56*(21), 8224–8256.

Horvath, B., Magid, L., Mukhopadhyay, P., Batkai, S., Rajesh, M., Park, O., et al. (2012). A new cannabinoid CB2 receptor agonist HU-910 attenuates oxidative stress, inflammation and cell death associated with hepatic ischaemia/reperfusion injury. *British Journal of Pharmacology*, *165*(8), 2462–2478.

Howlett, A. C., Barth, F., Bonner, T. I., Cabral, G., Casellas, P., Devane, W. A., et al. (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacological Reviews*, *54*(2), 161–202.

Huffman, J. W. (1999). Cannabimimetic indoles, pyrroles and indenes. *Current Medicinal Chemistry*, *6*(8), 705–720.

Huffman, J. W. (2000). The search for selective ligands for the CB2 receptor. *Current Pharmaceutical Design*, *6*(13), 1323–1337.

Kreitzer, F. R., & Stella, N. (2009). The therapeutic potential of novel cannabinoid receptors. *Pharmacology and Therapeutics*, *122*(2), 83–96.

Manera, C., Cascio, M. G., Benetti, V., Allara, M., Tuccinardi, T., Martinelli, A., et al. (2007). New 1,8-naphthyridine and quinoline derivatives as CB2 selective agonists. *Bioorganic and Medicinal Chemistry Letters*, *17*(23), 6505–6510.

Marx, I. E., DiMauro, E. F., Cheng, A., Emkey, R., Hitchcock, S. A., Huang, L. Y., et al. (2009). Discovery of alpha-amidosulfones as potent and selective agonists of CB(2): synthesis, SAR, and pharmacokinetic properties. *Bioorganic and Medicinal Chemistry Letters*, *19*(1), 31–35.

Mitchell, W. L., Giblin, G. M., Naylor, A., Eatherton, A. J., Slingsby, B. P., Rawlings, A. D., et al. (2009). Pyridine-3-carboxamides as novel CB(2) agonists for analgesia. *Bioorganic and Medicinal Chemistry Letters*, *19*(1), 259–263.

Murineddu, G., Lazzari, P., Ruiu, S., Sanna, A., Loriga, G., Manca, I., et al. (2006). Tricyclic pyrazoles. 4. Synthesis and biological evaluation of analogues of the robust and selective CB2 cannabinoid ligand 1-(2 ′,4 ′-dichlorophenyl)-6-methyl-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide. *Journal of Medicinal Chemistry*, *49*(25), 7502–7512.

Muthuppalanippan, M., Balasubramanian, G., Gullapalli, S., Joshi, N. K., & Narayanan, S. (2006). Novel cannabinoid receptor ligands, pharmaceutical compositions containing them, and process for their preparation. Patent No. WO2006129178.

Odan, M., Ishizuka, N., Hiramatsu, Y., Inagaki, M., Hashizume, H., Fujii, Y., et al. (2012). Discovery of S-777469: an orally available CB2 agonist as an antipruritic agent. *Bioorganic and Medicinal Chemistry Letters*, *22*(8), 2803–2806.

Ohta, H., Ishizaka, T., Tatsuzuki, M., Yoshinaga, M., Iida, I., Tomishima, Y., et al. (2007). N-Alkylidenearylcarboxamides as new potent and selective CB2 cannabinoid receptor agonists with good oral bioavailability. *Bioorganic and Medicinal Chemistry Letters*, *17*(22), 6299–6304.

Ohta, H., Ishizaka, T., Tatsuzuki, M., Yoshinaga, M., Iida, I., Yamaguchi, T., et al. (2008). Imine derivatives as new potent and selective CB2 cannabinoid receptor agonists with an analgesic action. *Bioorganic and Medicinal Chemistry*, *16*(3), 1111–1124.

Page, D., Balaux, E., Boisvert, L., Liu, Z., Milburn, C., Tremblay, M., et al. (2008). Novel benzimidazole derivatives as selective CB2 agonists. *Bioorganic and Medicinal Chemistry Letters*, *18*(13), 3695–3700.

Page, D., Yang, H., Brown, W., Walpole, C., Fleurent, M., Fyfe, M., et al. (2007). New 1,2,3,4-tetrahydropyrrolo[3,4-b]indole derivatives as selective CB2 receptor agonists. *Bioorganic and Medicinal Chemistry Letters*, *17*(22), 6183–6187.

Pasquini, S., De Rosa, M., Pedani, V., Mugnaini, C., Guida, F., Luongo, L., et al. (2011). Investigations on the 4-Quinolone-3-carboxylic acid motif. 4. Identification of new potent and selective ligands for the cannabinoid type 2 receptor with diverse substitution patterns and antihyperalgesic effects in mice. *Journal of Medicinal Chemistry*, *54*(15), 5444–5453.

Pertwee, R. G. (2005). Pharmacological actions of cannabinoids. *Handbook of Experimental Pharmacology*, *168*, 1–51.

Pertwee, R. G., Howlett, A. C., Abood, M. E., Alexander, S. P. H., Di Marzo, V., Elphick, M. R., et al. (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. *Pharmacological Reviews*, *62*(4), 588–631.

Ryckmans, T., Edwards, M. P., Horne, V. A., Correia, A. M., Owen, D. R., Thompson, L. R., et al. (2009). Rapid assessment of a novel series of selective CB(2) agonists using parallel synthesis protocols: a lipophilic efficiency (LipE) analysis. *Bioorganic and Medicinal Chemistry Letters*, *19*(15), 4406–4409.

Savinainen, J. R., Kokkola, T., Salo, O. M. H., Poso, A., Jarvinen, T., & Laitinen, J. T. (2005). Identification of WIN55212-3 as a competitive neutral antagonist of the human cannabinoid CB2 receptor. *British Journal of Pharmacology*, *145*(5), 636–645.

Sellitto, I., Le Bourdonnec, B., Worm, K., Goodman, A., Savolainen, M. A., Chu, G. H., et al. (2010). Novel sulfamoyl benzamides as selective CB2 agonists with improved in vitro metabolic stability. *Bioorganic and Medicinal Chemistry Letters*, *20*(1), 387–391.

Showalter, V. M., Compton, D. R., Martin, B. R., & Abood, M. E. (1996). Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. *Journal of Pharmacology and Experimental Therapeutics*, *278*(3), 989–999.

Trotter, B. W., Nanda, K. K., Burgey, C. S., Potteiger, C. M., Deng, J. Z., Green, A. I., et al. (2011). Imidazopyridine CB2 agonists: optimization of CB2/CB1 selectivity and implications for in vivo analgesic efficacy. *Bioorganic and Medicinal Chemistry Letters*, *21*(8), 2354–2358.

Verbist, B. M., De Cleyn, M. A., Surkyn, M., Fraiponts, E., Aerssens, J., Nijsen, M. J., et al. (2008). 5-Sulfonyl-benzimidazoles as selective CB2 agonists. *Bioorganic and Medicinal Chemistry Letters*, *18*(8), 2574–2579.

Vincenzi, F., Targa, M., Corciulo, C., Tabrizi, M. A., Merighi, S., Gessi, S., et al. (2013). Antinociceptive effects of the selective CB2 agonist

MT178 in inflammatory and chronic rodent pain models. *Pain*, *154*(6), 864–873.

Watson, C., Owen, D. R., Harding, D., Kon, I. K., Lewis, M. L., Mason, H. J., et al. (2011). Optimisation of a novel series of selective CNS penetrant CB(2) agonists. *Bioorganic and Medicinal Chemistry Letters*, *21*(14), 4284–4287.

Weissman, A., Milne, G. M., & Melvin, L. S. J. (1982). Cannabimimetic activity from CP-47,497, a derivative of 3-phenylcyclohexanol. *Journal of Pharmacology and Experimental Therapeutics*, *223*, 516–523.

Worm, K., Zhou, Q. J., Saeui, C. T., Green, R. C., Cassel, J. A., Stabley, G. J., et al. (2008). Sulfamoyl benzamides as novel CB2 cannabinoid receptor ligands. *Bioorganic and Medicinal Chemistry Letters*, *18*(9), 2830–2835.

Wu, Z., Green, A. I., & Hartnett, J. C. (2009). CB2 receptor ligands for the treatment of pain. Patent No. WO 2009025785.

Yao, B. B., Mukherjee, S., Fan, Y., Garrison, T. R., Daza, A. V., Grayson, G. K., et al. (2009). In vitro pharmacological characterization of AM1241: a protean agonist at the cannabinoid CB2 receptor? *British Journal of Pharmacology*, *149*, 145–154.

Zindell, R., Riether, D., Bosanac, T., Berry, A., Gemkow, M. J., Ebneth, A., et al. (2009). Morpholine containing CB2 selective agonists. *Bioorganic and Medicinal Chemistry Letters*, *19*(6), 1604–1609.

CHAPTER

63

The Endocannabinoid System as a Target for New Antiseizure Drugs

*L.R. Vilela**, *A.C.P. de Oliveira**, *M.F. Moraes*†, *F.A. Moreira**, *R.N. Takahashi***

***Department of Pharmacology, ICB, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; **Department of Pharmacology, CCB, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil, †Department of Physiology and Biochemistry, ICB, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil**

SUMMARY POINTS

- Epilepsy is a serious neurological syndrome characterized mainly by the repetitive occurrence of seizures.
- The treatment of epilepsy is limited due to the side effects and resistance to antiseizure compounds.
- *Cannabis sativa* and cannabinoids have been proposed for the treatment of epilepsy.
- Inhibition endocannabinoid hydrolysis promotes on-demand defense against excitotoxicity via cannabinoid receptors type 1 (CB1), and may pave the way for developing new pharmacological treatments for epilepsy.
- Blockade of transient receptor potential cation channel, subfamily vanilloid, member I (TRPV1) channel, similarly to CB1 receptor activation, may restrain seizures. Combining endocannabinoid-hydrolysis inhibition with TRPV1 blockade may lead to more efficacious medicines for controlling seizures and treating epilepsy.

KEY FACTS OF CANNABINOIDS AND SEIZURES

- The phytocannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC) is the main compound accounting for cannabis activity, including antiseizure effects, although also inducing side effects.
- Other phytocannabinoids, including cannabidiol (CBD), Δ^9-tetrahydrocannabivarin (Δ^9-THCV), and cannabidivarin (CBDV), may be explored for developing safer cannabis-based medicines efficacious against epilepsy.
- Synthetic Δ^9-THC analogs induce inconsistent effects.

KEYS FACTS OF ENDOCANNABINOID HYDROLYSIS INHIBITORS AND SEIZURES

- The endocannabinoid hydrolysis inhibitors facilitate the on-demand functioning of the endocannabinoid system.
- Enhancing the activity of the endocannabinoid system, represents a promising strategy for developing new treatments for epilepsy.
- The effects of fatty acid amide hydrolase (FAAH) inhibitors can be combined with simultaneous blockade of transient receptor potential cation channel, subfamily vanilloid, member I (TRPV1) channel.

LIST OF ABBREVIATIONS

2-AG	2-Arachidonoyl glycerol
Δ^9-THC	Δ^9-Tetrahydrocannabinol
Δ^9-THCV	Δ^9-Tetrahydrocannabivarin
AA-5-HT	*N*-arachidonoyl serotonin
ACEA	Arachidonoyl-2-chloroethylamide
AEA	*N*-arachidonoyl ethanolamide

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00073-9
Copyright © 2017 Elsevier Inc. All rights reserved.

AM251	1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-(1-piperidyl)pyrazole-3-carboxamide
AMG-9810	(2*E*)-*N*-(2,3-Dihydro-1,4-benzodioxin-6-yl)-3-[4-(1,1 dimethylethyl) phenyl]-2-propenamide
Ca^{2+}	Calcium
CB1	Cannabinoid receptor type 1
CB2	Cannabinoid receptor type 2
CBD	Cannabidiol
CBDV	Cannabidivarin
Cl^-	Chloride
CZP	Capsazepine
FAAH	Fatty acid amide hydrolase
GABA	γ-Amino butyric acid
GABA-T	4-Aminobutyrate transaminase
GAT-1	GABA transporter 1
ILAE	International League Against Epilepsy
JZL184	4-Nitrophenyl-4-[bis(1,3-benzodioxol-5-yl)(hydroxy) methyl]piperidine-1-carboxylate
K^+	Potassium
MGL	Lipase monoacyl glycerol
Na^+	Sodium
NMDA	*N*-methyl-D-aspartate
PTZ	Pentylenetetrazole
SB366791	4′-Chloro-3-methoxycinnamanilide
SR141716 (Rimonabant)	5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-1-yl)-1H-pyrazole-3-carboxamide
TRP	Transient potential receptor family
TRPV1	Transient receptor potential cation channel, subfamily vanilloid, member I
URB597	[3-(3-Carbamoylphenyl)phenyl] *N*-cyclohexylcarbamate
WAR	Wistar audiogenic rats
WIN55212-2	(R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-napthalenylmethanone

INTRODUCTION

The herb *Cannabis sativa* (“marijuana”, “hemp”) has been known for centuries due to its abuse-related effects and possible therapeutic uses, including for the treatment of epilepsies. The experimental and clinical studies in this field, however, have remained scant. The compound Δ^9-tetrahydrocannabinol (Δ^9-THC), which accounts for most of the typical cannabis actions, may have efficacy in attenuating seizures, although its therapeutic use is limited by its psychoactive properties. Other phytocannabinoids, including cannabidiol (CBD), Δ^9-tetrahydrocannabivarin (Δ^9-THCV), and cannabidivarin (CBDV) induce more favorable effects in animal models predictive of antiseizure activity, and in a few and small clinical studies. Synthetic cannabinoids have also been investigated in experimental settings, although with mixed results. As for their mechanisms, Δ^9-THC and other cannabinoids act by directly activating the CB1 cannabinoid receptor. The brain’s own agonists acting upon this target are the lipid compounds *N*-arachidonoyl ethanolamide (anandamide) and 2-arachidonoyl glycerol (2-AG), termed endocannabinoids, recruited on-demand to confer protections against excitotoxicity. Thus, inhibiting the enzymes responsible for terminating their action represents an interesting possibility for developing new antiseizure compounds. Anandamide, however, may also facilitate seizures through transient receptor potential vanilloid type-1 (TRPV1) channels, whose blockade represents another possible potential pharmacological approach. The present review expands on the aforementioned points, and discusses critically whether phytocannabinoids, synthetic cannabinoids, and endocannabinoid-hydrolysis inhibitors represent promising strategies for developing new treatments for epilepsies and related disorders.

EPILEPSY—DEFINITIONS, CLASSIFICATIONS, AND MECHANISMS

Epilepsy is a group of neurological diseases affecting approximately 2–3% of individuals worldwide. It can occur without a well-defined cause, or result from traumatic brain lesions, infections, tumors, and cerebrovascular events (Berg et al., 2010; Fisher et al., 2014). According to the *International League Against Epilepsy* (ILAE), it is a disease of the brain defined by any of the following conditions (Fisher et al., 2014):

- At least two unprovoked (or reflex) seizures occurring >24 h apart;
- One unprovoked (or reflex) seizure, and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years;
- Diagnosis of an epilepsy syndrome.

In addition, the ILAE (Berg et al., 2010) has proposed an international classification for seizures, as presented in Table 63.1:

Partial or focal seizures are characterized by electroencephalographic activity limited to a defined portion of the central nervous system. They can occur with the preservation or loss of consciousness, termed simple or

TABLE 63.1 International Classification of Seizures

1. Partial seizure (focal)
   - 1.1 Simple partial seizure
   - 1.2 Complex partial seizure
   - 1.3 Partial seizure secondarily generalized
2. Generalized seizure
   - 2.1 Absence
     - 2.1.1 Typical/classical
     - 2.1.2 Atypical/abnormal
   - 2.2 Myoclonic
   - 2.3 Clonic
   - 2.4 Tonic
   - 2.5 Tonic-clonic
3. Unclassified

The classification of seizures accordingly to the International League Against Epilepsy (ILAE).
Modified from Berg et al. (2010).

complex, respectively. Generalized seizures are those presenting a wide spectrum of electroencephalographic activity, indicating that a substantial portion of both sides of the brain was committed (Berg et al., 2010). During seizure events, the patient may show lack of consciousness, with no motor activity (simple absence), or diverse types of motor activity along with autonomic alterations and automatisms (Berg et al., 2010). According to the ILAE glossary (Blume-Chair et al., 2001), these motor expressions can occur as follows:

- myoclonic: sudden, brief involuntary muscle contractions with variable topography;
- clonic: myoclonus that is regularly repetitive, and extends to the exact same group of muscles from myoclonic;
- tonic: sustained contraction that lasts a few seconds to minutes;
- tonic-clonic: bilateral symmetric tonic followed by clonic contractions of members.

Regarding the molecular and cellular mechanisms, both synaptic and nonsynaptic processes seem to be involved (McNamara, 1994). Among the synaptic mechanisms, there may occur loss or facilitation of γ-aminobutyric acid (GABA)- and glutamate-mediated neurotransmissions, respectively, resulting in long term potentiation of excitatory synapses. Nonsynaptic mechanisms can be exemplified by the increase in extracellular potassium (K^+), responsible for triggering action potential on pyramidal neurons with a frequency much higher than the physiological rhythm (McNamara, 1994). One concept that has been critical for investigating the epilepsies is *epileptogenesis*. This term refers the dynamic process that begins after the occurrence of an insult, with modification of neuronal excitability that results in the first seizure, and continues with the disease. It may be characterized by neurodegeneration or neurogenesis, gliosis, axon budding, sprouting, and dendritic plasticity (Pitkänen & Lukasiuk, 2011).

THE CURRENT TREATMENT OF EPILEPSIES

Unfortunately, there is no definitive treatment for epilepsy. The main approach consists in the pharmacological control of seizures with substances termed antiseizure drugs, also called anticonvulsant or antiepileptic drugs. A second possibility is a surgical intervention to remove the epileptogenic focus, a far more invasive process, reserved for those cases who do not respond to the pharmacological treatment (Loscher, 2011; Kwan et al., 2010). Epilepsy is considered to be resolved for individuals who had an age-dependent epilepsy syndrome but are now past the applicable age, or those who have remained seizure-free for the last 10 years, with no antiseizure medicines for the last 5 years (Fisher et al., 2014). Currently, approximately 25 antiseizure drugs are available, and grouped according to their mechanisms of action and the type of the pathological condition. Some of them act by diminishing neuronal excitability, mainly though the blockage of voltage dependent sodium (Na^+) or calcium (Ca^{2+}) channels, and reduction of glutamatergic neurotransmission. Others facilitate inhibitory transmission, by indirectly or directly increasing GABA receptor functioning (Fig. 63.1) (Loscher, Klitgaard, Twyman, & Schmidt, 2013).

These medicines prevent or interrupt seizure (such as in status epilepticus), but they not properly "treat" epilepsy, since they are unable to interrupt or reverse the processes causing seizures. This results in the necessity of continuous and long-term treatments. In addition, pharmacoresistant or refractory epilepsy occurs in approximately one-third of patients, a fact defined as failure of adequate trials of two tolerated and appropriately chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom (Kwan et al., 2010). Therefore, there is an urgent need for new treatments that act by different mechanisms (Loscher et al., 2013). In this context, animal models for studying epilepsy and seizures are indispensable for allowing improvements toward the comprehension of the molecular mechanisms of epilepsy, particularly the course of epileptogenesis, and the characterization of new drug treatments, and their side effects (Loscher, 2011). Some of the main animal models are based on inducing seizures by the following stimuli:

- administration of drugs, such as kainic acid, pilocarpine and pentylenetetrazol (PTZ);
- brain electric stimulation;
- posttraumatic processes;
- brain hypoxia;
- hyperthermia;
- genetic models, such as the Wistar audiogenic rats (WAR).

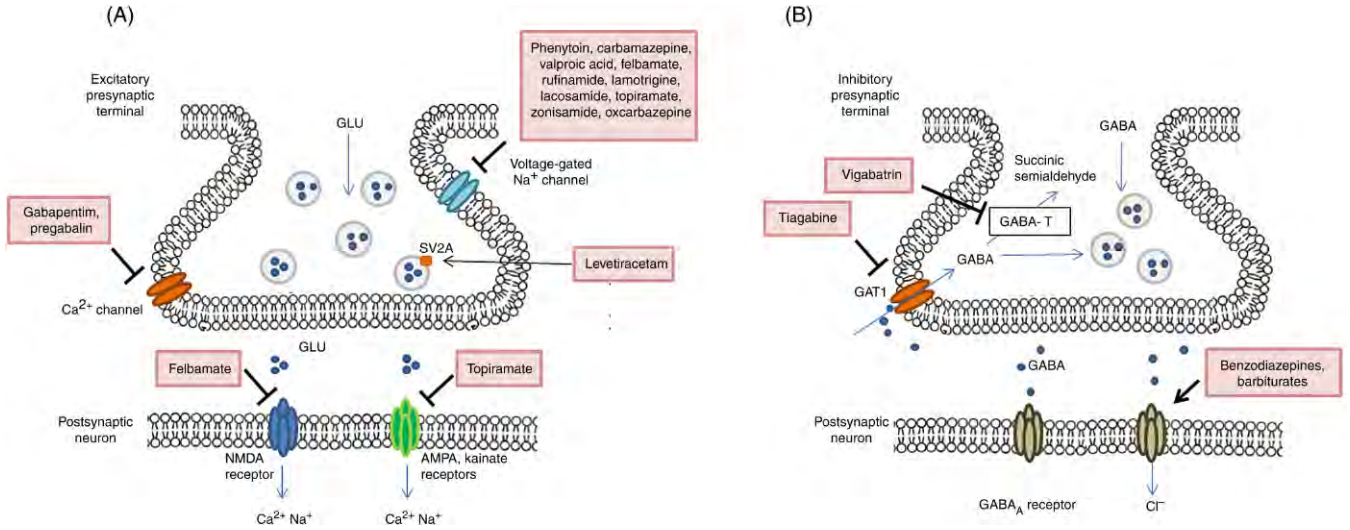


FIGURE 63.1 Mechanisms of action of currently available anticonvulsants through the excitatory and inhibitory synapses. (A) Anticonvulsants that act at excitatory synapse by blocking Na^+ and Ca^{2+} voltage-gated channels, synaptic vesicle glycoprotein and glutamatergic receptors (AMPA and NMDA channels). Some of them have multiple mechanisms of action, but their overall effect is to decrease glutamate (GLU) action; (B) anticonvulsants that acting at inhibitory synapse enhance GABAergic neurotransmission by inhibiting the uptake (mediated by a transporter, GAT) or metabolism (mediated by a transaminase, GABA-T) or by activating GABA-A receptor at specific sites.

THE ENDOCANNABINOID SYSTEM

The endocannabinoid system has been extensively reviewed regarding its biochemistry and physiology. It comprises, in its simplest form, the cannabinoid type-1 (CB1) and type-2 (CB2) receptors; at least two endogenous ligands (endocannabinoids), anandamide and 2-AG; and their metabolizing enzymes (Piomelli, 2003; Pertwee et al., 2010). The endocannabinoids are atypical neurotransmitters in several aspects. Their biosyntheses occur in postsynaptic terminals, where an increase in neural excitability and Ca^{2+} influx promotes the cleavage of membrane lipids, after which the endocannabinoids are immediately released to the synaptic cleft. Thereafter, they activate CB1 receptors located mainly at the presynaptic terminals. Anandamide has other molecular targets, such as TRPV1 channel. The actions of anandamide and 2-AG are terminated by a reuptake process followed by hydrolyses mediated by fatty acid hydrolase (FAAH) and monoacyl glycerol lipase (MGL), respectively (Piomelli, 2003; Pertwee et al., 2010). They serve as substrates for other enzymes, such as those that metabolize arachidonic acid, including cyclooxygenase and lipoxygenase, although the functions of the resulting compounds remain to be understood (Pertwee et al., 2010). The endocannabinoid system, with its main components, is illustrated in Fig. 63.2.

Some of the important compounds that act on the endocannabinoid system are the CB1/CB2 agonist, (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-napthalenylmethanone (WIN55212-2); the selective CB1 receptor agonist, arachidonoyl-2-chloroethylamide (ACEA); the CB1 receptor antagonists 5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-*N*-(piperidin-1-yl)-1H-pyrazole-3-carboxamide (SR141716, rimonabant), and 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-(1-piperidyl)pyrazole-3-carboxamide (AM251). The main compounds that act on endocannabinoid metabolic pathways are [3-(3-carbamoylphenyl)phenyl] *N*-cyclohexylcarbamate (URB597), an FAAH inhibitor, and 4-nitrophenyl-4-[bis(1,3-benzodioxol-5-yl)(hydroxy)methyl] piperidine-1-carboxylate (JZL184), an MAGL inhibitor. Also relevant are the TRPV1 blockers, capsazepine, and 4′-chloro-3-methoxycinnamanilide (SB399791); and the dual FAAH/TRPV1 blocker, arachidonoyl-serotonin (AA-5-HT) (Pertwee et al., 2010).

CANNABINOIDS AND THE ENDOCANNABINOID SYSTEM IN SEIZURES AND EPILEPSY

Effects of Phytocannabinoids

Some experimental studies and early small clinical reports have suggested the efficacy of cannabis and Δ^9-THC against seizures. Marihuana smoking protected patients against tonic-clonic and partial seizures (Consroe, Wood, & Buchsbaum, 1975), although conflicting results have been reported with Δ^9-THC, depending on the dose and types of seizures (Feeney, 1978; Karler, Calder, Sangdee, & Turkanis, 1984). In addition, results also document "rebound" effect following treatment with this compound, facilitating the occurrence of new seizures

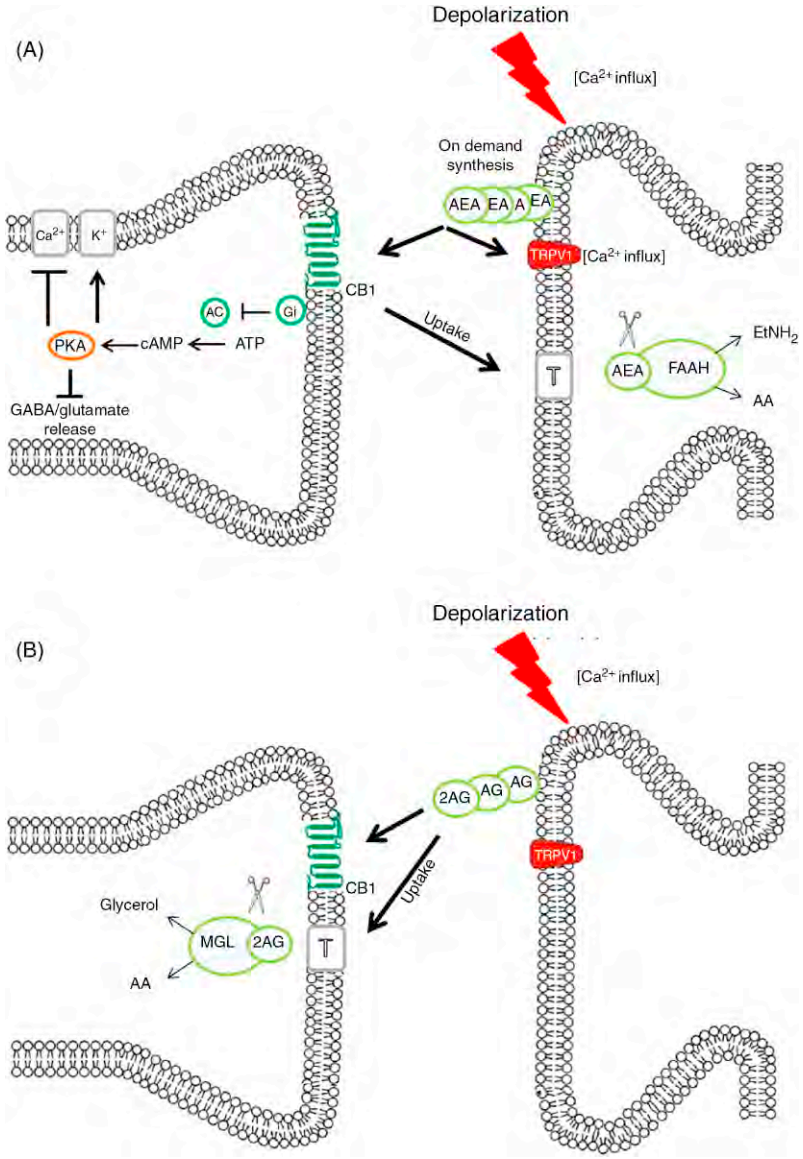


FIGURE 63.2 **Schematic representation of the endocannabinoid system.** (A) Anandamide (*N*-arachidonoyl ethanolamide, AEA) and (B) 2-arachidonoyl glycerol (2-AG) are synthesized from phospholipids at the postsynaptic neuron membrane, after calcium influx. They are immediately released to the synaptic cleft, and bind to presynaptically located cannabinoid type 1 receptors (CB1), whose activation results in decrease in calcium influx and neurotransmitter release. AEA and 2-AG are metabolized by the enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGL), respectively.

(Karler, Calder, & Turkanis, 1986). Finally, some patients may present significant central and peripheral side effects during treatment with Δ^9-THC, further indicating its therapeutic limitations (Gordon & Devinsky, 2001; Schneir & Baumbacher, 2012). Additional drawbacks are the amnestic, psychotomimetic effects of this and other compounds that act as CB1 receptor agonists (Pertwee et al., 2010).

An alternative could be CBD, the major nonpsychotomimetic component of *Cannabis sativa*, which proved to be effective in certain small experimental clinical reports. Most of them, however, date back to the 1970s, and this compound remained underinvestigated during several years, in part due to legal restrictions (Zuardi, 2008). More recently, interest in CBD has been renewed, with reports of its efficacy in children with pharmacoresistant epilepsy (Cortesi & Fusar-Poli, 2007). In experimental models, it inhibited epileptiform activity in brain slices, and exerted antiseizure activities against PTZ-induced generalized seizures in rats (Jones et al., 2010), in the pilocarpine model temporal lobe seizures, and the penicillin model of partial seizures (Jones et al., 2012), as well as in the PTZ- and electroshock-induced seizures in rats (Shirazi-zand, Ahmad-Molaei, Motamedi, &

Naderi, 2013). Thus, considering the efficacy of CBD, and the fact that it does not share the psychotomimetic, amnestic, and sedative effects of Δ^9-THC, it is an interesting potential treatment for certain cases of epilepsy. A recent clinical study observed that CBD-enriched cannabis extracts significantly reduced seizure frequency in children with treatment-resistant epilepsy, indicating that it could be used as a strategy when other pharmacological treatments fail (Porter & Jacobson, 2013).

It should be noted, however, that more extensive studies are still required to characterize the efficacy of CBD against diverse types of seizures and epileptic syndromes, preferentially in comparison with currently used antiseizure drugs. In addition, the mechanisms of action of this compound have remained elusive, and should be further investigated. CBD is a compound with a plethora of pharmacological actions, including anxiolytic and antipsychotic effects, which may act through diverse mechanisms, including facilitation of the endocannabinoid system, and binding to TRPV1 channels and serotonin type-1A receptors, among others (Izzo, Borrelli, Capasso, Di Marzo, & Mechoulam, 2009; Zuardi, 2008). Finally, other phytocannabinoids have also been investigated regarding their potential application for treating epilepsies, particularly Δ^9- THCV and CBDV (Hill et al., 2010, 2013). Since over 60 phytocannabinoids have been described, they should be further investigated in experimental models. Compounds that act though CB1-independent mechanisms deserve particular attention.

Effects of Synthetic Cannabinoids

Similarly to Δ^9-THC, its synthetic derivatives may either induce favorable effects or facilitate seizures, depending on the animal model and the doses tested (Lutz, 2004). The nonselective CB1 agonist, WIN-55,212-2, had anticonvulsant effects on electroshock model of generalized seizures (Wallace, Wiley, Martin, & DeLorenzo, 2001). In addition, WIN-55,212-2 and ACEA potentiated the effects of the classical anticonvulsants carbamazepine, phenytoin, and phenobarbital in electroshock-induced seizures (Luszczki et al., 2010). WIN-55,212-2 was also effective in the amygdala kindling model of temporal lobe epilepsy in mice (Wendt, Soerensen, Wotjak, & Potschka, 2011).

Several groups have used different models of seizures and a variety of experimental procedures (in vivo and in vitro) to demonstrate that CB1 receptor agonists can modulate both the frequency and severity of seizures (Blair et al., 2006; Bhaskaran & Smith, 2010; Jones et al., 2010; Shafaroodi et al., 2004, 2007). Other studies, however, provide evidence that CB1 receptor activation could have proconvulsant effects (Vilela et al., 2013). Furthermore, long term exposition to WIN-55,212-2 resulted in tolerance and exacerbation of seizures (Blair et al., 2009). Considering these controversies and their possible side effects, CB1 agonists are unlikely to represent the most attractive approach for developing new treatments. Since their effects on experimental seizures have remained poorly investigated, the reasons for these discrepancies are still unclear (Lutz, 2004).

Role of the Endocannabinoid System on Seizure Control, and the Effects of Endocannabinoid Hydrolysis Inhibitors

Some specific features of the endocannabinoid system suggest that it represents an attractive target for developing new drugs for treating epilepsy. The endocannabinoids are produced on-demand after neuronal depolarization to dampen excessive excitatory activity. In line with this notion, some studies have proposed that the endocannabinoid actions upon CB1 receptor may be as a tonic mechanism protecting the brain against seizures. Accordingly, the deletion or pharmacological blockade of this receptor increased KA-induced excitotoxicity (Marsicano et al., 2003). Moreover, conditional deletion of the CB1 receptors in forebrain glutamatergic neurons also facilitated seizure (Monory et al., 2006). Other studies have pointed out that the treatment with CB1 receptor antagonists increases the frequency and duration of seizures, as well as epileptiform discharges (Kozan, Ayyildiz, & Agar, 2009; Deshpande et al., 2007). Reduced levels of anandamide were found in patients (Romigi et al., 2010), and data from positron emission tomography showed that the expression of CB1 receptors was altered in epileptogenic regions (Goffin, Van Paesschen, & Van Laere, 2011). These results support the hypothesis of a primary dysfunction of the endocannabinoid system in patients with epilepsy.

Some contradictory results, however, have been observed. For instance, the treatment with the CB1 antagonists AM251 and SR141716 also prevented antiepileptogenic properties in hippocampal neuron culture in which glutamate agonists were added (Karr, Pan, & Rutecki, 2010). Furthermore, low doses of AM251 enhanced the anticonvulsant effects of ACEA against PTZ-induced seizures in mice (Gholizadeh et al., 2007). The reasons for these discrepancies could be related to the modulation of excitatory and inhibitory neurotransmissions by this receptor, as discussed earlier.

Despite these contrasting results, the observations that anandamide-CB1 signaling may act as an endogenous antiseizure mechanism indicate that FAAH inhibition represents a promising strategy for developing new treatments. Indeed, experimental studies with the kainic acid model of seizures indicate that FAAH inhibitors reduced both in vivo and in vitro excitotoxicity, in addition to inducing neuroprotection

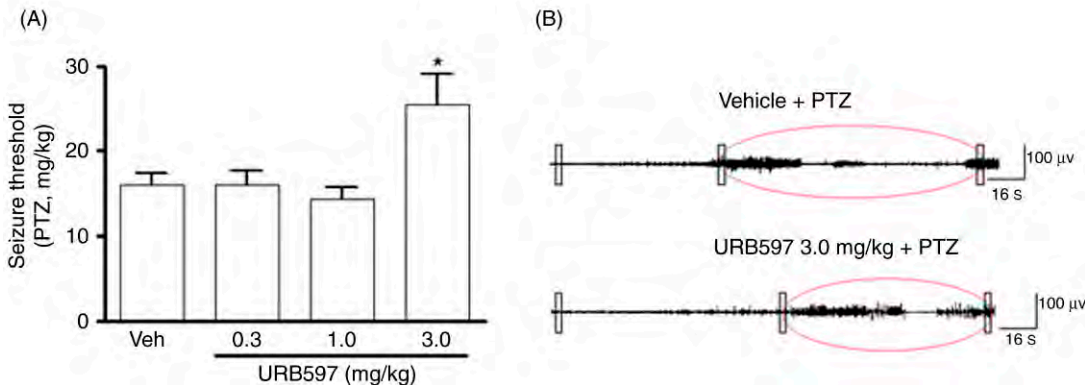


FIGURE 63.3 **Effect of anandamide hydrolysis inhibition on PTZ-induced behavioral and electroencephalographic seizures in rats.** (A) Administration of the FAAH inhibitor, URB597, increased dose of PTZ required to induce seizure in rats; (B) URB597 also reduced the electroencephalographic seizure duration, as shown in this representative EEG recording. The animals received intraperitoneal injections of URB597 30 min before continuous intravenous infusion of PTZ. Bars represent means and standard errors. *$p < 0.05$ as revealed by analysis of variance followed by the Newman–Keuls test; $n = 7–8$/group (for further details, see Vilela et al., 2013).

(Naidoo et al., 2011). Moreover, the FAAH inhibitor, URB597, had an anticonvulsant effect, reducing both seizures and electroencephalographic (EEG) activity induced by PTZ in rats (Fig. 63.3) (Vilela et al., 2013). In addition to anandamide, 2-AG hydrolysis can also be targeted to provide anticonvulsant and neuroprotective effects (Naidoo et al., 2012). Their neuroprotective mechanisms may be explained by a reduced excitatory signaling, reduced calcium influx, buffering of oxidative stress, and activation of intracellular pathways related to cellular survival associated with the production of neurotrophic factors (Bisogno & Di Marzo, 2010; Fernández Ruiz, Garcia, Sagredo, Gomez-Ruiz, & de Lago, 2010). An earlier study, however, observed that FAAH knockout mice exhibited a higher susceptibility to develop seizures after the administration of kainic acid (Clement, Hawkins, Lichtman, & Cravatt, 2003). The reasons for these discrepancies remain unclear.

Interactions Between the Endocannabinoid System and TRPV1 Channel

Although facilitation of the endocannabinoid system represents a promising strategy for controlling seizures, this approach can be hampered by the fact that anandamide binds to other targets, particularly the TRPV1 channel. This ion channel was first characterized as an "orphan receptor" on primary afferents sensory neurons. It is cation permeable, and activated by capsaicin (8-methyl-*N*-vanilil-6-nonanamide, the most irritant component of *Capsicum* sp, popularly known as red pepper), heat and low pH. In the brain, this receptor is thought to be activated mainly by anandamide, resulting in opposite effects to those ascribed to CB1 receptors (Ross et al., 2001; Starowicz, Nigam, & Di Marzo, 2007). Thus, whereas CB1 activation causes inhibition of cation influx, reduction of neural activity, and neurotransmitters release, TRPV1 activation promotes the opposite effects (Starowicz et al., 2007). Remarkably, the events facilitated by TRPV1 are associated with the development of epileptogenic events. Fig. 63.4 illustrates the dual effects of anandamide upon CB1/TRPV1 receptors activation.

In this way, pharmacological targeting of TRPV1 could represent an additional mechanism to modulate seizures. Activation of TRPV1 channels by capsaicin leads to epileptiform activity in vitro and in vivo, while antagonism with capsazepine effectively suppresses electrographic seizures (Gonzalez-Reyes, Ladas, Chiang, & Durand, 2013). Capsaicin also increased the frequency of excitatory postsynaptic potential through the activation of TRPV1 in pilocarpine-treated mice that survived status epilepticus (Bhaskaran & Smith, 2010). In addition, anandamide, at high doses, can facilitate seizures by the activation of TRPV1 in the PTZ model in mice (Manna & Umathe, 2012).

Considering these aspects, a possible strategy for designing new antiseizure drugs is to focus on compounds that simultaneously block TRPV1 and FAAH. A compound that acts through such a mechanism is *N*-arachidonoyl-serotonin (AA-5-HT), which is therefore able to increase the levels of anandamide and redirect the actions of this substance entirely to CB1 receptors (Micale et al., 2009a). Accordingly, the administration of AA-5-HT had efficacious anticonvulsant effect on tonic-clonic seizures in mice (Micale et al., 2009b; Vilela, Medeiros, de Oliveira, Moraes, & Moreira, 2014). This effect was prevented by the CB1 receptor selective agonist AM251. The TRPV1 antagonist, SB366791, however, only partially mimicked the action of the AA-5-HT. These results demonstrate that both TRPV1 blockade and indirect CB1 agonism contribute to the overt effects of AA-5-HT (Fig. 63.5) (Vilela et al., 2014).

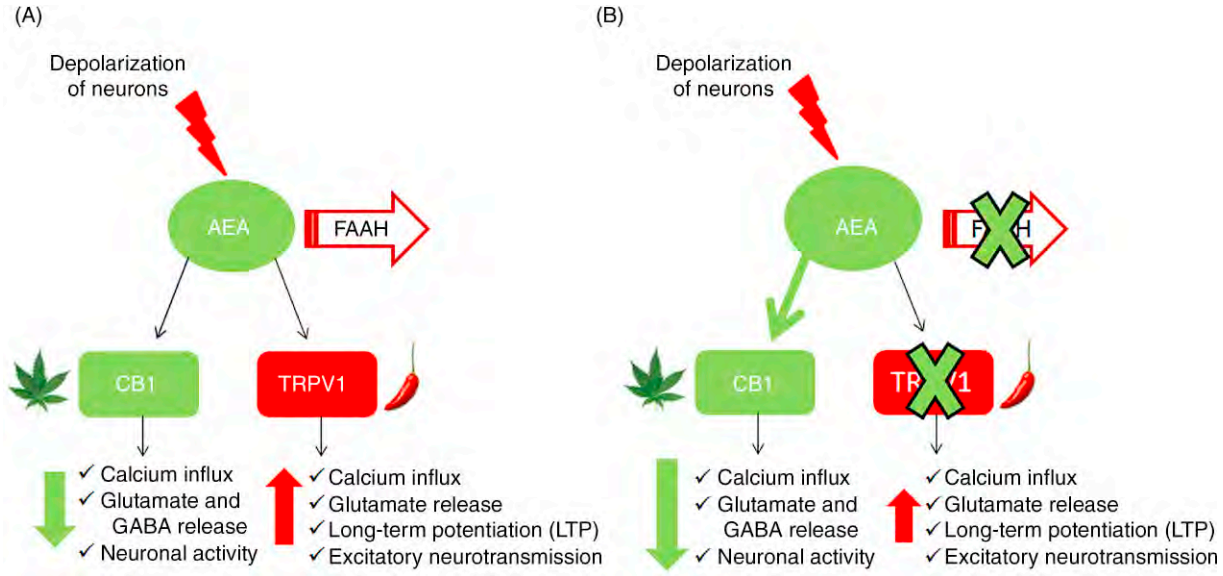


FIGURE 63.4 **Dual effects of anandamide (AEA) on neurotransmission through activation of CB1 receptors and TRPV1 channels.** (A) AEA can exert either anti- or proconvulsant activities, by activating CB1 receptors or TRPV1 channels, respectively. (B) Pharmacological modulation through dual blocking of FAAH and TRPV1 increases on-demand anandamide levels and redirects its actions entirely to CB1 receptor, possibly representing an attractive approach against excitotoxic events.

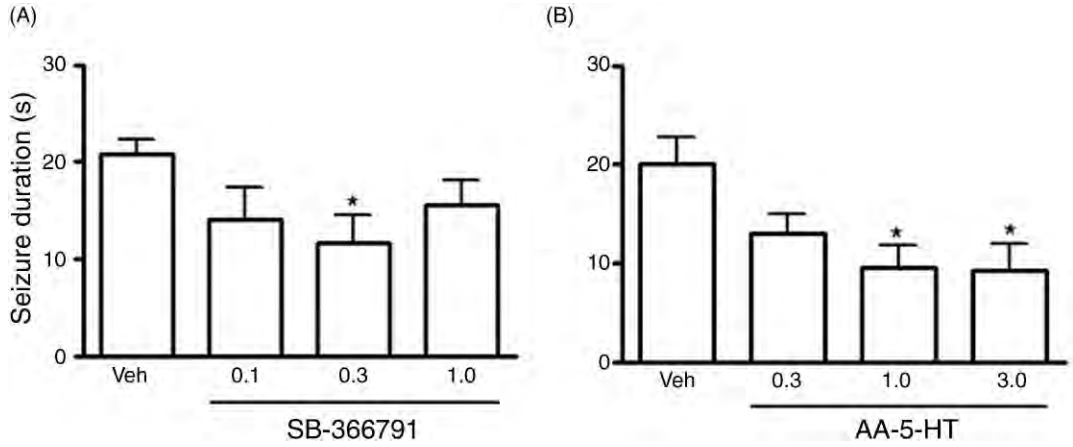


FIGURE 63.5 **Effects of compound that block FAAH and/or TRPV1 on PTZ-induced seizure in mice.** (A) The selective TRPV1 blocker, SB-366791, reduced PTZ-induced seizure. (B) The dual FAAH/TRPV1 blocker, AA-5-HT, reduced PTZ-induced seizure more consistently than the selective TRPV1 blocker. The animals received intraperitoneal injections of SB-366791 or AA-5-HT 30 min before intraperitoneal PTZ. Bars represent means and standard errors. *$p < 0.05$ as revealed by analysis of variance followed by the Newman–Keuls test; n = 9–10/group (for further details, see Vilela et al., 2014).

CONCLUSIONS

In this chapter, we discussed evidence from basic and clinical studies suggesting that cannabinoids and the endocannabinoid system should be further explored for the development of new treatments for epilepsies and related syndromes. Δ^9-THC and synthetic cannabinoids that act as CB1 agonists have discrepant effects, depending on the model and species employed. A possible reason for some seemingly divergent results might be related to the complex role of CB1 receptors in modulating both excitatory glutamatergic and inhibitory GABAergic neurotransmission. As an alternative, other phytocannabinoids, such as CBD, Δ^9-THCV, and CBDV, may represent an interesting strategy. Furthermore, considering that anandamide has been linked to on-demand protection against excitotoxicity, FAAH inhibitors may be employed to induce more favorable effects, as compared to direct CB1 receptor agonists. Finally, considering the possible actions of anandamide upon TRPV1 channels, which counteract CB1-mediated effects, compounds that simultaneously block FAAH and TRPV1 could be

an effective new drug class for seizure treatment. These compounds should be investigated particularly in comparison with clinically used drugs, in terms of both efficacy and side effects. Finally, it should be tested whether they can treat pharmacoresistant epilepsy, and reverse epileptogenesis, hopefully paving the way for new treatments for this devastating syndrome.

MINI-DICTIONARY

Antiseizure drug Any drug efficacious in preventing or interrupting seizures.
Convulsion Rapid and repeated contractions and relaxation of body muscles, usually bilateral, resulting in uncontrolled movements.
Electroencephalography Recording of electrical activity resulting from total ionic current flows in neurons of the brain.
Epilepsy A neurological syndrome characterized mainly by the repetitive occurrence of seizures.
Epileptogenesis Molecular and cellular processes that lead to the increase in neuronal excitability.
Kindling Sensitization to seizure-inducing stimuli after successive exposure.
Pharmacoresistant epilepsy Epilepsy characterized by crises that occurs in spite of the treatment with antiseizure drugs.
Seizure Brief episodes of abnormal excessive or synchronous neuronal activity in the brain.
Status epilepticus A life-threatening condition in which seizures last longer than 5 min, or recurrent seizures without regaining consciousness between seizures for longer than 5 min.
Synchronization Simultaneous firing of groups of neurons in the brain.

References

Berg, A. T., Berkovic, S. F., Brodie, M. J., Buchhalter, J., Cross, J. H., van Emde Boas, W., Engel, J., French, J., Glauser, T. A., Mathern, G. W., Moshé, S. L., Nordli, D., Plouin, P., & Scheffer (2010). Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. *Epilepsia*, *51*, 676–685.

Bhaskaran, M. D., & Smith, B. N. (2010). Cannabinoid-mediated inhibition of recurrent excitatory circuitry in the dentate gyrus in a mouse model of temporal lobe epilepsy. *PLoS One*, *5*, 10683.

Bisogno, T., & Di Marzo, V. (2010). Cannabinoid receptors and endocannabinoids: role in neuroinflammatory and neurodegenerative disorders. *CNS Neurological Disorders and Drug Targets*, *9*, 564–973.

Blair, R. E., Deshpande, L. S., Sombati, S., Elphick, M. R., Martin, B. R., & DeLorenzo, R. J. (2009). Prolonged exposure to WIN55,212-2 causes downregulation of the CB1 receptor and the development of tolerance to its anticonvulsant effects in the hippocampal neuronal culture model of acquired epilepsy. *Neuropharmacology*, *57*, 208–218.

Blair, R. E., Deshpande, L. S., Sombati, S., Falenski, K. W., Martin, B. R., & DeLorenzo, R. J. (2006). Activation of the cannabinoid type-1 receptor mediates the anticonvulsant properties of cannabinoids in the hippocampal neuronal culture models of acquired epilepsy and status epilepticus. *Journal of Pharmacology and Experimental Therapeutics*, *317*, 1072–1078.

Blume-Chair, W., Luders, H. O., Mizrahi, E., Tassinari, C., Boas, W. E., & Engel, J., Jr. (2001). Glossary of descriptive terminology for ictal semiology: report of the ILAE task force on classification and terminology. *Epilepsia*, *42*, 1212–1218.

Clement, A. B., Hawkins, E. G., Lichtman, A. H., & Cravatt, B. F. (2003). Increased seizure susceptibility and proconvulsant activity of anandamide in mice lacking fatty acid amide hydrolase. *Journal of Neuroscience*, *23*, 3916–3923.

Consroe, P. F., Wood, G. C., & Buchsbaum, H. (1975). Anticonvulsant nature of marihuana smoking. *Journal of the American Medical Association*, *234*, 306–307.

Cortesi, M., & Fusar-Poli, P. (2007). Potential therapeutical effects of cannabidiol in children with pharmacoresistant epilepsy. *Medical Hypotheses*, *68*, 920–921.

Deshpande, L. S., Blair, R. E., Ziobro, J. M., Sombati, S., Martin, B. R., & DeLorenzo, R. J. (2007). Endocannabinoids block status epilepticus in cultured hippocampal neurons. *European Journal of Pharmacology*, *558*, 52–59.

Feeney, D. M. (1978). Marihuana and epilepsy: paradoxical anticonvulsant and convulsant effects. *Advances in Biosciences*, *22*, 643–657.

Fernández-Ruiz, J., Garcia, C., Sagredo, O., Gomez-Ruiz, M., & de Lago, E. (2010). The endocannabinoid system as a target for the treatment of neuronal damage. *Expert Opinion in Therapeutic Targets*, *14*, 387–404.

Fisher, R. S., Acevedo, C., Arzimanoglou, A., Bogacz, A., Cross, J. H., Elger, C. E., Engel, J., Jr., Forsgren, L., French, J. A., Glynn, M., Hesdorffer, D. C., Lee, B. I., Mathern, G. W., Moshé, S. L., Perucca, E., Scheffer, I. E., Tomson, T., Watanabe, M., & Wiebe, S. (2014). ILAE Official Report: a practical clinical definition of epilepsy. *Epilepsia*, *55*, 475–482.

Gholizadeh, S., Shafaroodi, H., Ghasemi, M., Bahremand, A., Sharifzadeh, M., & Dehpour, A. R. (2007). Ultra-low dose cannabinoid antagonist AM251 enhances cannabinoid anticonvulsant effects in the pentylenetetrazole-induced seizure in mice. *Neuropharmacology*, *53*, 763–770.

Goffin, K., Van Paesschen, W., & Van Laere, K. (2011). In vivo activation of endocannabinoid system in temporal lobe epilepsy with hippocampal sclerosis. *Brain*, *134*, 1033–1040.

Gonzalez-Reyes, L. E., Ladas, T. P., Chiang, C. C., & Durand, D. M. (2013). TRPV1 antagonist capsazepine suppresses 4-AP-induced epileptiform activity in vitro and electrographic seizures in vivo. *Experimental Neurology*, *250*, 321–332.

Gordon, E., & Devinsky, O. (2001). Alcohol and marijuana: effects on epilepsy and use by patients with epilepsy. *Epilepsia*, *42*, 1266–1272.

Hill, A. J., Weston, S. E., Jones, N. A., Smith, I., Bevan, S. A., Williamson, E. M., Stephens, G. J., Williams, C. M., & Whalley, B. J. (2010). Δ-9-Tetrahydrocannabivarin suppresses in vitro epileptiform and in vivo seizure activity in adult rats. *Epilepsia*, *51*, 1522–1532.

Hill, T. D., Cascio, M. G., Romano, B., Duncan, M., Pertwee, R. G., Williams, C. M., Whalley, B. J., & Hill, A. J. (2013). Cannabidivarin-rich cannabis extracts are anticonvulsant in mouse and rat via a CB1 receptor-independent mechanism. *British Journal of Pharmacology*, *170*, 679–692.

Izzo, A. A., Borrelli, F., Capasso, R., Di Marzo, V., & Mechoulam, R. (2009). Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends in Pharmacological Sciences*, *30*, 515–527.

Jones, N. A., Glyn, S. E., Akiyama, S., Hill, T. D., Hill, A. J., Weston, S. E., Burnett, M. D., Yamasaki, Y., Stephens, G. J., Whalley, B. J., & Williams, C. M. (2012). Cannabidiol exerts anti-convulsant effects in animal models of temporal lobe and partial seizures. *Seizure*, *21*, 344–352.

Jones, N. A., Hill, A. J., Smith, I., Bevan, S. A., Williams, C. M., Whalley, B. J., & Stephens, G. J. (2010). Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo. *Journal of Pharmacology and Experimental Therapeutics*, *332*, 569–577.

Karler, R., Calder, L. D., Sangdee, P., & Turkanis, S. A. (1984). Interaction between delta-9-tetrahydrocannabinol and kindling by electrical and chemical stimuli in mice. *Neuropharmacology*, *23*, 1315–1320.

Karler, R., Calder, L. D., & Turkanis, A. S. (1986). Prolonged CNS hyperexcitability in mice after a single exposure to delta-9-tetrahydrocannabinol. *Neuropharmacology*, *25*, 441–446.

Karr, L., Pan, Y. Z., & Rutecki, P. A. (2010). CB1 antagonism impairs the induction of epileptiform activity by group I metabotropic glutamate receptor activation. *Epilepsia*, *51*(Suppl. 3), 121–125.

Kozan, R., Ayyildiz, M., & Agar, E. (2009). The effects of intracerebroventricular AM-251, a CB1-receptor antagonist, and ACEA, a CB1-receptor agonist, on penicillin-induced epileptiform activity in rats. *Epilepsia*, *50*, 1760–1767.

Kwan, P., Arzimanoglou, A., Berg, A. T., Brodie, M. J., Hauser, W. A., Mathern, G., Moshe, S. L., Perruca, E., Wiebe, S., & French, J. (2010). Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on therapeutic strategies. *Epilepsia*, *51*, 1069–1077.

Loscher, W. (2011). Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. *Seizure*, *20*, 359–368.

Loscher, W., Klitgaard, H., Twyman, R. E., & Schmidt, D. (2013). New avenues for anti-epileptic drug discovery and development. *Nature Reviews Drug Discovery*, *12*, 757–776.

Luszczki, J. J., Czuczwar, P., Cioczek-Czuczwar, A., Dudra-Jastrzebska, M., Andres-Mach, M., & Czuczwar, S. J. (2010). Effect of arachidonyl-2′-chloroethylamide, a selective cannabinoid CB1 receptor agonist, on the protective action of the various antiepileptic drugs in the mouse maximal electroshock-induced seizure model. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *34*, 18–25.

Lutz, B. (2004). On-demand activation of the endocannabinoid system in the control of neuronal excitability and epileptiform seizures. *Biochemical Pharmacology*, *68*, 1691–1698.

Manna, S. S., & Umathe, S. N. (2012). Involvement of transient receptor potential vanilloid type 1 channels in the pro-convulsant effect of anandamide in pentylenetetrazole-induced seizures. *Epilepsy Research*, *100*, 113–124.

Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., Azad, S. C., Cascio, M. G., Gutierrez, S. O., van der Stelt, M., Lopez-Rodriguez, M. L., Casanova, E., Schutz, G., Zieglgansberger, W., Di Marzo, V., Behl, C., & Lutz, B. (2003). CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science*, *302*, 84–88.

McNamara, J. O. (1994). Cellular and molecular basis of epilepsy. *The Journal of Neuroscience*, *14*, 3413–3425.

Micale, V., Cristino, L., Tamburella, A., Petrosino, S., Leggio, G. M., Drago, F., & Di Marzo, V. (2009a). Anxiolytic effects in mice of a dual blocker of fatty acid amide hydrolase and transient receptor potential vanilloid type-1 channels. *Neuropsychopharmacology*, *34*, 593–606.

Micale, V., Cristino, L., Tamburella, A., Petrosino, S., Leggio, G. M., Drago, F., & Di Marzo, V. (2009b). Altered responses of dopamine D3 receptor null mice to excitotoxic or anxiogenic stimuli: possible involvement of the endocannabinoid and endovanilloid systems. *Neurobiology of Diseases*, *36*, 70–80.

Monory, K., Massa, F., Egertova, M., Eder, M., Blaudzun, H., Westenbroek, R., Kelsch, W., Jacob, W., Marsch, R., Ekker, M., Long, J., Rubenstein, J. L., Goebbels, S., Nave, K. A., During, M., Klugmann, M., Wolfel, B., Dodt, H. U., Zieglgansberger, W., Wotjak, C. T., Mackie, K., Elphick, M. R., Marsicano, G., & Lutz, B. (2006). The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron*, *51*, 455–466.

Naidoo, V., Karanian, D. A., Vadivel, S. K., Locklear, J. R., Wood, J. T., Nasr, M., Quizon, P. M., Graves, E. E., Shukla, V., Makriyannis, A., & Bahr, B. A. (2012). Equipotent inhibition of fatty acid amide hydrolase and monoacylglycerol lipase–dual targets of the endocannabinoid system to protect against seizure pathology. *Neurotherapeutics*, *9*, 801–813.

Naidoo, V., Nikas, S. P., Karanian, D. A., Hwang, J., Zhao, J., Wood, J. T., Alapafuja, S. O., Vadivel, S. K., Butler, D., Makriyannis, A., & Bahr, B. A. (2011). A new generation fatty acid amide hydrolase inhibitor protects against kainate-induced excitotoxicity. *Journal of Molecular Neurosciences*, *43*, 493–502.

Pertwee, R. G., Howlett, A. C., Abood, M. E., Alexander, S. P., Di Marzo, V., Elphick, M. R., Greasley, P. J., Hansen, H. S., Kunos, G., Mackie, K., Mechoulam, R., & Ross, R. A. (2010). InternationalUnion of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. *Pharmacological Reviews*, *62*, 588–631.

Piomelli, D. (2003). The molecular logic of endocannabinoid signalling. *Nature Reviews Neuroscience*, *4*, 873–884.

Pitkänen, A., & Lukasiuk, K. (2011). Mechanisms of epileptogenesis and potential treatment targets. *Lancet Neurology*, *10*, 173–186.

Porter, B. E., & Jacobson, C. (2013). Report of a parent survey of cannabidiol-enriched cannabis use in pediatric treatment-resistant epilepsy. *Epilepsy and Behavior*, *29*, 574–577.

Romigi, A., Bari, M., Placidi, F., Marciani, M. G., Malaponti, M., Torelli, F., Izzi, F., Prosperetti, C., Zannino, S., Corte, F., Chiaramonte, C., & Maccarrone, M. (2010). Cerebrospinal fluid levels of the endocannabinoid anandamide are reduced in patients with untreated newly diagnosed temporal lobe epilepsy. *Epilepsia*, *51*, 768–772.

Ross, R. A., Gibson, T. M., Brockie, H. C., Leslie, M., Pashmi, G., Craib, S. J., Di Marzo, V., & Pertwee, R. G. (2001). Structure-activity relationship for the endogenous cannabinoid, anandamide, and certain of its analogues at vanilloid receptors in transfected cells and vas deferens. *British Journal of Pharmacology*, *132*, 631–640.

Schneir, A. B., & Baumbacher, T. (2012). Convulsions associated with the use of a synthetic cannabinoid product. *Journal of Medical Toxicology*, *8*, 62–64.

Shafaroodi, H., Asadi, S., Sadeghipour, H., Ghasemi, M., Ebrahimi, F., Tavakoli, S., Hajrasouliha, A. R., & Dehpour, A. R. (2007). Role of ATP-sensitive potassium channels in the biphasic effects of morphine on pentylenetetrazole-induced seizure threshold in mice. *Epilepsy Research*, *75*, 63–69.

Shafaroodi, H., Samini, M., Moezi, L., Homayoun, H., Sadeghipour, H., Tavakoli, S., Hajrasouliha, A. R., & Dehpour, A. R. (2004). The interaction of cannabinoids and opioids on pentylenetetrazole-induced seizure threshold in mice. *Neuropharmacology*, *47*, 390–400.

Shirazi-zand, Z., Ahmad-Molaei, L., Motamedi, F., & Naderi, N. (2013). The role of potassium BK channels in anticonvulsant effect of cannabidiol in pentylenetetrazole and maximal electroshock models of seizure in mice. *Epilepsy and Behavior*, *28*, 1–7.

Starowicz, K., Nigam, S., & Di Marzo, V. (2007). Biochemistry and pharmacology of endovanilloids. *Pharmacology and Therapeutics*, *114*, 13–33.

Vilela, L. R., Medeiros, D. C., de Oliveira, A. C., Moraes, M. F., & Moreira, F. A. (2014). Anticonvulsant effects of N-arachidonoylserotonin, a dual FAAH enzyme and TRPV1 channel blocker, on experimental seizures: the roles of cannabinoid CB1 receptors and TRPV1 channels. *Basic and Clinical Pharmacology and Toxicology*, *115*, 330–334.

Vilela, L. R., Medeiros, D. C., Rezende, G. H., de Oliveira, A. C., Moraes, M. F., & Moreira, F. A. (2013). Effects of cannabinoids and endocannabinoid hydrolysis inhibition on pentylenetetrazole-induced seizure and electroencephalographic activity in rats. *Epilepsy Research*, *104*, 195–202.

Wallace, M. J., Wiley, J. L., Martin, B. R., & DeLorenzo, R. J. (2001). Assessment of the role of CB1 receptors in cannabinoid anticonvulsant effects. *European Journal of Pharmacology*, *428*, 51–57.

Wendt, H., Soerensen, J., Wotjak, C. T., & Potschka, H. (2011). Targeting the endocannabinoid system in the amygdala kindling model of temporal lobe epilepsy in mice. *Epilepsia*, *52*, e62–e65.

Zuardi, A. W. (2008). Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. *Revista Brasileira de Psiquiatria*, *30*, 271–280.

CHAPTER

64

Pharmacological Aspects of Anandamide and 2-Arachidonoyglycerol as Bioactive Lipids

M. Alhouayek, G.G. Muccioli

Bioanalysis and Pharmacology of Bioactive Lipids Research Group, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium

SUMMARY POINTS

- The endocannabinoids *N*-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) are involved in many pathophysiological processes.
- Classically, endocannabinoids exert their actions by activating the CB_1 and CB_2 cannabinoid receptors, and their actions are terminated by their hydrolysis by specific hydrolases.
- More receptors mediating the effects of AEA and 2-AG have been added over the years.
- Endocannabinoids can also be metabolized by cyclooxygenase (COX)-2, which leads to the synthesis of other bioactive lipids.
- Endocannabinoids and their COX-2 derived metabolites can exert different effects in some settings, a fact that increases the complexity of endocannabinoid signaling.
- Interfering with the metabolism of endocannabinoids opens up a lot of therapeutic opportunities.

KEY FACTS ABOUT ENDOCANNABINOID-RELATED COMPOUNDS

- They are bioactive lipids belonging to the families of *N*-arachidonoylethanolamine (AEA) or 2-arachidonoylglycerol (2-AG) that do not bind cannabinoid receptors.
- They have long been considered as acting via an "entourage effect," by protecting endocannabinoids from degradation, and potentiating their actions; however, they also have receptor-specific effects.
- Among *N*-acylethanolamines: *N*-palmitoylethanolamine (PEA) exerts antiinflammatory, analgesic, and neuroprotective effects, and is a PPAR-α agonist, while *N*-oleoylethanolamine (OEA) is a GPR119 and a PPAR-α ligand known for its anorexigenic properties, and the control of glucagon secretion.
- 2-Oleoylglycerol (2-OG) is a monoacylglycerol related to 2-AG that acts as a GPR119 agonist to increase secretion of glucagon-like peptide 1 (GLP-1).

LIST OF ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
ABHD6	α/β Hydrolase domain 6
ABHD12	α/β Hydrolase domain 12
AEA	*N*-arachidonoylethanolamine or anandamide
CB_1 and CB_2	Cannabinoid receptor 1 and 2
COX-2	Cyclooxygenase-2
cPLA2	Cytosolic phospholipase A2
CYP450	Cytochrome P450
DAGL	Diacylglycerol lipase

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00074-0
Copyright © 2017 Elsevier Inc. All rights reserved.

EET	Epoxyeicosatrienoic acid
EET-EA	Epoxyeicosatrienoic acid-ethanolamide
EET-G	Epoxyeicosatrienoic acid-glycerol ester
FAAH	Fatty acid amide hydrolase
GDE1	Glycerophosphodiester phosphodiesterase 1
HETE	Hydroxyeicosatetraenoic acid
HETE-EA	Hydroxyeicosatetraenoic acid-ethanolamine
HETE-G	Hydroxyeicosatetraenoic acid-glycerol ester
LOX	Lipoxygenase
MAGL	Monoacylglycerol lipase
NAAA	*N*-acylethanolamine-hydrolyzing acid amidase
NAE	*N*-acylethanolamine
NAPE	*N*-acylphosphatidylethanolamine
NAPE-PLD	*N*-acylphosphatidylethanolamine phospholipase D
PG-EA	Prostaglandin-ethanolamine or prostamide
PG-G	Prostaglandin-glycerol ester
PPAR	Peroxisome proliferator activated receptor
TRPV1	Transient receptor potential vanilloid 1

INTRODUCTION

The concept of bioactive lipids stems from the recognition that lipids are not only involved in cell membrane structure and energy storage. Indeed, some lipids bind to specific receptors, leading to signal transduction and biological effects. Accordingly, variations in the levels of such lipids lead to pathophysiological consequences. These are considered bioactive lipids. Eicosanoids, such as the arachidonic acid-derived prostaglandins, are commonly known bioactive lipids.

The endocannabinoids *N*-arachidonoylethanolamine (anandamide or AEA) and 2-arachidonoylglycerol (2-AG) are bioactive lipids that are involved in many physiological processes, such as the regulation of food intake, the control of anxiety, and the modulation of pain and inflammation (Borrelli & Izzo, 2009; Ruehle, Rey, Remmers, & Lutz, 2012; Guindon & Hohmann, 2009; Alhouayek & Muccioli, 2012). As for many bioactive lipids, the activity of endocannabinoids is regulated by their tissue levels and, therefore by the balance between their biosynthesis and their degradation (Muccioli, 2010). In this context, pharmacological modulation of endocannabinoid levels represents an attractive therapeutic avenue for many diseases.

This chapter presents an overview of endocannabinoid metabolism, with many of the intricacies that characterize it, in order to describe the available pharmacological modulators of endocannabinoid signaling, as well as the potential therapeutic opportunities of such modulators.

OVERVIEW OF THE EFFECTS OF ANANDAMIDE AND 2-ARACHIDONOYLGLYCEROL

These endocannabinoids have several beneficial effects that could be used in potential treatments. For instance, endocannabinoids have antiinflammatory effects, which can be beneficial in diseases such as inflammatory bowel diseases and arthritis (Alhouayek & Muccioli, 2012; Mecs, Tuboly, Toth, Nagy, & Nyari, 2010). They are also valuable in neurodegenerative diseases such as multiple sclerosis, or following cerebral trauma, due to their antiinflammatory and neuroprotective properties (Lourbopoulos, Grigoriadis, Lagoudaki, Touloumi, & Polyzoidou, 2011; Panikashvili, Shein, Mechoulam, Trembovler, & Kohen, 2006). Endocannabinoids also exert analgesic effects in many settings (Guindon & Hohmann, 2009), modulate the stress response, reduce anxiety and depression (Ruehle et al., 2012), promote sleep (Murillo-Rodriguez, Poot-Ake, Arias-Carrion, Pacheco-Pantoja, & Fuente-Ortegon, 2011), and decrease nausea and vomiting (Sharkey, Darmani, & Parker, 2014). Generally, increasing endocannabinoid levels is thought to be beneficial, therefore endocannabinoid degradation enzymes constitute interesting targets, and many inhibitors of these enzymes have been developed. However, the endocannabinoid system also exerts some unwanted effects. As an example, cannabis use is associated with psychotropic effects that are reproduced following a chronic increase of 2-AG levels in animals (Long, Li, Booker, Burston, & Kinsey, 2009a; Schlosburg, Blankman, Long, Nomura, & Pan, 2010). Moreover, cannabis use is associated with impaired memory, and this is also observed with administration of cannabinoid receptor agonists to mice. Finally, endocannabinoids have been shown to exert deleterious effects in some settings, such as obesity. Indeed, AEA and 2-AG are orexigenic and adipogenic agents (Bisogno, Mahadevan, Coccurello, Chang, & Allara, 2013; Muccioli, Naslain, Backhed, Reigstad, & Lambert, 2010). In this context, inhibitors of endocannabinoid biosynthesis seem to be interesting therapeutic tools.

CLASSICAL METABOLISM OF ANANDAMIDE AND 2-ARACHIDONOYLGLYCEROL

Although both 2-AG and AEA are arachidonic acid derivatives synthetized from membrane phospholipids, their biosynthetic and degradation routes are quite different, with diverse enzymes implicated.

The most described biosynthetic route for 2-AG (Fig. 64.1A) is through the sequential actions of an activity-dependent phospholipase C-β (releasing diacylglycerols from phosphatidylinositols-4,5-bisphosphate) and diacylglycerol lipases (DAGLα and DAGLβ) to produce 2-AG. However, DAGLs do not constitute the only biosynthesis pathway for 2-AG, as this bioactive lipid is synthetized in DAGL-knockout mice (Aaltonen, Riera,

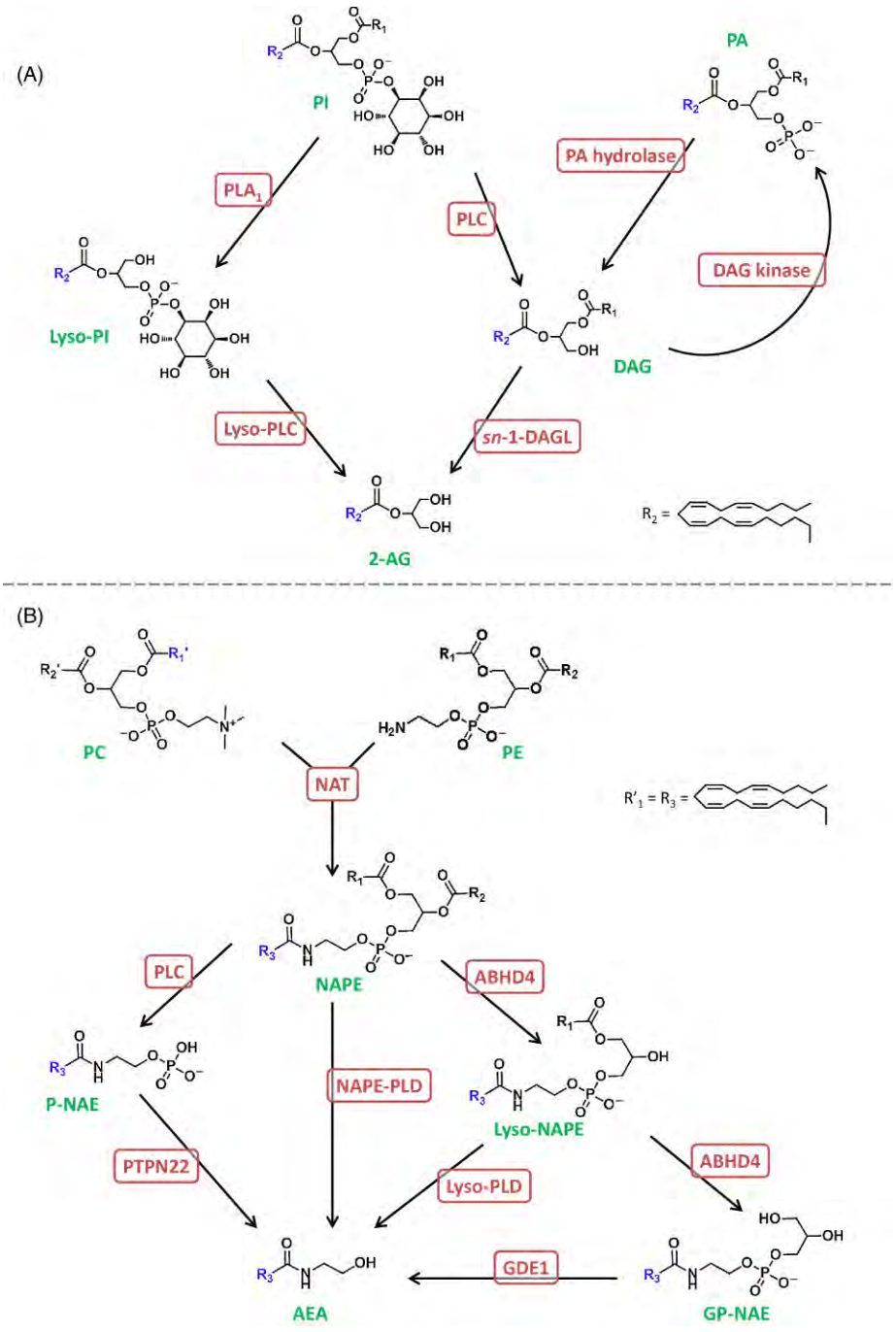


FIGURE 64.1 Biosynthesis of the endocannabinoids 2-AG and AEA. (A) 2-AG is produced through the sequential action of either a phospholipase C (PLC) and diacylglycerol lipase (sn-1-DAGL), or a phospholipase A (PLA1) and a lysophospholipase C (LysoPLC). Diacylglycerol (DAG) levels are also regulated by a phosphatidic acid hydrolase (PA hydrolase) and a diacylglycerol kinase (DAG kinase); (B) *N*-acylphosphatidylethanolamine (NAPE), the key intermediate in the synthesis of AEA, is synthesized from phosphatidylcholine (PC) and phosphatidylethanolamine (PE) by a calcium-dependent *N*-acyltransferase activity (NAT). Several pathways are responsible for the production of AEA from NAPE: (1) directly via a *N*-acylphosphatidylethanolamine preferring phospholipase D (NAPE-PLD); (2) or through a phosphate intermediate (P-NAE) generated by a phospholipase C (PLC) and hydrolyzed by the phosphatase PTPN22; (3) or through a lyso-*N*-acylphosphatidylethanolamine (lyso-NAPE), produced by ABHD4, and which in turn can be hydrolyzed into AEA by a lyso-phospholipase D (lyso-PLD), or converted into the glycerophosphate intermediate (GP-NAE) before its conversion into AEA by glycerophosphodiester phosphodiesterase 1 (GDE1).

Lehtonen, Savinainen, & Laitinen, 2014). 2-AG is then inactivated through its hydrolysis by several lipases into arachidonic acid and glycerol (Fig. 64.2). Monoacylglycerol lipase (MAGL) was the first enzyme described as hydrolyzing 2-AG. This enzyme plays a prevalent role in the brain, where it controls around 80% of 2-AG hydrolysis (Blankman, Simon, & Cravatt, 2007). Two additional α/β-hydrolases—α/β-hydrolase domain 6 (ABHD6) and 12 (ABHD12)—were also shown to hydrolyze 2-AG (Marrs, Blankman, Horne, Thomazeau, & Lin, 2010; Navia-Paldanius, Savinainen, & Laitinen, 2012). These three enzymes exhibit different subcellular localizations, which led to the hypothesis that they have access to different pools of 2-AG (Savinainen, Saario, & Laitinen, 2012). However, contrary to MAGL which only hydrolyzes monoacylglycerols, ABHD6 and ABHD12 also hydrolyze other bioactive lipids, such as lysoglycerophospholipids (Thomas, Betters, Lord, Brown, & Marshall, 2013; Blankman, Long, Trauger, Siuzdak, & Cravatt, 2013).

The primary biosynthetic route for AEA (Fig. 64.1B) is through the sequential actions of a Ca^{2+}-dependent *N*-acyltransferase (to generate *N*-acylphosphatidylethanolamines or NAPEs), and a NAPE-preferring phospholipase D (NAPE-PLD) that leads to the production of *N*-acylethanolamines (NAEs), including AEA (Muccioli, 2010). While NAPE-PLD was first described as responsible for the transition from NAPEs to NAEs, other less direct routes have been described over the years (Fig. 64.1B). AEA is hydrolyzed by two enzymes (Fig. 64.3), the fatty acid amide hydrolase (FAAH) and the more recently described *N*-acylethanolamine-hydrolyzing acid amidase (NAAA), which differ in particular by their catalytic properties and substrate specificity (Ueda, Tsuboi, & Uyama, 2010). FAAH is a serine hydrolase active at neutral and alkaline pH, and has the highest reactivity against AEA, while NAAA is a cysteine amidase active at acidic pH, inactive at alkaline pH, and is more efficient at hydrolyzing another NAE, *N*-palmitoylethanolamine (Ueda et al., 2010; Muccioli, 2010). However, recent studies point to a potential differential role for these enzymes in the regulation of NAE levels, depending on the tissue, or even the condition (Alhouayek & Muccioli, 2014b).

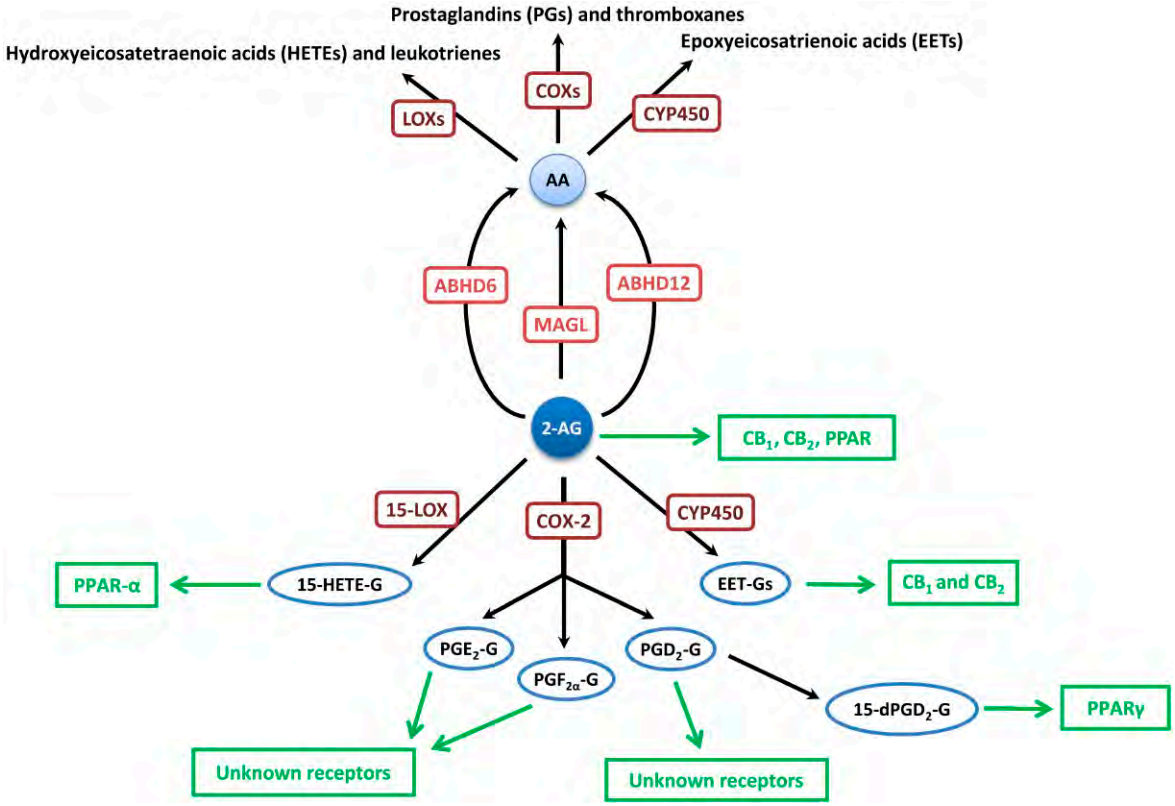


FIGURE 64.2 2-AG is at the center of a bioactive lipid network. 2-AG can be either hydrolyzed, by MAGL, ABHD6, or ABHD12, into arachidonic acid (AA) or oxidized, by 15-lipoxygenase (15-LOX), cyclooxygenase-2 (COX-2), or cytochrome P450 (CYP450) enzymes, to generate 2-AG-derived bioactive lipids. Thus, 15-LOX will generate 15-hydroxyeicosatetraenoic acid-glycerol ester (15-HETE-G), CYP450 will generate epoxyeicosatrienoic acid-glycerol esters (EET-Gs), and COX-2 will produce several prostaglandin-glycerol esters (PG-Gs), depending on the prostaglandin synthase expressed. The AA produced upon 2-AG hydrolysis can also be metabolized through the same pathways. The molecular targets identified so far for these bioactive lipids are presented in *green*.

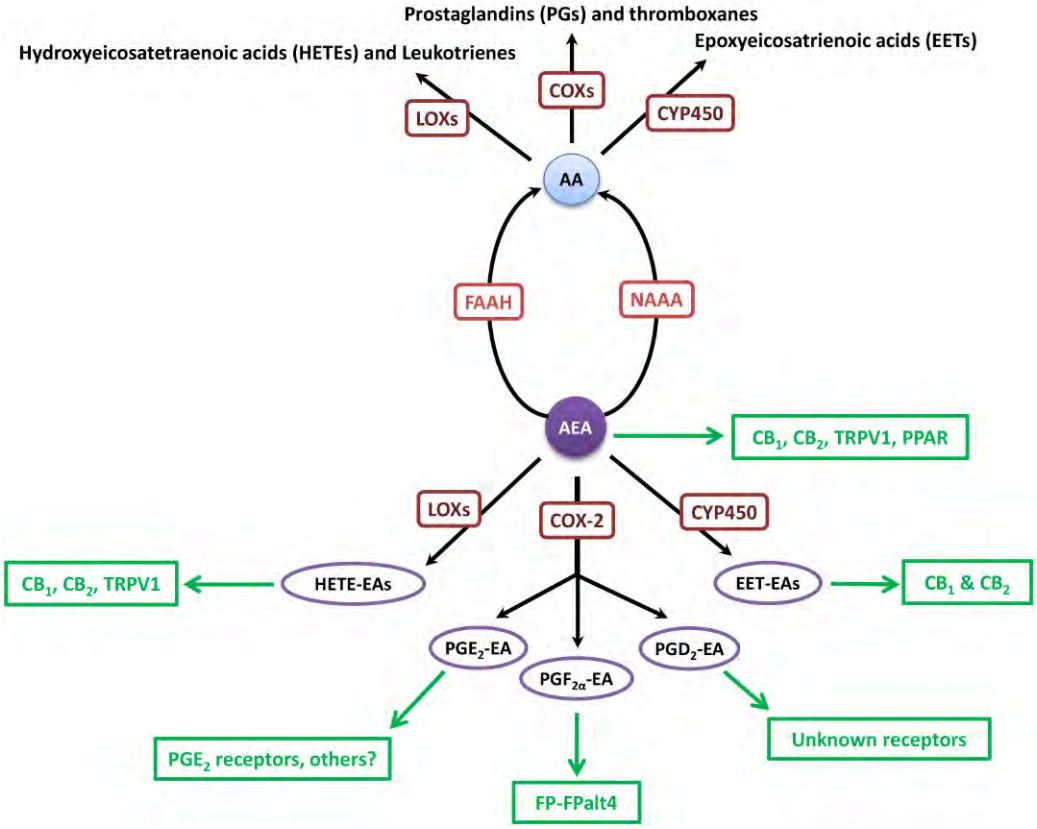


FIGURE 64.3 **AEA is at the center of a bioactive lipid network.** AEA can be either hydrolyzed, by FAAH or NAAA, into arachidonic acid (AA) or oxidized, by lipoxygenases (LOXs), cyclooxygenase-2 (COX-2), or cytochromes P450 (CYP450) enzymes, to generate AEA-derived bioactive lipids. Thus, LOXs will generate hydroxyeicosatetraenoic acid-ethanolamines (HETE-EAs), CYP450 will generate epoxyeicosatrienoic acid-ethanolamide (EET-EAs), and COX-2 will produce several prostaglandin-ethanolamines or prostamides (PG-EAs), depending on the prostaglandin synthase expressed. The AA produced upon AEA hydrolysis can also be metabolized through the same pathways. The molecular targets identified so far for these bioactive lipids are presented in *green*.

ANANDAMIDE AND 2-ARACHIDONOYLGLCEROL AT THE CENTER OF A NETWORK OF BIOACTIVE LIPIDS

The role of MAGL in hydrolyzing 2-AG in the brain was reinforced with the studies by Nomura, Morrison, Blankman, Long, and Kinsey (2011), showing that MAGL inhibition in the brain leads to a drastic increase in 2-AG levels, but also to a reduction in arachidonic acid levels. This study also demonstrated that inhibiting MAGL affects bioactive lipids levels at a larger scale than endocannabinoid hydrolysis, by reducing the levels of arachidonic acid, a major bioactive lipid implicated in inflammation (Nomura et al., 2011). But arachidonic acid is not the only connection between the endocannabinoid and eicosanoid systems. Seminal studies by the Marnett lab put forth the ability of COX-2 to metabolize the endocannabinoids, similarly to arachidonic acid. This is due to the arachidonoyl moiety shared by 2-AG and AEA, and to the ability of COX-2 to accommodate not only free fatty acids, but also their amide and ester derivatives (Rouzer & Marnett, 2011). This COX-2 mediated metabolism of the endocannabinoids will lead to ester (prostaglandin-glycerol esters or PG-Gs for 2-AG) and amide (prostaglandin-ethanolamides, also known as prostamides or PG-EAs for AEA) derivatives of the prostaglandins. These PG-Gs and PG-EAs are bioactive lipids in their own right that exert biological actions different from those of endocannabinoids and prostaglandins (Alhouayek & Muccioli, 2014a).

$PGF_{2\alpha}$-EA is probably the most studied of these metabolites. It has been used, along with its more stable analogs such as bimatoprost, for the treatment of high intraocular pressure (Woodward, Wang, & Poloso, 2013). More recently, $PGF_{2\alpha}$-EA was also shown to negatively regulate adipogenesis (Silvestri, Martella, Poloso, Piscitelli, & Capasso, 2013), and induce hyperalgesic effects in mice (Gatta, Piscitelli, Giordano, Boccella, & Lichtman, 2012). AEA, on the other hand, has analgesic effects, and stimulates adipogenesis (Muccioli et al., 2010; Guindon & Hohmann, 2009). This example is a perfect illustration of

the differential effects exerted by endocannabinoids and their COX-2-derived metabolites.

Adding to the complexity of this system is the fact that all PG-Gs and PG-EAs do not exert the same effects (Alhouayek & Muccioli, 2014a). PGE_2-EA and PGD_2-G exhibit antiinflammatory effects (Alhouayek, Masquelier, Cani, Lambert, & Muccioli, 2013; Brown, Davidson, & Rotondo, 2013), while PGE_2-G and $PGF_{2\alpha}$-G exert proinflammatory and hyperalgesic effects (Hu, Bradshaw, Chen, Tan, & Walker, 2008; Valdeolivas, Pazos, Bisogno, Piscitelli, & Iannotti, 2013; Alhouayek et al., 2013; Ligresti, Martos, Wang, Guida, & Allara, 2014). So, while 2-AG exerts antiinflammatory effects, two of its COX-2-derived metabolites are proinflammatory, and one is antiinflammatory. Therefore, it becomes important to determine under which conditions these PG-Gs are formed, and in which tissues, in order to determine if 2-AG levels should be increased or decreased, and which enzyme(s) to target.

These few examples highlight the intricacy of endocannabinoid metabolism, and the importance of using the right tools, at the right moment, in the right conditions. And this is only the tip of the iceberg. Other enzymes of the eicosanoid system, such as cytochromes P450 (CYP450) and lipoxygenases (LOX), are also able to metabolize the endocannabinoids, leading to glycerol esters and ethanolamide derivatives of epoxyeicosatrienoic acid (EETs) and hydroxyeicosatetraenoic acid (HETEs), respectively (Rouzer & Marnett, 2011; Alhouayek & Muccioli, 2014a).

As the number of endocannabinoid-hydrolyzing enzymes increases, it becomes very important, from a drug discovery standpoint, to understand where and under which circumstances each enzyme is responsible for controlling 2-AG or AEA levels. This entails the availability of potent and selective inhibitors for these enzymes, in order to dissect their contributions.

RECEPTORS AND TARGETS OF ANANDAMIDE, 2-ARACHIDONOYLGLYCEROL, AND THEIR DERIVATIVES

Classically, endocannabinoids exert their actions by activating two G protein-coupled receptors, the cannabinoid receptors 1 and 2 (CB_1 and CB_2). However, over the years, several other targets have been identified. These include the peroxisome proliferator activated receptors (PPARs) for both AEA (PPARα and PPARγ) and 2-AG (PPARγ), and the transient potential vanilloid receptor TRPV1 for AEA (Pertwee, Howlett, Abood, Alexander, & Di Marzo, 2010).

The complexity of endocannabinoid targets and biological effects is increased, when considering the oxidative derivatives of these compounds through enzymes such as COX-2, LOX, and CYP450s. Indeed, some of these derivatives, such as LOX-derived metabolites of AEA, are also agonists of the cannabinoid receptors or the TRPV1 receptor (Fig. 64.3) (Rouzer & Marnett, 2011; Alhouayek & Muccioli, 2014a). Similarly, CYP450-derived metabolites of AEA and 2-AG exhibit high affinity at cannabinoid receptors, while a 15-LOX derived metabolite of 2-AG is a PPARα agonist (Figs. 64.2 and 64.3) (Rouzer & Marnett, 2011; Alhouayek & Muccioli, 2014a). Moreover, the receptors mediating the effects of the endocannabinoid-derived prostaglandins remain elusive for the most part. $PGF_{2\alpha}$-EA was shown to act through a heterodimer of the $PGF_{2\alpha}$ receptor, and one of its splice variants (FP-FPalt4) (Woodward et al., 2013). PGE_2-EA can bind to all four of the PGE_2 receptors, but it is not clear yet whether they mediate its effects (Ross, Craib, Stevenson, Pertwee, & Henderson, 2002). Receptors for the other PG-Gs and PG-EAs have not been described yet, although 15-deoxy-$\Delta^{12,14}$-PGJ_2-G, a metabolite of PGD_2-G, was shown to be a PPARγ agonist (Raman, Kaplan, Thompson, Vanden Heuvel, & Kaminski, 2011).

These numerous targets of the endocannabinoids and their metabolites also add to the complexity of the endocannabinoid system. As interfering with endocannabinoid levels also affects a network of related bioactive lipids, it can be expected that, in some situations, the effects of administering cannabinoid receptor agonists will not have the same effects as increasing the levels of endocannabinoids.

TOOLS AND CONSEQUENCES OF ENDOCANNABINOID LEVELS MODULATION

Interfering with endocannabinoid metabolism offers an array of potential therapeutic applications in the fields of pain, inflammation, anxiety, and obesity, to cite a few. Inhibitors are available to interfere with endocannabinoid biosynthesis or degradation. However, similarly to FAAH which hydrolyzes AEA, the enzymes implicated in the metabolism of 2-AG—that is, DAGL, MAGL, ABHD6, and ABHD12—are all serine hydrolases. This made the identification of selective inhibitors of these enzymes more challenging. Considerable effort is being done to obtain more selective inhibitors. The most studied of these inhibitors, and the latest developments, are summarized in Tables 64.1 and 64.2 and Fig. 64.4. In this section, we will try to highlight the benefits and drawbacks of inhibiting the different enzymes responsible for the metabolism of endocannabinoids.

Inhibitors of the Metabolism of 2-AG

Inhibitors for several enzymes involved in the metabolism of 2-AG have been synthetized and characterized, such as inhibitors for DAGLs, MAGL, and ABHD6.

TABLE 64.1 Selected Modulators of 2-AG Metabolism

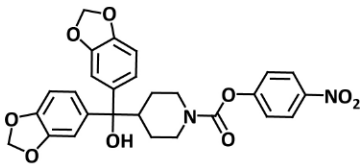
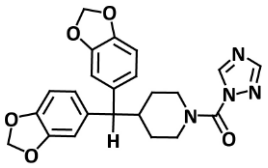
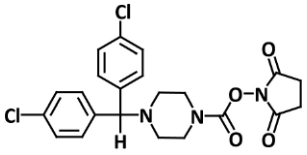
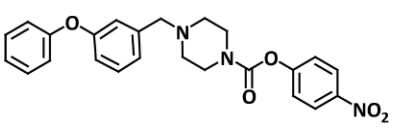
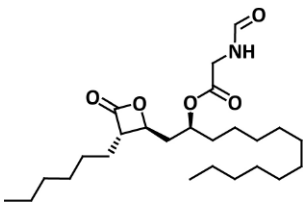
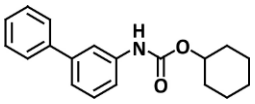
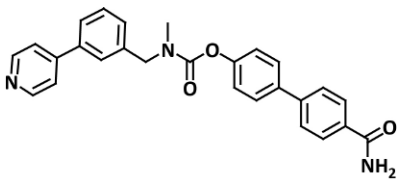
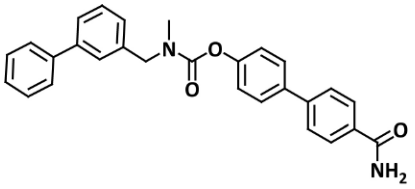
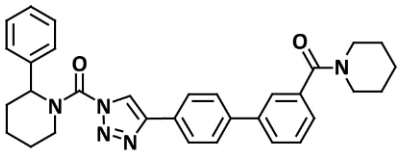
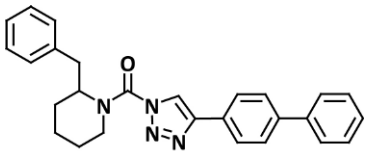
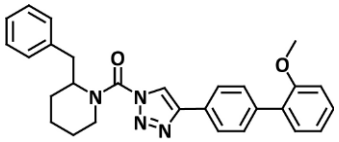
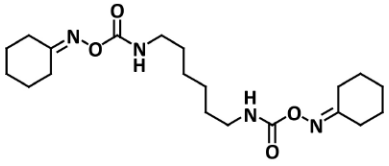
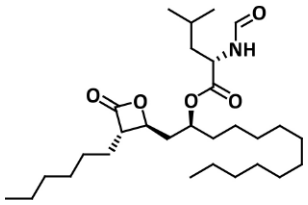
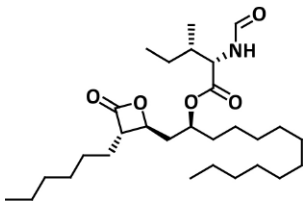
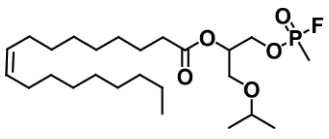
	Inhibitor	Structure	MAGL (IC_{50})	ABHD6 (IC_{50})	Other serine hydrolases	DAGLα (IC_{50})	DAGLβ (IC_{50})
MAGL	JZL184		8 nM mouse 262 nM rat 4 nM human	3.3 μM	FAAH: 4 μM ABHD12: no significant inhibition	—	—
	JJKK-048		0.3 nM mouse 0.2 nM rat 0.4 nM human	229 nM	FAAH: 2 μM ABHD12: no significant inhibition	—	—
	MJN110		9.5 nM mouse 9 nM human	260 nM	FAAH: no significant inhibition	—	—
	JZL195		4 nM	50 nM	FAAH: 2 nM	—	—
	OMDM169		1.5 μM mouse 0.9 μM human	—	FAAH: 3 μM	2.8 μM	—
	URB602		30–200 μM	—	FAAH: 17 μM	—	—
ABHD6	WWL70		No inhibition up to 1–10 μM	70–85 nM	FAAH: no inhibition at 10 μM; ABHD12: no inhibition at 1 μM	—	—
	WWL123		No significant inhibition	0.43 μM	FAAH: no significant inhibition; ABHD12: no significant inhibition	—	—

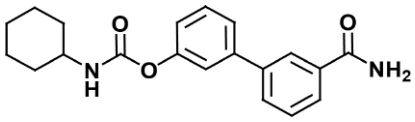
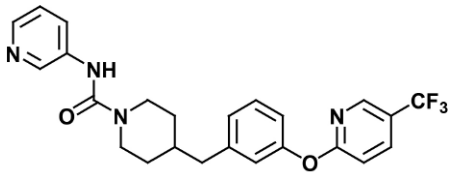
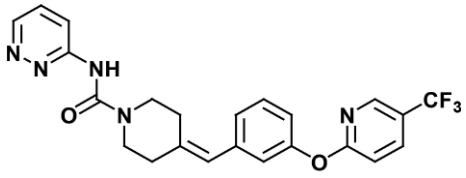
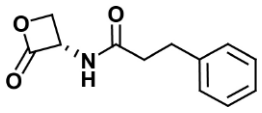
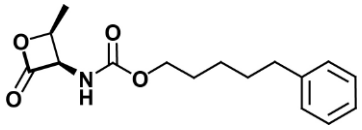
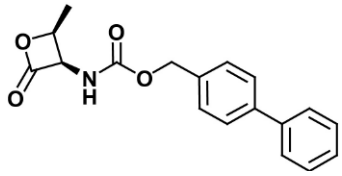
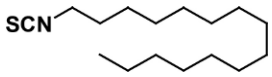
TABLE 64.1 Selected Modulators of 2-AG Metabolism (*cont.*)

	Inhibitor	Structure	MAGL (IC_{50})	ABHD6 (IC_{50})	Other serine hydrolases	DAGLα (IC_{50})	DAGLβ (IC_{50})
	KT185		No significant inhibition	1.3 nM	FAAH: no significant inhibition; ABHD12: no significant inhibition	—	No significant inhibition at 1 μM
DAGLβ	KT109		No significant inhibition	16 nM	FAAH: no significant inhibition; ABHD12: no significant inhibition	2300 nM	82 nM
	KT172		5 μM	5 nM	FAAH: no significant inhibition; ABHD12: no significant inhibition	140 nM	71 nM
DAGLα	RHC80267		No significant inhibition	No significant inhibition	FAAH: 70 μM; ABHD12: no significant inhibition	30–50 μM[a]	
	THL		No significant inhibition	No significant inhibition	FAAH: no significant inhibition; ABHD12: 80 nM	60 nM	100 nM
	OMDM-188		No significant inhibition	—	FAAH: no significant inhibition	16 nM	—
	O-7460		Partial inhibition at 10 μM	Partial inhibition at 10 μM	FAAH: no significant inhibition	690 nM	—

[a] *Not an IC_{50} value, concentrations typically used to inhibit DAGLs activities.*

The structures and pharmacological properties of several tools useful to modulate 2-AG hydrolysis and biosynthesis are summarized here.

TABLE 64.2 Selected Modulators of AEA Metabolism

	Inhibitor	Structure	FAAH (IC_{50})	NAAA (IC_{50})	MAGL (IC_{50})
FAAH	URB597		IC_{50} = 5 nM K_{inact}/K_i = 1590 $M^{-1}.s^{-1}$[a]	No significant inhibition	No significant inhibition
	PF 3845		K_{inact}/K_i = 12600 $M^{-1}.s^{-1}$	—	No significant inhibition
	PF 04457845		IC_{50} = 7–50 nM[b] K_{inact}/K_i = 40300 $M^{-1}.s^{-1}$	—	No significant inhibition
NAAA	(S)-OOPP		No inhibition	IC_{50} = 420 nM	No significant inhibition
	ARN077 (URB913)		No inhibition	IC_{50} = 50–130 nM[b]	—
	14q		No inhibition	IC_{50} = 7 nM	—
	AM9023		No inhibition	IC_{50} = 600 nM	No significant inhibition

[a] K_{inact}/K_i *values are the more accurate way to characterize irreversible inhibitors potency.*
[b] *Depending on preincubation time.*
The structures and pharmacological properties of several tools useful to modulate AEA hydrolysis are summarized here.

DAGL inhibition has been proposed as a promising anti-obesity strategy, as reducing 2-AG levels is expected to reduce food intake and adipogenesis (Bisogno et al., 2013). However, as 2-AG is at the center of a network of bioactive lipids, the levels of other lipids can also be affected. DAGLβ inhibition in peritoneal macrophages, for instance, leads to a decrease in 2-AG and arachidonic acid levels (Hsu, Tsuboi, Adibekian, Pugh, & Masuda, 2012). As these two COX-2 substrates are decreased, it is also expected that the levels of prostaglandins and PG-Gs will also be reduced. The reduction of arachidonic acid levels is interesting during inflammation, or in the context of macrophage activation, where a decrease in prostaglandin levels is desired. It remains to be seen whether this decrease in arachidonic acid and prostaglandins will have the same gastric and cardiovascular side effects as nonsteroidal antiinflammatory drugs (NSAIDs). However, as discussed further, several elements point to a

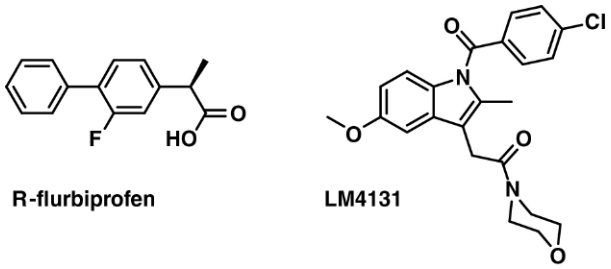


FIGURE 64.4 **Representative substrate-selective COX-2 inhibitors.** R-flurbiprofen and LM4131 preferentially inhibit the oxidative metabolism of endocannabinoids by COX-2, compared to the metabolism of arachidonic acid.

differential regulation of arachidonic acid levels by 2-AG metabolism, or the classical phospholipase A2 (cPLA2) pathway, depending on the tissues.

Indeed, studies with *MAGL inhibitors* led to a drastic increase in 2-AG levels in all the tissues assessed (Long, Nomura, & Cravatt, 2009b; Nomura et al., 2011). However, this was accompanied by a decrease in arachidonic acid levels in some tissues (eg, brain, liver, and lung) but not in others (eg, the gut) (Nomura et al., 2011). Accordingly, MAGL inhibition did not induce gastric ulcers (Nomura et al., 2011), and actually protects against NSAID-induced gastric ulcers, through a CB_1-dependent mechanism (Kinsey, Nomura, O'Neal, Long, & Mahadevan, 2011b). MAGL inhibition was therefore proposed as a potent antiinflammatory and neuroprotective strategy, devoid of the classical side effects of NSAIDs. Accordingly, MAGL inhibition was shown to exert beneficial effects in many models of inflammatory diseases, such as IBD and Parkinson disease in mice (Nomura et al., 2011; Alhouayek, Lambert, Delzenne, Cani, & Muccioli, 2011).

However, things are not so simple, and while MAGL inhibition increased the levels of 2-AG in a murine model of Parkinson disease, and decreased proinflammatory prostaglandin levels, leading to an overall protective effect, this was not the case in a murine model of Huntington's disease (Valdeolivas et al., 2013). In this latter case, MAGL inhibition had a neurotoxic effect. This was blocked by COX-2 inhibition and replicated by PGE_2-G administration, suggesting that the neurotoxic effect is the result of a COX-2 and prostaglandin E synthase metabolism of 2-AG. Accordingly, DAGL inhibition reduced 2-AG levels, and led to a neuroprotective effect, probably due to the decrease in PGE_2-G (Valdeolivas et al., 2013). It is therefore crucial to further understand the pathways mediating the effects of 2-AG in different pathologies.

MAGL inhibition, although very efficient at increasing 2-AG levels and reducing nausea, depression, anxiety, pain, and inflammation in some settings, comes with two major drawbacks: psychotropic side effects due to the drastic increase in 2-AG levels in the brain, as well as functional antagonism of cannabinoid receptors (Long et al., 2009a; Schlosburg et al., 2010). Two strategies can be proposed to circumvent these side effects: the development of peripherally-restricted MAGL inhibitors, and the inhibition of another 2-AG hydrolase, such as ABHD6, to increase peripheral 2-AG levels. Indeed, ABHD6 was shown to control less than 5% of 2-AG hydrolysis in mouse brain (Blankman et al., 2007) and ABHD6 inhibitors, while increasing 2-AG levels in various tissues did not affect 2-AG levels in the brain (Alhouayek et al., 2013). Also, as ABHD6 controls only a fraction of the 2-AG pool, its inhibition does not affect arachidonic acid levels (Alhouayek et al., 2013). Therefore, ABHD6 was proposed as a means for fine-tuning 2-AG levels, and thus avoiding the side effects due to the drastic increase of 2-AG levels. However, both these strategies lead to a loss of some of the beneficial central effects of 2-AG, such as the reduction of nausea, anxiety, and depression. Perhaps one way to deal with that is to increase the levels of the other endocannabinoid, AEA. Indeed, FAAH inhibition is associated with antiemetic and antidepressive effects, without all the side effects observed following MAGL inhibition (Long, Nomura, Vann, Walentiny, & Booker, 2009c).

ABHD6 inhibition increased 2-AG levels in the liver and lung of mice with lipopolysaccharide (LPS)-induced inflammation, and reduced inflammatory parameters (Alhouayek et al., 2013). This effect was partly dependent on cannabinoid receptors activation, and partly on COX-2 metabolism of 2-AG. Indeed, in macrophages activated with LPS, ABHD6 inhibition led to a decrease of several parameters of macrophage activation, and this effect was due to the production of PGD_2-G. Moreover, PGD_2-G was shown to exert antiinflammatory effects in LPS-induced inflammation in mice (Alhouayek et al., 2013). So, in this case, we have a COX-2-derived metabolite of 2-AG with antiinflammatory properties, in opposition with PGE_2-G that was formed in the brain, following MAGL inhibition. It remains to be seen whether this difference in the production of PG-Gs following the inhibition of two different 2-AG hydrolases is due to a different subcellular localization of the prostaglandin synthases, leading to a differential coupling with one enzyme or the other, or to a different regulation of prostaglandin synthases, in different tissues and different disease settings.

Recently, ABHD6 was shown to be more than a monoacylglycerol hydrolase, as it is able to hydrolyze lysoglycerophospholipids (Thomas et al., 2013). In this context, knockdown of ABHD6 in the liver and adipose tissue exerted beneficial effects on high fat diet-induced obesity and hepatic steatosis, probably due to the increase in the levels of other substrates than 2-AG (Thomas et al., 2013).

Identification of selective *inhibitors of ABHD12* proved more difficult. The first identified inhibitor of ABHD12

was tetrahydrolipstatin, which also inhibited DAGL (Hoover, Blankman, Niessen, & Cravatt, 2008). This made it difficult to identify the contribution of ABHD12 to the metabolism of 2-AG. Perhaps the more recently described triterpenoid derivatives, having no effect on DAGL, will allow for a more thorough understanding of this role of ABHD12 (Parkkari, Haavikko, Laitinen, Navia-Paldanius, & Rytilahti, 2014). However, studies with ABHD12 knockout mice point to a role for this enzyme in controlling the levels of lysophosphatidylserines, rather than 2-AG (Blankman et al., 2013).

Inhibitors of the Metabolism of AEA

Currently, there are no known inhibitors of *AEA biosynthesis*. Redundancy in the biosynthetic pathways of NAEs might be a reason for this lack of inhibitors, as several enzymes can make the transition from NAPEs to NAEs (Fig. 64.1B). In this context, it is interesting to note that some NAPE-PLD knockout mice exhibit altered NAE levels, while another NAPE-PLD knockout strain does not exhibit alterations in AEA levels (Leung, Saghatelian, Simon, & Cravatt, 2006; Tsuboi, Okamoto, Ikematsu, Inoue, & Shimizu, 2011). The same goes for GDE1 knockout mice in the brain (Simon & Cravatt, 2010).

In the context of interfering with AEA hydrolysis, the most developed and characterized inhibitors are *FAAH inhibitors* (Table 64.2). FAAH and NAAA profoundly differ in their catalytic mechanisms (Ueda et al., 2010) which allowed for the development of selective inhibitors of FAAH, devoid of activity at NAAA, and vice versa. FAAH inhibitors were shown to increase AEA levels in vivo, in physiological and pathological settings. These can be used for the treatment of pain and inflammatory diseases such as arthritis, as well as more central effects of the endocannabinoids, such as nausea and vomiting and mood disorders (Ahn, Johnson, Mileni, Beidler, & Long, 2009; Kinsey, Naidu, Cravatt, Dudley, & Lichtman, 2011a; Bluett, Gamble-George, Hermanson, Hartley, & Marnett, 2014). FAAH inhibition, and the subsequent increase in AEA levels in the central nervous system, was not associated with the same psychotropic effects observed with MAGL inhibition (Long et al., 2009c). As increasing AEA levels can be accompanied by a subsequent increase in prostamide levels in inflammatory settings, novel FAAH inhibitors that can double as antagonists of the $PGF_{2\alpha}$-EA receptor (AGN 211335) are being considered for the treatment of inflammatory pain (Ligresti et al., 2014). Indeed, as we mentioned earlier, AEA has analgesic and antiinflammatory properties, while $PGF_{2\alpha}$-EA is hyperalgesic and proinflammatory. However, antagonism of the effects of $PGF_{2\alpha}$-EA is not the goal in every situation, as this compound has proven very effective in the treatment of glaucoma (Woodward et al., 2013).

Regarding *NAAA inhibition*, much fewer inhibitors are available, probably due to the more recent molecular identification of NAAA, and the fact that less is known when it comes to the molecular mechanisms of cysteine hydrolases and their inhibitors, compared to serine hydrolases. Among the first described NAAA inhibitors, β-lactone derivatives are relatively potent, but exhibit limited plasma stability (Ponzano, Bertozzi, Mengatto, Dionisi, & Armirotti, 2013). These compounds were mostly tested in local administration schemes, where they increased *N*-palmitoylethanolamine levels, and exerted antiinflammatory and analgesic effects (Solorzano, Zhu, Battista, Astarita, & Lodola, 2009; Sasso, Moreno-Sanz, Martucci, Realini, & Dionisi, 2013). In these cases, the NAAA inhibitor did not affect AEA levels, or this was not reported (Solorzano et al., 2009; Sasso et al., 2013). Therefore, the impact of NAAA inhibition on AEA levels remains to be elucidated. As *N*-palmitoylethanolamine is a well-known antiinflammatory bioactive lipid (Alhouayek & Muccioli, 2014b), NAAA inhibitors are potential therapies in inflammatory diseases.

Dual Inhibitors of the Metabolism of 2-AG and AEA

Following the characterization of the MAGL inhibitor JZL184, another related carbamate inhibitor was developed as a dual FAAH/MAGL inhibitor, JZL195. While increasing the levels of both endocannabinoids seems promising to tackle numerous pathologies, the use of this inhibitor was accompanied by similar psychotropic side effects as those observed following cannabis use (Long et al., 2009c).

Another means to increase the levels of both AEA and 2-AG is *COX-2 inhibition*. Indeed, following the discovery that endocannabinoids can be oxidized by COX-2, it was hypothesized that some of the effects of NSAIDs were not only due to the decreased prostaglandin production, but also to increased endocannabinoid levels. This hypothesis was supported by several observations that COX-2 is able to control AEA levels in the brain, and COX inhibitors can increase endocannabinoid levels in murine models of inflammation (Telleria-Diaz, Schmidt, Kreusch, Neubert, & Schache, 2010). Moreover, some of the effects of NSAIDs can be blocked by CB_1 antagonists (Telleria-Diaz et al., 2010; Staniaszek, Norris, Kendall, Barrett, & Chapman, 2010). Thus, exploiting the endocannabinoid component of COX-2 inhibition might be a way to enhance the benefits of NSAIDs, and reduce their side effects. Indeed, endocannabinoids are analgesic and antiinflammatory compounds, therefore increasing their levels concomitantly with COX-2 inhibition has a synergistic effect, and would allow for a reduction of the doses of NSAIDs. In this context, compounds with

dual inhibitory activity at FAAH and COX are currently being evaluated (Cipriano, Bjorklund, Wilson, Congiu, & Onnis, 2013).

Another way to exploit the endocannabinoid component of COX-2 is the development of substrate-selective inhibitors of COX-2, that is, inhibitors of endocannabinoid metabolism by COX-2, without affecting the metabolism of arachidonic acid. R-flurbiprofen, the R-enantiomer of the NSAID S-flurbiprofen, was shown to increase endocannabinoid levels in vivo, and reduce nerve damage in a murine model of multiple sclerosis (Schmitz, de Bruin, Bishay, Mannich, & Haussler, 2014). Another COX-2 substrate-selective inhibitor, LM-4131, increased endocannabinoid levels, and reduced anxiety in mice (Hermanson, Hartley, Gamble-George, Brown, & Shonesy, 2013).

CONCLUDING REMARKS

The studies outlined in this review point to an ever-increasing complexity of the endocannabinoid system. While, in the early days of its exploration, the system looked relatively simple, it is nowadays clear that it is both complex and highly interconnected with the eicosanoid pathways. Also, what seemed to be two "redundant lipids," with AEA and 2-AG activating the same cannabinoid receptors, now appears as two distinct bioactive lipids characterized by numerous unique properties. While the complexity of the system might appear as a hurdle to its therapeutic exploitation, it is actually a unique opportunity to fine-tune the desired effect depending on the lipid that is modulated.

MINI-DICTIONARY

COX-2 substrate-selective inhibitors COX-2 inhibitors that selectively block endocannabinoid oxygenation by COX-2, without affecting the oxygenation of arachidonic acid. This is in opposition with other COX inhibitors, such as the NSAIDs which inhibit the oxygenation of arachidonic acid and endocannabinoids by COX-2.

Eicosanoids Biologically active lipid mediators, including prostaglandins, thromboxanes, leukotrienes, and other oxygenated derivatives, produced primarily by three classes of enzymes, cyclooxygenases, lipoxygenases, and cytochrome P450. The most studied of the fatty acids is arachidonic acid (C20:4), mostly found in esterified form, mainly to phospholipids and phosphatidylinositol, in particular. Arachidonate is released by the action of the enzyme phospholipase A2 (PLA2). 2-AG and MAGL have also been implicated in the release of arachidonic acid in some tissues and conditions.

Endocannabinoids Endogenous ligands of the two G-protein coupled receptors activated by Δ9-THC, one of the main constituents of cannabis. The two most studied endocannabinoids are the N-acylethanolamine N-arachidonoylethanolamine (AEA or anandamide), and the monoacylglycerol 2-arachidonoylglycerol (2-AG).

References

Aaltonen, N., Riera, R. C., Lehtonen, M., Savinainen, J. R., & Laitinen, J. T. (2014). Brain regional cannabinoid CB(1) receptor signalling and alternative enzymatic pathways for 2-arachidonoylglycerol generation in brain sections of diacylglycerol lipase deficient mice. *European Journal of Pharmaceutical Science*, *51*, 87–95.

Ahn, K., Johnson, D. S., Mileni, M., Beidler, D., Long, J. Z., et al. (2009). Discovery and characterization of a highly selective FAAH inhibitor that reduces inflammatory pain. *Chemical Biology*, *16*, 411–420.

Alhouayek, M., Lambert, D. M., Delzenne, N. M., Cani, P. D., & Muccioli, G. G. (2011). Increasing endogenous 2-arachidonoylglycerol levels counteracts colitis and related systemic inflammation. *FASEB Journal*, *25*, 2711–2721.

Alhouayek, M., Masquelier, J., Cani, P. D., Lambert, D. M., & Muccioli, G. G. (2013). Implication of the anti-inflammatory bioactive lipid prostaglandin D2-glycerol ester in the control of macrophage activation and inflammation by ABHD6. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 17558–17563.

Alhouayek, M., & Muccioli, G. G. (2012). The endocannabinoid system in inflammatory bowel diseases: from pathophysiology to therapeutic opportunity. *Trends in Molecular Medicine*, *18*, 615–625.

Alhouayek, M., & Muccioli, G. G. (2014a). COX-2-derived endocannabinoid metabolites as novel inflammatory mediators. *Trends in Pharmacological Sciences*, *35*, 284–292.

Alhouayek, M., & Muccioli, G. G. (2014b). Harnessing the anti-inflammatory potential of palmitoylethanolamide. *Drug Discovery Today*, *19*, 1632–1639.

Bisogno, T., Mahadevan, A., Coccurello, R., Chang, J. W., Allara, M., et al. (2013). A novel fluorophosphonate inhibitor of the biosynthesis of the endocannabinoid 2-arachidonoylglycerol with potential anti-obesity effects. *British Journal of Pharmacology*, *169*, 784–793.

Blankman, J. L., Long, J. Z., Trauger, S. A., Siuzdak, G., & Cravatt, B. F. (2013). ABHD12 controls brain lysophosphatidylserine pathways that are deregulated in a murine model of the neurodegenerative disease PHARC. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 1500–1505.

Blankman, J. L., Simon, G. M., & Cravatt, B. F. (2007). A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chemical Biology*, *14*, 1347–1356.

Bluett, R. J., Gamble-George, J. C., Hermanson, D. J., Hartley, N. D., Marnett, L. J., et al. (2014). Central anandamide deficiency predicts stress-induced anxiety: behavioral reversal through endocannabinoid augmentation. *Translational Psychiatry*, *4*, e408.

Borrelli, F., & Izzo, A. A. (2009). Role of acylethanolamides in the gastrointestinal tract with special reference to food intake and energy balance. *Best Practice and Research: Clinical Endocrinology and Metabolism*, *23*, 33–49.

Brown, K. L., Davidson, J., & Rotondo, D. (2013). Characterisation of the prostaglandin E2-ethanolamide suppression of tumour necrosis factor-alpha production in human monocytic cells. *Biochimica et Biophysica Acta*, *1831*, 1098–1107.

Cipriano, M., Bjorklund, E., Wilson, A. A., Congiu, C., Onnis, V., et al. (2013). Inhibition of fatty acid amide hydrolase and cyclooxygenase by the N-(3-methylpyridin-2-yl)amide derivatives of flurbiprofen and naproxen. *European Journal of Pharmacology*, *720*, 383–390.

Gatta, L., Piscitelli, F., Giordano, C., Boccella, S., Lichtman, A., et al. (2012). Discovery of prostamide F2alpha and its role in inflammatory pain and dorsal horn nociceptive neuron hyperexcitability. *PLoS One*, *7*, e31111.

Guindon, J., & Hohmann, A. G. (2009). The endocannabinoid system and pain. *CNS and Neurological Disorders - Drug Targets*, *8*, 403–421.

Hermanson, D. J., Hartley, N. D., Gamble-George, J., Brown, N., Shonesy, B. C., et al. (2013). Substrate-selective COX-2 inhibition decreases anxiety via endocannabinoid activation. *Nature Neuroscience*, *16*, 1291–1298.

Hoover, H. S., Blankman, J. L., Niessen, S., & Cravatt, B. F. (2008). Selectivity of inhibitors of endocannabinoid biosynthesis evaluated by activity-based protein profiling. *Bioorganic and Medicinal Chemistry Letters*, *18*, 5838–5841.

Hsu, K. L., Tsuboi, K., Adibekian, A., Pugh, H., Masuda, K., et al. (2012). DAGLbeta inhibition perturbs a lipid network involved in macrophage inflammatory responses. *Nature Chemical Biology*, *8*, 999–1007.

Hu, S. S., Bradshaw, H. B., Chen, J. S., Tan, B., & Walker, J. M. (2008). Prostaglandin E2 glycerol ester, an endogenous COX-2 metabolite of 2-arachidonoylglycerol, induces hyperalgesia and modulates NFkappaB activity. *British Journal of Pharmacology*, *153*, 1538–1549.

Kinsey, S. G., Naidu, P. S., Cravatt, B. F., Dudley, D. T., & Lichtman, A. H. (2011a). Fatty acid amide hydrolase blockade attenuates the development of collagen-induced arthritis and related thermal hyperalgesia in mice. *Pharmacology Biochemistry and Behavior*, *99*, 718–725.

Kinsey, S. G., Nomura, D. K., O'Neal, S. T., Long, J. Z., Mahadevan, A., et al. (2011b). Inhibition of monoacylglycerol lipase attenuates nonsteroidal anti-inflammatory drug-induced gastric hemorrhages in mice. *Journal of Pharmacologic and Experimental Therapeutics*, *338*, 795–802.

Leung, D., Saghatelian, A., Simon, G. M., & Cravatt, B. F. (2006). Inactivation of N-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids. *Biochemistry*, *45*, 4720–4726.

Ligresti, A., Martos, J., Wang, J., Guida, F., Allara, M., et al. (2014). Prostamide F(2) alpha receptor antagonism combined with inhibition of FAAH may block the pro-inflammatory mediators formed following selective FAAH inhibition. *British Journal of Pharmacology*, *171*, 1408–1419.

Long, J. Z., Li, W., Booker, L., Burston, J. J., Kinsey, S. G., et al. (2009a). Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. *Nature Chemical Biology*, *5*, 37–44.

Long, J. Z., Nomura, D. K., & Cravatt, B. F. (2009b). Characterization of monoacylglycerol lipase inhibition reveals differences in central and peripheral endocannabinoid metabolism. *Chemical Biology*, *16*, 744–753.

Long, J. Z., Nomura, D. K., Vann, R. E., Walentiny, D. M., Booker, L., et al. (2009c). Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 20270–20275.

Lourbopoulos, A., Grigoriadis, N., Lagoudaki, R., Touloumi, O., Polyzoidou, E., et al. (2011). Administration of 2-arachidonoylglycerol ameliorates both acute and chronic experimental autoimmune encephalomyelitis. *Brain Research*, *1390*, 126–141.

Marrs, W. R., Blankman, J. L., Horne, E. A., Thomazeau, A., Lin, Y. H., et al. (2010). The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. *Nature Neuroscience*, *13*, 951–957.

Mecs, L., Tuboly, G., Toth, K., Nagy, E., Nyari, T., et al. (2010). Peripheral antinociceptive effect of 2-arachidonoyl-glycerol and its interaction with endomorphin-1 in arthritic rat ankle joints. *Clinical and Experimental Pharmacology and Physiology*, *37*, 544–550.

Muccioli, G. G. (2010). Endocannabinoid biosynthesis and inactivation, from simple to complex. *Drug Discovery Today*, *15*, 474–483.

Muccioli, G. G., Naslain, D., Backhed, F., Reigstad, C. S., Lambert, D. M., et al. (2010). The endocannabinoid system links gut microbiota to adipogenesis. *Molecular Systems Biology*, *6*, 392.

Murillo-Rodriguez, E., Poot-Ake, A., Arias-Carrion, O., Pacheco-Pantoja, E., Fuente-Ortegon, A. L., et al. (2011). The emerging role of the endocannabinoid system in the sleep-wake cycle modulation. *Central Nervous System Agents in Medicinal Chemistry*, *11*, 189–196.

Navia-Paldanius, D., Savinainen, J. R., & Laitinen, J. T. (2012). Biochemical and pharmacological characterization of human alpha/beta-hydrolase domain containing 6 (ABHD6) and 12 (ABHD12). *Journal of Lipid Research*, *53*, 2413–2424.

Nomura, D. K., Morrison, B. E., Blankman, J. L., Long, J. Z., Kinsey, S. G., et al. (2011). Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. *Science*, *334*, 809–813.

Panikashvili, D., Shein, N. A., Mechoulam, R., Trembovler, V., Kohen, R., et al. (2006). The endocannabinoid 2-AG protects the blood-brain barrier after closed head injury and inhibits mRNA expression of proinflammatory cytokines. *Neurobiology of Disease*, *22*, 257–264.

Parkkari, T., Haavikko, R., Laitinen, T., Navia-Paldanius, D., Rytilahti, R., et al. (2014). Discovery of triterpenoids as reversible inhibitors of alpha/beta-hydrolase domain containing 12 (ABHD12). *PLoS One*, *9*, e98286.

Pertwee, R. G., Howlett, A. C., Abood, M. E., Alexander, S. P., Di Marzo, V., et al. (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB(1) and CB(2). *Pharmacological Reviews*, *62*, 588–631.

Ponzano, S., Bertozzi, F., Mengatto, L., Dionisi, M., Armirotti, A., et al. (2013). Synthesis and structure-activity relationship (SAR) of 2-methyl-4-oxo-3-oxetanylcarbamic acid esters, a class of potent N-acylethanolamine acid amidase (NAAA) inhibitors. *Journal of Medical Chemistry*, *56*, 6917–6934.

Raman, P., Kaplan, B. L., Thompson, J. T., Vanden Heuvel, J. P., & Kaminski, N. E. (2011). 15-Deoxy-delta12, 14-prostaglandin J2-glycerol ester, a putative metabolite of 2-arachidonyl glycerol, activates peroxisome proliferator activated receptor gamma. *Molecular Pharmacology*, *80*, 201–209.

Ross, R. A., Craib, S. J., Stevenson, L. A., Pertwee, R. G., Henderson, A., et al. (2002). Pharmacological characterization of the anandamide cyclooxygenase metabolite: prostaglandin E2 ethanolamide. *Journal of Pharmacological and Experimental Therapeutics*, *301*, 900–907.

Rouzer, C. A., & Marnett, L. J. (2011). Endocannabinoid oxygenation by cyclooxygenases, lipoxygenases, and cytochromes P450: cross-talk between the eicosanoid and endocannabinoid signaling pathways. *Chemical Reviews*, *111*, 5899–5921.

Ruehle, S., Rey, A. A., Remmers, F., & Lutz, B. (2012). The endocannabinoid system in anxiety, fear memory and habituation. *Journal of Psychopharmacology*, *26*, 23–39.

Sasso, O., Moreno-Sanz, G., Martucci, C., Realini, N., Dionisi, M., et al. (2013). Antinociceptive effects of the N-acylethanolamine acid amidase inhibitor ARN077 in rodent pain models. *Pain*, *154*, 350–360.

Savinainen, J. R., Saario, S. M., & Laitinen, J. T. (2012). The serine hydrolases MAGL, ABHD6 and ABHD12 as guardians of 2-arachidonoylglycerol signalling through cannabinoid receptors. *Acta Physiologica (Oxford)*, *204*, 267–276.

Schlosburg, J. E., Blankman, J. L., Long, J. Z., Nomura, D. K., Pan, B., et al. (2010). Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. *Nature Neuroscience*, *13*, 1113–1119.

Schmitz, K., de Bruin, N., Bishay, P., Mannich, J., Haussler, A., et al. (2014). R-flurbiprofen attenuates experimental autoimmune encephalomyelitis in mice. *EMBO Molecular Medicine*, *6*, 1398–1422.

Sharkey, K. A., Darmani, N. A., & Parker, L. A. (2014). Regulation of nausea and vomiting by cannabinoids and the endocannabinoid system. *European Journal of Pharmacology*, *722*, 134–146.

Silvestri, C., Martella, A., Poloso, N. J., Piscitelli, F., Capasso, R., et al. (2013). Anandamide-derived prostamide F2alpha negatively regulates adipogenesis. *Journal of Biological Chemistry*, *288*, 23307–23321.

Simon, G. M., & Cravatt, B. F. (2010). Characterization of mice lacking candidate N-acyl ethanolamine biosynthetic enzymes provides evidence for multiple pathways that contribute to endocannabinoid production in vivo. *Molecular Biosystems*, *6*, 1411–1418.

Solorzano, C., Zhu, C., Battista, N., Astarita, G., Lodola, A., et al. (2009). Selective N-acylethanolamine-hydrolyzing acid amidase inhibition reveals a key role for endogenous palmitoylethanolamide in inflammation. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 20966–20971.

Staniaszek, L. E., Norris, L. M., Kendall, D. A., Barrett, D. A., & Chapman, V. (2010). Effects of COX-2 inhibition on spinal nociception: the role of endocannabinoids. *British Journal of Pharmacology*, *160*, 669–676.

Telleria-Diaz, A., Schmidt, M., Kreusch, S., Neubert, A. K., Schache, F., et al. (2010). Spinal antinociceptive effects of cyclooxygenase inhibition during inflammation: involvement of prostaglandins and endocannabinoids. *Pain*, *148*, 26–35.

Thomas, G., Betters, J. L., Lord, C. C., Brown, A. L., Marshall, S., et al. (2013). The serine hydrolase ABHD6 Is a critical regulator of the metabolic syndrome. *Cell Reports*, *5*, 508–520.

Tsuboi, K., Okamoto, Y., Ikematsu, N., Inoue, M., Shimizu, Y., et al. (2011). Enzymatic formation of N-acylethanolamines from N-acylethanolamine plasmalogen through N-acylphosphatidylethanolamine-hydrolyzing phospholipase D-dependent and -independent pathways. *Biochimica et Biophysica Acta*, *1811*, 565–577.

Ueda, N., Tsuboi, K., & Uyama, T. (2010). N-acylethanolamine metabolism with special reference to N-acylethanolamine-hydrolyzing acid amidase (NAAA). *Progress in Lipid Research*, *49*, 299–315.

Valdeolivas, S., Pazos, M. R., Bisogno, T., Piscitelli, F., Iannotti, F. A., et al. (2013). The inhibition of 2-arachidonoyl-glycerol (2-AG) biosynthesis, rather than enhancing striatal damage, protects striatal neurons from malonate-induced death: a potential role of cyclooxygenase-2-dependent metabolism of 2-AG. *Cell Death and Disease*, *4*, e862.

Woodward, D. F., Wang, J. W., & Poloso, N. J. (2013). Recent progress in prostaglandin F2alpha ethanolamide (prostamide F2alpha) research and therapeutics. *Pharmacological Reviews*, *65*, 1135–1147.

CHAPTER

65

Pharmacological Aspects of NMDA Receptors, mGluR5, and Endocannabinoids

Y. Izumi, C.F. Zorumski

Department of Psychiatry; The Taylor Family Institute for Innovative Psychiatric Research, Washington University School of Medicine, St. Louis, MO, United States

SUMMARY POINTS

- Synaptic depression is often mediated by endocannabinoids (eCBs) serving as retrograde messengers at cannabinoid receptor 1 (CB1), and other cannabinoid receptors.
- eCB production and CB1 activation on inhibitory synaptic terminals are essential for DSI.
- Several forms of long-term depression (LTD) are mediated by eCBs serving as retrograde messengers.
- Binding of glutamate to postsynaptic metabotropic glutamate receptors (mGluRs), leading to eCB formation and release, results in activation of specific presynaptic eCB receptors, and induction of several forms of LTD.
- Whereas exogenously administered cannabinoids disrupt long-term potentiation (LTP), eCBs may paradoxically augment LTP induction.
- NMDA glutamate receptors (NMDAR) activation may have additional actions, including adverse effects on CB1 signaling.
- Some NMDAR effects may involve transient receptor potential vanilloid 1 (TRPV1) activation.

KEY FACTS ABOUT ENDOCANNABINOIDS AS RETROGRADE MESSENGERS

- At synapses, neurotransmitters are released from presynaptic sites to activate postsynaptic receptors.
- However, synaptic transmission is not limited to anterograde (forward) transmission.
- Retrograde messengers travel from postsynaptic sites to presynaptic sites and modulate neurotransmitter release.
- Endcannabinoids are known to serve as retrograde messengers.
- Activation of postsynaptic glutamate receptors triggers endocannabinoid release from postsynaptic sites.
- Endocannabinoids play pivotal roles in synaptic plasticity as retrograde messengers.

LIST OF ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
ACPD	Aminocyclopentane-1,3-dicarboxylic acid
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
AP5	2-Amino-5-phosphonovalerate
CB1	Cannabinoid receptor 1
CB2	Cannabinoid receptor 2
CHPG	2-Chloro-5-hydroxyphenylglycine
CNQX	6-Cyano-7-nitroquinoxaline-2,3-dione

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00075-2
Copyright © 2017 Elsevier Inc. All rights reserved.

CNS	Central nervous system
CPCCOEt	7-(Hydroxyimino)cyclopropa[*b*]chromen-1a-carboxylate ethyl ester
DAG	Diacylglycerol
DHPG	3,5-Dihydroxyphenylglycine
DSE	Depolarization induced suppression of excitation
DSI	Depolarization-induced suppression of inhibition
eCB	Endocannabinoid
EPSP	Excitatory post synaptic potential
FAAH	Fatty acid amide hydrolase
GPCR	G protein coupled receptor
KO	Knockout
LPI	L-alpha-lysophosphatidylinositol
I-LTD	Long-term depression of inhibitory postsynaptic currents
IPSC	Inhibitory postsynaptic current
IPSP	Inhibitory postsynaptic potential
LTD	Long-term depression
LTP	Long-term potentiation
MAGL	Monoacylglycereol lipase
MAPK	Mitogen-activated protein kinases
mGluR	Metabotropic glutamate receptors
MPEP	2-Methyl-6-phenylethynyl)pyridine
NADA	*N*-arachidonoyl dopamine
NBQX	2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzoquinoxaline-7-sulfonamide
NMDA	*N*-methyl-D-aspartate
NMDAR	NMDA glutamate receptors
PLC	Phospholipase C
PND	Postnatal date
SLM	Stratum lacunosum moleculare
THC	Δ^9-Tetrahydrocannabinol
TRPV1	Transient receptor potential vanilloid 1

INTRODUCTION

In this chapter, we review interactions between metabotropic and NMDA glutamate receptors (NMDARs) and the endocannabinoid (eCB) system. We also discuss neuropharmacological aspects of eCBs in the central nervous system (CNS), and the roles of metabotropic glutamate receptors (mGluRs), NMDARs, and eCBs in synaptic plasticity.

OVERVIEW OF GLUTAMATE RECEPTORS AND THE CNS eCB SYSTEM

Glutamate Receptors

Glutamate receptors are divided into two major groups: ionotropic receptors, and mGluRs. Ionotropic receptors are further classified according to specific agonists: AMPA, kainate, and NMDA. AMPA/kainate receptors participate in basal synaptic transmission, whereas NMDARs trigger intracellular events via calcium influx. Calcium, in turn, promotes synaptic plasticity [including long-term potentiation (LTP) and long-term depression (LTD)], but can also trigger excitotoxic neuronal death. mGluRs were previously called quisqualate receptors or ACPD receptors; quisqualate is a potent group I mGluR agonist, with additional actions at AMPA/kainate receptors, whereas aminocyclopentane-1,3-dicarboxylic acid (ACPD) is a group I and group II mGluR agonist. Group I mGluRs include mGluR1 and mGluR5, and activate phospholipase C (PLC) to form inositol 1,4,5-triphosphate and diacylglycerol (DAG). DAG is a precursor of 2-arachidonoylglycerol (2-AG), a major eCB. 3,5-Dihydroxyphenylglycine (DHPG) is an agonist for mGluR1 and mGluR5, whereas 2-chloro-5-hydroxyphenylglycine **(**CHPG) is a selective mGluR5 agonist. In the CA1 region of rat hippocampal slices, 100 μM DHPG induces chemical LTD, even in the presence of 2-methyl-6-phenylethynyl)pyridine (MPEP), a selective mGluR5 antagonist (Izumi & Zorumski, 2012), but chemical LTD induced by 10 μM DHPG is blocked by MPEP (Purgert et al., 2014), suggesting that LTD induced by high concentrations of DHPG involves mechanisms other than mGluR5 activation. CHPG (250 μM) fails to induce chemical LTD in the presence of MPEP (Purgert et al., 2014), suggesting that CHPG is a more specific mGluR5 agonist, though DHPG is the more commonly used agent to induce LTD. LY367385 and 7-(hydroxyimino)cyclopropa[*b*]chromen-1a-carboxylate ethyl ester (CPCCOEt) are specific antagonists against mGluR1. Recent evidence indicates that most mGluRs in the CNS are localized intracellularly, rather than at the cell membrane (Jong, Schwetye, & O'Malley, 2007), and these intracellular receptors contribute to LTD, but not LTP (Purgert et al., 2014).

Endocannabinoids

Endocannabinoids (eCBs) are endogenous lipids similar to active components in cannabinoids (Table 65.1). Anandamide was the first eCB identified (Devane et al., 1992), and is also called *N*-arachidonoylethanolamine (AEA). Anandamide is formed via hydrolysis of *N*-arachidonyl phosphatidylethanolamine, or from arachidonate and ethanolamine, via fatty acid amide hydrolyase (FAAH). FAAH also rapidly degrades anandamide, and is inhibited by URB597. AM404, an active metabolite of acetaminophen, is an uptake inhibitor that can be used to investigate physiological effects of anandamide.

2-AG was the second eCB identified. In the CNS, 2-AG is more abundant than anandamide (Sugiura, Kishimoto, Oka, & Gokoh, 2006). Because 2-AG formation is mediated

TABLE 65.1 Endocannabinoids (eCBs) in the CNS

Endocannabinoids (eCBs)
Anandamide (arachidonylethanolamine, AEA)
2-Arachidonyl glyceryl (2-AG)
2-Arachidonyl glyceryl ether (Noladin ether)
N-Arachidonyldopamine (NADA)
Oleamide

TABLE 65.2 Cannabinoid Receptors and Widely Used Antagonists

Cannabinoid receptor	Commonly used agonist	Commonly used antagonist
CB1	ACEA	Rimonabant (SR141716) AM251 (also agonist of GPR55) AM281
CB2		AM630
GPR55		Cannabidiol
TRPV1	Capsaicin	Capsazepine

by phospholipase C and DAG, it is likely that group I mGluRs stimulate 2-AG production. 2-AG is mainly metabolized by monoacylglycereol lipase (MAGL), which can be inhibited by UR602.

Other possible eCBs include 2-arachidonyl glyceryl ether (noladin ether), an agent that is present at levels lower than 2-AG, but is more stable than 2-AG. In rat substantia nigra, *N*-arachidonoyl dopamine (NADA) is another potential eCB generated by high potassium depolarization (Freestone et al., 2014). Oleamide may also be an eCB, because oleamide activates cannabinoid receptors. Oleamide accumulates in CSF, following sleep-deprivation, and produces sleep if administered to animals.

Synthetic Cannabinoids

In addition to phytocannabinoids, such as Δ^9-tetrahydrocannabinol (THC), other synthetic cannabinoids include CP 55,940, WIN55,212-2, and HU210. CP 55,940 activates classical cannabinoid receptors, but also antagonizes GPR55, a nonclassical cannabinoid receptor (Kapur et al., 2009).

Cannabinoid Receptors

THC induces various symptoms in animals and humans by activating specific CNS receptors. The cannabinoid receptor 1 (CB1), first described by Matsuda, Bonner, and Lolait (1992), is the main CNS cannabinoid receptor, and is densely expressed in cerebellum, hippocampus, cerebral cortex, and basal ganglia (Table 65.2). CB1 localizes presynaptically on glutamatergic axon terminals of most excitatory pathways in the hippocampus (Katona et al., 2006), and inhibits voltage-sensitive calcium channels and adenylyl cyclase, while stimulating mitogen-activated protein kinases (MAPKs) (Turu & Hunyady, 2010). CB1 is also present on mitochondrial membranes, which directly control energy metabolism, and is involved in depolarization-induced suppression of inhibition (DSI) in the hippocampus (Bénard et al., 2012). Rimonabant (SR141716), AM251, and AM281 are CB1 antagonists.

Cannabinoid receptor 2 (CB2), another cannabinoid receptor, is mainly expressed in the immune system and hematopoietic cells. CB2 is also found in CNS microglia and neurons (Gong et al., 2006). In the CA1 hippocampal region and substantia nigra, CB2 is mainly postsynaptic (Brusco, Tagliaferro, Saez, & Onaivi, 2008), and inhibits voltage-gated calcium channels (Atwood, Wager-Miller, Haskins, Straiker, & Mackie, 2012b). In mice, JWH203, a CB2 receptor agonist, reverses MK-801-induced disruptions in sensorimotor gating, suggesting that CB2 may protect against NMDAR hypofunction (Khella, Short, & Malone, 2014).

Nonclassical cannabinoid receptors may also exist in the CNS. GPR55 is one example, and L-alpha-lysophosphatidylinositol (LPI), an agonist of GPR55, transiently increases glutamate release at CA3-CA1 hippocampal synapses via calcium release from presynaptic stores (Sylantyev, Jensen, Ross, & Rusakov, 2013). Cannabidiol, a GPR55 antagonist, blocks this effect. GPR18, another nonclassical receptor, is expressed in microglia (McHugh, 2012).

Transient receptor potential vanilloid 1 (TRPV1) is another nonclassical candidate receptor that is activated by several lipid signaling molecules, including anandamide, NADA, and 12-(S)-HPETE (Marinelli et al., 2007). Capsaicin and capsazepine are selective TRPV1 agonists and antagonists, respectively. In the dorsal horn, TRPV1 and mGluR5 colocalize on presynaptic terminals of nociceptive neurons, suggesting that coupling between TRPV1 and mGluR5 serves spinal nociceptive neurotransmission (Kim et al., 2000). Furthermore, DHPG-mediated calcium responses in the spinal cord involve TRPV1 activation by DAG. In the CA1 region of CB1 knockout (KO) mice, reduction of excitatory postsynaptic potentials (EPSPs) by WIN55,212-2 and CP55940 is capasazepine sensitive, suggesting TRPV1 participates in eCB mediated synaptic depression (Benninger, Freund, & Hájos, 2008).

There may be other cannabinoid receptors. In interneurons of the rat hippocampus, the nonhydrolysable anandamide analog, R-methanadamide, depresses excitatory transmission (Edwards et al., 2012). This depression is not blocked by AM251 or capsazepine, suggesting the existence of CB1/TRPV1-independent, anandamide receptors (Fig. 65.1).

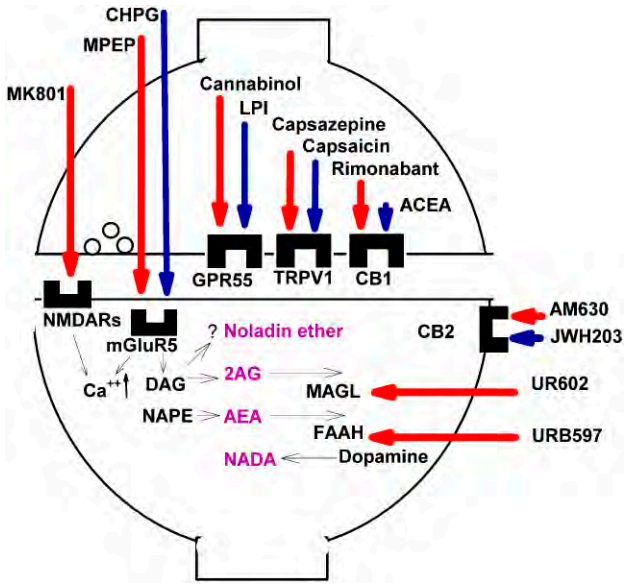


FIGURE 65.1 Pharmacological approach for understanding the eCB system. The upper part depicts the presynaptic site with neurotransmitter vesicles, and the lower part represents the postsynaptic site expressing glutamate receptors. Agonists for each receptor are shown with *blue arrows*, whereas antagonists and inhibitors are shown with *red arrows*. eCBs are shown in *purple*.

SYNAPTIC PLASTICITY AND mGluR5

eCBs are typically formed in postsynaptic neurons, and serve as retrograde messengers to activate presynaptic CB1. The activation of metabotropic receptors, especially mGluRs, plays an important role in eCB formation. In addition to CB1, other receptors may be involved in modulating synaptic transmission, participating in depolarization-induced suppression, and LTP. Furthermore, alteration of inhibitory synaptic transmission may affect LTP induction. Both genetic modifications and pharmacological approaches have helped to define the role of eCBs in synaptic plasticity.

Depolarization-Induced Suppression of Inhibition and the eCB System

Depolarization-induced suppression of inhibition (DSI) is a transient decrease in inhibitory GABAergic postsynaptic potentials or currents (IPSPs or IPSCs), triggered by depolarizing pulses. In hippocampal cultures, DSI is augmented by group I mGluR activation, and is prevented by AM 281 (Ohno-Shosaku, Maejima, & Kano, 2010). Application of 1.5–5 μM DHPG facilitates DSI (Ohno-Shosaku, Shosaku, Tsubokawa, & Kano, 2002). Given that CB1 is expressed presynaptically, retrograde eCB signaling is proposed to play a role in DSI, according to the following scheme: activation of postsynaptic mGluRs, eCB formation, activation of presynaptic CB1, and reduction of GABA release.

In a neuron/synaptic bouton preparation from the basolateral amygdala, a region critical for fear conditioning, a 4 s depolarizing step (–60 to 0 mV) produces robust suppression in the frequency and amplitude of spontaneous IPSPs (Zhu & Lovinger, 2005). MPEP shortens DSI duration but does not block DSI, while AM251 completely prevents DSI. Furthermore, WIN55,212-2 suppresses spontaneous IPSCs, and mimics the effects of depolarization (Zhu & Lovinger, 2005).

In neuron/synaptic boutons from the CA1 region, depolarization alone does not induce DSI, but depolarization combined with DHPG induces short-term depression. WIN55,212-2 depresses this short-term depression in spontaneous IPSCs. Similarly, 2-AG and anandamide also depress spontaneous IPSCs, with anandamide being more effective than 2-AG. The eCB-dependent short term depression is mGluR5-dependent, based on block by MPEP. These observations suggest that the depression in the hippocampus is more dependent on mGluR activation than that in the amygdala (Sheinin, Talani, Davis, & Lovinger, 2008).

In midbrain slices containing substantia nigra, IPSPs are facilitated by rimonabant. This facilitation is blocked by CPCCOEt but not MPEP, indicating that mGluR1, not mGluR5, is linked with eCB-mediated alteration of inhibitory synaptic transmission (Freestone et al., 2014).

Taken together, the role of mGluRs in DSI is variable among brain regions, but eCB production and CB1 activation on inhibitory terminals are essential for temporary reduction of GABA release. When a single neuron is grown on a small island of permissive substrate, it forms autapses (synapses) onto itself. In autaptic neurons from CB1 KO mice, depolarization to 0 mM from –60 mV fails to induce DSI. However, in CB2 transfected autaptic neurons from CB1 KO mice, DSI can be induced, and is prevented by AM630, indicating a possible role for CB2 (Atwood, Straiker, & Mackie, 2012a).

eCBs can also mediate depolarization-induced suppression of excitation (DSE; Ohno-Shosaku et al., 2002). 2-AG is the most likely endocannabinoid mediating DSE in autaptic neurons (Straiker & Mackie, 2005).

Some effects of eCBs in the CNS are not explained by activation of CB1 or CB2, and TRPV1 may be linked with glutamate receptors. The acute depression induced by DHPG in the CA1 region is facilitated by capsazepine, suggesting that TRPV1 may be involved in modulating mGluR-dependent short-term depression (Bennion et al., 2011; Fig. 65.2).

Heterosynaptic LTD

Chevaleyre and Castillo (2003) provided early evidence for the involvement of eCBs in heterosynaptic LTD. In the CA1 region of rat hippocampal slices, high frequency stimulation (HFS) (100 Hz 1 s × 2 strains) in

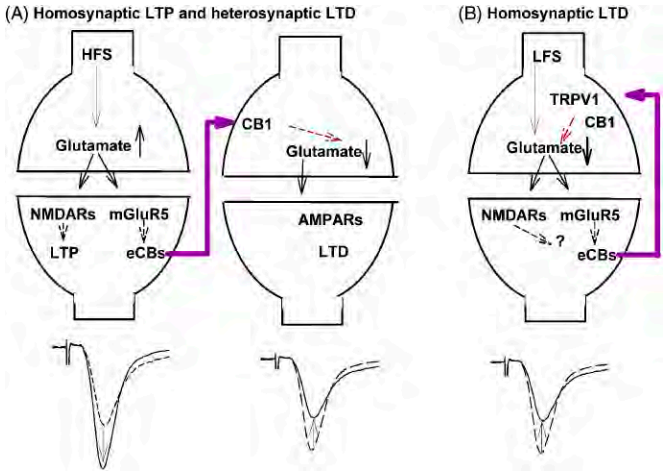


FIGURE 65.2 Schematic representation of heterosynaptic and homosynaptic LTD. (A) Glutamate released following presynaptic HFS activates NMDARs, resulting in homosynaptic LTP. Glutamate also activates postsynaptic mGluR5, leading to eCB formation. As retrograde messengers, eCBs activate presynaptic CB1, and suppress glutamate release on other neurons. The heterosynaptic pathway is independent from NMDARs. (B) Glutamate released during LFS activates both NMDARs and mGluR5 at postsynaptic sites. eCBs formed after activation of these glutamate receptors serve as retrograde messengers at the same synapses. In the CA1 region, eCBs activate presynaptic CB1, and suppress glutamate release. In the dentate gyrus, TRPV1 may be responsible for homosynaptic LTD induction. Traces show how EPSPs are altered after HFS and LFS.

stratum radiatum induces LTD of IPSCs (I-LTD). I-LTD induction is prevented by MPEP plus LY367385, and by U73122, a PLC inhibitor, suggesting that PLC stimulation following activation of group 1 mGluRs is essential. HFS-mediated I-LTD and chemical I-LTD induced by DHPG are not additive. HFS fails to induce I-LTD in the presence of AM251, but AM251 administered after HFS does not reverse I-LTD, indicating that CB1 helps to initiate but not sustain LTD. Chemical I-LTD by DHPG is also blocked by AM251. HFS-mediated I-LTD, and chemical I-LTD induced by WIN55,212-2 are not additive (Chevaleyre & Castillo, 2003). From these findings, a mechanism for I-LTD induction is proposed: glutamate release after HFS activates mGluRs1/5, leading to PLC activation and DAG formation; DAG is then converted into 2-AG, which inhibits GABA release by acting on heteropresynaptic CB1 (Chevaleyre & Castillo, 2004).

Yasuda, Huang, and Tsumoto (2008) showed that, prior to postnatal day (PND) 14, HFS (20 pulses at 100 Hz x 5 trains) in the presence of picrotoxin induced heterosynaptic LTD, in addition to homosynaptic LTP in the CA1 region. The heterosynaptic LTD is not induced in the presence of MPEP plus LY367385. This LTD is not blocked by 2-amino-5-phosphonovaleric acid (AP5), a selective NMDAR antagonist, whereas AP5 blocks homosynaptic LTP, indicating that the LTP is dependent on, but LTD is independent of NMDAR activation. AM251 inhibited heterosynaptic LTD, but did not affect homosynaptic LTP. At PND 2-5, CB1 inhibition is necessary for HFS to induce homosynaptic LTP (Yasuda et al., 2008). Similar findings were observed in mouse visual cortex (Huang, Yasuda, Sarihi, & Tsumoto, 2008). In layer II/III neurons in the visual cortex of young mice (PND 14-20), theta-bust stimulation with postsynaptic depolarization induces homosynaptic LTP and heterosynaptic LTD. AP5 abolishes homosynaptic LTP, but not heterosynaptic LTD; MPEP abolished heterosynaptic LTD, but not homosynaptic LTP. Similarly, AM251 abolishes heterosynaptic LTD, but not homosynaptic LTP. From these findings, the authors proposed a scheme to explain heterosynaptic LTD: postsynaptic activation of group 1 mGluRs stimulates release of eCBs as retrograde messengers that activate heteropresynaptic CB1, and depress neurotransmitter release (Huang et al., 2008).

Homosynaptic LTD

eCBs are involved in autaptic LTD (autLTD) (Kellogg, Mackie, & Straiker, 2009). The autLTD resulting from repeated 4 Hz stimulation is induced in the presence of AP5/CNQX, or even in the presence of 4-carboxyphenylglycine, a group I mGluR antagonist. However, administration of all the three antagonists blocks autLTD. Administration of 2-AG for 3–5 min induces acute suppression, and long-lasting depression of excitatory postsynaptic currents (EPSCs). The 2-AG mediated LTD is reversed by U73122, a PLC inhibitor. This finding suggests that autLTD occurs via activation of presynaptic PLC downstream of CB1 (Kellogg et al., 2009).

In the CA1 region, electrical LTD induced by low frequency stimulation (LFS), and chemical LTD induced by DHPG are both sensitive to chemical and genetic inhibition of CB1 (Xu, Chen, Zhang, & Chen, 2010). CB1 is abundant in stratum radiatum, but is less prevalent in stratum lacunosum moleculare (SLM) (Xu et al., 2010). This may explain why LTD is easier to induce in stratum radiatum than SLM. Consistent with this, LFS of direct perforant path inputs to CA1 SLM does not induce homo- or heterosynaptic LTD, but depotentiates stratum radiatum LTP (Izumi & Zorumski, 2008).

Péterfi et al. (2012) found that excitatory synaptic inputs to both CA1 interneurons and pyramidal cells readily undergo spike timing-dependent LTD. This form of LTD is blocked by a DAG lipase inhibitor, and also by MPEP, suggesting that eCBs formed via DAG lipase after mGluR5 activation are involved in hippocampal LTD induction. Furthermore, LTD was not seen in CB1 KO mice.

In the CA1 region of rat hippocampal slices, WIN55,212-2 and ACEA induce chemical LTD of Schaffer collateral EPSPs (Izumi & Zorumski, 2012). The LTD induced by WIN55,212-2 is blocked by AM251, but not by AM630, indicating that CB1, but not CB2, is required

for LTD induction. Conventional LTD of EPSPs induced by low frequency electrical stimulation is also blocked by AM251. The effect of WIN55,212-2 was best mimicked by noladin ether, but less by 2-AG or anandamide, suggesting that noladin ether rather than 2-AG may be involved in CA1 LTD. These observations also suggest that anandamide and 2-AG may serve roles other than LTD in CA1.

In the rat nucleus tractus solitarius, LTD is prevented by both AP5 and AM 251. Interestingly, LTD was not blocked by intracellular perfusion of MK801, implying an involvement of NMDARs on neighboring cells or presynaptic sites. In addition, FAAH inhibition lowers the threshold for LFS mediated LTD, suggesting the participation of anandamide as the eCB in this nucleus (Khlaifia, Farah, Gackiere, & Tell, 2013). In the striatum, eCB release is also involved in LTD of excitatory transmission (Gerdeman, Ronesi, & Lovinger, 2002).

In addition to CB1, TRPV1 may be involved in LTD induction. In the dentate gyrus, capsaicin triggers LTD that is mediated by anandamide in a CB1-independent manner (Chávez, Chiu, & Castillo, 2010). This TRPV1-mediated LTD also requires mGluR5 activation (Chávez et al., 2010).

Taken together, it is likely that mGluR activation, followed by eCB production and activation of CB1 or TRPV1, is necessary for homosynaptic LTD induction.

Synaptic Potentiation and the eCB System

Compared to the clear participation of eCBs in synaptic depression, their role in synaptic potentiation is less certain. Cannabinoids, however, are known to impair memory acquisition and maintenance (Ranganathan & D'Souza, 2006). Because LTP is thought to be a cellular mechanism of memory, it is hypothesized that cannabinoids block LTP. Indeed, HU201, a synthetic cannabinoid, inhibits LTP induction in rat hippocampal slices (Collins, Pertwee, & Davies, 1994), effects mimicked by WIN55,212-2 and anandamide (Terranova, Michaud, Le Fur, & Soubrié, 1995). Because these effects of WIN55,212-2 and anandamide are overcome by a CB1 antagonist, CB1 is believed to be pivotal for LTP inhibition (Terranova et al., 1995), and LTP is facilitated in CB1 KO mice (Jacob, Marsch, Marsicano, Lutz, & Wotjak, 2012). Another form of LTP induced by exogenous ATP (10 μM for 10 min) is blocked by WIN55,212-2. This inhibitory effect of WIN55,212-2 is overcome by AM251, suggesting again the involvement of CB1 (Ievglevskyi, Palygin, Kondratskaya, Grebenyuk, & Krishtal, 2012).

While exogenous cannabinoids disrupt LTP induction, it is less clear whether endogenously formed eCBs suppress LTP. Administration of URB597, an inhibitor of FAAH, doubles anandamide levels in the hippocampus (30–40 pmol/g) but only partially inhibits LTP (Basavarajappa, Nagre, Xie, & Subbanna, 2014). This depression of LTP is not seen in slices from CB1 KO mice. Although this inhibitory effect of anandamide is mediated by CB1, it is still possible that TRPV1, also activated by anandamide, may be involved in LTP modulation, and capsaicin enhances excitatory LTP in the hippocampus (Bennion et al., 2011).

Because eCBs suppress IPSCs at least transiently, and because GABAergic inhibition modulates excitatory LTP in the hippocampus, an opposite scenario is also plausible: eCBs may paradoxically augment LTP induction. Consistent with this, eCB release by rat hippocampal neurons during DSI allows subthreshold trains of EPSCs to induce LTP in individual cells (Carlson, Wang, & Alger, 2002). The enhancement of LTP can be mimicked by facilitation of iLTD. In area CA1, 10 Hz stimulation induces iLTD via CB1, but does not produce excitatory LTD. After 10 Hz stimulation, weak theta burst stimulation can induce excitatory LTP, suggesting an influence of iLTD on theta burst sensitivity (Chevaleyre & Castillo, 2004). Lin et al. (2011) have reported that AM281, a CB1 inhibitor, blocks LTP induction, and this inhibition was not seen in the presence of picrotoxin. These results suggest that CB1 activation participates in LTP induction via a GABA-A receptor mediated mechanism. However, AM404, an anandamide transport inhibitor, failed to alter LTP, suggesting that anandamide is not the eCB involved in LTP induction.

LFS priming prior to repeated HFS inhibits late phase LTP. This inhibition is prevented by AM251, and mimicked by WIN55,212-2, suggesting that CB1 activation is involved in this form of metaplasiticity (Slanina, Roberto, & Schweitzer, 2005).

In the amygdala, capsaicin inhibits LTP by activating both neuronal nitric oxide synthase (NOS) and CB1 (Zschenderlein, Gebhardt, von Bohlen Und Halbach, Kulisch, & Albrecht, 2011). This study suggests that TRPV1 activation alters LTP induction in a complex manner.

Glutamate receptors are not limited to the CNS. Peripheral injection of capsaicin into rat hind instep causes an increase in glutamate levels in the subcutaneal perfusate. This increase is suppressed by MK801, NBQX, or CPCCOEt (Jin et al., 2009), suggesting that three types of glutamate receptors are involved in nociceptive glutamate release triggered by TRPV1.

eCBS AND NMDARs

Effects of NMDARs on eCB Systems

NMDAR activation enhances eCB formation. This enhancement of eCBs synthesis likely involves calcium influx, and perhaps stimulation of PLC and DAG lipase. Intracranial infusion of NMDA in neonatal rats enhances

anandamide concentrations 13-fold, but does not increase 2-AG levels (Hansen et al., 2001). Similarly, mild brain injury increases anandamide levels via NMDAR activation (Hansen et al., 2001). In rat cortical neurons, administration of 300 μM NMDA alone fails to enhance anandamide formation, but increases anandamide levels 10-fold, when administered in combination with carbachol. This effect is also observed with glutamate and carbachol, and is blocked by MK801, an NMDAR antagonist. These observations indicate that enhanced anandamide formation likely involves synergistic activation of nicotinic receptors and NMDARs (Stella & Piomelli, 2001). In the same setting, NMDA also enhances 2-AG formation but the enhancement does not require carbachol (Stella & Piomelli, 2001).

NMDAR activation may not be sufficient for certain forms of LTD, and may require coincidental CB1 activation. In the rat nucleus tractus solitarius, for example, presynaptic LTD resulting from visceral fiber stimulation is induced by coactivation of NMDARs and CB1 (Khlaifia et al., 2013).

NMDARs may be more closely linked to TRPV1 than CB1. Low doses of anandamide have anxiolytic effects via CB1 (Fogaça, Gomes, Moreira, Guimarães, & Aguiarogaça, 2013). However, a high dose of anandamide masks these anxiolytic effects in a capsazepine-sensitive fashion, suggesting that effects of high dose anandamide involve TRPV1. The masking effects are also prevented by an NMDAR antagonist, suggesting that the actions of TRPV1 are mediated by NMDAR activation. It is also possible that TRPV1 activation and NMDAR activation have synergistic effects against the anxiolytic actions. TRPV1 activation not only masks the anxiolytic effects of anandamide, but also induces depressive behaviors in rats (Fogaça et al., 2013), and TRPV1 activation with resiniferatoxin enhances immobility of rats in the forced swim test. This enhanced immobility is blocked by ketamine. Because ketamine antagonizes NMDARs, it is possible that TRPV1-mediated depressive behaviors depend on NMDARs (Abdelhamid, Kovács, Nunez, & Larson, 2014).

NMDAR Actions May be Affected by eCBs

Just as the eCB system can be modified by NMDARs, the effects of NMDARs are modulated by cannabinoids. For example, anandamide inhibits NMDAR-mediated calcium influx in rat brain slices (Hampson et al., 1998), via effects on CB1. This effect suggests that CB1 activation may be neuroprotective against NMDAR-induced toxicity. Indeed, neuronal death in the dentate gyrus by NMDA is ameliorated by NADA, in a fashion that is blocked by AM251 but not by AM630 or 6-iodonordihydrocapsaicin, indicating a role for CB1, but not CB2 or TRPV1 (Grabiec et al., 2012).

Although anandamide inhibits NMDAR-mediated calcium influx via CB1, anandamide paradoxically enhances NMDA-mediated calcium influx when CB1 is blocked by rimonabant (Hampson et al., 1998). This enhancement is not mimicked by THC. A role for TRPV1 was not examined, although NMDAR activation sensitizes TRPV1 via receptor phosphorylation (Lee, Chung, & Ro, 2012). In the retina, neuronal death after intravitreal injections of capsaicin is partially prevented by AP5 (Leonelli, Martins, & Britto, 2013), suggesting not only participation of NMDARs in TRPV1-mediated retinal cell death, but also that NMDAR-mediated neuronal degeneration is partially mediated by TRPV1. However, actions of TRPV1 in the retina are likely complex, and TRPV1 activation can be neuroprotective. Ganglion cell death by intravitreal NMDA injection is completely prevented by capsaicin (Sakamoto et al., 2014). It is important to note, however, that ketamine was used as an anesthetic during NMDA injection, in this study. Because ketamine may alter TRPV1's actions (Abdelhamid et al., 2014), it is possible that the neuroprotective effect of TRPV1 on NMDAR-meditated ganglion cell death involves interactions with ketamine.

Taken together, it seems likely that both CB1 and TRPV1 are involved in NMDAR-mediated neuronal degeneration. Furthermore, cannabinoids can alter NMDAR-mediated actions, and NMDAR activation modulates the effects of eCBs.

CONCLUSIONS

Current evidence suggests that both mGluRs and NMDARs may alter eCB signaling. Synaptic depression is often mediated by eCBs serving as synaptic retrograde messengers at CB1 and other cannabinoid receptors. While it is known that postsynaptic mGluR5 is likely important for eCB formation, the role of NMDARs is less certain. NMDAR activation may have additional effects, including adverse effects on CB1 actions. Some of these NMDAR actions may involve TRPV1.

MINI-DICTIONARY

DSI A transient suppression of inhibitory GABAergic postsynaptic potentials or currents triggered by a depolarizing pulse. DSI may increase the likelihood of LTP induction.
EPSP An electrical response in postsynaptic neurons in response to the release of glutamate. EPSPs stimulate neurons and promote the firing of postsynaptic action potentials.
IPSP An electrical response in postsynaptic neurons following release of GABA at synapses. IPSPs suppress the occurrence of postsynaptic action potentials.
LTP A form of synaptic plasticity in which synaptic function is persistently enhanced. Hippocampal LTP is a cellular model of learning and memory.

LTD A form of synaptic plasticity that leads to persistently diminished synaptic transmission. Hippocampal LTD may play a role in novelty processing.
Metabotropic receptors A class of neurotransmitter receptors that act on second messengers, such as G-proteins, and alter intracellular signaling.
mGluR5 A type of G-protein coupled glutamate receptor. Like mGluR1, mGluR5 activates PLC. Dysfunction of mGluR5 may be involved in neuropsychiatric illnesses, including depression, drug abuse, and Fragile X syndrome.
NMDARs A type of ionotropic glutamate receptor. NMDARs are voltage dependent, and allow calcium influx into neurons. The calcium influx is critical for many forms of synaptic plasticity.
Retrograde messenger A substance released from postsynaptic sites that travels back to reach presynaptic sites, and activate specific receptors. Endocannabinoids serve as retrograde messengers for some forms of synaptic plasticity.
Synaptic plasticity The adaptability of a synapse to activity-dependent changes in strength.

ACKNOWLEDGMENTS

This work was supported by NIH grants MH077791 and AA017413, and the Bantly Foundation.

References

Abdelhamid, R. E., Kovács, K. J., Nunez, M. G., & Larson, A. A. (2014). Depressive behavior in the forced swim test can be induced by TRPV1 receptor activity and is dependent on NMDA receptors. *Pharmacological Research*, *79*, 21–27.

Atwood, B. K., Straiker, A., & Mackie, K. (2012a). CB_2 cannabinoid receptors inhibit synaptic transmission when expressed in cultured autaptic neurons. *Neuropharmacology*, *63*, 514–523.

Atwood, B. K., Wager-Miller, J., Haskins, C., Straiker, A., & Mackie, K. (2012b). Functional selectivity in CB(2) cannabinoid receptor signaling and regulation: implications for the therapeutic potential of CB(2) ligands. *Molecular Pharmacology*, *81*, 250–263.

Basavarajappa, B. S., Nagre, N. N., Xie, S., & Subbanna, S. (2014). Elevation of endogenous anandamide impairs LTP, learning, and memory through CB1 receptor signaling in mice. *Hippocampus*, *24*, 808–818.

Bénard, G., Massa, F., Puente, N., Lourenço, J., Bellocchio, L., Soria-Gómez, E., Matias, I., Delamarre, A., Metna-Laurent, M., Cannich, A., Hebert-Chatelain, E., Mulle, C., Ortega-Gutiérrez, S., Martín-Fontecha, M., Klugmann, M., Guggenhuber, S., Lutz, B., Gertsch, J., Chaouloff, F., López-Rodríguez, M. L., Grandes, P., Rossignol, R., & Marsicano, G. (2012). Mitochondrial CB_1 receptors regulate neuronal energy metabolism. *Nature Neuroscience*, *15*, 558–564.

Benninger, F., Freund, T. F., & Hájos, N. (2008). Control of excitatory synaptic transmission by capsaicin is unaltered in TRPV1 vanilloid receptor knockout mice. *Neurochemistry International*, *52*, 89–94.

Bennion, D1, Jensen, T., Walther, C., Hamblin, J., Wallmann, A., Couch, J., Blickenstaff, J., Castle, M., Dean, L., Beckstead, S., Merrill, C., Muir, C., St Pierre, T., Williams, B., Daniel, S., & Edwards, J. G. (2011). Transient receptor potential vanilloid 1 agonists modulate hippocampal CA1 LTP via the GABAergic system. *Neuropharmacology*, *61*, 730–738.

Brusco, A., Tagliaferro, P. A., Saez, T., & Onaivi, E. S. (2008). Ultrastructural localization of neuronal brain CB2 cannabinoid receptors. *Annals of the New York Academy of Sciences*, *1139*, 450–457.

Carlson, G., Wang, Y., & Alger, B. E. (2002). Endocannabinoids facilitate the induction of LTP in the hippocampus. *Nature Neuroscience*, *5*, 723–724.

Chávez, A. E., Chiu, C. Q., & Castillo, P. E. (2010). TRPV1 activation by endogenous anandamide triggers postsynaptic long-term depression in dentate gyrus. *Nature Neuroscience*, *13*, 1511–1518.

Chevaleyre, V., & Castillo, P. E. (2003). Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. *Neuron*, *38*, 461–472.

Chevaleyre, V., & Castillo, P. E. (2004). Endocannabinoid-mediated metaplasticity in the hippocampus. *Neuron*, *43*, 871–881.

Collins, D. R., Pertwee, R. G., & Davies, S. N. (1994). The action of synthetic cannabinoids on the induction of long-term potentiation in the rat hippocampal slice. *European Journal of Pharmacology*, *259*, R7–R8.

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., & Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*, *258*, 1946–1949.

Edwards, J. G., Gibson, H. E., Jensen, T., Nugent, F., Walther, C., Blickenstaff, J., & Kauer, J. A. (2012). A novel non-CB1/TRPV1 endocannabinoid-mediated mechanism depresses excitatory synapses on hippocampal CA1 interneurons. *Hippocampus*, *22*, 209–221.

Fogaça, M. V., Gomes, F. V., Moreira, F. A., Guimarães, F. S., & Aguiar, D. C. (2013). Effects of glutamate NMDA and TRPV1 receptor antagonists on the biphasic responses to anandamide injected into the dorsolateral periaqueductal grey of Wistar rats. *Psychopharmacology (Berlin)*, *226*, 579–587.

Freestone, P. S., Guatteo, E., Piscitelli, F., di Marzo, V., Lipski, J., & Mercuri, N. B. (2014). Glutamate spillover drives endocannabinoid production and inhibits GABAergic transmission in the substantia nigra pars compacta. *Neuropharmacology*, *79*, 467–475.

Gerdeman, G. L., Ronesi, J., & Lovinger, D. M. (2002). Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nature Neuroscience*, *5*, 446–451.

Gong, J. P., Onaivi, E. S., Ishiguro, H., Liu, Q. R., Tagliaferro, P. A., Brusco, A., & Uhl, G. R. (2006). Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Research*, *1071*, 10–23.

Grabiec, U., Koch, M., Kallendrusch, S., Kraft, R., Hill, K., Merkwitz, C., Ghadban, C., Lutz, B., Straiker, A., & Dehghani, F. (2012). The endocannabinoid *N*-arachidonoyldopamine (NADA) exerts neuroprotective effects after excitotoxic neuronal damage via cannabinoid receptor 1 (CB(1)). *Neuropharmacology*, *62*, 1797–1807.

Hampson, A. J., Bornheim, L. M., Scanziani, M., Yost, C. S., Gray, A. T., Hansen, B. M., Leonoudakis, D. J., & Bickler, P. E. (1998). Dual effects of anandamide on NMDA receptor-mediated responses and neurotransmission. *Journal of Neurochemistry*, *70*, 671–676.

Hansen, H. H., Schmid, P. C., Bittigau, P., Lastres-Becker, I., Berrendero, F., Manzanares, J., Ikonomidou, C., Schmid, H. H., Fernández-Ruiz, J. J., & Hansen, H. S. (2001). Anandamide, but not 2-arachidonoylglycerol, accumulates during in vivo neurodegeneration. *Journal of Neurochemistry*, *78*, 1415–1427.

Huang, Y., Yasuda, H., Sarihi, A., & Tsumoto, T. (2008). Roles of endocannabinoids in heterosynaptic long-term depression of excitatory synaptic transmission in visual cortex of young mice. *Journal of Neuroscience*, *28*, 7074–7083.

Ievglevskyi, O., Palygin, O., Kondratskaya, E., Grebenyuk, S., & Krishtal, O. (2012). Modulation of ATP-induced LTP by cannabinoid receptors in rat hippocampus. *Purinergic Signalling*, *8*, 705–713.

Izumi, Y., & Zorumski, C. F. (2008). Direct cortical inputs erase long-term potentiation at Schaffer collateral synapses. *Journal of Neuroscience*, *28*, 9557–9563.

Izumi, Y., & Zorumski, C. F. (2012). NMDA receptors, mGluR5, and endocannabinoids are involved in a cascade leading to hippocampal long-term depression. *Neuropsychopharmacology*, *37*, 609–617.

Jacob, W., Marsch, R., Marsicano, G., Lutz, B., & Wotjak, C. T. (2012). Cannabinoid CB1 receptor deficiency increases contextual fear memory under highly aversive conditions and long-term potentiation in vivo. *Neurobiology of Learning and Memory*, *98*, 47–55.

Jin, Y. H., Yamaki, F., Takemura, M., Koike, Y., Furuyama, A., & Yonehara, N. (2009). Capsaicin-induced glutamate release is implicated in nociceptive processing through activation of ionotropic glutamate receptors and group I metabotropic glutamate receptor in primary afferent fibers. *Journal of Pharmacological Sciences*, *109*, 233–241.

Jong, Y. J., Schwetye, K. E., & O'Malley, K. L. (2007). Nuclear localization of functional metabotropic glutamate receptor mGlu1 in HEK293 cells and cortical neurons: role in nuclear calcium mobilization and development. *Journal of Neurochemistry*, *101*, 458–469.

Kapur, A., Zhao, P., Sharir, H., Bai, Y., Caron, M. G., Barak, L. S., & Abood, M. E. (2009). Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands. *Journal of Biological Chemistry*, *284*, 29817–29827.

Katona, I., Urbán, G. M., Wallace, M., Ledent, C., Jung, K. M., Piomelli, D., Mackie, K., & Freund, T. F. (2006). Molecular composition of the endocannabinoid system at glutamatergic synapses. *Journal of Neuroscience*, *26*, 5628–5637.

Kellogg, R., Mackie, K., & Straiker, A. (2009). Cannabinoid CB1 receptor-dependent long-term depression in autaptic excitatory neurons. *Journal of Neurophysiology*, *102*, 1160–1171.

Khella, R., Short, J. L., & Malone, D. T. (2014). CB2 receptor agonism reverses MK-801-induced disruptions of prepulse inhibition in mice. *Psychopharmacology (Berlin)*, *231*, 3071–3087.

Khlaifia, A., Farah, H., Gackiere, F., & Tell, F. (2013). Anandamide, cannabinoid type 1 receptor, and NMDA receptor activation mediate non-Hebbian presynaptically expressed long-term depression at the first central synapse for visceral afferent fibers. *Journal of Neuroscience*, *33*, 12627–12637.

Kim, Y. H., Park, C. K., Back, S. K., Lee, C. J., Hwang, S. J., Bae, Y. C., Na, H. S., Kim, J. S., Jung, S. J., & Oh, S. B. (2000). Membrane-delimited coupling of TRPV1 and mGluR5 on presynaptic terminals of nociceptive neurons. *Journal of Neuroscience*, *29*, 10000–10009.

Lee, J., Chung, M. K., & Ro, J. Y. (2012). Activation of NMDA receptors leads to phosphorylation of TRPV1 S800 by protein kinase C and A-Kinase anchoring protein 150 in rat trigeminal ganglia. *Biochemical and Biophysical Research Communications*, *424*, 358–363.

Leonelli, M., Martins, D. O., & Britto, L. R. (2013). Retinal cell death induced by TRPV1 activation involves NMDA signaling and upregulation of nitric oxide synthases. *Cellular and Molecular Neurobiology*, *33*, 379–392.

Lin, Q. S., Yang, Q., Liu, D. D., Sun, Z., Dang, H., Liang, J., Wang, Y. X., Chen, J., & Li, S. T. (2011). Hippocampal endocannabinoids play an important role in induction of long-term potentiation and regulation of contextual fear memory formation. *Brain Research Bulletin*, *86*, 139–145.

Marinelli, S., Di Marzo, V., Florenzano, F., Fezza, F., Viscomi, M. T., van der Stelt, M., Bernardi, G., Molinari, M., Maccarrone, M., & Mercuri, N. B. (2007). N-arachidonoyl-dopamine tunes synaptic transmission onto dopaminergic neurons by activating both cannabinoid and vanilloid receptors. *Neuropsychopharmacology*, *32*, 298–308.

Matsuda, L. A., Bonner, T. I., & Lolait, S. J. (1992). Cannabinoid receptors: which cells, where, how, and why? *NIDA Research Monograph*, *126*, 48–56.

McHugh, D. (2012). GPR18 in microglia: implications for the CNS and endocannabinoid system signalling. *British Journal of Pharmacology*, *167*, 1575–1582.

Ohno-Shosaku, T., Maejima, T., & Kano, M. (2001). Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron*, *29*, 729–738.

Ohno-Shosaku, T., Shosaku, J., Tsubokawa, H., & Kano, M. (2002). Cooperative endocannabinoid production by neuronal depolarization and group I metabotropic glutamate receptor activation. *European Journal of Neuroscience*, *15*, 953–961.

Péterfi, Z., Urbán, G. M., Papp, O. I., Németh, B., Monyer, H., Szabó, G., Erdélyi, F., Mackie, K., Freund, T. F., Hájos, N., & Katona, I. (2012). Endocannabinoid-mediated long-term depression of afferent excitatory synapses in hippocampal pyramidal cells and GABAergic interneurons. *Journal of Neuroscience*, *32*, 14448–14463.

Purgert, C. A., Izumi, Y., Jong, Y. J., Kumar, V., Zorumski, C. F., & O'Malley, K. L. (2014). Intracellular mGluR5 can mediate synaptic plasticity in the hippocampus. *Journal of Neuroscience*, *34*, 4589–4598.

Ranganathan, M., & D'Souza, D. C. (2006). The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berlin)*, *188*, 425–444.

Sakamoto, K., Kuroki, T., Okuno, Y., Sekiya, H., Watanabe, A., Sagawa, T., Ito, H., Mizuta, A., Mori, A., Nakahara, T., & Ishii, K. (2014). Activation of the TRPV1 channel attenuates *N*-methyl-D-aspartic acid-induced neuronal injury in the rat retina. *European Journal of Pharmacology*, *733C*, 13–22.

Sheinin, A., Talani, G., Davis, M. I., & Lovinger, D. M. (2008). Endocannabinoid- and mGluR5-dependent short-term synaptic depression in an isolated neuron/bouton preparation from the hippocampal CA1 region. *Journal of Neurophysiology*, *100*, 1041–1052.

Slanina, K. A., Roberto, M., & Schweitzer, P. (2005). Endocannabinoids restrict hippocampal long-term potentiation via CB1. *Neuropharmacology*, *49*, 660–668.

Stella, N., & Piomelli, D. (2001). Receptor-dependent formation of endogenous cannabinoids in cortical neurons. *European Journal of Pharmacology*, *425*, 189–196.

Straiker, A., & Mackie, K. (2005). Depolarization-induced suppression of excitation in murine autaptic hippocampal neurones. *Journal of Physiology*, *569*(Pt 2), 501–517.

Sugiura, T., Kishimoto, S., Oka, S., & Gokoh, M. (2006). Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. *Progress in Lipid Research*, *45*, 405–446.

Sylantyev, S., Jensen, T. P., Ross, R. A., & Rusakov, D. A. (2013). Cannabinoid- and lysophosphatidylinositol-sensitive receptor GPR55 boosts neurotransmitter release at central synapses. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 5193–5198.

Terranova, J. P., Michaud, J. C., Le Fur, G., & Soubrié, P. (1995). Inhibition of long-term potentiation in rat hippocampal slices by anandamide, WIN55 212-2: reversal by SR 141716 A, a selective antagonist of CB1 cannabinoid receptors. *Naunyn Schmiedebergs Archives of Pharmacology*, *352*, 576–579.

Turu, G., & Hunyady, L. (2010). Signal transduction of the CB1 cannabinoid receptor. *Journal of Molecular Endocrinology*, *44*, 75–85.

Xu, J. -Y., Chen, R., Zhang, J., & Chen, C. (2010). Endocannabinoids differentially modulate synaptic plasticity in rat hippocampal CA1 pyramidal neurons. *PLoS One*, *5*, e10306.

Yasuda, H., Huang, Y., & Tsumoto, T. (2008). Regulation of excitability and plasticity by endocannabinoids and PKA in developing hippocampus. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 3106–3111.

Zhu, P. F., & Lovinger, DM (2005). Retrograde endocannabinoid signaling in a postsynaptic neuron/synaptic bouton preparation from basolateral amygdala. *Journal of Neuroscience*, *25*, 6199–6207.

Zschenderlein, C., Gebhardt, C., von Bohlen Und Halbach, O., Kulisch, C., & Albrecht, D. (2011). Capsaicin-induced changes in LTP in the lateral amygdala are mediated by TRPV1. *PLoS One*, *6*, e16116.

CHAPTER

66

Peripheral CB1 Receptors and Ghrelin in Feeding Regulation: Pharmacological Implications

L. Orio, R. Gómez de Heras, F. Rodríguez de Fonseca

Department of Psychobiology, Faculty of Psychology, Complutense University of Madrid, Pozuelo de Alarcón, Madrid, Spain

SUMMARY POINTS

- Peripheral signals arising from the gut, and metabolically relevant organs, can control appetite and energy expenditure.
- Ghrelin is an orexigenic signal arising from the gut, mainly from the stomach. It stimulates appetite, acting both at peripheral (vagus nerve) and central (hypothalamus) sites.
- In all peripheral organs, the endogenous cannabinoid system contributes to the regulation of both, appetite and energy expenditure, through the regulatory actions exerted by cannabinoid receptors.
- Drugs modulating signals in the periphery are likely devoid of central nervous system-derived side effects. Orexigenic signals, such as ghrelin and endocannabinoids, are being studied for pharmaceutical development.
- Cannabinoid receptor antagonism in the periphery counteracts the orexigenic actions of ghrelin, suggesting that a combinatory therapy targeting both systems might have synergistic effects in controlling appetite in the periphery.

KEY FACTS OF FEEDING REGULATION

- Nutrients and their components released in the blood can regulate appetite and satiety.
- When these nutrients reach the gut (from the stomach to the small intestine), they promote the release of signals that reach the brain centers controlling appetite.
- These signals can be either orexigenic (like ghrelin or anandamide) or anorexigenic (like cholecystokinin or oleoylethanolamide).
- Peripheral signals arising from the gut, and metabolically relevant organs, such as the muscles, the pancreas, the liver, and the adipose tissue can either reach directly the brain or activate the peripheral sensory system.
- In the brain, the main structure that controls appetite and energy expenditure is the hypothalamus.
- The hypothalamus, in association with the autonomic nervous system to which is heavily connected, also communicates to metabolically relevant organs in order to control the onset and termination of feeding behavior.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00077-6
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
AEA	Anandamide
AgRP	Agouti-related protein
AMPK	AMP-activated protein kinase
BBB	Blood brain barrier
CNS	Central nervous system
ECS	Endocannabinoid system
GH	Growth hormone
GHRH	Growth hormone releasing hormone
GHS-R1a	Growth hormone secretagogue receptor
NPY	Neuropeptide Y
NTS	Nucleus of the tractus solitarius
PNS	Peripheral nervous system
POMC	Proopiomelanocortin
PVN	Paraventricular nucleus

INTRODUCTION: FEEDING REGULATION BY PERIPHERAL SIGNALS

The maintenance of body weight and the control of energy expenditure is a complex function that stands on a bidirectional flow of information between the brain and the body (Gao & Horvath, 2007). The control of feeding behavior, the management of nutrient processing and storage, the delivery of both fuel molecules and structural components to the different tissues of the body, as well as the maintenance of temperature through heat production, are tightly regulated by dozens of different signals that come and go from the body to the brain. On top of that, motivational processes controlled by the monoaminergic network of the reward circuit help to sustain food search and food selection (Dietrich & Horvath, 2009).

Peripheral signals are either nutrients, including bioactive lipids, such as the endocannabinoids, gut peptide hormones, such as ghrelin or glucagon-like peptide-1, pancreatic hormones, such as insulin, or adipocyte-linked signals, such as leptin or adiponectin (Dietrich & Horvath, 2009; Shirazi et al., 2013). These signaling molecules can either reach directly the brain or activate the peripheral sensory system. In turn, the brain can directly deliver controlling commands to metabolically relevant organs, through the autonomic nervous system (Gao & Horvath, 2007; Merchenthaler, 1991). Because most functions related to body weight and energy expenditure oscillate around homeostatic set points, these signals can be divided on the basis of the direction of the modulatory activity (either activation or inhibition) that they exert.

In the brain, the main structure that controls appetite and energy expenditure is the hypothalamus, in association with the autonomic nervous system to which it is heavily connected (Dietrich & Horvath, 2009; Diéguez, Vazquez, Romero, López, & Nogueiras, 2011). In the ventral hypothalamus, several neuronal groups, especially those from the arcuate nucleus, control the onset and termination of feeding behavior, thanks to the ability to detect these peripheral signals that reach the hypothalamus through the blood. These arcuate neurons are basically distributed into two majors groups, one expressing the neuropeptide Y (NPY), together with agouti-related protein (AgRP), and the other one expressing the opioid gene proopiomelanocortin (POMC), in association with the neuropeptide cocaine, and amphetamine-related transcript, CART. While NPY neurons stimulate appetite, POMC neurons inhibit it. Both NPY/AgRP and POMC/CART neurons are activated by peripheral signals, thanks to the convergent mechanisms on lipid metabolism-regulatory proteins, including the AMP-activated protein kinase, AMPK. These neurons have the ability to reprogram their metabolic substrate utilization away from glycolysis, and toward lipid oxidation, depending first on substrate availability, and second on AMPK activity. AMPK is regulated both by metabolic status and incoming signals and, ultimately, it will regulate the production of orexigenic NPY or anorexigenic POMC. While fasting, endocannabinoids, glucocorticoids, adiponectin, ghrelin, or AgRP activates AMPK, thus promoting feeding, signals, such as leptin, refeeding, insulin, GLP-1, or melanocortin receptor agonists that reduce AMPK activity, resulting in feeding inhibition. Nutrients, such as glucose, citrate, lactate, or alpha-lipoic acid can also modulate AMPK activity and feeding (Gao & Horvath, 2007; Diéguez et al., 2011).

Although this simple mechanism allows to start and stop feeding behavior, additional mechanisms centered in the motivational properties of food are also working in parallel to ensure a correct associative learning (ie, to learn caloric value, organoleptic properties, and adequacy of nutrients), and to provide reinforcing signals that help to organize food choice and meal patterns, whose temporal organization is further arranged by the internal clocks located in the suprachiasmatic nuclei of the hypothalamus. A relevant role for dopaminergic and serotonergic neurons in organizing these aspects of feeding behavior has been clearly determined (Abizaid et al., 2006; Dietrich & Horvath, 2009; Gao & Horvath, 2007).

The existence of these well-identified brain networks that control appetite and energy expenditure led to the development of therapies based on drugs capable to interfere with those central mechanisms (Dietrich & Horvath, 2012). Although being effective in preclinical models, and being apparently safe in early phases of clinical trials, these therapies were either not sufficiently safe, after extensive phase III studies, or withdrawn from

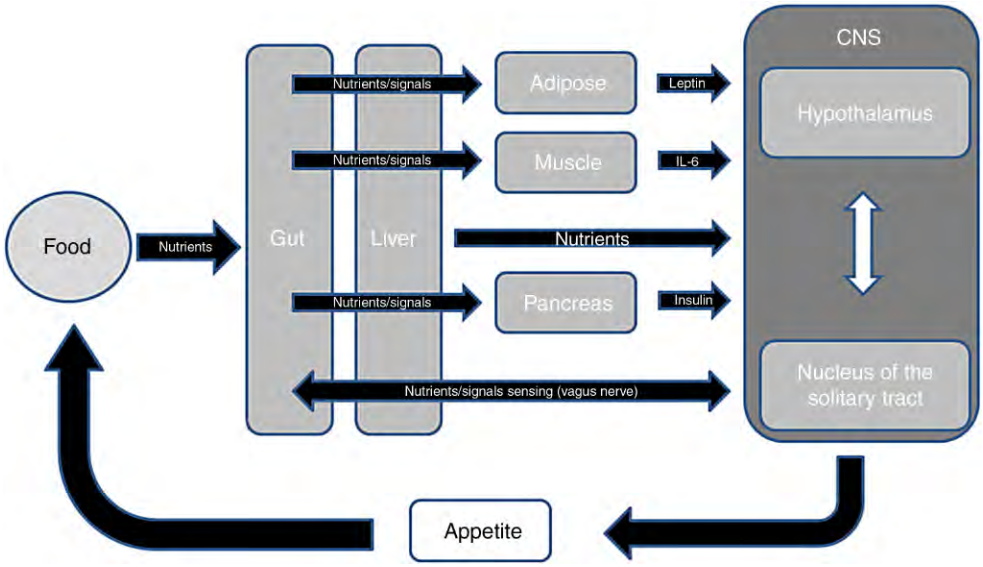


FIGURE 66.1 **Peripheral control of appetite through nutrients/blood-borne signals.** Peripheral control of appetite. Nutrients arriving to the gut elicit multiple signals (peptides, lipids) that, by direct access through the blood stream, by modulation of vagal afferents, or through their action on peripheral organs, such as the adipose tissue, the endocrine pancreas, or the muscles, modulate the activity of CNS nuclei involved in appetite control. In addition, these peripheral organs send additional signals (adipokines such as leptin, myokines, such as IL-6, or pancreatic hormones, such as insulin), that contribute to inform the brain on the metabolic status of the body, and the availability of nutrients.

the market because of unwanted side effects, especially of cardiovascular or psychiatric nature.

Because the control of appetite and energy expenditure is an unmet medical need, a considerable effort has been placed on the development of alternative therapies that avoid the unwanted central effects. A new strategy for treating obesity has been centered in understanding and controlling the actions of orexigenic/anorexigenic signals arising from the peripheral tissues, trying to moderate or even reverse the brain-oriented information that the metabolically relevant signals send from the gut, the adipose tissue, the liver, the pancreas, or the muscles.

As depicted in Fig. 66.1, upon the arrival of nutrients to the gut, a complex set of signals starts to emerge from the different sections of the gut, including mouth, stomach, and intestine. This information is derived from the direct effect of nutrients on sensors located along the different portions of the gut (Ariyasu et al., 2001; Gao & Horvath, 2007; Langhans, Leitner, & Arnold, 2011). In addition, the nutrients that reach the blood are delivered to metabolically relevant organs, where secondary signals help to orchestrate the adaptation to meals, or the response to fasting, in case there were long periods without food availability (Ellingsgaard et al., 2011). All these primary and secondary signals, together with specific nutrients (ie, glucose, aminoacids, fatty acids, such as lipoic acid) are either delivered to the hypothalamus or directly to the sensory terminals of the vagus nerve, that will inform, via the nucleus of the solitary tract, the hypothalamic network (Berthoud, 2008; Dietrich & Horvath, 2009). Because these signals can be either orexigenic or anorexigenic, it is reasonable to think that targeting this activity on the peripheral sites of origin may affect feeding behavior, without producing the unwanted adverse effects observed when brain target-designed drugs were used.

Among these signals, a special attention has been put on both the endocannabinoids and the stomach peptide ghrelin. In the present chapter, we review the interactions of endocannabinoids and ghrelin, both at peripheral and central levels, to control appetite. Combinatorial therapies aimed to antagonize these orexigenic signals might offer alternative approaches devoid of the unwanted side effects of classical central targets (Dietrich & Horvath, 2012).

THE PERIPHERAL ENDOCANNABINOID SYSTEM AND FEEDING REGULATION

The endocannabinoid system (ECS) is composed of a number of endogenous ligands synthesized from membrane phospholipids, mainly anandamide (AEA) and 2-arachidonoylglycerol (2-AG), the corresponding G protein coupled cannabinoid receptors CB1 and CB2, and a large number of enzymes involved in synthesis and degradation. The ECS is widely expressed throughout the body, especially in areas responsible for regulating the homeostatic control (Pagotto, Marsicano, Cota, Lutz, & Pasquali, 2006), helping to keep the tone of response to the requirements of the environment.

The CB1 receptor was originally described as brain receptor because of its wide expression in this organ. These receptors are located in brain areas that play a critical role in the homeostatic regulation of food intake, such as the hypothalamus, brainstem, and mesocorticolimbic system. Presently, we know that CB1 receptors are also expressed in peripheral organs, especially in those related to metabolic control. By contrast, CB2 receptor shows a more restricted distribution, and is mainly present in the immune system, although its presence in the gut and metabolically relevant tissues is documented (Bermúdez-Silva et al., 2008; Piomelli, 2003).

Activation of the ECS induces a large number of physiological responses in the main organs responsible for the regulation of food intake and metabolism, due to the coexistence of ECS in all signaling that regulates central and peripheral control of feeding. The central control of food intake is governed mainly by the hypothalamus, as a structure that receives sympathetic efferent inputs and peripheral hormones (leptin, CCK, ghrelin, GLP-1, glucocorticoids) reporting on the peripheral energy status. Peripheral response also depends on the different hormonal signals and sympathetic efferents. CB receptors are expressed widely in the hypothalamus, the reward system, the brainstem, the nucleus of the tractus solitarius (NTS), and other brain structures involved in feeding.

Increasing evidence indicates that the PNS (both sympathetic efferent branches and parasympathetic vagal afferents) mediates, through the engagement of the ECS, the crosstalk between the CNS and peripheral organs (Fig. 66.2). Endocannabinoids induce overfeeding, delaying the natural satiety induced by the arrival of food at the gut (Kirkham, Williams, Fezza, & Di Marzo, 2002). This effect is elicited by activation of CB1 receptors located in hypothalamic brain areas involved in appetite regulation. However, the peripheral ECS appears to contribute in a significant way to the regulation of feeding behavior. Intestinal AEA levels increase during fasting, and are abrogated by refeeding. Interestingly, the peripheral but not central administration of either anandamide or the synthetic agonist WIN 55,212-2 produce hyperphagia in rats partially satiated, and this effect disappears after vagotomy, indicating that cannabinoids promote food intake beyond the restricted hypothalamic control of the hunger and satiety mechanism (Gomez et al., 2002). Therefore, the administration of CB1 receptor antagonists has suppressive effect on feeding, as a result of the decline of endogenous cannabinoid tone. This reasoning was the basis of the development of clinical trials and the approval of the CB1 receptor antagonist rimonabant for the treatment of obesity, although its central effects generated adverse reactions that eventually led to its withdrawal from the market.

It is important to stress that the effects of pharmacological blockade of CB1 receptors on food intake and body weight are not due exclusively to a central hypothalamic and mesolimbic action. When the administration of the CB1 receptor antagonist, rimonabant, has no restriction effect on feeding by the development of tolerance to the drug, the rats still keep losing weight for 2 weeks (Colombo et al., 1998). Clinical studies have replicated these findings with alternative CB1 antagonists (Addy et al., 2008). All these data show that changes in the regulation of ECS produce a variety of central and peripheral metabolic actions that may be independent of food intake, and that are directly involved in the regulation of lipid and carbohydrate metabolism.

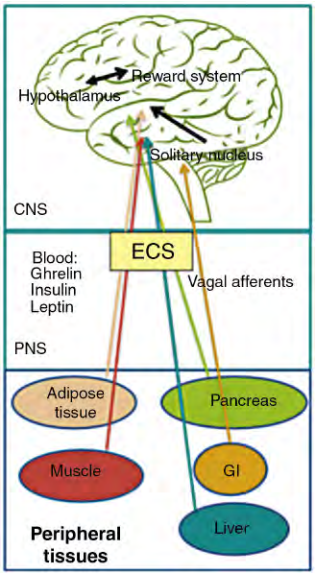


FIGURE 66.2 Schematic representation of the involvement of the endocannabinoid system in the peripheral and central regulation of food intake. The *arrows* represent connections between CNS and peripheral tissues, mediated by endocrine and the PN systems. In all these connections, the ECS plays a fundamental role based on the presence of CB receptors and their endogenous ligands. *CNS*, Central nervous system; *ECS*, endocannabinoid system; *GI*, gastrointestinal system; *PNS*, peripheral nervous system.

As mentioned before, CB1 receptors are extensively expressed in peripheral organs. In the gut, CB1 receptors are at even higher concentrations than in the brain, and they control intestinal motility, facilitate the inhibition of gastric emptying, and regulate from a distance the gustatory neural activity elicited by the sweet taste. This sensorial signal reaches the brain via the vagus nerve (cranial nerve X), which contains branches that communicate efferent and afferent food-related signals between brain and gut. In the adipose tissue, CB1 receptor stimulation facilitates fatty acid accumulation, and an increase in lipoprotein lipase activity, is reversed by CB1 antagonism (Cota et al., 2003). There is also an overexpression of CB1 receptors in adipocytes of obese rats. CB1 activation promotes neolipogenesis in the liver, the release of insulin by the pancreas, or a decrease of

glucose uptake induced by insulin in the skeletal muscle. In general, activation of the ECS occurs in the periphery, in situations where an energy deficit happens in order to activate a set of responses designed to obtain energy storage (Bermúdez-Silva et al., 2008).

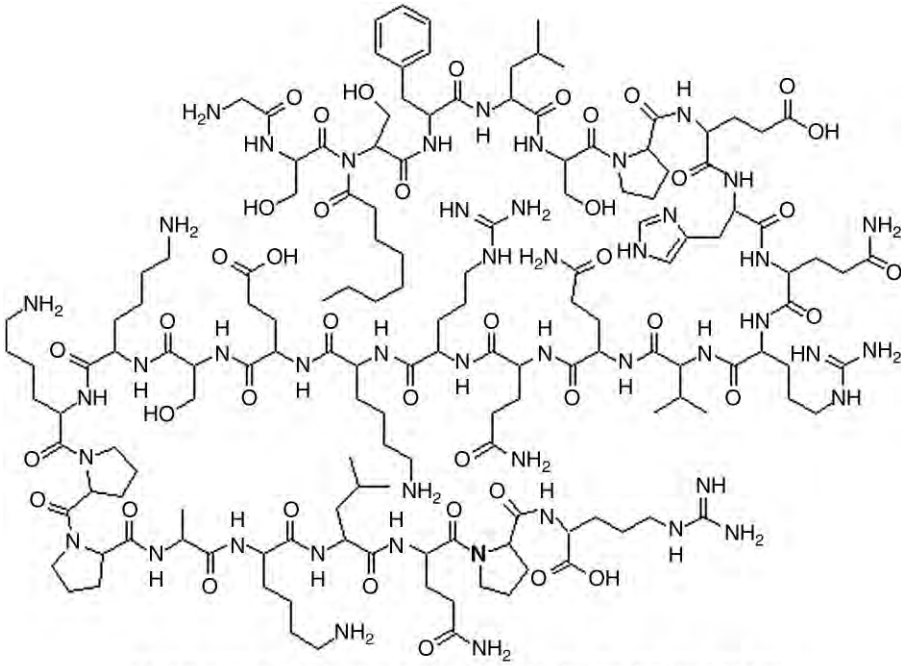
In conclusion, the ECS is expressed in all structures related to feeding, both in the central and peripheral nervous system. Peripheral organs contain their own ECS machinery which modulates its physiology. In general, cannabinoid activity exerts an anabolic effect that, on one hand, promotes food intake and, on the other hand, reduces intestinal and gastric motility, gastric acid secretion, vomiting and nausea, is antidiarrheal, and generates a prolipogenic action. In contrast, agents that block the CB1 receptors (antagonists) cause the opposite effects. All this activity makes the ECS an attractive and potentially effective multidrug target for many diseases involving the gut, the liver, and the control of metabolism and energy expenditure, such as obesity, diabetes, and appetite-related syndromes.

GHRELIN AND INTEGRATIVE SIGNALS FOR APPETITE

One of the peripheral signals that target the brain in order to control our feeding behavior is the hormone ghrelin (Fig. 66.3). Ghrelin is the only peripheral peptide hormone known to date which has orexigenic function, acting in a similar way than anandamide, as described earlier. The major source of ghrelin production is the stomach. Small quantities of ghrelin are also synthesized in other peripheral organs, such as the lower parts of the gastrointestinal tract, the pancreas or thymus, and in the brain, although the major contribution to circulating ghrelin levels appears to come from the stomach (Kojima et al., 1999; Ariyasu et al., 2001).

Levels of circulating ghrelin increase during fasting, and decrease immediately after food ingestion, which suggests that ghrelin may participate in meal initiation (Cummings et al., 2001). Conversely, a drop in circulating ghrelin levels is understood by the brain as a satiety signal. Ghrelin has also a role in the long-term control of body energy homeostasis by suppressing fat utilization, reducing energy expenditure, or promoting gluconeogenesis and adipose tissue deposition. The body mass index of one individual is negatively correlated with ghrelin levels. Indeed, levels of ghrelin are reduced in obese individuals and, conversely, increased in lean subjects, including anorectic patients.

Peripherally-produced ghrelin targets the CNS, specifically hypothalamic areas related to appetite regulation, via the vagus nerve, in order to transmit the hunger signal. Ghrelin may also cross the blood brain barrier (BBB), or reach the brain though circumventricular organs (BBB open areas) surrounding the hypothalamus (Banks, Tschop, Robinson, & Heiman, 2002; Merchenthaler, 1991). Ghrelin was originally found to



Gly-Ser-Ser(octanoyl)-Phe-Leu-Ser-Pro-Glu-His-Gln-Arg-Val-Gln-Gln-Arg-Lys-Glu-Ser-Lys-Lys-Pro-Pro-Ala-Lys-Leu-Gln-Pro-Arg-OH

FIGURE 66.3 **Chemical structure of human ghrelin.** The chemical structure of human ghrelin is represented in the figure. Molecular formula: $C_{149}H_{249}N_{47}O_{42}$. *Source: Reproduced with permission from http://www.chemblink.com/*

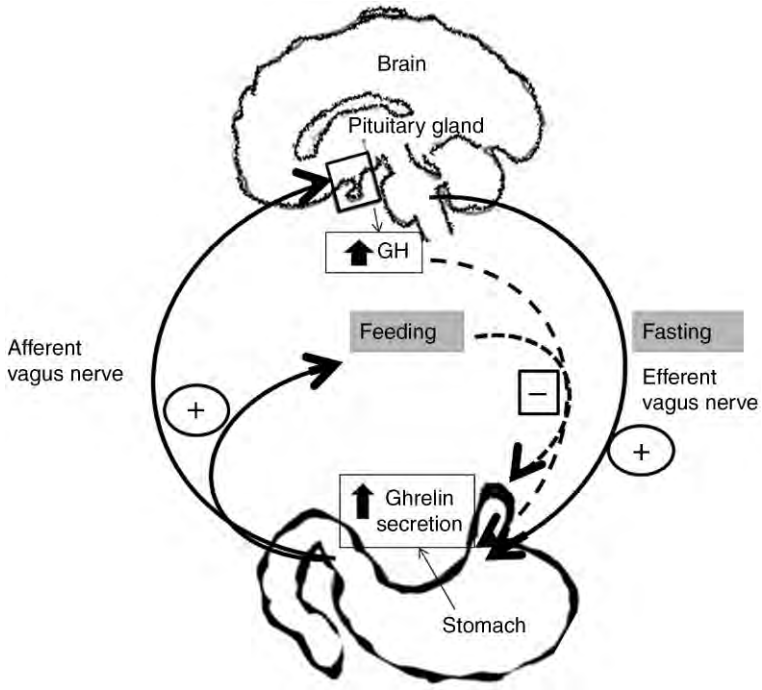


FIGURE 66.4 **Role of the vagus nerve in ghrelin actions and level regulation.** Ghrelin is mainly produced by the stomach, and released in the blood. Circulating ghrelin is increased during fasting, and decreased after feeding. The afferent vagus nerve contains ghrelin receptors that transmit ghrelin signal from the stomach to the hypothalamus, where the centers of appetite are located. Vagotomy eliminates both the ability of ghrelin to stimulate feeding, and GH release from the pituitary gland. The efferent vagus nerve has a role in increasing ghrelin secretion by the stomach, in fasting conditions, but it is not involved in the suppression of ghrelin levels after feeding. This is supported by the observation that vagotomy suppresses the increase in ghrelin production induced by food deprivation, but not baseline blood levels or reduction of ghrelin levels by nutrient infusion. Pituitary GH inhibits ghrelin secretion by the stomach as a feedback mechanism. *GH*, Growth hormone; continuous line and (+), means activation; discontinuous line and (–), means inhibition.

stimulate the release of growth hormone (GH) by binding to growth hormone secretagogue receptor (GHS-R1a) (Kojima et al., 1999). There is a regulatory system that controls GH and ghrelin: while ghrelin stimulates the release of GH from the pituitary, GH decreases ghrelin synthesis by the stomach as a feedback mechanism. The efferent vagus nerve contributes to ghrelin secretion by the stomach in fasting conditions, since vagotomy completely prevents fasting-induced ghrelin increase (Williams, Grill, Cummings, & Kaplan, 2003). Basal levels of ghrelin are not affected by vagotomy (Fig. 66.4).

Ghrelin receptors are located in hypothalamic areas that control appetite, such as the arcuate nucleus or the paraventricular nucleus, but also in the vagal nodose ganglia and the gastrointestinal tract, and their stimulation, as mentioned before, increases food consumption, reduces energy expenditure, and has adipogenic actions (Burdyga, Varro, Dimaline, Thompson, & Dockray, 2006; Guan et al., 1997; Kojima et al., 1999). Ghrelin receptors located in the nodose ganglia of the vagus nerve might be responsible of transmission of ghrelin signal from the stomach to the brain. Both peripheral and central administration of ghrelin increase feeding (Wren et al., 2000), and, inversely, blockade of GHS-R1a reduces food intake (Salome et al., 2009). The availability of nutrients in the gastrointestinal tract (but not its biodisponibility in blood) regulates the release of ghrelin. For example, glucose suppresses ghrelin levels (Overduin, Frayo, Grill, Kaplan, & Cummings, 2005), and interestingly, ghrelin may maintain circulating glucose levels (via GH release) during extreme fasting conditions (Zhao et al., 2010).

At a central level, ghrelin receptors located in NPY/AGPR-expressing neurons in the hypothalamus appear to be responsible of the orexigenic effect of this hormone. Ghrelin may also modulate feeding behavior by affecting the corticostriatal-limbic circuitry related to motivational behavior and reward, with the ventral hippocampus (Kanoski, Fortin, Ricks, & Grill, 2013), amygdala (Alvarez-Crespo et al., 2012), and ventral tegmental area (Abizaid et al., 2006) as the main neurobiological targets for these effects.

INTERACTIONS BETWEEN GHRELIN AND PERIPHERAL CB1 RECEPTORS IN FEEDING REGULATION

As mentioned before, both endocannabinoids (through CB1 receptors) and ghrelin modulate feeding behavior potently. There is a functional interaction between both

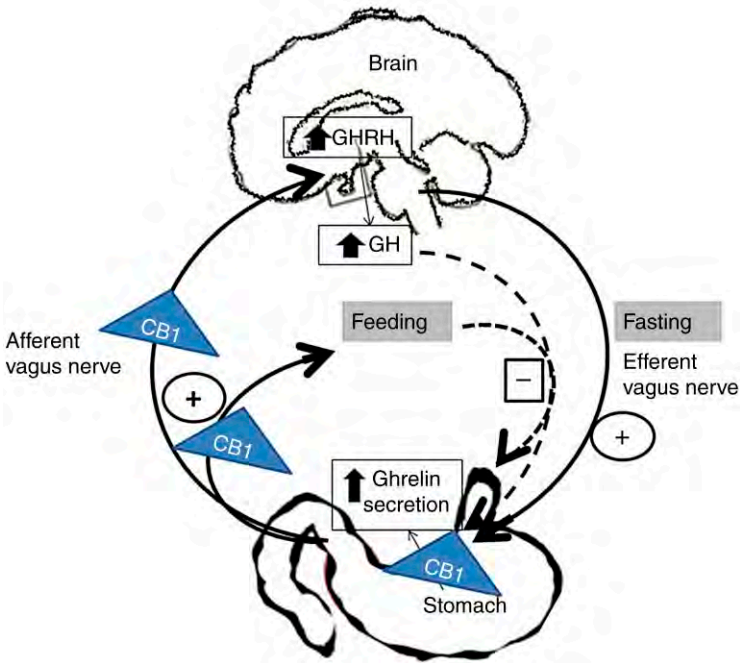


FIGURE 66.5 **Role of peripheral CB1 receptors in the release and activity of ghrelin.** Peripheral CB1 receptors located in the neuroendocrine cells of the stomach modulate gastric ghrelin secretion. CB1 receptors located in the afferent branch of the vagus nerve influence ghrelin-induced feeding, hypothalamic GHRH expression, and pituitary GH release. *GHRH*, Growth hormone releasing hormone; *GH*, growth hormone.

systems that has been described in vivo, although the specific site of such interaction is still not clear. The metabolic status influences both the endocannabinoid system and ghrelin. For example, food restriction increases endocannabinoid levels in the brain (Kirkham et al., 2002), modulates vagal CB1 receptor expression (Burdyga et al., 2006), and influences ghrelin circulating levels. It has been described that the orexigenic effects of ghrelin require the existence of functional CB1 receptors (Kola et al., 2008; Tucci, Rogers, Korbonits, & Kirkham, 2004) and, inversely, endocannabinoid-induced activation of the energy homeostasis regulator AMPK requires GHS-R1 mediated ghrelin signaling in hypothalamus, liver, and adipose tissue (Lim et al., 2013).

The interplay between CB1 receptors and ghrelin has been proposed to take place at central or peripheral levels. Ghrelin increases the endocannabinoid content in the hypothalamus, and a CB1 pharmacological blockade inhibits ghrelin hyperphagic activity in the PVN (Tucci et al., 2004). Additionally, genetic ablation of CB1 receptors abolishes ghrelin's orexigenic effects (Kola et al., 2008). Recently, it has been proposed that central CB1 receptors may simultaneously stimulate or inhibit food intake, depending on the neural circuits activated (Bellocchio et al., 2010). Although the widely assumed role for endocannabinoids is to increase appetite, endocannabinoids may also induce hypophagia, under stimulated conditions, by acting in the PVN. Interestingly, ghrelin influences the neuronal activity in the PVN, and might determine the positive or negative effects of CB1 receptors in feeding (Soria-Gómez, Bellocchio, & Marsicano, 2014).

Peripheral CB1 receptors may also interplay with ghrelin, in the control of food intake (Fig. 66.5). Several studies support a crosstalk between peripheral CB1 receptors and ghrelin, in appetite. Firstly, gastric CB1 receptors modulate the secretion of ghrelin by the stomach (Senin et al., 2013). Secondly, peripheral CB1 receptors located in the vagus nerve influence central ghrelin activity. The vagus nerve has a dominant role in the transmission of the hunger signal induced by endocannabinoids and ghrelin to hypothalamic areas and, ultimately, in the regulation of energy balance. The nodose ganglia (inferior ganglion of vagus nerve) coexpress CB1 and GHS-R1 receptors (Burdyga et al., 2006; Date et al., 2005). Food administration induces a downregulation of CB1 receptors in the human nodose ganglia, prevented by ghrelin administration (Burdyga et al., 2006). Al-Massadi et al. (2010) showed that systemic administration of a CB1 antagonist decreases ghrelin-induced release of GH and hypothalamic growth hormone releasing hormone (GHRH) mRNA expression. Surgical vagotomy or central CB1 antagonist administration abolishes this effect. According to this, peripheral CB1 receptors located in the vagus nerve afferent branch facilitate the hypothalamic release of GHRH, and posterior ghrelin-mediated release of GH by the pituitary gland, which is implicated in energy homeostasis (Al-Massadi et al., 2010). Interestingly, peripheral CB1 receptors may also mediate ghrelin-induced hyperphagia, since an ultra-low dose of a CB1

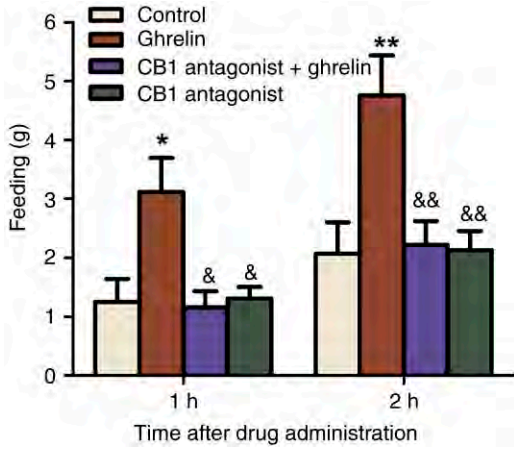


FIGURE 66.6 Peripherally restricted CB1 antagonist (LH-21) counteracts ghrelin-induced hyperphagia in free feeding rats. Intracerebroventricular administration of ghrelin induces an increase in food intake in animals with free access to food. Pretreatment with the compound LH-21, a CB1 antagonist that does not cross the BBB, reduces ghrelin-induced orexigenic effect, with no effect in control animals. *, Statistically different from control group; &, statistically different from ghrelin-treated group. *Source: Modified from data in Alen et al. (2013) (with permission of PLoS One).*

antagonist (that is not centrally active) or a peripherally-restricted CB1 antagonist inhibit the orexigenic effect of ghrelin under balanced energy balance conditions (Alen et al., 2013; Fig. 66.6).

As a conclusion, while targeting CB1 receptors to fight against obesity would be an appropriate pharmacological alternative, the associated central side effects have to be considered. CB1 antagonist-based pharmacotherapies are efficient in reducing body weight and improving metabolic parameters (Pagotto et al., 2006), but they induce undesirable relevant side effects in the CNS, such as depression, anxiety, and even suicidal ideas (Christensen, Kristensen, Bartels, Bliddal, & Astrup, 2007). Because of the psychiatric collateral side effects, CB1 antagonists are no longer allowed as a pharmacotherapy for obesity. In this context, targeting CB1 receptors outside of the blood brain barrier emerges as an attractive alternative to treat obesity, since peripheral restricted CB1 antagonists maintain the benefits on energy homeostasis regulation, avoiding undesirable psychiatric effects. Interestingly, the association of a peripheral cannabinoid CB1 receptor antagonist with a drug targeting additional peripheral signals that regulate feeding offers unexplored alternatives that might lead to safer but effective therapies. In the present review, the identification of ghrelin as a signal that might be counteracted by cannabinoid receptor antagonists indicates that a potential association of these peripheral cannabinoid receptor antagonists with a ghrelin antagonist might have synergistic effects as feeding inhibitors. Other gut peptide that can be considered for this strategy is cholecystokinin (Orio et al., 2011), although the multiplicity of gut peptides controlling appetite and metabolism opens additional possibilities. In addition, other endocannabinoid-like substances that target noncannabinoid receptors may have a beneficial profile to control obesity, when combined with peripheral ghrelin antagonists. For example, oleoylethanolamide (OEA) is a fatty acid ethanolamide that does not bind CB1 receptors, and is emerging as a good candidate to treat obesity, due to the metabolic benefits and its ability to control peptides regulating food intake, including ghrelin (Cani, Montoya, Neyrinck, Delzenne, & Lambert, 2004; Serrano et al., 2008, 2011). A whole research area on combinatorial therapies based in these peripheral signals demands to be considered for future studies.

MINI-DICTIONARY

Combinational therapy A therapy where several targets, usually two, are being modulated by the concurrent administration of several drugs. A combinational therapy searches for synergistic effects. A combinational therapy might reduce unwanted effects without a loss of effectiveness.

Endocannabinoid system A lipid signaling system which includes the cannabinoid receptors, the endogenous lipid ligands (endocannabinoids), and the enzymatic machinery for their synthesis and inactivation. This system regulates a wide range of biological functions, including nociception, motor activity, cognitive and emotional processes, immune function, and energy balance.

Endocannabinoids Family of biologically active lipids that bind to CB1 and CB2 receptors. They include several derivatives of arachidonic acid, generated on demand by neurons and other cells, in response to physiological or pathological stimuli. The two best characterized endocannabinoids are 2-arachidonoyl-*sn*-glycerol (2-AG) and anandamide (AEA).

Ghrelin Peptidic hormone released mainly by endocrine cells of the stomach; it has orexigenic functions. There are receptors for ghrelin in the hypothalamus.

Hypothalamus Brain area located in the diencephalon which integrates central neuropeptides and peripheral hormones involved in feeding and energy homeostasis.

Nodose ganglia Collection of sensorial neurons located in the caudal part of the vagus nerve.

Vagotomy The surgical cutting of the vagus nerve. In research, it is used as strategy to examine the peripheral versus central actions of compounds (such as ghrelin).

Vagus nerve The tenth pair and largest cranial nerve which sends to the brain afferent visceral information related to feeding. It is a mixed nerve, since it also conveys parasympathetic efferents from the brain to the thoracic and abdominal cavities.

References

Abizaid, A., Liu, Z. W., Andrews, Z. B., Shanabrough, M., Borok, E., Elsworth, J. D., Roth, R. H., Sleeman, M. W., Picciotto, M. R., Tschop, M. H., Gao, X. B., & Horvath, T. L. (2006). Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *Journal of Clinical Investigation, 116*, 3229–3239.

Addy, C., Wright, H., Van Laere, K., Gantz, I., Erondu, N., Musser, B. J., Lu, K., Yuan, J., Sanabria-Bohórquez, S. M., Stoch, A., Stevens, C.,

Fong, T. M., De Lepeleire, I., Cilissen, C., Cote, J., Rosko, K., Gendrano, I. N., III, Nguyen, A. M., Gumbiner, B., Rothenberg, P., de Hoon, J., Bormans, G., Depré, M., Eng, W. S., Ravussin, E., Klein, S., Blundell, J., Herman, G. A., Burns, H. D., Hargreaves, R. J., Wagner, J., Gottesdiener, K., Amatruda, J. M., & Heymsfield, S. B. (2008). The acyclic CB1R inverse agonist taranabant mediates weight loss by increasing energy expenditure and decreasing caloric intake. *Cell Metabolism*, *7*, 68–78.

Al-Massadi, O., Gabellieri, E., Trujillo, M. L., Señaris, R., Pagotto, U., Pasquali, R., Casanueva, F. F., & Seoane, L. M. (2010). Peripheral endocannabinoid system-mediated actions of rimonabant on growth hormone secretion are ghrelin-dependent. *Journal of Neuroendocrinology*, *22*, 1127–1136.

Alen, F., Crespo, I., Ramirez-Lopez, M. T., Jagerovic, N., Goya, P., de Fonseca, F. R., de Heras, R. G., & Orio, L. (2013). Ghrelin-induced orexigenic effect in rats depends on the metabolic status and is counteracted by peripheral CB1 receptor antagonism. *PLoS One*, *8*, e60918.

Alvarez-Crespo, M., Skibicka, K. P., Farkas, I., Molnar, C. S., Egecioglu, E., Hrabovszky, E., Liposits, Z., & Dickson, S. L. (2012). The amygdala as a neurobiological target for ghrelin in rats: neuroanatomical, electrophysiological and behavioral evidence. *PLoS One*, *7*, e46321.

Ariyasu, H., Takaya, K., Tagami, T., Ogawa, Y., Hosoda, K., Akamizu, T., Suda, M., Koh, T., Natsui, K., Toyooka, S., Shirakami, G., Usui, T., Shimatsu, A., Doi, K., Hosoda, H., Kojima, M., Kangawa, K., & Nakao, K. (2001). Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *Journal of Clinical Endocrinology and Metabolism*, *86*, 4753–4758.

Banks, W. A., Tschop, M., Robinson, S. M., & Heiman, M. L. (2002). Extent and direction of ghrelin transport across the blood–brain barrier is determined by its unique primary structure. *Journal of Pharmacology and Experimental Therapeutics*, *302*, 822–827.

Bellocchio, L., Lafenetre, P., Cannich, A., Cota, D., Puente, N., Grandes, P., Chaouloff, F., Piazza, P. V., & Marsicano, G. (2010). Bimodal control of stimulated food intake by the endocannabinoid system. *Nature Neuroscience*, *13*, 281–283.

Bermúdez-Silva, F. J., Suárez, J., Baixeras, E., Cobo, N., Bautista, D., Cuesta-Muñoz, A. L., Fuentes, E., Juan-Pico, P., Castro, M. J., Milman, G., Mechoulam, R., Nadal, A., & Rodríguez de Fonseca, F. (2008). Presence of functional cannabinoid receptors in human endocrine pancreas. *Diabetologia*, *51*, 476–487.

Berthoud, H. R. (2008). The vagus nerve, food intake and obesity. *Regulatory Peptides*, *149*, 15–25.

Burdyga, G., Varro, A., Dimaline, R., Thompson, D. G., & Dockray, G. J. (2006). Ghrelin receptors in rat and human nodose ganglia: putative role in regulating CB-1 and MCH receptor abundance. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *290*, G1289–G1297.

Cani, P. D., Montoya, M. L., Neyrinck, A. M., Delzenne, N. M., & Lambert, D. M. (2004). Potential modulation of plasma ghrelin and glucagon-like peptide-1 by anorexigenic cannabinoid compounds, SR141716A (rimonabant) and oleoylethanolamide. *British Journal of Nutrition*, *92*, 757–761.

Christensen, R., Kristensen, P. K., Bartels, E. M., Bliddal, H., & Astrup, A. (2007). Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. *Lancet*, *370*, 1706–1713.

Colombo, G., Agabio, R., Diaz, G., Lobina, C., Reali, R., & Gessa, G. L. (1998). Appetite suppression and weight loss after the cannabinoid antagonist SR 141716. *Life Science*, *63*, L113–L117.

Cota, D., Marsicano, G., Tschoep, M., Gruebler, Y., Flachskamm, C., Schubert, M., Auer, D., Yassouridis, A., Thone-Reineke, C., Ortmann, S., Tomassoni, F., Cervino, C., Nisoli, E., Linthorst, A. C., Pasquali, R., Lutz, B., Stalla, G. K., & Pagotto, U. (2003). The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripherallipogenesis. *Journal of Clinical Investigation*, *112*, 423–431.

Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E., & Weigle, D. S. (2001). A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*, *50*, 1714–1719.

Date, Y., Toshinai, K., Koda, S., Miyazato, M., Shimbara, T., Tsuruta, T., Niijima, A., Kangawa, K., & Nakazato, M. (2005). Peripheral interaction of ghrelin with cholecystokinin on feeding regulation. *Endocrinology*, *146*, 3518–3525.

Diéguez, C., Vazquez, M. J., Romero, A., López, M., & Nogueiras, R. (2011). Hypothalamic control of lipid metabolism: focus on leptin, ghrelin and melanocortins. *Neuroendocrinology*, *94*, 1–11.

Dietrich, M. O., & Horvath, T. L. (2009). Feeding signals and brain circuitry. *European Journal of Neuroscience*, *30*, 1688–1696.

Dietrich, M. O., & Horvath, T. L. (2012). Limitations in anti-obesity drug development: the critical role of hunger-promoting neurons. *Nature Reviews Drug Discovery*, *11*, 675–691.

Ellingsgaard, H., Hauselmann, I., Schuler, B., Habib, A. M., Baggio, L. L., Meier, D. T., EpplervE, Bouzakri, K., Wueest, S., Muller, Y. D., Hansen, A. M., Reinecke, M., Konrad, D., Gassmann, M., Reimann, F., Halban, P. A., Gromada, J., Drucker, D. J., Gribble, F. M., Ehses, J. A., & Donath, M. Y. (2011). Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. *Nature Medicine*, *17*, 1481–1489.

Gao, Q., & Horvath, T. L. (2007). Neurobiology of feeding and energy expenditure. *Annual Review of Neuroscience*, *30*, 367–398.

Gomez, R., Navarro, M., Ferrer, B., Trigo, J. M., Bilbao, A., Del Arco, I., Cippitelli, A., Nava, F., Piomelli, D., & Rodríguez de Fonseca, F. (2002). A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. *Journal of Neuroscience*, *22*, 9612–9617.

Guan, X. M., Yu, H., Palyha, O. C., McKee, K. K., Feighner, S. D., Sirinathsinghji, D. J., Smith, R. G., Van der Ploeg, L. H., & Howard, A. D. (1997). Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Research. Molecular Brain Research*, *48*, 23–29.

Kanoski, S. E., Fortin, S. M., Ricks, K. M., & Grill, H. J. (2013). Ghrelin signaling in the ventral hippocampus stimulates learned and motivational aspects of feeding via PI3K-Akt signaling. *Biological Psychiatry*, *73*, 915–923.

Kirkham, T. C., Williams, C. M., Fezza, F., & Di Marzo, V. (2002). Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *British Journal of Pharmacology*, *136*, 550–557.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., & Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, *402*, 656–660.

Kola, B., Farkas, I., Christ-Crain, M., Wittmann, G., Lolli, F., Amin, F., Harvey-White, J., Liposits, Z., Kunos, G., Grossman, A. B., Fekete, C., & Korbonits, M. (2008). The orexigenic effect of ghrelin is mediated through central activation of the endogenous cannabinoid system. *PLoS One*, *3*, e1797.

Langhans, W., Leitner, C., & Arnold, M. (2011). Dietary fat sensing via fatty acid oxidation in enterocytes: possible role in the control of eating. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *300*, R554–R565.

Lim, C. T., Kola, B., Feltrin, D., Perez-Tilve, D., Tschop, M. H., Grossman, A. B., & Korbonits, M. (2013). Ghrelin and cannabinoids require the ghrelin receptor to affect cellular energy metabolism. *Molecular and Cell Endocrinology*, *365*, 303–308.

Merchenthaler, I. (1991). Neurons with access to the general circulation in the central nervous system of the rat: a retrograde tracing study with fluoro-gold. *Neuroscience*, *44*, 655–662.

Orio, L., Crespo, I., López-Moreno, J. A., Reyes-Cabello, C., Rodríguez de Fonseca, F., & Gómez de Heras, R. (2011). Additive effects of

cannabinoid CB1 receptors blockade and cholecystokinin on feeding inhibition. *Pharmacology Biochemistry and Behavior*, *98*, 220–226.

Overduin, J., Frayo, R. S., Grill, H. J., Kaplan, J. M., & Cummings, D. E. (2005). Role of the duodenum and macronutrient type in ghrelin regulation. *Endocrinology*, *146*, 845–850.

Pagotto, U., Marsicano, G., Cota, D., Lutz, B., & Pasquali, R. (2006). The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocrine Reviews*, *27*, 73–100.

Piomelli, D. (2003). The molecular logic of endocannabinoid signalling. *Nature Reviews Neuroscience*, *4*, 873–884.

Salome, N., Haage, D., Perrissoud, D., Moulin, A., Demange, L., Egecioglu, E., Fehrentz, J. A., Martinez, J., & Dickson, S. L. (2009). Anorexigenic and electrophysiological actions of novel ghrelin receptor (GHS-R1A) antagonists in rats. *European Journal of Pharmacology*, *612*, 167–173.

Senin, L. L., Al-Massadi, O., Folgueira, C., Castelao, C., Pardo, M., Barja-Fernandez, S., Roca-Rivada, A., Amil, M., Crujeiras, A. B., Garcia-Caballero, T., Gabellieri, E., Leis, R., Dieguez, C., Pagotto, U., Casanueva, F. F., & Seoane, L. M. (2013). The gastric CB1 receptor modulates ghrelin production through the mTOR pathway to regulate food intake. *PLoS One*, *8*, e80339.

Serrano, A., Del Arco, I., Javier Pavon, F., Macias, M., Perez-Valero, V., & Rodriguez de Fonseca, F. (2008). The cannabinoid CB1 receptor antagonist SR141716A (rimonabant) enhances the metabolic benefits of long-term treatment with oleoylethanolamide in Zucker rats. *Neuropharmacology*, *54*, 226–234.

Serrano, A., Pavon, F. J., Tovar, S., Casanueva, F., Senaris, R., Dieguez, C., & de Fonseca, F. R. (2011). Oleoylethanolamide: effects on hypothalamic transmitters and gut peptides regulating food intake. *Neuropharmacology*, *60*, 593–601.

Shirazi, R., Palsdottir, V., Collander, J., Anesten, F., Vogel, H., Langlet, F., Jaschke, A., Schürmann, A., Prévot, V., Shao, R., Jansson, J. O., & Skibicka, K. P. (2013). Glucagon-like peptide 1 receptor induced suppression of food intake, and body weight is mediated by central IL-1 and IL-6. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 16199–16204.

Soria-Gómez, E., Bellocchio, L., & Marsicano, G. (2014). New insights on food intake control by olfactory processes: the emerging role of the endocannabinoid system. *Molecular and Cellular Endocrinology*, *397*, 59–66.

Tucci, S. A., Rogers, E. K., Korbonits, M., & Kirkham, T. C. (2004). The cannabinoid CB1 receptor antagonist SR141716 blocks the orexigenic effects of intrahypothalamic ghrelin. *British Journal of Pharmacology*, *143*, 520–523.

Williams, D. L., Grill, H. J., Cummings, D. E., & Kaplan, J. M. (2003). Vagotomy dissociates short- and long-term controls of circulating ghrelin. *Endocrinology*, *144*, 5184–5187.

Wren, A. M., Small, C. J., Ward, H. L., Murphy, K. G., Dakin, C. L., Taheri, S., Kennedy, A. R., Roberts, G. H., Morgan, D. G., Ghatei, M. A., & Bloom, S. R. (2000). The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology*, *141*, 4325–4328.

Zhao, T. J., Liang, G., Li, R. L., Xie, X., Sleeman, M. W., Murphy, A. J., Valenzuela, D. M., Yancopoulos, G. D., Goldstein, J. L., & Brown, M. S. (2010). Ghrelin O-acyltransferase (GOAT) is essential for growth hormone-mediated survival of calorie-restricted mice. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 7467–7472.

CHAPTER

67

Pharmacological Aspects of Novel Antiobesity Agents Related to Cannabinoids

L. Hernandez-Folgado

Institute of Medicinal Chemistry, CSIC, Madrid, Spain

SUMMARY POINTS

- Obesity appears to be associated with a dysregulated and hyperactive endocannabinoid system, in rodents and humans.
- Animal studies and clinical trials have shown that blockade of CB1 cannabinoid receptor induces weight loss, improves cardiometabolic risk factors, and insulin resistance, and causes metabolic benefits in mammals, so blockade of this receptor is considered a challenged strategy in obesity.
- Since rimonabant was withdrawn in 2009, weight-loss strategies were aimed to design and prepare CB1 neutral antagonists, acting either centrally or peripherally, or CB1 inverse agonists acting only at a peripheral level.
- CB1 neutral antagonists and CB1 inverse agonists structurally related to rimonabant, such as AM6545, LH21, NESS038C6, otenabant, and so on, have been reported.
- In addition, novel cannabinoid targets to develop antiobesity drugs, such as CB2 cannabinoid receptor, the putative cannabinoid GPR55 receptor, and 2-monoacylglycerol lipase, are being taken into account.

KEY FACTS OF OBESITY

- Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health.
- Obesity is one of the greatest public health challenges of the 21st century.
- Its prevalence has tripled in many countries of the WHO European Region since the 1980s, and the numbers of those affected continue to rise at an alarming rate.
- More than 40 million children under the age of five were overweight in 2011.
- Current treatment for obese patients is based on diet and practicing exercise, combined with pharmacotherapy.

LIST OF ABBREVIATIONS

AA	Arachidonic acid
BMI	Body mass index
CB1, CB2	Cannabinoid receptors type 1 and 2
CNS	Central nervous system
DIO	Diet-induced obesity
ECS	Endocannabinoid system
EMA	European Medicines Agency

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00078-8
Copyright © 2017 Elsevier Inc. All rights reserved.

ICR	Imprinting control region
KO	Knockout
LPI	Lysophosphatidylinositol
MDMA	3,4-Methylenedioxymethamphetamine
MGL	2-Monoacylglycerol lipase
PET	Positron emission tomography
PPARα	Peroxisome proliferator-activated receptor-α
PUFA	Polyunsaturated fatty acids
THCV	Δ^9-Tetrahydrocannabivarin
WAT	White adipose tissue
WHO	World Health Organization

OBESITY AND WEIGHT-LOSS DRUGS

The global epidemic of overweight and obesity—"globesity"—is rapidly becoming a major public health problem in many parts of the world. According to the WHO (http://www.who.int/mediacentre/factsheets/fs311/en/), obesity is one of the greatest public health challenges of the 21st century. Its prevalence has tripled in many countries of the WHO European Region since the 1980s, and the numbers of those affected continue to rise at an alarming rate.

Obesity is a complex metabolic disorder characterized by an imbalance in energy homeostasis, abnormal increase of adipose tissue, and dysregulation of hormones, cytokines, and other important signaling systems. The increasing prevalence of overweight and obesity is associated with many diet-related chronic diseases, including diabetes mellitus, hepatic steatosis, cardiovascular risk, sleep apnea, stroke, hypertension, and certain cancers.

Current treatment for overweight or obese patients is based on diet and practicing exercise, combined with pharmacotherapy (Table 67.1). In the extreme cases of morbid obesity (BMI > 40), gastric bypass surgery is also considered. A successful weight-loss drug should reduce energy intake and/or increase energy expenditure, without adverse side effects. However, most of the antiobesity drugs commercialized in the last 20 years were withdrawn due to their severe side effects (Adan, 2013). Phentermine, an amphetamine, was combined with fenfluramine in FenPhen. This successful drug was withdrawn because it often induced pulmonary hypertension. Sibutramine, a noradrenalin and serotonin reuptake inhibitor, was withdrawn in 2010 because of cardiovascular side effects, which resulted in increased heart attacks and strokes.

Orlistat is currently the only widely available monotherapy for a long-term treatment of obesity. It is a gastric and pancreatic lipase inhibitor that has poor compliance because of its side effects (eg, fecal incontinence, oily stools). Lorcaserin and Qsymia are novel additions to the limited repertoire of antiobesity drugs. Lorcaserin, a serotonin (5-HT2c) agonist, is not free of side effects such as headache, dizziness, and nausea. Whereas the anticonvulsant topiramate as monotherapy results in 6–7% more body weight loss compared to placebo, when it is combined with phentermine (in Qsymia), it has an efficacy of 9–10% more body weight-loss, compared to placebo, after 1 year of treatment. This combination has a noradrenalin releaser-anticonvulsant mechanism of action, and little is known about where and how it works. It has considerable side effects, which are mainly related to brain function, and even possible teratogenic effects.

Two upcoming drugs are being under clinical trials: one is Contrave, a combination of bupropion (noradrenalin/dopamine reuptake inhibitor) and naltrexone (opioid receptor antagonist), with nausea its most common side effect; and Tesofensine, a serotonin/noradrenalin/dopamine reuptake inhibitor which shows depressed mood and cardiovascular side effects.

As shown earlier, targeting monoaminergic systems (serotoninergic, noradrenergic, and dopaminergic) was the main approach to develop antiobesity drugs. But, in the 1990s, the endocannabinoid system was found

TABLE 67.1 Past and Current Antiobesity Drugs

Drug	Approval	Efficacy	Remarks
FenPhen	In the 1990s	Up to 10% body weight loss	Withdrawn
Sibutramine	In the late 1990s	Loss of 4–5 kg over 1 year	Withdrawn in 2010
Rimonabant	2006 (Europe)	Decrease in body weight and waist circumference	Withdrawn in 2009
Orlistat	1998	Loss of 2–3 kg over 1 year	Only monotherapy for a long-term treatment
Lorcaserin	2012	3–4% weight loss over 1 year	—
Qsymia	2012	9–10% weight loss over 1 year	—
Contrave	In clinical trials	4–5% weight loss over 1 year	Declined in 2011 and refiled
Tesofensine	In Phase III	—	—

Data of antiobesity drugs that were withdrawn, are currently available, or are in advanced clinical trials.

to be involved in food intake and energy homeostasis, so it was considered a promising target for weight-loss drugs.

ENDOCANNABINOID SYSTEM AND OBESITY

Obesity appears to be associated with a dysregulated and hyperactive endocannaboboid system (ECS), in rodents and humans. There is an increase in circulating endocannabinoid levels and altered expression of the components of this system, in cerebral areas such as hypothalamus, hindbrain, and limbic areas, but also in peripheral organs, including adipose tissue, liver, and pancreas.

The widespread presence of the ECS indicates its physiological relevance in the regulation of a variety of biological processes. In the last years, there has been substantial interest in investigating the role of endocannabinoid signaling in the regulation of metabolism and energy homeostasis, mainly due to the abundance of cannabinoid receptor type 1 (CB1) expression in brain regions and peripheral tissues involved in the control of feeding behavior and energy balance (Serrano et al., 2012). CB1 receptors, as well as endocannabinoid producing machinery, have been described in the gut, liver, muscle, endocrine pancreas, and white adipose tissue (WAT). In this regard, animal studies and clinical trials have shown that blockade of CB1 receptor induces weight loss, improves cardiometabolic risk factors and insulin resistance, and causes metabolic benefits in mammals. Therefore, all these data have emphasized the potential of CB1 receptor blockade as a therapeutic strategy in obesity.

Consequently, rimonabant (trade name Acomplia), an inverse agonist at the cannabinoid CB1 receptor acting mainly at the central nervous system (CNS), was developed by Sanofi-Aventis in the 1990s (Rinaldi-Carmona et al., 1994). It was approved by the European Medicines Agency (EMA) and introduced in Europe in 2006 (Table 67.1). Rimonabant, when combined with a hypocaloric diet over 1–2 years, can promote a clinically significant decrease in body weight and waist circumference, an increase in HDL cholesterol, a reduction in triglycerides, and an improvement of cardiovascular risk factors (Pi-Sunyer, Aronne, Heshmati, Devin, & Rosenstock, 2006). Due to its central action, adverse events of severe depression and suicidal thoughts were frequently reported with its use. Therefore, it was withdrawn in 2009 by the European Regulatory Authorities.

It was clear that peripheral blockade of CB1 receptors may induce metabolic advantages for complicated obesity. However, a major goal was to avoid the unwanted central effects. Thus, alternative approaches were searched for blocking CB1 receptors preventing the side effects of rimonabant. One option was to design and prepare CB1 neutral antagonists, acting either centrally or peripherally, or CB1 inverse agonists acting only at a peripheral level; and another option was to develop dual ligands or combinatorial therapies that exploit synergies between CB1 antagonists/inverse agonists and additional targets, by enhancing thermogenesis and blocking lipid mobilization (Fernández-Fernández et al., 2013). Regarding the first option, in the literature, there is a controversy about whether CB1 receptor blocking at only peripheral level achieves the same effects on feeding behavior than at both central and peripheral levels at the same time. Moreover, some authors suggest that, among peripherally restricted compounds, CB1 inverse agonists may offer distinct advantages over neutral antagonists; particularly as far as weight reduction and insulin sensitization are concerned. Meanwhile, other authors demonstrate that a CB1 neutral antagonist, which leaves the constitutive CB1 activity intact is just as efficient in reducing weight gain as a CB1 inverse agonist, but lacks potentially harmful effects on anxiety and motivation.

To further deepen in these issues, the following sections cover the main results reported from 2010 to the beginning of 2014 about developing novel antiobesity agents related to cannabinoids.

ANTIOBESITY DRUGS STRUCTURALLY RELATED TO RIMONABANT

As it was already mentioned, rimonabant, a diarylpyrazole derivative (Fig. 67.1), was the first selective CB1 inverse agonist to be reported (Rinaldi-Carmona et al., 1994). It is a very useful pharmacological tool, being to date the CB1 inverse agonist of reference in most of the preclinical in vitro and in vivo assays. Even after its withdrawal from the European market, many structural analogs have been evaluated in feeding behavior. They are being synthesized and characterized to prevent adverse effects observed with rimonabant while maintaining antiobesity properties.

In this section, the design, molecular structure, and pharmacology of these so-called second generation CB1 antagonists/inverse agonists will be described.

CB1 Antagonists

One of the most interesting compound is AM6545 (Fig. 67.1), a neutral CB1 antagonist with relatively poor penetrability into the brain (Cluny et al., 2010). This drug reduces food intake in animals fed high-fat and high-carbohydrate diets, but it is less effective in reducing

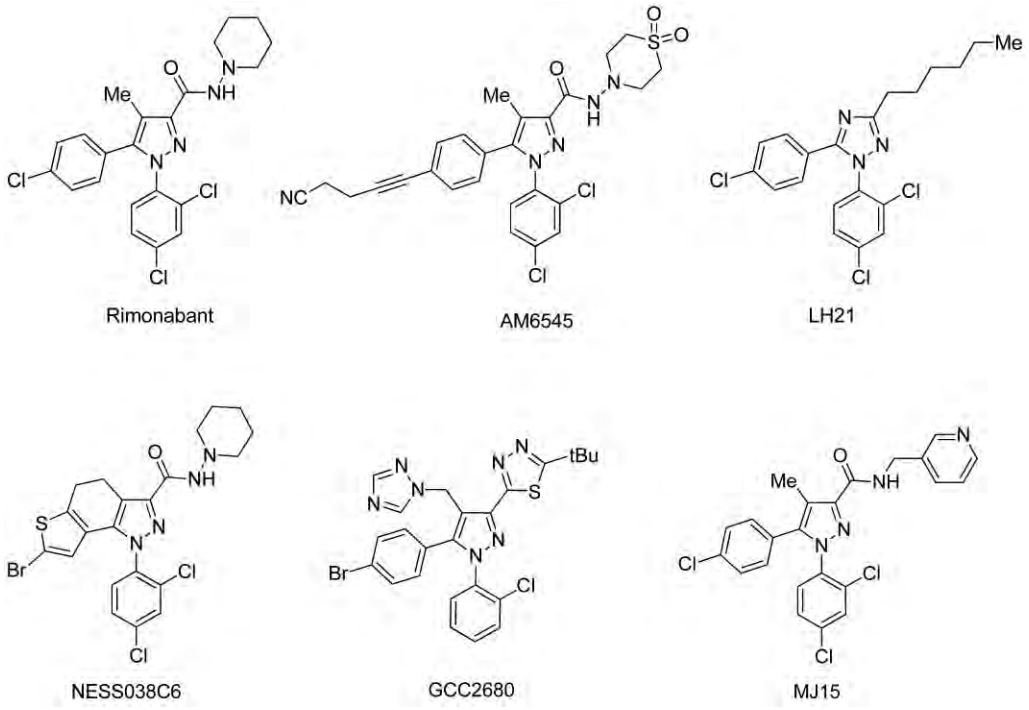


FIGURE 67.1 **Rimonabant and novel CB1 antagonists.** Structures of rimonabant and novel CB1 antagonists related to rimonabant.

regular chow intake. Due to its low brain penetration, AM6545 does not affect behavioral responses mediated by CB1 receptors in the brain, such as catalepsy, hypomotility, hypothermia, and anxiety-like behaviors. AM6545 reduces body weight gain and the adiposity index, improving the metabolic profile of obese mice fed a high-fat diet in a food intake–independent manner. In fact, this drug has been reported to decrease lipogenic and increase lipolytic gene expression in both liver and adipose tissue. Moreover, AM6545 treatment reverses the hepatic steatosis induced by high-fat diet, reducing liver triglycerides and hepatocellular damage. By contrast, AM6545 has no effect on body weight and adiposity in *ob/ob* mice, likely due to leptin signaling deficiency. In addition, AM6545 is also able to reduce food intake and body weight gain in CB1 receptor knockout (KO) mice, indicating the existence of other alternative pathways. Cluny et al. (2010) suggest that hypophagia mediated by cannabinoid receptors is not solely dependent on central activation. In fact, the feeding behavior of AM6545 seems to be dependent only on peripheral effects.

Jagerovic et al. (2004) synthesized and characterized LH21 (Fig. 67.1), a triazole analog of rimonabant, that acts as a CB1 neutral antagonist. LH21 displays a different pharmacological profile than rimonabant. Acute administration of this compound reduces food intake in fasted rats. Similarly, subchronic treatment causes hypophagia and reduces body weight gain in obese rats, but with no metabolic effects. LH21 is more efficacious as an antiobesity drug in obese rats exposed to enriched-fat diet than in control rats, likely due to the dysregulation and overactivation of the ECS associated to obesity. Contrary to the metabolic benefits of rimonabant, obese rats treated with LH21 do not display an improvement of the obesity-associated hepatic steatosis. The poor penetration of LH21 into the CNS indicates that its anorectic effects are mainly mediated by peripheral CB1 receptors located in gut sensory terminals that control satiety, and also with cannabinoid receptors located in metabolically relevant tissues. A study from 2012 has indicated that the antiobesity properties of LH21 may be mediated through modulation of the lipogenic pathways in adipose tissue (Alonso et al., 2012). In adipocytes, LH21 treatment also affects the gene expression of leptin, reducing its transcription, and improving the leptin resistance associated to obesity. In a recent study, Alen et al. (2013) investigated whether peripheral-acting CB1 antagonists, such as LH21, may modulate other signals controlling appetite, such as ghrelin. Ghrelin is an endogenous regulator of energy homeostasis synthesized by the stomach to stimulate appetite and positive energy balance. A subanxiogenic ultra-low dose of LH21 was tested on ghrelin orexigenic actions, finding that it blocked ghrelin-induced hyperphagia in free feeding animals. This study suggests that the peripheral ECS may interact with ghrelin signal in the control of food intake, under equilibrate energy balance conditions, and that therapies using peripheral-restricted CB1 antagonist might be efficacious for the treatment of obesity when ghrelin-inducing food intake signal is overactivated.

NESS038C6 (Fig. 67.1) was highlighted as a novel CB1 antagonist without central effects (Mastinu, Pira, Pani, Pinna, & Lazzari, 2012). Although Mastinu et al. (2012) reported this compound as a CB1 antagonist, no functional assay data were shown both in Mastinu's paper and in the US patent where its chemical synthesis was described (Lazzari, Murineddu, Pinna, & Ruiu, 2009). Thus, there was no accurate information about whether it acted as a neutral antagonist or an inverse agonist. Anyway, the effects induced by a chronic treatment with NESS038C6 in DIO mice were studied. NESS038C6 chronic treatment (30 mg/kg per day for 31 days) determined a significant reduction in DIO mice weight, relative to that of vehicle fat diet. The entity of the effect was comparable to that detected in rimonabant fat diet. Moreover, NESS038C6 fat diet evidenced an improvement of cardiovascular risk factors; a significant decrease in adipose tissue leptin expression; an increase in mRNA expression of hypothalamic orexigenic peptides, and a decrease of anorexigenic peptides; an expression increase of metabolic enzymes and PPARα in the liver; and a normalization of monoaminergic transporters and neurotrophic expression in mesolimbic area. However, in contrast to the case of rimonabant, this CB1 antagonist improved the disrupted expression profile of genes linked to the hunger-satiety circuit, without altering monoaminergic transmission.

Green Cross Corporation disclosed GCC2680 (Fig. 67.1) as a potent, selective and orally efficacious CB1 antagonist with antiobesity properties (Lee et al., 2010). Through a process of lead optimization based on modeling data, it was selected as a candidate for development as a weight-loss agent. Although Lee et al. (2010) claimed GCC2680 as a CB1 antagonist, functional data of this compound were not reported, so the functionality of the compound should be called into question. However, its in vivo efficacy was deeply studied on high fat DIO mice and rats. GCC2680 is substantially more efficacious than rimonabant in both animal models. Moreover, it displayed significant antidiabetic activity through oral administration in various animal models, including *ob/ob* mice, *db/db* mice, and DIO mice. Furthermore, the pharmacokinetic properties of GCC2680 were measured in Sprague Dawley rats. Thus, the off-target activity of this compound was evaluated, demonstrating not to be a significant inhibitor of cytochrome p450 enzymes. Its activity in the hERG potassium channel assay was determined to be higher than that of rimonabant, indicating less risk of increasing QT interval. Besides, GCC2680 proved not to be mutagenic in comet and Ames mutagenicity assays. In addition, when the cytotoxicity of GCC2680 was tested on a panel of cells (Huh-7, Vero, HCT116, and HEK-293) it demonstrated to be safe. Subsequently, in vivo toxicity studies were performed, estimating an LD_{50} greater than 2 g/kg.

Also, through a process of lead optimization, CB1 antagonist MJ15 (Fig. 67.1) was identified as a precandidate for further development as an antiobesity molecule (Chen et al., 2010). Its affinity to CB1 receptor was almost equivalent to that of rimonabant, and its efficacy in selectively antagonizing the CB1 receptor in mammalian cells was a little inferior to that of rimonabant. MJ15 was studied in two animal models, DIO rats and DIO mice. In both models, it demonstrated remarkable effects from 20 to 40 mg/kg, including promoting small intestine peristalsis in ICR mice, and inhibiting food intake and body weight increase. Interestingly, MJ15 not only could prevent the body weight and adipose gain in DIO rats, but could also reduce body weight and fat mass in obesity DIO mice. Moreover, to the accompanied dyslipidemia, MJ15 completely ameliorated it in DIO rats, but only corrected the hypertriglyceridemia of the DIO mice. Additionally, preliminary pharmacokinetics studies showed less distribution of MJ15 in the brain of mice, which indicated that MJ15 displays lowered propensity to pass the blood–brain barrier, and thus may avoid some CNS side effects seen with rimonabant.

CB1 Inverse Agonists

An interesting central acting inverse agonist, developed by Pfizer, is Otenabant (Fig. 67.2) (Hadcock et al., 2010b). In vivo, Otenabant reverses four cannabinoid agonist-mediated CNS-driven responses (hypolocomotion, hypothermia, analgesia, and catalepsy), which involves central effects. This compound exhibits dose and concentration-dependent anorectic activity in two models of acute food intake in rodents, fast-induced refeeding, and spontaneous, nocturnal feeding. It also acutely stimulates energy expenditure in rats, and decreases the respiratory quotient, indicating a metabolic switch to increased fat oxidation. Concentration/effect relationships combined with ex vivo brain CB1 receptor occupancy data were used to evaluate efficacy in behavioral, food intake, and energy expenditure studies.

Three Phase III trials were conducted to evaluate the long-term efficacy and safety of Otenabant in the treatment of overweight or obese patients with or without type 2 diabetes (Aronne et al., 2011). In 2008, Pfizer, as sponsor, elected to discontinue the three trials and the Otenabant development program. The decision was based on changing regulatory perspectives with regard to the risk/benefit profile of CB1 receptor-related drugs, and the likely difficulties of obtaining regulatory approval for them. The most frequent adverse events found for Otenabant in these trials were diarrhea, nausea, nasopharyngitis, and headache. Furthermore, self-reported experiences of anxiety and suicidal thoughts

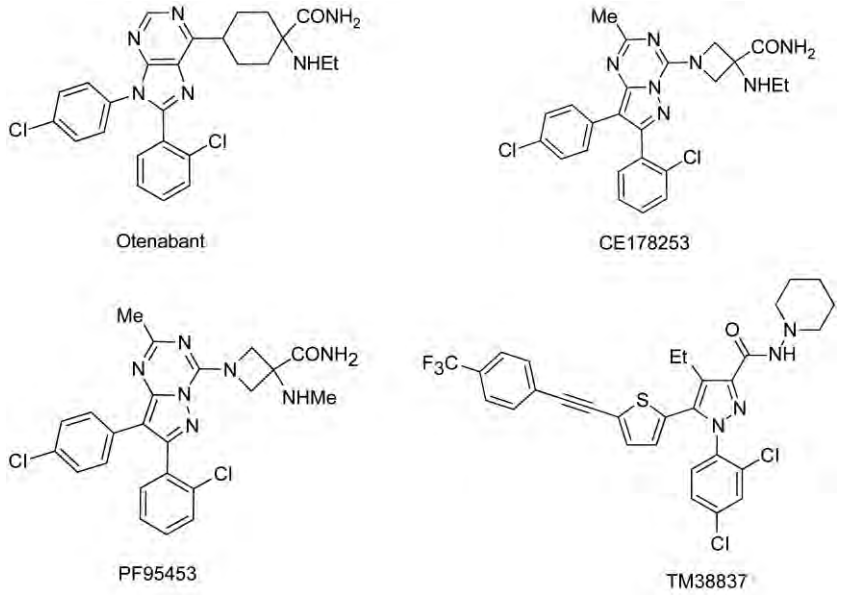


FIGURE 67.2 **Novel CB1 inverse agonists.** Structures of novel CB1 inverse agonists related to rimonabant.

were higher with Otenabant than placebo, as were the incidence of depression and depressed mood.

After discontinuing Otenabant, Pfizer reported the pharmacology and food intake properties of two of their analogs. CE178253 (Fig. 67.2) was described as a centrally-acting CB1 inverse agonist, and its quantitative in vitro and in vivo pharmacological profile was widely reported by Hadcock et al. (2010a). PF95453 (Fig. 67.2) was evaluated in vivo in weight-stable, diet-standardized obese cynomolgus monkeys, using a protocol that involved both a vehicle-treated control group and a pair-fed control group to optimize the ability to differentiate variances related to anorectic behaviors from those related to food intake-independent actions of the compound. Wagner et al. (2010) reported PF95453 as a selective CB1 antagonist, but it was not clear if it was a neutral antagonist or an inverse agonist, like its counterpart compounds. Both CE178253 and PF95453 were discontinued like Otenabant, and no further research about them can be found in the literature. Nevertheless, Pfizer researchers suggested an interesting conclusion from these studies. They found that appetite suppression of CB1 antagonists, and the corresponding anxiety and depressed mood probably were inexorably linked. However, evidence from animal studies indicated that the beneficial metabolic effects of CB1 receptor antagonism were mediated in part by CB1 receptor blockade in peripheral tissues.

A second generation CB1 inverse agonist, TM38837 (Fig. 67.2), was demonstrated to induce comparably significant weight-loss in mice, at the same dose of rimonabant (10 mg/kg), while the dose of TM38837 showed only around 5% of CB1 receptor occupancy in the CNS (Hung et al., 2010). Moreover, TM38837 was tentatively recognized as a peripheral inverse agonist, based on its negative central effects in CB1 receptor-induced hypothermia and analgesia models. Consequently, Hung et al. (2010) suggested that similar levels of antiobesity effects could be achieved even when central CB1 receptor occupancy is rather low. In 2014, a PET study was published, in which in vivo brain CB1 receptor occupancy of TM38837, in comparison with rimonabant in nonhuman primates, was investigated (Takano et al., 2014). Expected clinical therapeutic doses of TM38837 (20 mg) demonstrated only low levels of in vivo brain CB1 receptor occupancy, compared with rimonabant at the comparable doses in nonhuman primates. This low CNS occupancy by TM38837 would reduce the risk of CNS side effects in clinical situations. According to the authors, this nonhuman primate PET study supports further development of TM38837 in clinical trials.

ANTIOBESITY AGENTS NOT RELATED TO RIMONABANT

Phytocannabinoids

Wargent et al. (2013) reported pharmacological in vitro and in vivo experiments of Δ^9-tetrahydrocannabivarin (THCV). THCV is a naturally occurring analog of Δ^9-tetrahydrocannabinol, which behaves as a CB1 neutral antagonist (Pertwee et al., 2007) and, depending on the assays used, also as a CB2 agonist or antagonist (Bolognini et al., 2010). THCV produced therapeutic metabolic effects in two different mouse models of obesity, DIO and genetically (*ob/ob*) obese mice. In particular,

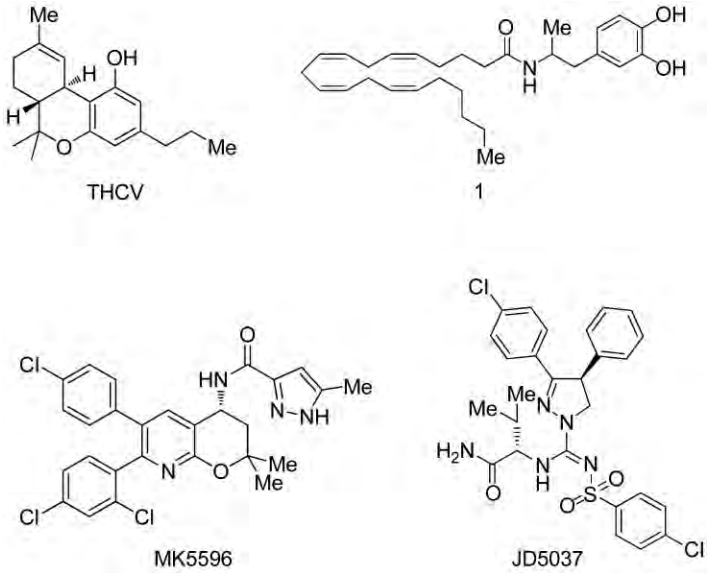


FIGURE 67.3 Novel antiobesity agents not related to rimonabant. Structure of novel antiobesity agents not related to rimonabant.

its strongest effects are exerted on plasma glucose and insulin levels in DIO mice, and on liver triglycerides in *ob/ob* mice. Based on these data, Wargent et al. (2013) suggested that THCV may be useful for the treatment of the metabolic syndrome and/or type 2 diabetes, either alone or in combination with existing treatments.

Fatty Acid Derivatives

Interestingly, studies performed in both rodents and humans have demonstrated that dietary lipids can modulate endocannabinoid levels (Kim, Li, & Watkins, 2013). Specifically, diet n-3 polyunsaturated fatty acids (PUFA) could potentially alter the levels of endocannabinoids (mainly AEA and 2-AG), and the ECS tone. Introducing n-3 PUFA to the diet directly reduced the substrate amount of arachidonic acid (AA) available in membrane phospholipids for the synthesis of AEA and 2-AG. Specific hypothesis tried to explain how long chain n-3 PUFA may function to alter the homeostatic induction of the ECS. The long chain n-3 PUFA derivatives may bind with varying affinities to the cannabinoid receptors, and act as modifiers of AEA and 2-AG bound to receptors in order to diminish overstimulation of this signaling pathway contributing to obesity. In addition, the long chain n-3 PUFA may increase or decrease the expression or activity of enzymes in the synthesis and degradation of endocannabinoids.

Thus, PUFA derivatives have been recently developed as antiobesity drugs. Some fatty acid amides, specifically oleic and arachidonic ones, of several metabolites of 3,4-methylenedioxymethamphetamine (MDMA) resulted in negative modulation of food intake in rats (Almeida et al., 2010). The most active compound (see "1" in Fig. 67.3) showed some affinity for the CB1 receptor, but no data about its functionality were reported. Moreover, it displayed an acute food intake modulation profile.

Heterocycles

From 2010, some heterocyclic structures not related to rimonabant have been reported. At around the same time that rimonabant was withdrawn, Merck announced the withdrawal of its CB1 inverse agonist taranabant from Phase II and III studies for the indications of smoking cessation and obesity, also due to psychiatric side effects including depression, irritability, anxiety, and suicidality. Afterwards, this pharmaceutical company claimed another inverse agonist for the treatment of obesity. It was MK5596 (Fig. 67.3), also a CB1 inverse agonist related to taranabant (Yan et al., 2010). It reduced body weight and food intake in a DIO rat model, at a very low dose of 1 mg/kg. An extensive preclinical study revealed that MK5596 possessed attenuated off-target hERG activity, CB1 receptor-related pharmacological efficacy similar to that of taranabant, good pharmacokinetic profiles in preclinical species (rat, monkey, and dog), acceptable safety margins, and a potentially balanced metabolism profile in human. Despite this excellent preclinical profile, Merck decided to discontinue the clinical evaluation of MK5596 as an antiobesity agent due to the rimonabant and taranabant side effects.

JD5037 (Fig. 67.3), an analog of the brain-penetrant CB1 inverse agonist ibipinabant, was investigated as a peripheral CB1 inverse agonist with weight-loss properties (Tam et al., 2012). The absence of in vivo brain CB1 receptor occupancy following acute or chronic

administration of JD5037 was documented by PET. In mice with DIO, JD5037 was equieffective with its brain-penetrant parent compound ibipinabant in reducing appetite, body weight, hepatic steatosis, and insulin resistance, even though it does not occupy central CB1 receptor or induce related behaviors. Appetite and weight reduction by JD5037 were mediated by resensitizing DIO mice to endogenous leptin through reversing hyperleptinemia. It was performed by decreasing leptin expression and secretion by adipocytes, and increasing leptin clearance via the kidney. Thus, inverse agonism at peripheral CB1 receptor not only improves cardiometabolic risk in obesity, but has antiobesity effects by reversing leptin resistance. Tam et al. (2012) claimed that a key finding of their study was the greater efficacy of peripheral CB1 inverse agonism, compared to neutral antagonism in reducing food intake and body weight. Hyperleptinemia, the target of these effects, was partially reduced by the neutral antagonist AM6545, but completely normalized by JD5037. It is possible that, in obesity, the fraction of CB1 receptor present in the active conformation is increased in peripheral tissues involved in metabolic regulation, a fact that could account for the greater efficacy of inverse agonists over neutral antagonists. Moreover, this study seemed to confirm earlier suggestions that the hypophagic effect of brain-penetrant CB1 inverse agonists may be mediated by peripheral CB1 receptor (Gomez et al., 2002).

CONCLUSIONS AND PERSPECTIVES

As it was shown in the previous sections, some considerable debate remains about the best way in which to target CB1 receptors for weight and metabolic control. Some authors claim that inverse agonism of CB1 receptors at a central and peripheral level is the best approach to achieve weight loss, and others suggest that blocking the CB1 receptor with nonbrain penetrating compounds and neutral antagonism should be the strategy to be chosen. Consequently, many questions remain unanswered about the ideal methods by which to inhibit CB1 receptors for weight and metabolic control, and even novel cannabinoid targets to develop antiobesity drugs are being taken into account.

Cannabinoid CB2 receptors were first described in immune system cells, being involved in modulating immune responses. However, subsequent analysis revealed that this receptor is also expressed in organs controlling metabolism, such as the liver, adipose tissue, skeletal muscle, and the endocrine pancreas. In agreement, changes in their expression have been reported in obesity in both the liver and adipose tissue, and CB2 specific activation potentiated inflammatory markers, insulin resistance, and liver steatosis in obese mice, whereas its blockade did the opposite (Deveaux et al., 2009). Indeed, animals genetically lacking the CB2 receptor in the whole body have shown to increase food intake and body weight with age, displaying less insulin resistance and inflammatory markers than the wild-type. These CB2 KO mice were also more resistant to the development of DIO.

In addition, CB2 receptor has been also detected in several brain areas (Van Sickle et al., 2005), so the putative role of central CB2 receptors in modulating body weight and glucose homeostasis is still under study (Romero-Zerbo et al., 2012).

Recent pharmacological data has demonstrated that certain cannabinoid ligands mediate their biological effects through the GPCR GPR55 (Lauckner et al., 2008), considering it as a novel putative cannabinoid receptor. Regarding obesity, GPR55 exerts some metabolic actions on insulin secretion and gut movement. Importantly, this receptor may be a clinically relevant target because GPR55 expression in WAT is increased in obese subjects, and even more in obese patients with type 2 diabetes. Moreover, LPI, the endogenous ligand of GPR55, showed increased circulating levels in obese patients, and a positive correlation with fat percentage and BMI in women (Imbernon et al., 2014). These preliminary but interesting results require investigating the potential role of GPR55 agonists/antagonists in obesity and insulin sensitivity.

In brain, 2-monoacylglycerol lipase (MGL) works as a primary regulator of the endocannabinoid 2-AG, signaling action via the ECS. Chon et al. (2012) showed that intestinal MGL expression and activity were significantly induced by a high fat diet, but not by fasting, suggesting a potential role for MGL in dietary lipid assimilation. Moreover, they generated transgenic mice that overexpressed MGL in the small intestine, finding that, after 3 weeks of a high fat diet, these mice showed a significant increase of body weight gain and body fat accumulation, accompanied by hyperphagia, as well as reduced energy expenditure. Thus, preliminarily, intestinal MGL may be envisioned as a new metabolic regulator involved in the control of the whole body energy homeostasis.

MINI-DICTIONARY

Adipocytes Cells of the adipose tissue.
Anorectic drug Substance which reduces appetite and food consumption.
Dyslipidemia An abnormal amount of lipids in the blood.
Energy homeostasis It measures the energy intake, so the sum of energy stored, work, and heat.
Hyperleptinemia High serum levels of leptin.
Hypertriglyceridemia High blood levels of triglycerides.
Hypophagia Reduction of food intake.

Leptin Hormone regulating the amount of fat stored in the body.
Liver/hepatic steatosis A process in which fat accumulates in liver cells via an abnormal retention of lipids.
Rimonabant Antiobesity drug whose mechanism involves CB1 receptor blocking.

References

Adan, R. A. H. (2013). Mechanisms underlying current and future anti-obesity drugs. *Trends in Neuroscience*, *36*, 133–140.

Alen, F., Crespo, I., Ramirez-Lopez, M. T., Jagerovic, N., Goya, P., de Fonseca, F. R., de Heras, R. G., & Orio, L. (2013). Ghrelin-induced orexigenic effect in rats depends on the metabolic status and is counteracted by peripheral CB1 receptor antagonism. *PLoS One*, *8*, e60918.

Almeida, B., Joglar, J., Luque Rojas, M. J., Decara, J. M., Bermúdez-Silva, F. J., Macias-González, M., Fitó, M., Romero-Cuevas, M., Farré, M., Covas, M. I., Rodríguez de Fonseca, F., & de la Torre, R. (2010). Synthesis of fatty acid amides of catechol metabolites that exhibit antiobesity properties. *ChemMedChem*, *5*, 1781–1787.

Alonso, M., Serrano, A., Vida, M., Crespillo, A., Hernandez-Folgado, L., et al. (2012). Anti-obesity efficacy of LH-21, a cannabinoid CB1 receptor antagonist with poor brain penetration, in diet-induced obese rats. *British Journal of Pharmacology*, *165*, 2274–2291.

Aronne, L. J., Finer, N., Hollander, P. A., England, R. D., Klioze, S. S., Chew, R. D., Fountaine, R. J., Powell, C. M., & Obourn, J. D. (2011). Efficacy and safety of CP-945,598, a selective cannabinoid CB1 receptor antagonist, on weight loss and maintenance. *Obesity*, *19*, 1404–1414.

Bolognini, D., Costa, B., Maione, S., Comelli, F., Marini, P., Di Marzo, V., Parolaro, D., Ross, R. A., Gauson, L. A., Cascio, M. G., & Pertwee, R. G. (2010). The plant cannabinoid Δ^9-tetrahydrocannabivarin can decrease signs of inflammation and inflammatory pain in mice. *British Journal of Pharmacology*, *160*, 677–687.

Chen, W., Tang, H., Liu, H., Long, L., Gong, Z., Zheng, J., Chi, M., Xie, Y., Zheng, Z., Li, S., & Wang, L. (2010). Novel selective antagonist of the cannabinoid CB1 receptor, MJ15, with prominent anti-obesity effect in rodent models. *European Journal of Pharmacology*, *637*, 178–185.

Chon, S. -H., Douglass, J. D., Zhou, Y. X., Malik, N., Dixon, J. L., Brinker, A., Quadro, L., & Storch, J. (2012). Over-expression of monoacylglycerol lipase (MGL) in small intestine alters endocannabinoid levels and whole body energy balance, resulting in obesity. *PLoS One*, *7*, e43962.

Cluny, N. L., Vemuri, V. K., Chambers, A. P., Limebeer, C. L., Bedard, H., Wood, J. T., Lutz, B., Zimmer, A., Parker, L. A., Makriyannis, A., & Sharkey, K. A. (2010). A novel peripherally restricted cannabinoid receptor antagonist, AM6545, reduces food intake and body weight, but does not cause malaise, in rodents. *British Journal of Pharmacology*, *161*, 629–642.

Deveaux, V., Cadoudal, T., Ichigotani, Y., Teixeira-Clerc, F., Louvet, A., et al. (2009). Cannabinoid CB2 receptor potentiates obesity-associated inflammation, insulin resistance and hepatic steatosis. *PLoS One*, *4*, e5844.

Fernández-Fernández, C., Decara, J., Bermúdez-Silva, F. J., Sánchez, E., Morales, P., et al. (2013). Description of a bivalent cannabinoid ligand with hypophagic properties. *Archiv der Pharmazie (Weinheim)*, *346*, 171–179.

Gomez, R., Navarro, M., Ferrer, B., Trigo, J. M., Bilbao, A., Del Arco, I., Cippitelli, A., Nava, F., Piomelli, D., & Rodriguez de Fonseca, F. (2002). A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. *Journal of Neuroscience*, *22*, 9612–9617.

Hadcock, J., Carpino, P., Iredale, P., Dow, R., Gautreau, D., et al. (2010a). Quantitative in vitro and in vivo pharmacological profile of CE-178253, a potent and selective cannabinoid type 1 (CB1) receptor antagonist. *BMC Pharmacology*, *10*, 9.

Hadcock, J. R., Griffith, D. A., Iredale, P. A., Carpino, P. A., Dow, R. L., Black, S. C., O'Connor, R., Gautreau, D., Lizano, J. S., Ward, K., Hargrove, D. M., Kelly-Sullivan, D., & Scott, D. O. (2010b). In vitro and in vivo pharmacology of CP-945,598, a potent and selective cannabinoid CB1 receptor antagonist for the management of obesity. *Biochemical and Biophysical Research Communications*, *394*, 366–371.

Hung, M. -S., Chang, C. -P., Li, T. -C., Yeh, T. -K., Song, J. -S., Lin, Y., Wu, C. -H., Kuo, P. -C., Amancha, P. K., Wong, Y. -C., Hsiao, W. -C., Chao, Y. -S., & Shia, K. -S. (2010). Discovery of 1-(2,4-dichlorophenyl)-4-ethyl-5-(5-(2-(4-(trifluoromethyl)phenyl)ethynyl)thiophen-2-yl)-*N*-(piperidin-1-yl)-1H-pyrazole-3-carboxamide as a potential peripheral cannabinoid-1 receptor inverse agonist. *ChemMedChem*, *5*, 1439–1443.

Imbernon, M., Whyte, L., Diaz-Arteaga, A., Russell, W. R., Moreno, N. R., Vazquez, M. J., Gonzalez, C. R., Diaz-Ruiz, A., Lopez, M., Malagon, M. M., Ross, R. A., Dieguez, C., & Nogueiras, R. (2014). Regulation of GPR55 in rat white adipose tissue and serum LPI by nutritional status, gestation, gender and pituitary factors. *Molecular and Cellular Endocrinology*, *383*, 159–169.

Jagerovic, N., Hernandez-Folgado, L., Alkorta, I., Goya, P., Navarro, M., Serrano, A., Rodriguez de Fonseca, F., Dannert, M. T., Alsasua, A., Suardiaz, M., Pascual, D., & Martin, M. I. (2004). Discovery of 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-3-hexyl-1H-1,2,4-triazole, a novel in vivo cannabinoid antagonist containing a 1,2,4-triazole motif. *Journal of Medical Chemistry*, *47*, 2939–2942.

Kim, J., Li, Y., & Watkins, B. A. (2013). Fat to treat fat: emerging relationship between dietary PUFA, endocannabinoids, and obesity. *Prostaglandins and Other Lipid Mediators*, *104–105*, 32–41.

Lauckner, J. E., Jensen, J. B., Chen, H. -Y., Lu, H. -C., Hille, B., & Mackie, K. (2008). GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 2699–2704.

Lazzari, P., Murineddu, G., Pinna, G. A., & Ruiu, S. (2009). Pharmaceutical compounds. Patent No. US7,485,730

Lee, J., Seo, H. J., Lee, S. H., Kim, J., Jung, M. E., et al. (2010). Discovery of 2-(4-((1H-1,2,4-triazol-1-yl)methyl)-5-(4-bromophenyl)-1-(2-chlorophenyl)-1H-pyrazol-3-yl)-5-tert-butyl-1,3,4-thiadiazole (GCC2680) as a potent, selective and orally efficacious cannabinoid-1 receptor antagonist. *Bioorganic and Medicinal Chemistry*, *18*, 6377–6388.

Mastinu, A., Pira, M., Pani, L., Pinna, G. A., & Lazzari, P. (2012). NESS038C6, a novel selective CB1 antagonist agent with anti-obesity activity and improved molecular profile. *Behavioural Brain Research*, *234*, 192–204.

Pertwee, R. G., Thomas, A., Stevenson, L. A., Ross, R. A., Varvel, S. A., Lichtman, A. H., Martin, B. R., & Razdan, R. K. (2007). The psychoactive plant cannabinoid, Δ^9-tetrahydrocannabinol, is antagonized by Δ^8- and Δ^9-tetrahydrocannabivarin in mice in vivo. *British Journal of Pharmacology*, *150*, 586–594.

Pi-Sunyer, F., Aronne, L. J., Heshmati, H. M., Devin, J., & Rosenstock, J. (2006). Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. *JAMA*, *295*, 761–775.

Rinaldi-Carmona, M., Barth, F., Heaulme, M., Shire, D., Calandra, B., et al. (1994). SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Letters*, *350*, 240–244.

Romero-Zerbo, S. Y., Garcia-Gutierrez, M. S., Suárez, J., Rivera, P., Ruz-Maldonado, I., Vida, M., Rodriguez de Fonseca, F., Manzanares, J., & Bermúdez-Silva, F. J. (2012). Overexpression of cannabinoid CB2 receptor in the brain induces hyperglycaemia and a lean phenotype in adult mice. *Journal of Neuroendocrinology*, *24*, 1106–1119.

Serrano, A., Pavon, F., Suarez, J., Romero-Cuevas, M., Baixeras, E., Goya, P., & Fonseca, F. R. (2012). Obesity and the endocannabinoid system: is there still a future for CB1 antagonists in obesity? *Current Obesity Reports*, *1*, 216–228.

Takano, A., Gulyás, B., Varnäs, K., Little, P. B., Noerregaard, P. K., Jensen, N. O., Elling, C. E., & Halldin, C. (2014). Low brain CB1 receptor occupancy by a second generation CB1 receptor antagonist TM38837 in comparison with rimonabant in nonhuman primates: a PET study. *Synapse*, *68*, 89–97.

Tam, J., Cinar, R., Liu, J., Godlewski, G., Wesley, D., et al. (2012). Peripheral cannabinoid-1 receptor inverse agonism reduces obesity by reversing leptin resistance. *Cell Metabolism*, *16*, 167–179.

Van Sickle, M. D., Duncan, M., Kingsley, P. J., Mouihate, A., Urbani, P., et al. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science*, *310*, 329–332.

Wagner, J. D., Zhang, L., Kavanagh, K., Ward, G. M., Chin, J. E., Hadcock, J. R., Auerbach, B. J., & Harwood, H. J. (2010). A selective cannabinoid-1 receptor antagonist, PF-95453, reduces body weight and body fat to a greater extent than pair-fed controls in obese monkeys. *Journal of Pharmacology and Experimental Therapeutics*, *335*, 103–113.

Wargent, E. T., Zaibi, M. S., Silvestri, C., Hislop, D. C., Stocker, C. J., Stott, C. G., Guy, G. W., Duncan, M., Di Marzo, V., & Cawthorne, M. A. (2013). The cannabinoid [Delta]^{9}-tetrahydrocannabivarin (THCV) ameliorates insulin sensitivity in two mouse models of obesity. *Nutrition and Diabetes*, *3*, e68.

Yan, L., Huo, P., Debenham, J. S., Madsen-Duggan, C. B., Lao, J., et al. (2010). Discovery of *N*-[(4R)-6-(4-chlorophenyl)-7-(2,4-dichlorophenyl)-2,2-dimethyl-3,4-dihydro-2H-pyrano[2,3-b]pyridin-4-yl]-5-methyl-1H-pyrazole-3-carboxamide (MK-5596) as a novel cannabinoid-1 receptor (CB1R) inverse agonist for the treatment of obesity. *Journal of Medicinal Chemistry*, *53*, 4028–4037.

CHAPTER

68

Cannabinoid Reward and Dependence: Focus on the Main Psychoactive Ingredients of Marijuana in Preclinical Studies

G. Panagis

University of Crete, Department of Psychology, Laboratory of Behavioral Neuroscience, Rethymnon, Crete, Greece

SUMMARY POINTS

- This chapter focuses on the effects of the major phytocannabinoids, Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) in brain reward processes that are believed to play a pivotal role in the development and maintenance of drug abuse and addiction.
- Very low doses of Δ^9-THC lower brain stimulation reward thresholds. The ability of drugs to lower brain stimulation reward thresholds correlates with high abuse potential.
- Δ^9-THC is self-administered intravenously by experimental animals, although the range of conditions under which this occurs is more limited than with other drugs of abuse.
- Δ^9-THC is self-administered intracerebroventricularly, or into the posterior ventral tegmental area and the shell of the nucleus accumbens by experimental animals.
- Depending on a number of experimental parameters (dose, strain of the animal, number of conditioning sessions, timing between injections, previous drug exposure), Δ^9-THC induces both conditioned place preference (CPP) and aversion.
- In experimental animals, abrupt discontinuation of chronic Δ^9-THC treatment does not result in a robust spontaneous withdrawal syndrome. However, challenge with the CB_1 receptor antagonist SR141716A elicits withdrawal signs in animals treated with Δ^9-THC.
- Cannabidiol is not rewarding in experimental animals, since it neither decreases brain stimulation reward thresholds, nor induces conditioned place preference.

KEY FACTS OF CANNABINOIDS AND THE ENDOCANNABINOID SYSTEM

- The plant *Cannabis sativa* contains over 400 chemical compounds, including about 80 terpeno-phenols, unique to the plant of cannabis, known as plant cannabinoids (phytocannabinoids).
- Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the major psychoactive constituent of the plant.
- Plant cannabinoids with very weak or no psychotropic and psychotomimetic activity include cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), Δ^9-tetrahydrocannabivarin (Δ^9-THCV), cannabidivarin (CBDV), Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA), and cannabidiolic acid (CBDA).

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00079-X
Copyright © 2017 Elsevier Inc. All rights reserved.

- Δ^9-THC exerts its effects by interacting with specific receptors. Two major cannabinoid (CB) receptors have been cloned (CB_1 and CB_2), both of which belong to the superfamily of G-protein coupled receptors.
- CB_1 receptors are expressed ubiquitously, but they are preferentially located in the brain and the spinal cord. They can be found in the highest concentrations in areas involved in motor function and coordination (eg, basal ganglia and cerebellum), memory (eg, hippocampus), emotionality (eg, neocortex, and amygdala), and motivation and reward (eg, prefrontal cortex, nucleus accumbens, amygdala). CB_1 receptors are localized preferentially at the presynaptic level, and mediate inhibition of ongoing release of other neurotransmitters, including dopamine.
- The CB_2 receptor has been categorized classically as the peripheral cannabinoid receptor, since it is expressed principally in the immune system. However, recent findings suggest that CB_2 receptors are also expressed in the brain under normal and, in particular, under pathological conditions.
- Endogenous ligands for CB receptors have been discovered and characterized. These endocannabinoids include anandamide (AEA), 2-arachidonoylglycerol (2-AG), noladine ether, virodhamine, and *N*-arachidonoyldopamine (NADA).
- Endocannabinoids are released upon demand from lipid precursors, and their concentrations are regulated in a strict manner through a reuptake process into cells, and a rapid enzymatic inactivation.
- The endocannabinoid system is an important lipid signaling system which modulates various physiological functions, not only in the central nervous system, but also in the autonomic nervous system, the endocrine, the reproductive, the immune, and the cardiovascular system.

LIST OF ABBREVIATIONS

CB_1 receptors	Cannabinoid 1 receptors
CBD	Cannabidiol
CPP	Conditioned place preference
ICSS	Intracranial self-stimulation
KO	Knockout
Δ^9-THC	Δ^9-Tetrahydrocannabinol

INTRODUCTION

Cannabis is by far the most commonly consumed illegal drug worldwide, and it is generally considered to have only modest addictive potential, compared to other drugs of abuse (Ramo, Liu, & Prochaska, 2012). In many countries, more than 50% of young people have used it at least once. The popularity of cannabis as a recreational drug is due to its ability to alter sensory processing, as well as producing clear subjective motivational responses accompanied by a sense of mild euphoria and wellbeing. Indeed, this elation, along with the sense of relaxation produced by cannabis, are among the most cited subjective psychotropic properties of the drug (Green, Kavanagh, & Young, 2003). In Europe today there is a trend toward relaxation of the criminal penalties associated with cannabis use and cannabis users, mainly because it is perceived as relatively safe. However, there is evidence that some users do become dependent on cannabis, consuming the drug in a compulsive fashion, and experiencing withdrawal symptoms upon cessation.

In spite of the well-established abuse properties of cannabis preparations in humans, rewarding effects of cannabinoids in animal models of drug abuse and dependence have been rather difficult to demonstrate. However, several recent findings have demonstrated many commonalities between the rewarding and addictive effects of cannabinoids, and those of other drugs of abuse. Here, we will briefly describe and evaluate the actions of cannabis, its main psychoactive ingredient Δ^9-tetrahydrocannabinol (Δ^9-THC), and cannabidiol (CBD) on brain reward circuitry, that are believed to play a pivotal role in the development and maintenance of cannabinoid addiction.

Δ^9-THC and Cannabidiol Effects on Brain Stimulation Reward

A number of studies have been conducted on the effects of Δ^9-THC in the intracranial self-stimulation (ICSS) paradigm (Table 68.1). According to Gardner and coworkers, 1 and 1.5 mg/kg of Δ^9-THC decreased ICSS thresholds in Lewis rats, but not in Fisher 344 rats, whereas in Sprague-Dawley rats the effect was only marginal (Gardner et al., 1988; Lepore, Liu, Savage, Matalon, & Gardner, 1996). In contrast, other studies failed to show an enhancement of brain stimulation reward with Δ^9-THC in the dose range from 0.5 to 10 mg/kg in Sprague-Dawley rats, under baseline conditions (Katsidoni, Kastellakis, & Panagis, 2013b; Kwilasz & Negus, 2012; Vlachou, Nomikos, Stephens, & Panagis, 2007), or in animals preexposed to stress (Fokos & Panagis, 2010). Similar results have been reported in Long-Evans rats with oral administration of 10 mg/kg Δ^9-THC and various doses of three synthetic analogs structurally related to Δ^9-THC, namely levonantradol, nabilone, and canbisol (Kucharski, Williams, & Kornetsky, 1983; Stark & Dews, 1980). Surprisingly, however, in a recent study we showed that Δ^9-THC can induce both rewarding and anhedonic effects in the ICSS paradigm in

TABLE 68.1 Studies Regarding the Role of Δ^9-Tetrahydrocannabinol (Δ^9-THC) in Reward and Dependence

Model	Dose of Δ^9-THC	Brief result	Animals	References
ICSS	0.12–10 mg/kg	Increased ICSS threshold	Long-Evans rats	Stark and Dews (1980)
ICSS	0.2, 0.3 mg/kg	Increased ICSS threshold	Albino CDF rats	Kucharski et al. (1983)
ICSS	1.5 mg/kg	Decreased ICSS threshold	Lewis rats	Gardner et al. (1988)
ICSS	1 mg/kg	Marginal decrease in ICSS threshold No effect Decreased ICSS threshold	Sprague-Dawley rats Fischer 344 rats Lewis rats	Lepore et al. (1996)
ICSS	1–2 mg/kg	Increased ICSS threshold	Sprague-Dawley rats	Vlachou et al. (2007)
ICSS	0.5, 1 mg/kg	No effect	Sprague-Dawley rats	Fokos and Panagis (2010)
ICSS	0.32–1 mg/kg	No effect	Sprague-Dawley rats	Kwilasz and Negus (2012)
ICSS	0.1 mg/kg 1 mg/kg	Decreased threshold Increased threshold	Sprague-Dawley rats	Katsidoni et al. (2013b)
Intravenous self-administration	0.02–0.3 mg/kg	No effect (self-administration in no higher than vehicle levels)	Rhesus monkeys (with previous drug experience)	Harris, Waters, and McLendon (1974)
Intravenous self-administration	6.25–50 µg/kg per injection	Self-administration in food restricted rats	Wistar albino rats	Takahashi and Singer (1979)
Intravenous self-administration	12.5 µg/kg per injection	Self-administration in food restricted rats	Wistar albino rats	Takahashi and Singer (1980)
Intravenous self-administration	0.4 mg/kg per injection	Self-administration after withdrawal from forced automatic intravenous injections of Δ^9-THC (drug naive monkeys did not self-administer Δ^9-THC)	Rhesus monkeys (with previous drug experience)	Kaymakcalan (1981)
Intravenous self-administration	17–100 µg/kg per injection	No effect (self-administration in no higher than vehicle levels)	Rhesus monkeys (with previous drug experience)	Mansbach, Nicholson, Martin, and Balster (1994)
Intravenous self-administration	2 and 4 mg/kg per injection	Self-administration	Squirrel monkeys (with previous drug experience)	Tanda, Munzar, and Goldberg (2000)
Intravenous self-administration	2, 4, 8 mg/kg per injection	Self-administration	Naive squirrel monkeys	Justinova, Tanda, Redhi, and Goldberg (2003)
Intravenous self-administration	2–8 µg/kg per injection	Self-administration	Squirrel monkeys (with previous drug experience)	Justinova, Tanda, Munzar, and Goldberg (2004)
Intravenous self-administration	20–80 µg/kg	Self-administration under second order schedule	Squirrel monkeys (with previous drug experience)	Justinova et al. (2008)
Intravenous self-administration	0.0032–0.032 mg/kg per infusion	No effect (self-administration in no higher than vehicle levels)	Rhesus monkeys (with previous drug experience)	Li, Koek, and France (2012)
Intravenous self-administration	4 µg/kg per injection	Self-administration	Squirrel monkeys (with previous drug experience)	Justinova et al. (2013)
Intracerebroventricular self-administration	0.01–1 µg/2 µL per infusion	Self-administration	Wistar rats	Braida, Iosue, Pegorini, and Sala (2004)
Intracerebral (posterior ventral tegmental area and posterior shell of the nucleus accumbens) self-administration	100 nL injection of 66 or 200 pmol	Self-administration	Sprague-Dawley rats	Zangen, Solinas, Ikemoto, Goldberg, and Wise (2006)

(*Continued*)

TABLE 68.1 Studies Regarding the Role of Δ^9-Tetrahydrocannabinol (Δ^9-THC) in Reward and Dependence (*cont.*)

Model	Dose of Δ^9-THC	Brief result	Animals	References
CPP	1 mg/kg	No effect	Long-Evans rats	Lepore, Vorel, Lowinson, and Gardner (1995)
	2 and 4 mg/kg	CPP		
	48 h between sessions:			
	0.5 mg/kg	No effect		
	1 mg/kg	CPP		
	2 and 4 mg/kg	CPA		
CPP	0.2–1.5 mg/kg	CPA	Lewis and Sprague-Dawley rats	Parker and Gillies (1995)
CPP	48 h between sessions:		Sprague-Dawley rats	Sanudo-Pena et al. (1997)
	1.5 mg/kg	No effect		
	15 mg/kg	CPA		
CPP	0.5–10 mg/kg	No effect	CD1 mice	Hutcheson et al. (1998)
	20 mg/kg	CPA		
CPP	0.1, 0.5, 2, 4, 8 mg/kg	No effect	Wistar rats	Mallet and Beninger (1998)
	1 and 1.5 mg/kg	CPA		
CPP	5 mg/kg	CPA	CD1 mice	Valjent and Maldonado (2000)
	1 mg/kg	No effect		
	Pretreatment: 1 injection 24 h			
	5 mg/kg	No effect		
	1 mg/kg	CPP		
CPP	0.075–0.75 mg/kg	CPP	Wistar rats	Braida et al. (2004)
	0.015, 1, 3 mg/kg	No effect		
	6 mg/kg	CPA		
CPP	1 mg/kg Pretreatment: 1 injection 24 h	CPP	A_{2A} KO and wild-type mice	Soria et al. (2004)
	5 mg/kg	CPA		
CPP	0.01, 1, 2 mg/kg	No effect	Sprague-Dawley rats	Le Foll, Wiggins, and Goldberg (2006)
	0.1 mg/kg	CPP		
CPP	1, 3 mg/kg Pretreatment 1 injection 24 h	No effect	C57B1/6Lx129Sv mice	Vlachou et al. (2007)
CPP	1 mg/kg	No effect	ICR mice	Vann et al. (2008)
	10 mg/kg	CPA		
CPP	Pretreatment: 1 injection 24 h		Adolescent Wistar rats	Quinn et al. (2008)
	5 mg/kg	No effect	Adult Wistar rats	
	5 mg/kg	CPA		
CPP	10 mg/kg	No effect	Albino Wistar rats	Klein et al. (2011)
CPP	0.1–3 mg/kg	No effect	Sprague Dawley rats	Scherma et al. (2012)
Spontaneous withdrawal	10 mg/kg for 8 days after a chronic treatment with increasing doses of 1.8–10 mg/kg for 28 days	No spontaneous withdrawal symptoms	Pigeons	McMillan, Harris, Frankenheim, and Kennedy (1970)

TABLE 68.1 Studies Regarding the Role of Δ^9-Tetrahydrocannabinol (Δ^9-THC) in Reward and Dependence (*cont.*)

Model	Dose of Δ^9-THC	Brief result	Animals	References
Spontaneous withdrawal	Chronic treatment with Δ^9-THC	No spontaneous withdrawal symptoms	Dogs	Dewey, Jenkins, O'Rourke, and Harris (1972)
Spontaneous withdrawal	10, 20, 40, 80 mg/kg orally for 11, 13, 28 days	No spontaneous withdrawal symptoms	QS strain mice	Chesher and Jackson (1974)
Spontaneous withdrawal	0.025–0.3 mg/kg, 12 h access for 20 days	No spontaneous withdrawal symptoms	Rhesus monkeys	Harris et al. (1974)
Spontaneous withdrawal	0.1 mg/mL of cannabis resin for 126 days	No spontaneous withdrawal symptoms	Wistar rats	Leite and Carlini (1974)
Spontaneous withdrawal	Treatment for 5 weeks with increasing doses up to 40 mg/kg	Spontaneous withdrawal symptoms (teeth chattering, defecation, urination, dyspnea, complete palpebral closure) and increased locomotor activity	Rats (female)	Kaymakcalan, Ayhan, and Tulunay (1977)
Spontaneous withdrawal	0.5 mg/kg, every 6 h, for 3 weeks	Withdrawal reactions (increase in gross movement, eye contact, and tooth baring)	Rhesus monkeys	Fredericks and Benowitz (1980)
Spontaneous withdrawal	0.05 mg/kg for 10 days	No spontaneous withdrawal reactions, but suppression of operant behavior for food reinforcement	Rhesus monkeys	Beardsley, Balster, and Harris (1986)
Precipitated withdrawal by SR141716A	4 days: 0.5, 2.5, and 12.5 mg/kg for the 1st day. Each starting dose was doubled each day throughout the infusion.	Withdrawal signs (head shakes, facial tremors, tongue rolling, biting, wet-dog shakes, eyelid ptosis, facial rubbing, paw treading, retropulsion, immobility, ear twitch, chewing, licking, stretching and arched back)	Sprague Dawley rats	Aceto, Scates, Lowe, and Martin (1995)
Spontaneous withdrawal	4 days, 0.5 up to 100 mg/kg	No spontaneous withdrawal symptoms	Sprague Dawley rats	Aceto, Scates, Lowe, and Martin (1996)
Precipitated withdrawal by SR141716A	4 days, 0.5 up to 100 mg/kg	Withdrawal signs (scratching, rubbing face with paws, licking, wet-dog shakes, arched back and ptosis)		
Spontaneous withdrawal	15 mg/kg b.i.d for 6.5 days	No spontaneous withdrawal symptoms	Sprague Dawley albino rats	Diana, Melis, Muntoni, and Gessa (1998)
Precipitated withdrawal by SR141716A		Withdrawal signs (scratching, facial rubbing, licking, wet dog shakes)		
Precipitated withdrawal by SR141716A	10 mg/kg b.i.d. for 5.5 days	Withdrawal signs (wet-dog shakes, frontpaw tremor, ataxia, hunched posture, tremor, ptosis, piloerection, decreased locomotor activity and mastication)	CD-1 mice	Hutcheson et al. (1998)

(*Continued*)

TABLE 68.1 Studies Regarding the Role of Δ^9-Tetrahydrocannabinol (Δ^9-THC) in Reward and Dependence (*cont.*)

Model	Dose of Δ^9-THC	Brief result	Animals	References
Precipitated withdrawal by SR141716A	20 mg/kg b.i.d. for 5.5 days	Withdrawal symptoms (increased rearing and sniffing, wet-dog shakes, paw tremor, piloerection, hunched posture, body tremor) in wild-type mice but not in mutants	CB_1 KO mice	Ledent et al. (1999)
Precipitated withdrawal by SR141716A	20 mg/kg b.id. for 5.5 days	Withdrawal signs (ataxia, mastication, piloerection, front paw tremor, wet-dog shakes, tremor, ptosis)	CD-1 mice	Tzavara et al. (2000)
Precipitated withdrawal by SR141716A	30–50 mg/kg b.i.d. for 6 days	Suppression of operant behavior for food reinforcement	Long Evans Hooded rats	Beardsley and Martin (2000)
Precipitated withdrawal by SR141716A	2 days, 10 mg/kg, b.i.d. 3rd day 10 mg/kg	Paw tremors and head shakes	IRC mice	Lichtman, Fisher, and Martin (2001)
Precipitated withdrawal by SR141716A	11 times, every 12 h, 20 mg/kg	Forepaw licking, facial preening, grooming, forepaw tremor, head shakes and weight loss	ddY mice	Anggadiredja et al. (2003)
Precipitated withdrawal by SR141716A	8 days, 10 mg/kg	Reduced time spent in inactivity in the open-field test, turning, tremor and retropulsion	Wistar rats	Gonzalez et al. (2004)
Spontaneous withdrawal	5 days, 20 mg/kg, b.i.d. 6th day 20 mg/kg	No spontaneous withdrawal symptoms	A_{2A} KO mice	Soria et al. (2004)
Precipitated withdrawal by SR141716A		Precipitated withdrawal symptoms (wet dog shakes, paw tremor, tremor, ptosis, piloerection, genital licks, mastication)		
Precipitated withdrawal by SR141716A	4 days, 25 mg/kg, b.i.d. 5th day 25 mg/kg	Withdrawal reactions (hyperlocomotion and paw tremors)	C57BL/6 mice	Huang, Liu-Chen, Unterwald, and Cowan (2009)
Precipitated withdrawal by SR141716A	High-dose regimen: 50 mg/kg, b.i.d. for 5.5 days Low-dose regimen: 10 mg/kg for 6 days	Withdrawal reactions (paw tremors, head twitches	C57BL/6J mice	Schlosburg et al. (2009)
Precipitated withdrawal by SR141716A	Chronic treatment with 1 mg/kg per 12 h, s.c.	Discriminated stimulus effects of SR141716A and head shakes	Rhesus monkeys	Stewart and McMahon (2010)

TABLE 68.2 Studies Regarding the Role of Cannabidiol in Reward

Model	Dose of CBD	Brief result	Animals	References
ICSS	5 mg/kg	No effect	Sprague-Dawley rats	Katsidoni et al. (2013a)
	10, 20 mg/kg	Increased ICSS threshold		
CPP	5 mg/kg	No effect	Sprague-Dawley rats	Parker, Burton, Sorge, Yakiwchuk, and Mechoulam (2004)
CPP	1, 10 mg/kg	No effect (but reversed the CPA in mice treated with 10 mg/kg Δ^9-THC)	ICR mice	Vann et al. (2008)
CPP	1, 3, 10 mg/kg	Tendency for CPP only when administered in combination with 10 mg/kg Δ^9-THC	Albino Wistar rats	Klein et al. (2011)

Sprague-Dawley rats, depending on the dose used (Katsidoni et al., 2013b). Indeed, a low dose of 0.1 mg/kg decreased ICSS thresholds, and caused clear parallel leftward shifts in the rate-frequency function, whereas a higher dose of 1 mg/kg increased ICSS thresholds, and produce rightward shifts. These effects are specifically mediated by cannabinoid 1 (CB_1) receptors, since they have been reversed by a low dose of the CB_1 receptor antagonist SR141716A. These results therefore need to be interpreted with caution. These seemingly contradictory results could be attributed to differences in the dose range of the tested compounds, the strain of the animals used, and the methods followed. More importantly, it is possible that Lewis rats may have a differential sensitivity to Δ^9-THC, compared to Sprague-Dawley and Fisher 344 rats, and it could conceivably be hypothesized that the dose-response function of Δ^9-THC on brain stimulation reward is not linear, but rather biphasic.

CBD is a nonpsychotomimetic constituent of *Cannabis sativa*, which induces central effects in rodents. Although CBD has been shown to affect emotional responses, to date, only one study has been undertaken to evaluate its effects on brain stimulation reward (Table 68.2). According to the findings of this report, low doses of CBD did not affect brain stimulation reward, whereas higher doses increased ICSS thresholds, and produce rightward shifts (Katsidoni, Anagnostou, & Panagis, 2013a). Thus, CBD lacks hedonic properties and abuse potential, but can reduce brain reward function, producing an anhedonic-like state. Interestingly, it has been also shown that CBD blocks the reward-facilitating effect of morphine in the ICSS paradigm (Katsidoni et al., 2013a), and attenuates cue-induced reinstatement of heroin-seeking behavior (Ren, Whittard, Higuera-Matas, Morris, & Hurd, 2009).

Δ^9-THC Self-Administration

Most drugs abused by humans, including psychostimulants, opiates, ethanol, and nicotine, support reliable and persistent self-administration behavior in drug naive experimental animals, under normal experimental conditions. However, for many years, demonstrations of cannabis or Δ^9-THC self-administration in drug naive rodents or primates had been limited and unconvincing. Interestingly, in many studies, the procedures of the self-administration experiments have been varied in order to demonstrate reliable, persistent, and dose-dependent self-administration of Δ^9-THC in experimental animals (Table 68.1).

Kaymakcalan (1981) was the first who demonstrated successful self-administration of Δ^9-THC in two monkeys out of six studied, but only after withdrawal from forced automatic intravenous injections of Δ^9-THC, when signs of physical dependence on Δ^9-THC occurred. Furthermore, one monkey exhibited Δ^9-THC self-administration behavior following cocaine self-administration. In the same study, drug-naive monkeys did not self-administer Δ^9-THC (Kaymakcalan, 1981). Similarly, other studies have shown self-administration of Δ^9-THC in experimental animals with a previous self-administration experience of phencyclidine, amphetamine, cocaine, or food (Justinova et al., 2004; Justinova et al., 2008, 2013; Tanda et al., 2000; Thompson & Pickens, 1975). However, these studies suffer from some serious limitations. Indeed, previous drug exposure may affect acquisition and maintenance of self-administration of various abused drugs in experimental animals, since the motivational state in naive animals, compared with animals that self-administer other drugs, could be different, and therefore their corresponding response could also vary accordingly. Interestingly, there are studies that have failed to show robust Δ^9-THC self-administration, even in animals preexposed to other drugs of abuse, such as cocaine, phenobarbital, phencyclidine, ethanol, or amphetamine (Harris et al., 1974; Li et al., 2012; Mansbach et al., 1994).

Food deprivation was another frequently used manipulation in the study of Δ^9-THC self-administration. Indeed, Takahashi and Singer reported Δ^9-THC self-administration in food-restricted rats (Takahashi & Singer, 1979, 1980). Interestingly, however, self-administration immediately decreased to placebo levels, when food-restriction was discontinued. Thus, a criticism of this research is that self-administration Δ^9-THC

was demonstrated under special conditions that may have altered the functional or motivational state of the animals.

In a seminal article, Justinova et al. (2003) showed for the first time that low doses of Δ^9-THC can initiate and sustain high rates of intravenous self-administration in drug-naive squirrel monkeys. In their study, they used three drug naive squirrel monkeys, low doses of Δ^9-THC (1–8 μg/kg per injection) that, according to the authors, were several times lower than the doses generally used in previous attempts to demonstrate Δ^9-THC self-administration in monkeys, and comparable to those delivered by an average marijuana cigarette. The self-administration behavior was rapidly extinguished, either by substituting vehicle injections for Δ^9-THC injections, or by administering the CB_1 receptor antagonist SR141716A before the session, indicating that this effect was mediated through the CB_1 receptor. Interestingly, Justinova et al. (2008) were also the first to report Δ^9-THC self-administration using a drug-seeking procedure (second-order schedule), although in that study the monkeys had a previous experience with self-administration of amphetamine, cocaine, or food.

Two studies investigated the possibility that Δ^9-THC is self-administered intracerebrally by experimental animals. Braida et al. (2004) were the first to report intracerebroventricular self-administration of Δ^9-THC in water-deprived rats. Subsequently, Zangen et al. (2006) reported Δ^9-THC self-administration directly into the posterior ventral tegmental area and the shell of the nucleus accumbens.

To conclude, studies with self-administration of Δ^9-THC under normal experimental conditions have not always been consistent. Thus, it could be argued that self-administration of Δ^9-THC occurs under more limited conditions than self-administration of other drugs of abuse. However, a number of studies report robust self-administration of Δ^9-THC under normal conditions, either in squirrel monkeys or, intracerebrally, in rodents. These findings provide some evidence that Δ^9-THC produces rewarding effects through the same reward brain circuitry as other drugs of abuse.

Δ^9-THC and CBD Effects on Conditioned Place Preference (CPP)

Although most drugs abused by humans produce a clear CPP in experimental animals, the corresponding data on Δ^9-THC are not very consistent. Indeed, Δ^9-THC can induce appetitive effects under some conditions, but aversive effects under others (Table 68.1). This is determined at least partly by the particular dose used, the administration of a priming injection, and the timing between injections. Surprisingly, in the studies reporting CPP, the preference is connected to a single dose. Since this is not the case with other drugs of abuse, in which CPP can be obtained over a range of doses, it has been suggested that cannabis is an atypical or anomalous drug of abuse.

In the study by Lepore et al. (1995), 2–4 mg/kg Δ^9-THC produced CPP in Long Evans rats when the CPP pairing interval was 24 h; using the same experimental design, 1 mg/kg of Δ^9-THC did not produce any preference). However, when the schedule of daily injections was changed, allowing a longer washout interval between injections (48 h), Δ^9-THC produced a clear place aversion in the dose of 2–4 mg/kg, but CPP in the dose of 1 mg/kg. Thus, Δ^9-THC induced CPP was dependent upon the dose and the injection schedule. In this regard, increasing the time interval between Δ^9-THC injections might have changed the effects of Δ^9-THC due to a postdrug dysphoric rebound effect. That is, at the shorter pairing interval, the post Δ^9-THC rebound dysphoria might have attenuated Δ^9-THC's rewarding effect, while at the longer pairing interval, where the post Δ^9-THC rebound effect subsided, the effects of all doses were accentuated, allowing the 1 mg/kg to be rewarding, and the highest doses to be aversive.

Similar results have been reported in subsequent studies. Braida et al. (2004) demonstrated Δ^9-THC induced CPP in Wistar rats, in a dose range between 0.075 and 0.75 mg/kg; in the same study, higher doses produced aversive effects. The authors suggested that the low doses of Δ^9-THC used in their experiment could have minimized any dysphoric effects of the drug that might lead to aversion. In another study, Le Foll et al. (2006) reported that a low dose of Δ^9-THC (0.1 mg/kg) produced CPP, while doses lower (ie, 0.01 mg/kg) or higher (ie, 1 mg/kg) than this did not produce any preference. However, more recently, the Goldberg group did not find any rewarding or aversive effects of Δ^9-THC in the CPP paradigm in Sprague-Dawley rats, and in the dose range of 0.1–3 mg/kg (Scherma et al., 2012).

Valjent and Maldonado (2000) reported Δ^9-THC induced CPP bypassing the dysphoric/aversive effects of Δ^9-THC that has been reported in naive animals, with a priming injection 24 h before the first conditioning session. However, Vlachou et al. (2007), using the same experimental manipulation, did not found CPP with the 1 mg dose of Δ^9-THC. These differences can be explained, in part, by the different strain of animals used, the number of pairings, or the periods of conditioning and administration of the drugs. Although Maldonado's group replicated their findings in a subsequent study (Soria et al., 2004), it is worth noting that they also report conditioned place aversion with the dose of 5 mg/kg of Δ^9-THC.

A number of studies reported clear aversive effects of Δ^9-THC in the CPP paradigm, in rats or mice (Hutcheson et al., 1998; Mallet & Beninger, 1998; Parker & Gillies, 1995; Sanudo-Pena et al., 1997; Vann et al., 2008).

Two studies have investigated whether adolescent rats are more vulnerable to Δ^9-THC than adult rats. Quinn et al. (2008) found that Δ^9-THC (5 mg/kg) produced CPA in adult rats, whereas in adolescent rats there was only a nonsignificant trend toward aversion. Interestingly, the aversive effect reported in adult rats was long-lasting, since the animals still avoided Δ^9-THC paired environment 16 days following the last drug exposure. Surprisingly, in a more recent study in adolescent Wistar rats, although Δ^9-THC (10 mg/kg) did not induce CPP when administered alone, it tended to produce a preference when administered in combination with cannabidiol (Klein et al., 2011). Surprisingly, however, when administered alone, CBD lacks hedonic properties because it does not induce CPP (Parker et al., 2004; Vann et al., 2008) (Table 68.2).

In conclusion, while almost all drugs abused by humans are able to produce a clear and reliable CPP (ie, increase the time spent in the drug paired compartment) over a wide range of doses, results with Δ^9-THC have not always been consistent. The studies reporting a CPP associated with administration of Δ^9-THC have used particular experimental methodology, and the preference often occurs at only a single dose, complicating the interpretation of the results.

Δ^9-THC Physical Dependence and Withdrawal

Although experimental animals treated chronically with most drugs of abuse exhibit typical withdrawal reactions, either following drug cessation or upon the administration of the appropriate receptor antagonist, data on Δ^9-THC are largely controversial. Indeed, several studies failed to report spontaneous withdrawal symptoms following abrupt cessation of chronic Δ^9-THC administration in experimental animals (Aceto et al., 1996; Chesher & Jackson, 1974; Dewey et al., 1972; Diana et al., 1998; Harris et al., 1974; Leite & Carlini, 1974; McMillan et al., 1970). It has been proposed that the main reason for this is the particular pharmacokinetic properties of Δ^9-THC (high lipophilicity, long duration of action, and slow elimination) that may prevent the development of a pronounced withdrawal syndrome, when the drug is abruptly discontinued (Gonzalez et al., 2005). Kaymakcalan et al. (1977) published a paper in which they reported a spontaneous withdrawal reaction after chronic Δ^9-THC treatment. However, the authors treated the rats for 5 weeks with extremely high doses that, in the last 2 weeks, reached 40 mg/kg twice daily.

In spite of the failure to observe clear spontaneous withdrawal reactions following Δ^9-THC cessation, Beardsley et al. (1986) reported a suppression of operant behavior for food reinforcement in monkeys. The authors interpreted this finding as a manifestation of spontaneous withdrawal, since it returned to normal after readministration of the drug. In a latter study, they replicated the same behavioral pattern in rats (Beardsley & Martin, 2000). Similarly, Fredericks and Benowitz (1980) suggested a cannabis abstinence syndrome following cessation of Δ^9-THC in monkeys, with core symptoms being an increased frequency and/or duration of gross movement, eye contact, and tooth bearing. However, these manifestations deviate from the view of the classical withdrawal syndrome observed with other drugs of abuse.

In the absence of a clear spontaneous withdrawal from Δ^9-THC in experimental animals, a number of investigators have tried to demonstrate physical dependence and withdrawal signs by challenging chronically treated animals with a CB_1 receptor antagonist, such as SR141716A. Indeed, administration of the CB_1 receptor antagonist SR141716A in experimental animals chronically treated with Δ^9-THC precipitates specific withdrawal reactions. The signs observed are largely somatic (mainly of motor nature), with no relevant vegetative changes. The most characteristic withdrawal signs are wet-dog shakes, head shakes, facial rubbing, front paw tremor, body tremor, ataxia, eyelid ptosis, piloerection, hyperlocomotion, retropulsion, licking, scratching, and mastication (Aceto et al., 1995; Aceto et al., 1996; Anggadiredja et al., 2003; Gonzalez et al., 2004; Huang et al., 2009; Hutcheson et al., 1998; Ledent et al., 1999; Lichtman et al., 2001; Schlosburg et al., 2009; Soria et al., 2004; Stewart & McMahon, 2010; Tzavara et al., 2000). These somatic signs, even if they appear very mild, are mediated through the CB_1 receptor, since in CB_1 KO mice receiving chronic treatment with Δ^9-THC administration of the CB_1 receptor antagonist SR141716A did not precipitate any signs of abstinence (Ledent et al., 1999). However, the most serious disadvantage of many of the studies cited earlier is that the authors used extremely large doses of Δ^9-THC and/or SR141716A. This may have complicated the obtained results and, thus, the generalizability of much published research on this issue is problematic.

Although most drugs of abuse upon withdrawal after chronic treatment exhibit a clear "anhedonic" state, as reflected in elevated ICSS thresholds (Paterson & Markou, 2007), this has not been investigated properly with Δ^9-THC. Gardner and Vorel (1998) report that "withdrawal from as little as a single 1.0 mg/kg dose of Δ^9-THC produces significant elevations in electrical brain stimulation thresholds." However, one major criticism of this work is that we cannot explain how a single and relatively low dose of Δ^9-THC could produce dependence, so that withdrawal anhedonia can be observed. Since Gardner et al. (1988) adopt in their studies a very strict criterion of stable responding (ie, 0.01 log units for three consecutive days), it is possible that the observed effect of Δ^9-THC in their study could reflect normal baseline variation over days.

In summary, there is no conclusive evidence for a robust spontaneous withdrawal in experimental animals treated chronically with Δ^9-THC. The precipitated withdrawal has been shown using very high doses of Δ^9-THC, which exceed the relevant dose range in human cannabis users.

CONCLUSIONS

From a wide variety of data, it appears that Δ^9-THC activates brain reward circuitry and enhances reward-related behaviors in similar fashion to other drugs of abuse, although the range of conditions under which this occurs is more limited than with drugs such as cocaine, heroin, alcohol, and nicotine. The rewarding properties of Δ^9-THC are clearly shown by a decrease in brain stimulation reward thresholds, and self-administration behavior. However, particular experimental conditions are required to induce Δ^9-THC place preference, and many studies have reported aversive-like responses of Δ^9-THC in the CPP paradigm. Similarly, the doses of Δ^9-THC required to induce physical dependence and withdrawal in experimental animals are very high, and are not comparable with those consumed by humans. Moreover, there is no conclusive evidence for a robust spontaneous withdrawal in experimental animals treated chronically with Δ^9-THC. Thus, withdrawal reactions have been only observed after challenge with the CB_1 receptor antagonist SR141716A. On the other hand, the other major phytocannabinoid, cannabidiol, does not produce rewarding effects in experimental animals, as evident by a lack of effect on the ICSS and the CPP paradigm. Interestingly, cannabidiol exerts a wide range of pharmacological effects, many of which are of potential therapeutic interest, without exerting significant intrinsic activity upon CB_1 receptors. Presumably, for this reason, cannabidiol lacks hedonic properties. In general, these animal behavioral models have provided a better understanding of the rewarding effects of Δ^9-THC, and have revealed commonalities between Δ^9-THC and other drugs of abuse. We believe that more precise examination of the actions of Δ^9-THC in brain areas of the reward circuitry will advance our understanding of the process of drug addiction. On the other hand, cannabidiol which is devoid of Δ^9-THC-like psychoactive effects represents a phytocannabinoid with high potential for therapeutic use.

MINI-DICTIONARY

Antagonist-precipitated withdrawal Withdrawal from long term administration of an addictive drug, caused by the administration of an appropriate antagonistic drug. Precipitated withdrawal is similar to spontaneous withdrawal, but is more intense and has a much faster onset.

Cannabidiol (CBD) A major nonpsychotomimetic constituent of *Cannabis sativa*. It exerts a plethora of pharmacological effects, many of them associated with central actions.

Cannabinoid 1 (CB_1) receptor The most common G-protein coupled receptor in the brain through which Δ^9-THC elicits its psychological effects.

Cannabinoids A class of diverse chemical compounds that bind to cannabinoid receptors.

Cannabis sativa The hemp plant, which is the source of marijuana. It contains over 400 chemical compounds, including about 80 terpenophenols, known as plant cannabinoids (phytocannabinoids).

Conditioned place preference (CPP) paradigm A procedure that assesses an experimental animal's preference for environments (contexts) in which it has previously experienced drug effects. Although this procedure does not directly measure drug reinforcement, it can be used to infer the motivational value of addictive drugs. Thus, CPP produced by a drug indicates that it exhibits rewarding properties, and that it might therefore have abuse potential in humans.

Intracranial self-stimulation (ICSS) paradigm An operant behavioral paradigm in which animals press a lever to administer brief electrical pulses to their own brains. It has been shown that most drugs of abuse are able to lower brain stimulation reward threshold, indicating that they activate the same substrate with electrical stimulation in a synergistic manner.

Physical dependence A state in which the discontinuation of drug taking will induce typical withdrawal reactions.

Self-administration paradigm A behavioral paradigm in which animals are given the opportunity to self-administer a drug intravenously, orally, or intracerebrally, by making an operant response, such as pressing a lever in an operant chamber, or inserting their nose into a hole.

Spontaneous withdrawal A procedure in which the chronic administration of an addictive drug is abruptly discontinued, and the physical signs that emerge are evaluated.

SR141716A (also known as rimonabant) A highly potent and selective CB_1 receptor antagonist that has been used extensively in many studies as pharmacological tool.

Δ^9-Tetrahydrocannabinol (Δ^9-THC) The major psychoactive constituent of the plant *Cannabis sativa* that was isolated in 1964 by Gaoni and Mechoulam.

Withdrawal syndrome The appearance of symptoms opposite to those produced by an addictive drug when the drug is administered repeatedly and then is no longer readministered, or is administered in smaller quantities.

References

Aceto, M. D., Scates, S. M., Lowe, J. A., & Martin, B. R. (1995). Cannabinoid precipitated withdrawal by the selective cannabinoid receptor antagonist, SR 141716A. *European Journal of Pharmacology*, *282*(1–3), R1–R2.

Aceto, M. D., Scates, S. M., Lowe, J. A., & Martin, B. R. (1996). Dependence on delta 9-tetrahydrocannabinol: studies on precipitated and abrupt withdrawal. *Journal of Pharmacology and Experimental Therapeutics*, *278*(3), 1290–1295.

Anggadiredja, K., Yamaguchi, T., Tanaka, H., Shoyama, Y., Watanabe, S., & Yamamoto, T. (2003). Prostaglandin E2 attenuates SR 141716A-precipitated withdrawal in tetrahydrocannabinol-dependent mice. *Brain Research*, *966*(1), 47–53.

Beardsley, P. M., Balster, R. L., & Harris, L. S. (1986). Dependence on tetrahydrocannabinol in rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics*, *239*(2), 311–319.

Beardsley, P. M., & Martin, B. R. (2000). Effects of the cannabinoid CB(1) receptor antagonist, SR 141716A, after Delta(9)-tetrahydrocannabinol withdrawal. *European Journal of Pharmacology*, *387*(1), 47–53.

Braida, D., Iosue, S., Pegorini, S., & Sala, M. (2004). Delta9-tetrahydrocannabinol-induced conditioned place preference and intracerebroventricular self-administration in rats. *European Journal of Pharmacology*, *506*(1), 63–69.

Chesher, G. B., & Jackson, D. M. (1974). The effect of withdrawal from cannabis on pentylenetetrazol convulsive threshold in mice. *Psychopharmacologia*, *40*(2), 129–135.

Dewey, W. L., Jenkins, J., O'Rourke, T., & Harris, L. S. (1972). The effects of chronic administration of trans-9-tetrahydrocannabinol on behavior and the cardiovascular system of dogs. *Archives Internationales de Pharmacodynamie et de Therapie*, *198*(1), 118–131.

Diana, M., Melis, M., Muntoni, A. L., & Gessa, G. L. (1998). Mesolimbic dopaminergic decline after cannabinoid withdrawal. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(17), 10269–10273.

Fokos, S., & Panagis, G. (2010). Effects of delta9-tetrahydrocannabinol on reward and anxiety in rats exposed to chronic unpredictable stress. *Journal of Psychopharmacology*, *24*(5), 767–777.

Fredericks, A. B., & Benowitz, N. L. (1980). An abstinence syndrome following chronic administration of delta-9-terahydrocannabinol in rhesus monkeys. *Psychopharmacology (Berlin)*, *71*(2), 201–202.

Gardner, E. L., Paredes, W., Smith, D., Donner, A., Milling, C., Cohen, D., & Morrison, D. (1988). Facilitation of brain stimulation reward by delta 9-tetrahydrocannabinol. *Psychopharmacology (Berlin)*, *96*(1), 142–144.

Gardner, E. L., & Vorel, S. R. (1998). Cannabinoid transmission and reward-related events. *Neurobiology of Disease*, *5*(6 Pt B), 502–533.

Gonzalez, S., Cebeira, M., & Fernandez-Ruiz, J. (2005). Cannabinoid tolerance and dependence: a review of studies in laboratory animals. *Pharmacology Biochemistry and Behavior*, *81*(2), 300–318.

Gonzalez, S., Fernandez-Ruiz, J., Di Marzo, V., Hernandez, M., Arevalo, C., Nicanor, C., Ramos, J. A., et al. (2004). Behavioral and molecular changes elicited by acute administration of SR 141716 to Delta9-tetrahydrocannabinol-tolerant rats: an experimental model of cannabinoid abstinence. *Drug and Alcohol Dependence*, *74*(2), 159–170.

Green, B., Kavanagh, D., & Young, R. (2003). Being stoned: a review of self-reported cannabis effects. *Drug and Alcohol Reviews*, *22*(4), 453–460.

Harris, R. T., Waters, W., & McLendon, D. (1974). Evaluation of reinforcing capability of delta-9-tetrahydrocannabinol in rhesus monkeys. *Psychopharmacologia*, *37*(1), 23–29.

Huang, P., Liu-Chen, L. Y., Unterwald, E. M., & Cowan, A. (2009). Hyperlocomotion and paw tremors are two highly quantifiable signs of SR 141716-precipitated withdrawal from delta9-tetrahydrocannabinol in C57BL/6 mice. *Neuroscience Letters*, *465*(1), 66–70.

Hutcheson, D. M., Tzavara, E. T., Smadja, C., Valjent, E., Roques, B. P., Hanoune, J., & Maldonado, R. (1998). Behavioural and biochemical evidence for signs of abstinence in mice chronically treated with delta-9-tetrahydrocannabinol. *British Journal of Pharmacology*, *125*(7), 1567–1577.

Justinova, Z., Mascia, P., Wu, H. Q., Secci, M. E., Redhi, G. H., Panlilio, L. V., & Goldberg, S. R. (2013). Reducing cannabinoid abuse and preventing relapse by enhancing endogenous brain levels of kynurenic acid. *Nature Neuroscience*, *16*(11), 1652–1661.

Justinova, Z., Munzar, P., Panlilio, L. V., Yasar, S., Redhi, G. H., Tanda, G., & Goldberg, S. R. (2008). Blockade of THC-seeking behavior and relapse in monkeys by the cannabinoid CB(1)-receptor antagonist rimonabant. *Neuropsychopharmacology*, *33*(12), 2870–2877.

Justinova, Z., Tanda, G., Munzar, P., & Goldberg, S. R. (2004). The opioid antagonist naltrexone reduces the reinforcing effects of Delta 9 tetrahydrocannabinol (THC) in squirrel monkeys. *Psychopharmacology (Berlin)*, *173*(1–2), 186–194.

Justinova, Z., Tanda, G., Redhi, G. H., & Goldberg, S. R. (2003). Self-administration of delta9-tetrahydrocannabinol (THC) by drug naive squirrel monkeys. *Psychopharmacology (Berlin)*, *169*(2), 135–140.

Katsidoni, V., Anagnostou, I., & Panagis, G. (2013a). Cannabidiol inhibits the reward-facilitating effect of morphine: involvement of 5-HT1A receptors in the dorsal raphe nucleus. *Addiction Biology*, *18*(2), 286–296.

Katsidoni, V., Kastellakis, A., & Panagis, G. (2013b). Biphasic effects of Delta9-tetrahydrocannabinol on brain stimulation reward and motor activity. *International Journal of Neuropsychopharmacology*, *16*(10), 2273–2284.

Kaymakcalan, S. (1981). The addictive potential of cannabis. *Bulletin on Narcotics*, *33*(2), 21–31.

Kaymakcalan, S., Ayhan, I. H., & Tulunay, F. C. (1977). Naloxone-induced or postwithdrawal abstinence signs in delta9-tetrahydrocannabinol-tolerant rats. *Psychopharmacology (Berlin)*, *55*(3), 243–249.

Klein, C., Karanges, E., Spiro, A., Wong, A., Spencer, J., Huynh, T., & McGregor, I. S. (2011). Cannabidiol potentiates Delta(9)-tetrahydrocannabinol (THC) behavioural effects and alters THC pharmacokinetics during acute and chronic treatment in adolescent rats. *Psychopharmacology (Berlin)*, *218*(2), 443–457.

Kucharski, L. T., Williams, J. E., & Kornetsky, C. (1983). The effects of levonantradol on rewarding brain stimulation thresholds in the rat. *Pharmacology Biochemistry and Behavior*, *19*(1), 149–151.

Kwilasz, A. J., & Negus, S. S. (2012). Dissociable effects of the cannabinoid receptor agonists Delta9-tetrahydrocannabinol and CP55940 on pain-stimulated versus pain-depressed behavior in rats. *Journal of Pharmacology and Experimental Therapeutics*, *343*(2), 389–400.

Le Foll, B., Wiggins, M., & Goldberg, S. R. (2006). Nicotine pre-exposure does not potentiate the locomotor or rewarding effects of Delta-9-tetrahydrocannabinol in rats. *Behavioral Pharmacology*, *17*(2), 195–199.

Ledent, C., Valverde, O., Cossu, G., Petitet, F., Aubert, J. F., Beslot, F., & Parmentier, M. (1999). Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science*, *283*(5400), 401–404.

Leite, J. R., & Carlini, E. A. (1974). Failure to obtain "cannabis-directed behavior" and abstinence syndrome in rats chronically treated with cannabis sativa extracts. *Psychopharmacologia*, *36*(2), 133–145.

Lepore, M., Liu, X., Savage, V., Matalon, D., & Gardner, E. L. (1996). Genetic differences in delta 9-tetrahydrocannabinol-induced facilitation of brain stimulation reward as measured by a rate-frequency curve-shift electrical brain stimulation paradigm in three different rat strains. *Life Sciences*, *58*(25), PL365–PL372.

Lepore, M., Vorel, S. R., Lowinson, J., & Gardner, E. L. (1995). Conditioned place preference induced by delta 9-tetrahydrocannabinol: comparison with cocaine, morphine, and food reward. *Life Sciences*, *56*(23–24), 2073–2080.

Li, J. X., Koek, W., & France, C. P. (2012). Interactions between Delta(9)-tetrahydrocannabinol and heroin: self-administration in rhesus monkeys. *Behavioral Pharmacology*, *23*(8), 754–761.

Lichtman, A. H., Fisher, J., & Martin, B. R. (2001). Precipitated cannabinoid withdrawal is reversed by Delta(9)-tetrahydrocannabinol or clonidine. *Pharmacology Biochemistry and Behavior*, *69*(1–2), 181–188.

Mallet, P. E., & Beninger, R. J. (1998). Delta9-tetrahydrocannabinol, but not the endogenous cannabinoid receptor ligand anandamide, produces conditioned place avoidance. *Life Sciences*, *62*(26), 2431–2439.

Mansbach, R. S., Nicholson, K. L., Martin, B. R., & Balster, R. L. (1994). Failure of Delta(9)-tetrahydrocannabinol and CP 55, 940 to maintain intravenous self-administration under a fixed-interval schedule in rhesus monkeys. *Behavioral Pharmacology*, *5*(2), 219–225.

McMillan, D. E., Harris, L. S., Frankenheim, J. M., & Kennedy, J. S. (1970). l-Dgr9-trans-tetrahydrocannabinol in pigeons: tolerance to the behavioral effects. *Science*, *169*(3944), 501–503.

Parker, L. A., Burton, P., Sorge, R. E., Yakiwchuk, C., & Mechoulam, R. (2004). Effect of low doses of delta9-tetrahydrocannabinol and cannabidiol on the extinction of cocaine-induced and amphetamine-induced conditioned place preference learning in rats. *Psychopharmacology (Berlin)*, *175*(3), 360–366.

Parker, L. A., & Gillies, T. (1995). THC-induced place and taste aversions in Lewis and Sprague-Dawley rats. *Behavioral Neuroscience*, *109*(1), 71–78.

Paterson, N. E., & Markou, A. (2007). Animal models and treatments for addiction and depression co-morbidity. *Neurotoxicity Research*, *11*(1), 1–32.

Quinn, H. R., Matsumoto, I., Callaghan, P. D., Long, L. E., Arnold, J. C., Gunasekaran, N., & McGregor, I. S. (2008). Adolescent rats find repeated Delta(9)-THC less aversive than adult rats but display greater residual cognitive deficits and changes in hippocampal protein expression following exposure. *Neuropsychopharmacology*, *33*(5), 1113–1126.

Ramo, D. E., Liu, H., & Prochaska, J. J. (2012). Reliability and validity of young adults' anonymous online reports of marijuana use and thoughts about use. *Psychology of Addictive Behaviors*, *26*(4), 801–811.

Ren, Y., Whittard, J., Higuera-Matas, A., Morris, C. V., & Hurd, Y. L. (2009). Cannabidiol, a nonpsychotropic component of cannabis, inhibits cue-induced heroin seeking and normalizes discrete mesolimbic neuronal disturbances. *Journal of Neuroscience*, *29*(47), 14764–14769.

Sanudo-Pena, M. C., Tsou, K., Delay, E. R., Hohman, A. G., Force, M., & Walker, J. M. (1997). Endogenous cannabinoids as an aversive or counter-rewarding system in the rat. *Neuroscience Letters*, *223*(2), 125–128.

Scherma, M., Justinova, Z., Zanettini, C., Panlilio, L. V., Mascia, P., Fadda, P., & Goldberg, S. R. (2012). The anandamide transport inhibitor AM404 reduces the rewarding effects of nicotine and nicotine-induced dopamine elevations in the nucleus accumbens shell in rats. *British Journal of Pharmacology*, *165*(8), 2539–2548.

Schlosburg, J. E., Carlson, B. L., Ramesh, D., Abdullah, R. A., Long, J. Z., Cravatt, B. F., & Lichtman, A. H. (2009). Inhibitors of endocannabinoid-metabolizing enzymes reduce precipitated withdrawal responses in THC-dependent mice. *AAPS Journal*, *11*(2), 342–352.

Soria, G., Castane, A., Berrendero, F., Ledent, C., Parmentier, M., Maldonado, R., & Valverde, O. (2004). Adenosine A2A receptors are involved in physical dependence and place conditioning induced by THC. *European Journal of Neuroscience*, *20*(8), 2203–2213.

Stark, P., & Dews, P. B. (1980). Cannabinoids. I. Behavioral effects. *Journal of Pharmacology and Experimental Therapeutics*, *214*(1), 124–130.

Stewart, J. L., & McMahon, L. R. (2010). Rimonabant-induced Delta9-tetrahydrocannabinol withdrawal in rhesus monkeys: discriminative stimulus effects and other withdrawal signs. *Journal of Pharmacology and Experimental Therapeutics*, *334*(1), 347–356.

Takahashi, R. N., & Singer, G. (1979). Self-administration of delta 9-tetrahydrocannabinol by rats. *Pharmacology Biochemistry and Behavior*, *11*(6), 737–740.

Takahashi, R. N., & Singer, G. (1980). Effects of body weight levels on cannabis self-injection. *Pharmacology Biochemistry and Behavior*, *13*(6), 877–881.

Tanda, G., Munzar, P., & Goldberg, S. R. (2000). Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. *Nature Neuroscience*, *3*(11), 1073–1074.

Thompson, T., & Pickens, R. (1975). An experimental analysis of behavioral factors in drug dependence. *Federation Proceedings*, *34*(9), 1759–1770.

Tzavara, E. T., Valjent, E., Firmo, C., Mas, M., Beslot, F., Defer, N., & Maldonado, R. (2000). Cannabinoid withdrawal is dependent upon PKA activation in the cerebellum. *European Journal of Neuroscience*, *12*(3), 1038–1046.

Valjent, E., & Maldonado, R. (2000). A behavioural model to reveal place preference to delta 9-tetrahydrocannabinol in mice. *Psychopharmacology (Berlin)*, *147*(4), 436–438.

Vann, R. E., Gamage, T. F., Warner, J. A., Marshall, E. M., Taylor, N. L., Martin, B. R., & Wiley, J. L. (2008). Divergent effects of cannabidiol on the discriminative stimulus and place conditioning effects of Delta(9)-tetrahydrocannabinol. *Drug and Alcohol Dependence*, *94*(1–3), 191–198.

Vlachou, S., Nomikos, G. G., Stephens, D. N., & Panagis, G. (2007). Lack of evidence for appetitive effects of Delta 9-tetrahydrocannabinol in the intracranial self-stimulation and conditioned place preference procedures in rodents. *Behavioral Pharmacology*, *18*(4), 311–319.

Zangen, A., Solinas, M., Ikemoto, S., Goldberg, S. R., & Wise, R. A. (2006). Two brain sites for cannabinoid reward. *Journal of Neuroscience*, *26*(18), 4901–4907.

CHAPTER

69

Peroxisome Proliferator Activated Receptors and Cannabinoids

E. Muñoz[*], *F. Pollastro*[**], *O. Taglialatela-Scafati*[†], *G. Appendino*[**]

*Department of Cell Biology, Physiology and Immunology, University of Córdoba, Córdoba, Spain
**Department of Pharmaceutical Science, University of Piemonte Orientale, Novara, Italy
†Department of Pharmacy, University of Napoli Federico II, Napoli, Italy

SUMMARY POINTS

- This chapter focuses on cannabinoids as PPARγ ligands.
- Cannabidiol, a nonpsychotropic cannabinoid, is a promising candidate for the management of neuroinflammatory and neurodegenerative diseases.
- Cannabinoids are valid templates for the development of novel chemical entities with superior activities on well-defined molecular targets.
- Oxidation of CBD and CBG leads to the paraquinones HU-311 and VCE-003, respectively.
- HU-311 and VCE-003 bind and activate PPARγ.

KEY FACTS OF CANNABINOIDS

- Plant-derived cannabinoids represent a class of natural products endowed with pleiotropic activities and low toxicological profile.
- Endocannabinoids are produced in the brain and periphery.

KEY FACTS OF PPARγ

- PPARγ is expressed in all type of cells present in the CNS.
- PPARγ is a validated target for the development of novel therapies against neuroinflammatory and neurodegenerative diseases.
- There is evidence showing that cannabinoids exert their bioactivities through PPARγ-dependent and PPARγ-independent mechanisms.

KEY FACTS OF POLYPHARMACOLOGY

- Polypharmacology defines drug molecules interacting with multiple targets.
- Phytocannabinoids are pleiotropic by nature and polypharmacology is behind the benefits of this class of compounds.

KEY FACTS OF BLOOD–BRAIN BARRIER

- Blood–brain barrier is a diffusion barrier, which impedes influx of most compounds from blood to brain.
- Phytocannabinoids such as Δ^9-THC, CBD, and CBG are lipophilic, and penetrate into the brain where they can activate cannabinoid receptors, including PPARγ.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00080-6
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
AEA	Anandamide
AjA	Ajulemic acid
BBB	Blood–brain barrier
CB 2	Cannabinoid receptors type 1 and 2
CBD	Cannabidiol
CBG	Cannabigerol
EAE	Experimental autoimmune encephalopathy
eCB	Endocannabinoids
IL	Interleukin
NRs	Nuclear receptors
OLs	Oligodendrocytes
PPARs	Peroxisome proliferator activated receptors
ROS	Reactive oxygen species
RXR	Retinoid X receptor
T2D	Type 2 diabetes
Δ^9-THC	Δ^9-Tetrahydrocannabinol
TRP	Transient receptor potential

INTRODUCTION

Cannabinoids and Biological Receptors

Plant-derived cannabinoids (phytocannabinoids) refer to nearly 100 lipid-soluble compounds found exclusively in the plant *Cannabis sativa*, and different plant varieties differ in their content of phytocannabinoids. The two major substances, Δ^9-tetrahydrocannabinol (Δ^9-THC, the main psychoactive ingredient), and cannabidiol (CBD) are derived from the common synthetic precursor cannabigerol (CBG). These plant metabolites are synthesized from fatty acid precursors through metabolic pathways, involving specific transferases and synthases (de Meijer et al., 2003).

The use of *Cannabis sativa* preparations for ceremonial, recreational, and medical use has been common throughout history, but the psychotropic activity of Δ^9-THC has dampened the medical use of *Cannabis* since the early 20th century. Δ^9-THC was isolated, identified, and synthesized in 1964 (Gaoni & Mechoulam, 1964), and, 30-years later, the first cannabinoid receptor (CB), a G-protein coupled receptor named CB_1, was described and cloned from brain tissues (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990). Thereafter, a second cannabinoid receptor, CB_2, was cloned and found to have only a 45% homology to the CB_1 receptor (Munro, Thomas & Abu-Shaar, 1993). These CB receptors are widely distributed throughout mammalian tissue, and early studies have revealed that CB_1 receptors are the most abundant receptors in neural tissue, and responsible for the psychotropic effect of Δ^9-THC and *Cannabis* preparations. CB_2 is considered the peripheral cannabinoid receptor since it is expressed at high concentrations in cells and organs of the immune system (Mackie, 2006). However, evidence of neural CB_2 expression has been emerging only in the past few years, and its functionality is still largely unknown (Atwood, Straiker, & Mackie, 2012).

The cloning and characterization of endogenous CB_1 and CB_2 receptors led to the identification and isolation of the growing family of endogenous ligands collectively named endocannabinoids (eCBs), composed of amides, esters, and ethers of long chain polyunsaturated fatty acids. The first discovered eCBs were anandamide (AEA) and 2-arachidonoylglycerol (2-AG), but other endolipids such as noladin ether, virodhamine, *N*-palmitoylethanolamine (PEA), *N*-oleoylethanolamine, and *N*-arachidonoyl-dopamine are also considered relevant members of the eCBs family (Di Marzo, De Petrocellis, & Bisogno, 2005). eCBs are synthesized upon demand, and bind to and activate their target receptors mediating several biological effects on different tissues. The main target receptors for both phytocannabinoids and eCBs are CB_1 and CB_2, but many other receptors have been identified to respond to them (Pertwee et al., 2010). For instance, the transient receptor potential (TRP) superfamily of cation channels is targeted by different cannabinoids (Pertwee et al., 2010). Phytocannabinoids such as CBD and CBG were shown to act as full agonists of human TRPV1 (De Petrocellis et al., 2011). Recent evidence indicates that both AEA and NADA act as TRPM8 antagonists, while CBD, CBG, and Δ^9-THC were found to activate this receptor (De Petrocellis et al., 2011). In addition, TRPA1 is also activated by phytocannabinoids such as Δ^9-THC, cannabinol, and cannabichromene, as well as synthetic cannabinoids such as AM1231, HU331, and WIN55212 (Jordt et al., 2004; De Petrocellis et al., 2011).

In the past few years, it has been demonstrated that peroxisome proliferator activated receptors (PPARs), a family of nuclear receptors able to regulate lipid turnover and metabolism, are also targeted by cannabinoids of different nature (Pistis & Melis, 2010).

Nuclear Receptors

Nuclear receptors (NRs) comprise a family of 49 members sharing a highly conserved amino-terminal activation domain, a carboxy-terminal ligand binding domain, a zinc-finger DNA-binding domain, and a second activation C-terminal domain. NRs act as ligand-inducible transcription factors, and are classified into two categories (www.nursa.org). Type I NRs include estrogen, glucocorticoid, progesterone, mineralocorticoid, and androgen receptors, which undergo nuclear translocation upon ligand activation, and bind as homodimers to DNA. Type II NRs are localized in the nucleus, and bind to DNA as heterodimers with the retinoid X receptor (RXR). PPARs, thyroid hormone

receptor, liver X receptors and Vitamin D receptors fall into this category (Sladek, 2011).

PPARs

PPARs heterodimerize with RXR, and bind to PPAR responsive element (PPRE) in the regulatory region of target genes that function in diverse biological processes, such as lipid metabolism, insulin sensitivity, cell growth and differentiation, and immune response. They are also the targets of drugs that are effective in the treatment of metabolic syndrome, including insulin resistance, glucose intolerance, obesity, dyslipidemia, hypertension, atherosclerosis, and renal dysfunction (Chen, Jia, & Yang, 2014).

So far, three isoforms of PPARs have been identified, namely PPARα PPARδ (also known as PPARβ), and PPARγ (PPARγ_1 and PPARγ_2), all of which have been treated as attractive targets for developing drugs to treat type 2 diabetes (T2D). Although they share structural similarity, and exhibit high homology in amino acid sequence, the three isoforms are differentially expressed among tissues. In general, PPARα is abundant in the liver, brown adipose tissue, heart, and kidney; PPARγ is highly expressed in the adipose tissue and other tissues, and PPARδ is ubiquitously expressed. Their differential distributions, as well as different affinities to ligands, are attributed to their distinct roles. PPAR ligands vary from endogenous fatty acids such as leukotriene B4, 8-S-hydroxy eicosatetraenoic acid, and prostaglandin J2, to pharmaceutical drugs such as fibrates and thiazolidinediones (TZDs). Lipid-lowering fibrate drugs like clofibrate and fenofibrate have been shown to activate preferentially PPARα, and insulin sensitizers TZDs are selective agonists of PPARγ (Willson, Brown, Sternbach, & Henke, 2000). In addition to the beneficial effects in diabetes, specific PPARγ agonists have also been investigated as a potential therapy against several chronic diseases, such as rheumatoid arthritis, liver fibrosis, inflammatory bowel diseases, neurodegenerative and neuroinflammatory disorders, and some type of cancers (Lehrke & Lazar, 2005).

TZDs such as rosiglitazone and pioglitazone have been largely used so far in clinical practice. They provide similar effects on glycemic control, as well as a range of similar adverse effects, such as weight gain, fluid retention, and increased risk of heart failure, which seem to be PPARγ-mediated. Indeed, rosiglitazone was recently withdrawn in Europe, and its use has been restricted in the United States, as a consequence of increased risk of cardiovascular events in T2D patients.

The development and use of PPAR ligands in the past decades have greatly advanced our understanding of the physiological and pathological role of PPARs, and the therapeutic implication of targeting them. In this sense, although TZDs are potent PPARγ full agonists (PPARγ-fa) their mechanism-based side effects have limited the full therapeutic potential of those compounds (Ciudin, Hernandez, & Simó, 2012). Moreover, the use of TZDs for CNS treatments is also dampened because of their poor blood–brain barrier (BBB) permeability. Nevertheless, the physiologic and therapeutic relevance of the PPARγ pathway has promoted new studies to develop newer classes of molecules that reduce or eliminate adverse effects. Therefore, a lot of progress has been achieved in the discovery and development of selective PPARγ modulators (PPARγ-m), as safer alternatives to PPARγ-fa. This class of compounds can only activate a subset of the functions induced by the cognate ligand, or those that act in a cell-type-selective manner. The preclinical and clinical findings clearly suggest that selective PPARγ-m have the potential to become the next generation of PPARγ agonists: effective insulin sensitizers with a superior safety profile to that of PPARγ-fa (Doshi, Brahma, Bahirat, Dixit, & Nemmani, 2012). In this sense, natural and synthetic cannabinoids are considered PPARγ-m that readily transverse the BBB because of their lipophilicity. Preclinical evidence shows that cannabinoids alleviate neuroinflammatory and neurodegenerative processes through the activation of PPARγ (Fernández-Ruiz et al., 2013).

PPARγ and Neuroinflammation

The immune response evoked during noninfectious CNS diseases such as multiple sclerosis, Alzheimer disease, Parkinson disease, trauma, and ischemia/stroke often leads to the development of neuroinflammatory processes , which in turn, accelerate neurodegeneration in the majority of cases. In the last decade, there have been an increasing number of experimental studies supporting the use of PPARγ ligands to treat major disabling brain diseases.

The therapeutic effects of PPARγ agonists have been tested in many different neuroinflammatory diseases (Heneka & Landreth, 2007). Loss of function of PPARγ results in an exacerbated experimental autoimmune encephalopathy (EAE), with increased CNS inflammation and demyelination in mice (Natarajan, Muthian, Barak, Evans, & Bright, 2003), and treatment with PPARγ agonists such as glitazones and 15d-PGJ2 ameliorates EAE (Storer, Xu, Chavis, & Drew, 2005). On the other hand, PPARγ agonists reduce inflammation and the amyloid burden in animal models of Alzheimer disease (Esposito et al., 2011), regulate the inflammatory response in trauma, and reduce infarct size in animal models (Culman, Zhao, Gohlke, & Herdegen, 2007). Moreover, pioglitazone treatment effectively reduced inflammation, and restored mitochondrial function in different animal models of dopaminergic neurodegeneration (Heneka & Landreth, 2007).

The beneficial effects of PPARγ agonists in brain pathologies are mediated by different mechanisms that

may be disease-specific, and involve both peripheral and central antiinflammatory activities, by affecting crucial functions of peripheral (macrophages and/or lymphocytes) and central immune cells (microglial cells). As well as microglia, PPARγ agonists can act on other neural cell types, including astrocytes, neurons, and oligodendrocytes (Bernardo & Minghetti, 2008). While the exact mechanisms for the antiinflammatory activity of PPARγ agonists are not completely identified, it seems that this mechanism is multifactorial, and involves transcriptional and posttranscriptional mechanisms. In addition, PPARγ agonists inhibit interleukin (IL)-12 induced differentiation of Th1 cells, and alleviate EAE (Bright, Kanakasabai, Chearwae, & Chakraborty, 2008).

Oligodendrocytes (OLs) are the myelin-forming cells of the CNS. Their differentiation from precursor to mature cells occurs through a series of stages that can be defined by morphological and antigenic changes. Although PPARβ/δ has been long considered the PPAR type mainly expressed in OLs, and involved in myelination, recent findings suggest a key role of PPARγ in OL protection and differentiation (Bernardo, Bianchi, Magnaghi, & Minghetti, 2009).

Astrocytes are the most abundant glial cells in the CNS, and are essential to maintain brain homeostasis. In addition, astrocytes also exert important roles in brain inflammation and immunity. Similar to microglia, astrocytes react rapidly to external insults and/or damaging events, and represent an important target for antiinflammatory and neuroprotective therapeutic strategies. In this sense, there is large evidence showing that PPARγ agonists modulate astrocyte functions, and attenuate astrogliosis (Kielian & Drew, 2003; Drew, Xu, Storer, Chavis, & Racke, 2009). For instance, TZD compounds were reported to be neuroprotective in vivo (Luna-Medina et al., 2007). In addition, 15d-PGJ2, a natural ligand of PPARγ, suppresses the release of the proinflammatory cytokine IL-12 in LPS-stimulated astrocytes (Xu & Drew, 2007). In line with the role of PPARγ in regulating cytokines' mediated functions, it has also been observed that troglitazone increases IL-4 expression in glia cells (Loane et al., 2009).

Microglia in the CNS are the major population of brain resident macrophages. In the healthy brain, microglia maintain homeostasis by continuously interacting with the environment. When CNS infection and/or inflammation occurs, microglia respond appropriately by changing their activation state. Microglia exhibit a range of activation states associated with pathogen defense, wound healing, and tissue repair. PPARγ agonists have been extensively studied for their therapeutic potential to prevent the undesired toxic effects of microglial activation (Bright et al., 2008).

Cannabinoids as PPARs Ligands

In this review, we focus on cannabinoid-based PPARγ ligands that have been shown to exert neuroprotective activities. The effects of cannabinoids on other PPARs have been reviewed elsewhere (Mechoulam & Ross, 2010; Pertwee et al., 2010; O'Sullivan, 2007). Thus, a number of cannabinoids and endocannabinoids have been reported to be PPARγ agonists (Table 69.1). In general, the potency of cannabinoids is several orders of magnitude lower at PPARγ than the reference drugs pioglizatone and rosiglitazone, which act at low nanomolar concentrations. In addition, the activity of cannabinoids on PPARγ can be dissected from its ability to bind and activate or inhibit other cell surface receptors, such as CB_1, CB_2, GPR55, and TRPs (Pertwee et al., 2010).

It has been shown that Δ^9-THC binds to PPARγ, and activates its transcriptional activity at relatively high concentrations, compared to those required to activate CB_1 and CB_2 (Granja et al., 2012; O'Sullivan et al., 2005). Δ^9-THC activates PPARγ through CB_1- and CB_2-independent pathways, and causes PPARγ-dependent vasodilatation (O'Sullivan et al., 2005). It has been suggested that this mechanism is mediated by an increase in superoxide dismutase activity that scavenges NO by endogenous superoxides, and also to catalyze the conversion of

TABLE 69.1 Cannabinoids and Endocannabinoid-Like Molecules Acting on PPARγ

Molecule	References
Δ^9-THC	Granja et al. (2012); O'Sullivan, Tarling, Bennett, Kendall, and Randall (2005)
Cannabidiol	Granja et al. (2012); O'Sullivan and Kendall (2010)
Cannabigerol	Granja et al. (2012)
Cannabichromene	Granja et al. (2012)
Anandamide	Bouaboula et al. (2005)
Ajulemic acid	Ambrosio et al. (2007)
VCE-003	Granja et al. (2012)
HU-331	Granja et al. (2012)

superoxides to H_2O_2 (O'Sullivan et al., 2005; O'Sullivan, Kendall, & Randall, 2006). More recently, it has been reported that the PPARγ antagonist GW9662 prevented in vivo the antitumor effect of Δ^9-THC in hepatocarcinoma tumor xenografts (Vara, Morell, Rodríguez-Henche, & Diaz-Laviada, 2013). In the CNS, there is clear evidence that Δ^9-THC mediates neuroprotective effects by acting on CB_1 and PPARγ. For instance, Δ^9-THC showed neuroprotective effects through PPARγ activation in an in vitro model of Parkinson disease, where the CB_1 antagonist AM251 did not block this effect. In contrast, the PPARγ antagonist T0070907 blocked the neuroprotective effects of Δ^9-THC (Carroll, Zeissler, Hanemann, & Zajicek, 2012). Thus, it is possible that the neuroprotective effects of Δ^9-THC may be mediated not only through CB_1 signaling, but also through activation of PPARγ, thereby exemplifying the polypharmacological profile of the major phytocannabinoids.

CBD is a nonpsychotropic component of marijuana that binds to CB_1 receptors with very low affinity. Biochemical studies indicate that CBD may enhance endogenous AEA by inhibiting its intracellular degradation catalyzed by the enzyme fatty acid amide hydrolase (FAAH). However, CBD still remains enigmatic with respect to its precise mode of action. CBD targets PPARγ and some TRPs, and it is also an agonist of the $5\text{-}HT_{1A}$ receptor, as well as an antagonist of the GPR55 receptor (Pertwee et al., 2010). Although the PPARγ binding activity of CBD is relatively weak, with an EC50 ranging from 5 to 20 μM (O'Sullivan & Kendall, 2010; Granja et al., 2012), there is mounting evidence that many biological activities of CBD are mediated through PPARγ. In this sense, it cannot be discarded that CBD metabolites account for the PPARγ-mediated activity of CBD in vivo.

The interest of CBD as a therapeutic option to manage a plethora of different diseases has substantially increased in recent years. Thus, CBD is neuroprotective in rat Alzheimer disease models, and the blockade of PPARγ was able to significantly blunt CBD effects on reactive gliosis and, subsequently, on neuronal damage (Iuvone et al., 2004; Esposito et al., 2011). In the same study, CBD was observed to stimulate hippocampal neurogenesis through a PPARγ-dependent pathway (Iuvone et al., 2004). CBD, as well as Δ^9-THC, also induces adipogenesis, and causes a time-dependent vasorelaxant effect that is partially inhibited by a PPARγ antagonist (O'Sullivan, Sun, Bennett, Randall, & Kendall, 2009).

CBD has been shown to promote proapoptotic effects on different types of tumor cells, and it has been proven to exert antiinvasive and antimetastatic activities (Ramer, Merkord, Rohde, & Hinz, 2010; Ramer et al., 2012). More recently, it has been shown that CBD increases the expression of COX-2 and PPARγ at the protein level, in lung cancer cells, and both proteins converge in a coordinated fashion via activation of PPARγ by the COX-2 derived products PGD_2 and $15d\text{-}PGJ_2$, which in fact, may be responsible for the antitumor activity of CBD (Ramer et al., 2013).

CBG, the precursor of Δ^9-THC and CBD, is another major phytocannabinoid present in some varieties of *Cannabis sativa*. CBG has been less investigated than Δ^9-THC and CBD, but it is also endowed with pleiotropic activities. In vitro studies have revealed that CBG is an α2-adrenoceptor agonist, antagonizes 5HT1A receptor, activates TRPV1, TRPV2, and TRPA1, and is an antagonist of TRPM8 (Russo, 2011). Interestingly, CBG outperforms Δ^9-THC and CBD in the binding to PPARγ, showing an EC50 of 12.7 μM (Granja et al., 2012). However, no direct evidence of the involvement of CBG on PPARγ activation has been investigated in vivo. Several reports have suggested that PPARγ is a potential target for the development of novel anti-Huntington disease drugs. In this sense, we have shown that CBG was neuroprotective in mice intoxicated with 3-nitropropionate, improving motor deficits and preserving striatal neurons, and we also found that CBG partially reversed the downregulation of PPARγ in R6/2 transgenic mice expressing the HD gene with 150 CAG repeats. In the same study, it was shown that CBG activated PPARγ in cultured striatal cells expressing endogenous wild type and mutant huntingtin (Valdeolivas et al., 2014).

There is evidence that agents interacting with the endocannabinoid system in the body can also interact with PPARγ. For instance, CB_1 activation by 2-AG enhances PPARγ expression (Du, Chen, Zhang, & Chen, 2011) and, conversely, PPARγ agonists may enhance the tissue levels of AEA by inhibiting its catabolism (Lenman & Fowler, 2007). It has been described that AEA, as well as some other endocannabinoids, can mediate some of its biological activities through PPARγ signaling. AEA binds to PPARγ, induces its transcriptional activity with an EC50 of 8 μM, and mediates fibroblast differentiation into adipocytes (Bouaboula et al., 2005). In addition, AEA and *N*-arachydonoyl-dopamine cause a PPARγ-dependent mediated vasorelaxation in rat aorta, suggesting that activation of PPARγ in endothelial vascular cells may represent a novel mechanism by which endocannabinoids can regulate the vascular tone (O'Sullivan, Sun, Bennett, Randall, & Kendall, 2009). Palmitoylethanolamide (PEA), another endogenous fatty acid amide, reduces inflammation and pain associated to spinal cord trauma through mechanisms involving both PPARα and PPARγ signaling (Paterniti et al., 2013).

It is well known that PPARγ ligands are endowed with potent antiinflammatory and immunosuppressive activities. Accordingly, in mouse splenocytes, AEA evoked a concentration-dependent inhibition of IL-2 secretion that was blocked by the PPARγ antagonist T0070907 (Rockwell & Kaminski, 2004). In the same sense, 2-AG is metabolized by COX-2 to 15-Deoxy-$\Delta^{12,14}$-prostaglandin J2-glycerol ester that binds to PPARγ, and suppresses

IL-2 gene transcription by targeting the binding of the transcription factor NFAT to the IL-2 gene promoter (Raman, Kaplan, & Kaminski, 2012).

Ajulemic acid (AjA) is a nonpsychotropic analog of the Δ^9-THC metabolite THC-11-oic acid that was described more than 20 years ago (Burstein et al., 1992). AjA has potent analgesic and antiinflammatory activity, acting as a dual agonist for CB_2 and PPARγ. Activation of PPARγ by AjA requires the AF-2 helix of the receptor, suggesting that AjA activates PPARγ through its ligand-dependent AF-2 region and, therefore, AjA binding enables PPARγ to recruit nuclear receptor coactivators (Ambrosio et al., 2007). Phase II clinical trials have shown that AjA is orally available, and was effective in reducing chronic neuropathic pain, compared with placebo, and no major adverse effects were observed (Burstein, 2005). More recently, it has been shown that AjA prevents progression of fibrosis in vivo, and inhibits fibrogenesis in vitro, through a PPARγ-dependent pathway (Gonzalez et al., 2012).

Other synthetic cannabinoids, such as WIN55212-2 and CP55940, are able to activate PPARγ, as measured in reporter gene assays, but the pharmacological significance of these findings remains to be investigated (Pertwee et al., 2010).

Cannabinoid Quinones as a Novel Class of PPARγ Agonists

Hemp tissues have long been known to give a purple color when treated with bases (Beam, 1911). This unique color reaction is at the basis of the Beam test, one of the simplest forensic tests to detect marijuana. The chemistry underlying this color reaction was elucidated by Mechoulam, who, in a seminal work published almost 50 years ago (Mechoulam, Ben-Zvi, & Gaoni, 1968), identified the colored pigment as a mixture of monomeric and dimeric cannabinoid quinols, characterizing the structure of the compounds formed from CBD (Fig. 69.1). Paradoxically, Δ^9-THC and CBN, the psychoactive principles of marijuana, do not give the color reaction, which requires the presence of a resorcinyl moiety with free hydroxyls. Therefore, the Beam test actually detects the presence of the nonpsychotropic cannabinoids typical of fiber hemp, rather than the psychotropic cannabinoids typical of marijuana.

From a mechanistic standpoint, the reaction involves the activation of the resorcinyl system by deprotonation, and then reaction with atmospheric oxygen to form an α-hydroperoxy ketone. The nucleophilic distal hydroperoxyl oxygen next reacts with the keto form of the *p*-enolic hydroxyl, to generate a peroxyhemiacetal, whose fragmentation by cleavage of the weak oxygen–oxygen bond eventually affords the quinol derivative (Fig. 69.2). This mechanism rationalizes the need to have two free phenolic hydroxyls to give the color reaction, the first one being necessary for the activation of the aromatic system by deprotonation, and the second for the tautomerization and the generation of an electrophilic carbonyl sink paralocated to the hydroperoxyl group.

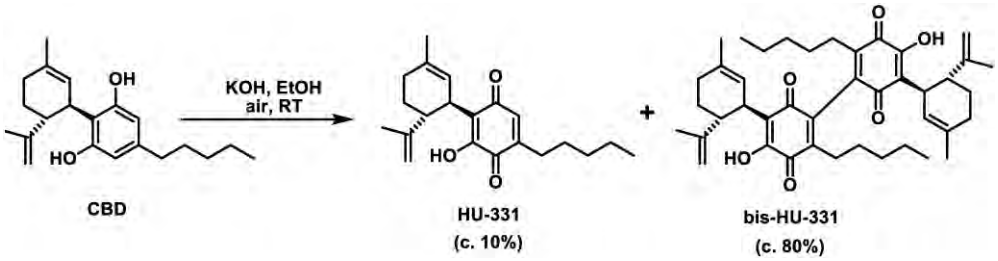


FIGURE 69.1 **Base-catalyzed air oxidation of CBD.**

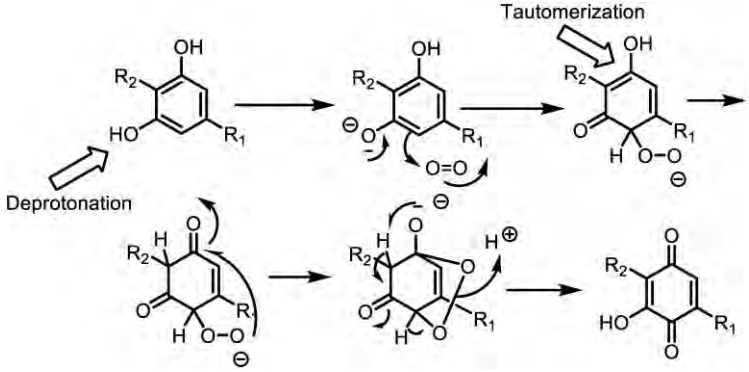


FIGURE 69.2 **Possible mechanism for the formation of quinol cannabinoids by base-promoted air oxidation of cannabinoids (R_1 = pentyl, R_2 = sesquiterpenyl).**

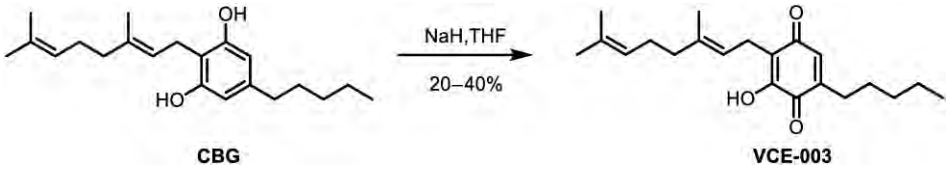


FIGURE 69.3 **Synthesis of VCE-003.**

Despite the forensic relevance of cannabinoid quinones, the biological profile of these compounds has long been overlooked because of the difficulty of their purification, their chemical instability, and uncertainties on their structure, long debated between a *p*- or *o*-quinone nature. Interest in cannabinoid quinols was rekindled in the past decade by Mechoulam, who confirmed their *p*-quinone structure by X-ray, improved their synthesis, and discovered the potent cytotoxic activity of the quinol from CBD, a compound known under the name of HU-331 (Kogan et al., 2004). HU-331 is a selective and potent (nanomolar IC_{50}) inhibitor of DNA topoisomerase II, with antitumor activity (Peters & Kogan, 2007). Interestingly, this compound was described previously to be generated during hepatic microsomal metabolism of CBD, and capable of generating reactive oxygen species (ROS) inducing cell toxicity (Watanabe, Usami, Yamamoto, & Yoshimura, 1991; Usami, Yamamoto, & Watanabe, 2008). We have also found that HU-331 induces ROS and the collapse of mitochondrial membrane potential in human leukemia cell lines (unpublished results).

The quinol derivative denbinobin occurs in some varieties of fiber hemp (Sánchez-Duffhues et al., 2008), but cannabinoid quinols have never been isolated from *Cannabis* plant samples, presumably because of their instability. However, an acetylated form of the quinol of CBG was described from a high-potency marijuana sample (Radwan et al., 2009). This report inspired the synthesis of the quinol of CBG (VCE-003), a compound that was prepared by oxidation of CBG under a variety of basic conditions, as well as with hypervalent iodine reagents (Granja et al., 2012) (Fig. 69.3).

We found that oxidation of CBD and CBG to HU-311 and VCE-003, respectively, greatly enhances its binding activity to PPARγ (Granja et al., 2012). VCE-003 has potent immunosuppressive activities by targeting both PPARγ and CB_2, and ameliorates the neurological defects and the severity of MOG-induced EAE in mice (Carrillo-Salinas et al., 2014). Regarding structure–activity relationships, the quinol hydroxyl is critical for activity, since its methylation or acetylation gave substantially inactive analogs (unpublished results).

More recently, a class of CBD quinones, chromenopyrazolediones, inspired by HU-345, have also been described and shown to exert antitumor activity on prostate cancer cells, and on tumor xenografts (Morales et al., 2013). In the same study, it was shown that chromenopyrazolediones 4 inhibits cell viability through a mechanism involving ROS generation and PPARγ activation.

MINI-DICTIONARY

Cannabinoids A complex group of lipophilic molecules that comprise phytocannabinoids, endocannabinoids, and synthetic cannabinoids.
Endocannabinoids A class of lipid mediators that are essential to maintain the homeostasis of the endocannabinoid system.
Nuclear receptors A superfamily of protein hormones that serve as transcription factors.
Peroxisome proliferator activated receptors A family of nuclear receptors able to regulate lipid turnover and metabolism. They have a role in the resolution of inflammatory processes.
Phytocannabinoids Defined as the terpenophenolic constituents of *Cannabis sativa* L.
PPARγ ligands Small molecules that bind and activate the nuclear receptor PPARγ
Thiazolidinediones (glitazones) Potent PPARγ agonists used in the treatment of diabetes.
TRP receptors Transient receptor potential multigene superfamily encoding integral membrane proteins that function as ion channels.

References

Ambrosio, A. L., Dias, S. M., Polikarpov, I., Zurier, R. B., Burstein, S. H., & Garratt, R. C. (2007). Ajulemic acid, a synthetic nonpsychoactive cannabinoid acid, bound to the ligand binding domain of the human peroxisome proliferator-activated receptor gamma. *Journal of Biological Chemistry*, *282*, 18625–18633.

Atwood, B. K., Straiker, A., & Mackie, K. (2012). CB_2: therapeutic target-in-waiting. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *38*, 16–20.

Beam, W. (1911). Report of the Chemical Section of the Wellcome Tropical Research Laboratories at the Gordon Memorial College Khartoum. *Fourth Report of the Wellcome Tropical Research Laboratories at the Gordon Memorial College Khartoum*. London, UK: Bailliere, Tindall and Cox, Vol. B: General Science.

Bernardo, A., Bianchi, D., Magnaghi, V., & Minghetti, L. (2009). Peroxisome proliferator-activated receptor-gamma agonists promote differentiation and antioxidant defenses of oligodendrocyte progenitor cells. *Journal of Neuropathology and Experimental Neurology*, *68*, 797–808.

Bernardo, A., & Minghetti, L. (2008). Regulation of glial cell functions by PPAR-gamma natural and synthetic agonists. *PPAR Research*, *2008*, 864140.

Bouaboula, M., Hilairet, S., Marchand, J., Fajas, L., Le Fur, G., & Casellas, P. (2005). Anandamide induced PPARgamma transcriptional activation and 3T3-L1 preadipocyte differentiation. *European Journal of Pharmacology*, *517*, 174–181.

Bright, J. J., Kanakasabai, S., Chearwae, W., & Chakraborty, S. (2008). PPAR regulation of inflammatory signaling in CNS diseases. *PPAR Research*, *2008*, 658520.

Burstein, S. (2005). Ajulemic acid (IP-751): synthesis, proof of principle, toxicity studies, and clinical trials. *AAPS Journal*, *7*, E143–E148.

Burstein, S. H., Audette, C. A., Breuer, A., Devane, W. A., Colodner, S., Doyle, S. A., & Mechoulam, R. (1992). Synthetic nonpsychotropic cannabinoids with potent antiinflammatory, analgesic, and leukocyte antiadhesion activities. *Journal of Medicinal Chemistry*, *35*, 3135–3141.

Carrillo-Salinas, F. J., Navarrete, C., Mecha, M., Feliú, A., Collado, J. A., Cantarero, I., Bellido, M. L., Muñoz, E., & Guaza, C. (2014). A cannabigerol derivative suppresses immune responses and protects mice from experimental autoimmune encephalomyelitis. *PLoS One*, *9*, e94733.

Carroll, C. B., Zeissler, M. L., Hanemann, C. O., & Zajicek, J. P. (2012). Δ^9-Tetrahydrocannabinol (Δ^9-THC) exerts a direct neuroprotective effect in a human cell culture model of Parkinson's disease. *Neuropathology and Applied Neurobiology*, *38*, 535–547.

Chen, L., Jia, Z., & Yang, G. (2014). PPARs and metabolic syndrome. *PPAR Research*, *2014*, 832606.

Ciudin, A., Hernandez, C., & Simó, R. (2012). Update on cardiovascular safety of PPARgamma agonists and relevance to medicinal chemistry and clinical pharmacology. *Current Topics in Medicinal Chemistry*, *12*, 585–604.

Culman, J., Zhao, Y., Gohlke, P., & Herdegen, T. (2007). PPAR-gamma: therapeutic target for ischemic stroke. *Trends in Pharmacological Sciences*, *28*, 244–249.

de Meijer, E. P., Bagatta, M., Carboni, A., Crucitti, P., Moliterni, V. M., Ranalli, P., & Mandolino, G. (2003). The inheritance of chemical phenotype in *Cannabis sativa* L. *Genetics*, *163*, 335–346.

De Petrocellis, L., Ligresti, A., Moriello, A. S., Allarà, M., Bisogno, T., Petrosino, S., Stott, C. G., & Di Marzo, V. (2011). Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. *British Journal of Pharmacology*, *163*, 1479–1494.

Di Marzo, V., De Petrocellis, L., & Bisogno, T. (2005). The biosynthesis, fate and pharmacological properties of endocannabinoids. *Handbook of Experimental Pharmacology*, *168*, 147–185.

Doshi, L. S., Brahma, M. K., Bahirat, U. A., Dixit, A. V., & Nemmani, K. V. (2012). Discovery and development of selective PPAR gamma modulators as safe and effective antidiabetic agents. *Expert Opinion on Investigational Drugs*, *19*, 489–512.

Drew, P. D., Xu, J., Storer, P. D., Chavis, J. A., & Racke, M. K. (2006). Peroxisome proliferator-activated receptor agonist regulation of glial activation: relevance to CNS inflammatory disorders. *Neurochemistry International*, *49*, 183–189.

Du, H., Chen, X., Zhang, J., & Chen, C. (2011). Inhibition of COX-2 expression by endocannabinoid 2-arachidonoylglycerol is mediated via PPAR-γ. *British Journal of Pharmacology*, *163*, 1533–1549.

Esposito, G., Scuderi, C., Valenza, M., Togna, G. I., Latina, V., De Filippis, D., Cipriano, M., Carratù, M. R., Iuvone, T., & Steardo, L. (2011). Cannabidiol reduces Aβ-induced neuroinflammation and promotes hippocampal neurogenesis through PPARγ involvement. *PLoS One*, *6*, e28668.

Fernández-Ruiz, J., Sagredo, O., Pazos, M. R., García, C., Pertwee, R., Mechoulam, R., & Martínez-Orgado, J. (2013). Cannabidiol for neurodegenerative disorders: important new clinical applications for this phytocannabinoid? *British Journal of Clinical Pharmacology*, *75*, 323–333.

Gaoni, Y., & Mechoulam, R. (1964). Isolation, structure and partial synthesis of an active constituent of hashish. *Journal of the American Chemical Society*, *86*, 1646–1647.

Gonzalez, E. G., Selvi, E., Balistreri, E., Akhmetshina, A., Palumbo, K., Lorenzini, S., Lazzerini, P. E., Montilli, C., Capecchi, P. L., Lucattelli, M., Baldi, C., Gianchecchi, E., Galeazzi, M., Pasini, F. L., & Distler, J. H. (2012). Synthetic cannabinoid ajulemic acid exerts potent antifibrotic effects in experimental models of systemic sclerosis. *Annals of the Rheumatic Diseases*, *71*, 1545–1551.

Granja, A. G., Carrillo-Salinas, F., Pagani, A., Gómez-Cañas, M., Negri, R., Navarrete, C., Mecha, M., Mestre, L., Fiebich, B. L., Cantarero, I., Calzado, M. A., Bellido, M. L., Fernandez-Ruiz, J., Appendino, G., Guaza, C., & Muñoz, E. (2012). A cannabigerol quinone alleviates neuroinflammation in a chronic model of multiple sclerosis. *Journal of Neuroimmune Pharmacology*, *7*, 1002–1016.

Heneka, M. T., & Landreth, G. E. (2007). PPARs in the brain. *Biochimica et Biophysica Acta*, *1771*, 1031–1045.

Iuvone, T., Esposito, G., Esposito, R., Santamaria, R., Di Rosa, M., & Izzo, A. A. (2004). Neuroprotective effect of cannabidiol, a nonpsychoactive component from *Cannabis sativa*, on beta-amyloid-induced toxicity in PC12 cells. *Journal of Neurochemistry*, *89*, 134–141.

Jordt, S. E., Bautista, D. M., Chuang, H. H., McKemy, D. D., Zygmunt, P. M., Högestätt, E. D., Meng, I. D., & Julius, D. (2004). Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature*, *427*, 260–265.

Kielian, T., & Drew, P. D. (2003). Effects of peroxisome proliferator-activated receptor-γ agonists on central nervous system inflammation. *Journal of Neuroscience Research*, *71*, 315–325.

Kogan, N. M., Rabinowitz, R., Levi, P., Gibson, D., Sandor, P., Schlesinger, M., & Mechoulam, R. (2004). Synthesis and antitumor activity of quinonoid derivatives of cannabinoids. *Journal of Medicinal Chemistry*, *47*, 3800–3806.

Lehrke, M., & Lazar, M. A. (2005). The many faces of PPARgamma. *Cell*, *123*, 993–999.

Lenman, A., & Fowler, C. J. (2007). Interaction of ligands for the peroxisome proliferator-activated receptor gamma with the endocannabinoid system. *British Journal of Pharmacology*, *151*, 1343–1351.

Loane, D. J., Deighan, B. F., Clarke, R. M., Griffin, R. J., Lynch, A. M., & Lynch, M. A. (2009). Interleukin-4 mediates the neuroprotective effects of rosiglitazone in the aged brain. *Neurobiology of Aging*, *30*, 920–931.

Luna-Medina, R., Cortes-Canteli, M., Sanchez-Galiano, S., Morales-Garcia, J. A., Martinez, A., Santos, A., & Perez-Castillo, A. (2007). NP031112, a thiadiazolidinone compound, prevents inflammation and neurodegeneration under excitotoxic conditions: potential therapeutic role in brain disorders. *Journal of Neuroscience*, *27*, 5766–5776.

Mackie, K. (2006). Cannabinoid receptors as therapeutic targets. *Annual Review of Pharmacology and Toxicology*, *46*, 101–122.

Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., & Bonner, T. I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature*, *346*, 561–564.

Mechoulam, R., Ben-Zvi, Z., & Gaoni, Y. (1968). Hashish—13. On the nature of the Beam test. *Tetrahedron*, *16*, 5615–5624.

Mechoulam, R., & Ross, R. A. (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. *Pharmacological Reviews*, *62*, 588–631.

Morales, P., Vara, D., Goméz-Cañas, M., Zúñiga, M. C., Olea-Azar, C., Goya, P., Fernández-Ruiz, J., Díaz-Laviada, I., & Jagerovic, N. (2013). Synthetic cannabinoid quinones: preparation, in vitro antiproliferative effects and in vivo prostate antitumor activity. *European Journal of Medicinal Chemistry*, *70*, 111–119.

Munro, S., Thomas, K. L., & Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, *365*, 61–65.

Natarajan, C., Muthian, G., Barak, Y., Evans, R. M., & Bright, J. J. (2003). Peroxisome proliferator-activated receptor-gamma-deficient heterozygous mice develop an exacerbated neural antigen-induced Th1 response and experimental allergic encephalomyelitis. *Journal of Immunology*, *171*, 5743–5750.

O'Sullivan, S. E. (2007). Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. *British Journal of Pharmacology*, *152*, 576–582.

O'Sullivan, S. E., & Kendall, D. A. (2010). Cannabinoid activation of peroxisome proliferator-activated receptors: potential for modulation of inflammatory disease. *Immunobiology*, *215*, 611–616.

O'Sullivan, S. E., Kendall, D. A., & Randall, M. D. (2006). Further characterization of the time-dependent vascular effects of delta9-tetrahydrocannabinol. *Journal of Pharmacology and Experimental Therapeutics*, *317*, 428–438.

O'Sullivan, S. E., Tarling, E. J., Bennett, A. J., Kendall, D. A., & Randall, M. D. (2005). Novel time-dependent vascular actions of Delta9-tetrahydrocannabinol mediated by peroxisome proliferator-activated receptor gamma. *Biochemical and Biophysical Research Communications*, *337*, 824–831.

O'Sullivan, S. E., Sun, Y., Bennett, A. J., Randall, M. D., & Kendall, D. A. (2009). Time-dependent vascular actions of cannabidiol in the rat aorta. *European Journal of Pharmacology*, *612*, 61–68.

Paterniti, I., Impellizzeri, D., Crupi, R., Morabito, R., Campolo, M., Esposito, E., & Cuzzocrea, S. (2013). Molecular evidence for the involvement of PPAR-δ and PPAR-γ in anti-inflammatory and neuroprotective activities of palmitoylethanolamide after spinal cord trauma. *Journal of Neuroinflammation*, *10*, 20.

Pertwee, R. G., Howlett, A. C., Abood, M. E., Alexander, S. P., Di Marzo, V., Elphick, M. R., Greasley, P. J., Hansen, H. S., Kunos, G., Mackie, K., Mechoulam, R., & Ross, R. A. (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. *Pharmacological Reviews*, *62*, 588–631.

Peters, M., & Kogan, N. (2007). HU-331: a cannabinoid quinone, with uncommon cytotoxic properties and low toxicity. *Expert Opinion on Investigational Drugs*, *16*, 1405–1413.

Pistis, M., & Melis, M. (2010). From surface to nuclear receptors: the endocannabinoid family extends its assets. *Current Medicinal Chemistry*, *17*, 1450–1467.

Radwan, M. M., Elsohly, M. A., Slade, D., Ahmed, S. A., Khan, I. A., & Ross, S. A. (2009). Biologically active cannabinoids from high-potency *Cannabis sativa*. *Journal of Natural Products*, *72*, 906–911.

Raman, P., Kaplan, B. L., & Kaminski, N. E. (2012). 15-Deoxy-$\Delta^{12,14}$ prostaglandin J_2-glycerol, a putative metabolite of 2-arachidonyl glycerol and a peroxisome proliferator-activated receptor γ ligand, modulates nuclear factor of activated T cells. *Journal of Pharmacology and Experimental Therapeutics*, *342*, 816–826.

Ramer, R., Bublitz, K., Freimuth, N., Merkord, J., Rohde, H., Haustein, M., Borchert, P., Schmuhl, E., Linnebacher, M., & Hinz, B. (2012). Cannabidiol inhibits lung cancer cell invasion and metastasis via intercellular adhesion molecule-1. *FASEB Journal*, *26*, 1535–1548.

Ramer, R., Heinemann, K., Merkord, J., Rohde, H., Salamon, A., Linnebacher, M., & Hinz, B. (2013). COX-2 and PPAR-γ confer cannabidiol-induced apoptosis of human lung cancer cells. *Molecular Cancer Therapeutics*, *12*, 69–82.

Ramer, R., Merkord, J., Rohde, H., & Hinz, B. (2010). Cannabidiol inhibits cancer cell invasion via upregulation of tissue inhibitor of matrix metalloproteinases-1. *Biochemical Pharmacology*, *79*, 955–966.

Rockwell, C. E., & Kaminski, N. E. (2004). A cyclooxygenase metabolite of anandamide causes inhibition of interleukin-2 secretion in murine splenocytes. *Journal of Pharmacology and Experimental Therapeutics*, *311*, 683–690.

Russo, E. B. (2011). Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *British Journal of Pharmacology*, *163*, 1344–1364.

Sánchez-Duffhues, G., Calzado, M. A., de Vinuesa, A. G., Caballero, F. J., Ech-Chahad, A., Appendino, G., Krohn, K., Fiebich, B. L., & Muñoz, E. (2008). Denbinobin, a naturally occurring 1,4-phenanthrenequinone, inhibits HIV-1 replication through an NF-kappaB-dependent pathway. *Biochemical Pharmacology*, *76*, 1240–1250.

Sladek, F. M. (2011). What are nuclear receptor ligands? *Molecular and Cellular Endocrinology*, *334*, 3–13.

Storer, P. D., Xu, J., Chavis, J., & Drew, P. D. (2005). Peroxisome proliferator-activated receptor-gamma agonists inhibit the activation of microglia and astrocytes: implications for multiple sclerosis. *Journal of Neuroimmunology*, *161*, 113–122.

Usami, N., Yamamoto, I., & Watanabe, K. (2008). Generation of reactive oxygen species during mouse hepatic microsomal metabolism of cannabidiol and cannabidiol hydroxy-quinone. *Life Sciences*, *83*, 717–724.

Valdeolivas, S., Navarrete, C., Cantarero, I., Bellido, M. L., Muñoz, E., & Sagredo, O. (2014). Neuroprotective properties of cannabigerol in Huntington's disease: studies in R6/2 mice and 3-Nitropropionate-lesioned mice. *Neurotherapeutics*, *12*, 185–199.

Vara, D., Morell, C., Rodríguez-Henche, N., & Diaz-Laviada, I. (2013). Involvement of PPARγ in the antitumoral action of cannabinoids on hepatocellular carcinoma. *Cell Death and Disease*, *4*, e618.

Watanabe, K., Usami, N., Yamamoto, I., & Yoshimura, H. (1991). Inhibitory effect of cannabidiol hydroxy-quinone, an oxidative product of cannabidiol, on the hepatic microsomal drug-metabolizing enzymes of mice. *Journal of Pharmacobiodynamics*, *7*, 421–427.

Willson, T. M., Brown, P. J., Sternbach, D. D., & Henke, B. R. (2000). The PPARs: from orphan receptors to drug discovery. *Journal of Medical Chemistry*, *43*, 527–550.

Xu, J., & Drew, P. D. (2007). Peroxisome proliferator-activated receptor-gamma agonists suppress the production of IL-12 family cytokines by activated glia. *Journal of Immunology*, *178*, 1904–1913.

CHAPTER

70

The Protein–Protein Interactions of Cannabinoid Receptor Interacting Protein 1a (CRIP1a) and Cannabinoid 1 Receptor: The Molecular Mechanism Study Through an Integrated Molecular Modeling Approach

M.H. Ahmed, Y. Zhang

Department of Medicinal Chemistry, School of Pharmacy and Institute for Structural Biology and Drug Discovery, Virginia Commonwealth University, Richmond, VA, United States

SUMMARY POINTS

- This chapter focuses on developing an atomic model for the interaction between cannabinoid receptor interacting protein 1a (CRIP1a) and the cannabinoid type-1 receptor (CB_1R).
- CRIP1a was experimentally shown to bind to, and decrease, the constitutive activity of CB_1R.
- Unfortunately, there is no experimental data for the details of how that interaction occurs.
- Molecular modeling offers valuable information that might explain the molecular mechanism of that interaction.
- The primary sequence of CRIP1a was used to generate a three-dimensional (3D) model that was subsequently used to dock the C-terminus of CB_1R, in order to construct a model of CRIP1a–CB_1R interaction.
- Several computational techniques were used during the process, most importantly: homology-modeling, protein threading, and ab initio, for construction of the CRIP1a model, followed by protein–protein docking to the CB_1R C-terminus and, finally, molecular dynamics for model refinement.
- The model suggests several putative key interactions that might contribute to the experimentally observed CRIP1a–CB_1R interaction.
- This model may be useful to guide future site directed mutagenesis, and to improve the crystallization process of CRIP1a protein.

KEY FACTS OF CANNABINOID RECEPTOR INTERACTING PROTEINS

- Cannabinoid receptor interacting proteins is a family of two structurally related proteins: CRIP1a and CRIP1b.
- They were first discovered by Niehaus et al. (2007).
- CRIP1a and CRIP1b are generated by alternative splicing of the *Cnrip1* gene located on chromosome 2 in humans.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00081-8
2017 Published by Elsevier Inc.

- CRIP1a is a protein 164 amino acids long, while CRIP1b is 128.
- Orthologs of CRIP1a occur throughout the vertebrates, while CRIP1b is unique to primates.
- Both proteins bind to the C-terminus of cannabinoid type-1 receptor.

KEY FACTS OF HOMOLOGY MODELING

- Also known as comparative modeling of protein, referring to a computational method for the construction of a 3D atomic-resolution model of a protein.
- Homology modeling relies on the concept that similar amino acid sequences adopt similar protein structures.
- An experimental 3D structure of a related homologous protein (template) to the target protein is required for this process.
- Homologous proteins (templates) are identified by having an amino acid sequence identity of at least 20% to the target protein.
- A sequence alignment between the template's and the target's amino acid sequences is produced that maps residues in the target's sequence to residues in the template's sequence, which is subsequently used for the actual model construction of the target protein.

KEY FACTS OF PROTEIN THREADING

- Also known as fold recognition, referring to a computational method for the construction of a 3D atomic-resolution model of a protein.
- This method is used for proteins that do not have homologous proteins (templates) with known structure.
- Protein threading relies on two concepts: (1) many unrelated sequences fold into similar structures, and (2) there is only a relatively small number of unique structural folds, when compared with the number of proteins in nature.
- An experimental 3D structure of a protein (template) of the same fold as the target protein is required for this process, regardless of their sequence identities.
- Protein threading relies on using statistical knowledge to analyze the relationship between the currently available experimentally determined 3D protein structures (potential templates) and the sequence of the protein in question (target).
- The 3D model of the target protein is constructed by threading each amino acid in the target sequence to a position in the template structure, and assessing how well the target fits the template.
- After selecting the best-fit template, the actual model of the target protein is constructed, based on its sequence alignment with the chosen template.

KEY FACTS OF PROTEIN–PROTEIN DOCKING

- Protein–protein docking is the computational modeling of the 3D structure of complexes formed by two or more interacting proteins.
- The 3D structures of the interacting proteins are used to produce plausible candidate structures that are subsequently ranked using scoring functions to identify structures most likely to occur in nature.
- The term "docking" originated in the late 1970s, a period that witnessed the development of scoring functions that were able to discriminate between good and bad candidate protein–protein interaction models, as well as the first computational attempts to predict protein–protein complexes' 3D structures.
- Protein–protein docking can be broadly categorized into rigid-body docking and flexible docking.
- Rigid-body docking is carried out with all bond angles, bond lengths, and torsion angles of the interacting proteins fixed, which is sometimes inadequate. However, it is computationally fast.
- Flexible docking permits conformational change by intelligently selecting a small subset of possible conformational changes of the interacting proteins for docking calculations, which is computationally expensive. However, it is more accurate than rigid-body docking.

KEY FACTS OF THE HINT FORCE FIELD

- HINT is short for hydropathic interactions.
- HINT is a novel empirical molecular modeling system with new methods for de novo drug design, and protein or nucleic acid structural analysis.
- HINT is being developed by Dr Glen Kellogg of the Medicinal Chemistry Department at Virginia Commonwealth University in Richmond, USA.
- HINT translates the well-developed Medicinal Chemistry and QSAR formalism of LogP and hydrophobicity into a free energy interaction model for all biomolecular systems, based on the experimental data from solvent partitioning.
- The partition coefficient (LogP) is a thermodynamic parameter that, because of its unprocessed and unbiased experimental nature, encodes all noncovalent interactions in the biological environment, as well as solvent effects, and entropy.

LIST OF ABBREVIATIONS

CB_1R	Cannabinoid type-1 receptor
CB_2R	Cannabinoid type-2 receptor
Cnrip1	Cannabinoid receptor interacting protein gene
CRIP1a	Cannabinoid receptor interacting protein 1a
CRIP1b	Cannabinoid receptor interacting protein 1b
HINT	Hydropathic interactions
MD	Molecular dynamics
ns	Nanoseconds
ps	Picoseconds
RhoGDI 2	Rho protein GDP dissociation inhibitor type-2
RMSD	Root mean square deviation
RMSF	Root mean square fluctuation

INTRODUCTION

Ever since their discovery in the mid-1980s, cannabinoid receptors have been receiving increasing attention due to the fact that their roles in an expanding array of vital human physiological processes are elucidated. For example, regulation of motivation, motor function, memory, appetite, energy homeostasis, pain perception, immune function, gastrointestinal and cardiovascular function, and bone mass maintenance have all been attributed to cannabinoid receptors. Most notably, cannabinoid type-1 receptor (CB_1R) signaling and regulation have been implicated in a wide range of diseases, including substance abuse disorders (as the major targets of Δ^9-tetrahydrocannabinol, the main psychoactive constituent in marijuana) and neurodegenerative diseases such as Parkinson, Alzheimer, Huntington diseases, cancer, obesity, inflammatory bowel disease, and neuropathic and inflammatory pain (Lee, Choi, & Pak, 2009; Miller & Devi, 2011; Oesch & Gertsch, 2009; Thakur, Tichkule, Bajaj, & Makriyannis, 2009).

The CB_1R signals mainly through the activation of $G_{i/o}$ proteins family, which inhibits adenylyl cyclases, and regulates ion channels, including calcium and potassium channels (Hudson, Hébert, & Kelly, 2010). Evidence also exists that, in certain cell types, CB_1Rs can stimulate adenylyl cyclase via G_s, which can induce receptor-mediated Ca^{2+} fluxes, and stimulate phospholipases (Turu & Hunyady, 2010). Moreover, stimulation of CB_1Rs results in the phosphorylation and activation of mitogen-activated protein kinases (MAPKs) that regulate nuclear transcription factors (Howlett, 2005). In recent years, it has become evident that CB_1Rs also interact with various non-G protein GPCR-interacting proteins that can modulate CB_1R function (Smith, Sim-Selley, & Selley, 2010). In addition, CB_1Rs have the ability to form homo- and heterodimers/oligomers, resulting in altered pharmacological properties, which might contribute to the diverse pharmacological effects of cannabinoids observed in various tissues (Turu & Hunyady, 2010). However, one mechanism that appears to be unique to CB_1Rs is related to their binding to CRIP1a and CRIP1b, the cannabinoid receptor interacting proteins (Niehaus et al., 2007).

CRIP1a/b are globular proteins first discovered by the Lewis group, when they observed that the deletion of the CB_1R C-terminus resulted in delaying the time required to peak Ca^{2+} current inhibition, augmented the tonic CB_1R-mediated inhibition of Ca^{2+} currents, and promoted the ability of CB_1R to sequester G-proteins (Nie & Lewis, 2001a,b). These findings suggested that the C-terminal tail could be serving as an autoinhibitor. Searching for additional proteins that might be involved in regulating CB_1R activity, they used the CB_1R distal C-terminus as bait in a yeast two-hybrid screen, and identified two proteins: CRIP1a and CRIP1b. Later, CRIP1a was shown to bind to a GST-labeled CB_1R-C-terminal tail fusion protein and to coimmunoprecipitate with CB_1R, although no interaction of CRIP1a with the CB_2R has been observed (Niehaus et al., 2007).

CRIP1a and CRIP1b are generated by alternative splicing of the *Cnrip1* gene, located on chromosome 2 in humans (Niehaus et al., 2007). CRIP1a is most highly expressed in the brain, and its homologs are found throughout the vertebrates. Interestingly, CRIP1a was shown to selectively reverse basal, but not CB_1R agonist-induced, inhibition of voltage-gated Ca^{2+} channels when cotransfected with CB_1R in superior cervical ganglion neurons, which suggests that CRIP1a inhibits constitutive CB_1R activity (Niehaus et al., 2007). Supporting this interpretation, the ability of the CB_1R inverse agonist rimonabant (SR141716A) to stimulate basal Ca^{2+} channel activity in CB_1R-transfected neurons was eliminated by coexpression of CRIP1a (Niehaus et al., 2007).

Interestingly, CRIP1a possesses a palmitoylation site and a C-terminus PDZ class I ligand domain (Niehaus et al., 2007; Howlett, Blume, & Dalton, 2010; Smith et al., 2010). The palmitoylation site may play a role in localizing CRIP1a to the plasma membrane (Niehaus et al., 2007). The PDZ ligand domain may play several roles: (1) allowing CRIP1a to interact with other proteins, act as a scaffolding site, and/or enabling the formation of heterodimers between CB_1Rs and other receptors; and (2) potentially modulating the localization, desensitization, or internalization of CB_1Rs (Howlett et al., 2010; Smith et al., 2010).

CRIP1a may also be involved in the balance between neuroprotection and degeneration. Katona's group employed quantitative PCR to compare the levels of CB_1R and CRIP1a mRNA in epileptic and healthy post-mortem human hippocampal tissue. Reduced levels of both CRIP1a and CB_1R mRNA were found in sclerotic hippocampi (Ludányi et al., 2008). Alternatively, CRIP1a mRNA was found to be elevated following kainic

acid–induced seizures in rats (Bojnik et al., 2012). Both reports suggest that CRIP1a plays a role in modulating CB_1R function in the pathogenesis or neuroadaptive response to epilepsy. Moreover, in a model of glutamate excitoxicity in cultured cortical neurons, virally mediated expression of CRIP1a inhibited the protective effects of a cannabinoid agonist while conferring a protective effect to an antagonist (Stauffer et al., 2011). In addition to its putative roles in the brain, CRIP1a is presynaptically expressed along with CB_1Rs in the retina, and the *Cnrip1* gene exhibits hypermethylation in a subset of colorectal carcinomas and adenomas (Hu et al., 2010; Lind et al., 2011; Oster et al., 2011).

While none are definitive, the previously mentioned findings suggest potentially important functions of CRIP1a in multiple physiological systems, yet very little is known about the exact mechanisms by which CRIP1a binds to the CB_1R, which is crucial to understanding its regulation of CB_1R signaling. In this chapter, we apply a palette of complementary computational techniques, including homology modeling, ab initio, and protein threading, to generate all-atom molecular models for CRIP1a. Then, using protein–protein docking methods, the resulting CRIP1a model is docked to the C-terminus of the CB_1R, and further refined by means of molecular dynamics to generate a model for the CRIP1a–CB_1R interaction.

Using the CRIP1a amino acid sequence, the secondary structure pattern of CRIP1a was predicted. Then, three different methods were used to build 3D models for CRIP1a (homology modeling, ab initio, and protein threading). Hydrogen atoms were added to the CRIP1a models followed by energy minimization. Next, all models were evaluated with multiple scoring paradigms, and compared against the predicted secondary structure patter, in order to choose the best model to carry forward into succeeding stages. The best model for CRIP1a was then docked to the C-terminus of the CB_1R. The protein–protein complex model was then refined and optimized. The final stage was to subject the model to a 10 ns all-atom molecular dynamics simulation in explicit solvent, for further refinement.

CRIP1A SECONDARY STRUCTURE PREDICATION

CRIP1a secondary structure pattern was predicted using the amino acid primary sequence by different algorithms available in the GeneSilico Metaserver (Kurowski & Bujnicki, 2003), which provides a consensus secondary structure. Predictions by the majority of algorithms suggest that CRIP1a is composed almost exclusively of β-sheets and loops, which make CRIP1a a member of all β proteins class (data not shown). This was supported by the prediction of CRIP1a structural class and fold type using the 1D protein structure prediction software from Kurgan's lab (Chen, Kurgan, & Ruan, 2008) that predicts structural class and fold type information from the primary sequence of a protein; it also predicted CRIP1a is of the concanavalin A-like lectins/glucanases all β strands sandwich fold.

CRIP1A 3D STRUCTURE PREDICTION

Several algorithms available in the GeneSilico Metaserver (Kurowski & Bujnicki, 2003) were used for identifying templates suitable for homology modeling of CRIP1a, in addition to BLAST (Altschul et al., 1997). However, all such identified protein templates were of low homology, relative to CRIP1a (data not shown). For the prediction of distant homologs, protein threading algorithms were used, which are pDomTHREADER (Lobley, Sadowski, & Jones, 2009), I-TASSER (Roy, Kucukural, & Zhang, 2010), RaptorX (Källberg et al., 2012), LOMETS (Local Meta-Threading-Server) (Wu & Zhang, 2007); and MUSTER (Multi-Sources Threader) (Wu & Zhang, 2008), in addition to the algorithms available in the GeneSilico Metaserver (Kurowski & Bujnicki, 2003). These methods yielded 111 different sequence alignments of 74 different protein chains. Each algorithm has its own scoring method, so it is difficult to compare the results directly. However, it was clear that most of the generated alignments were in the low range of their respective algorithm's scoring scales. Only pDomTHREADER, an algorithm generally regarded as precise and sensitive in discriminating superfamilies, yielded two alignments in the high range. pDomTHREADER combines information from both sequence and structure to produce domain alignments. The highest scoring alignment thus produced was for PDB ID 1DS6 Chain B, (human Rho-specific guanine nucleotide dissociation inhibitor 2, RhoGDI 2), which covers amino acids 1–152 of CRIP1a's 164 amino acids, that is, all but the last of CRIP1a's β-sheet (see Fig. 70.1).

RhoGDI is also an all β protein of sandwich fold type that plays an important role in G-protein coupled receptor (GPCR) signaling, including that of CB_1R (Dovas & Couchman, 2005). The pDomTHREADER analysis also suggested CRIP1a to be somewhat homologous to RhoGDI 1, implying the possibility of CRIP1a sharing a similar function with the RhoGDIs. RhoGDI decreases the activity of Rho by preventing guanine nucleotide exchange, and membrane association. RhoGDI may also act as a positive regulator for Rho activities by providing spatial restriction, guidance, and availability signals to effectors, functions that are essential for the correct targeting and regulation of these effectors (Dovas & Couchman, 2005). RhoGDI 2 has an identity of 15.9%

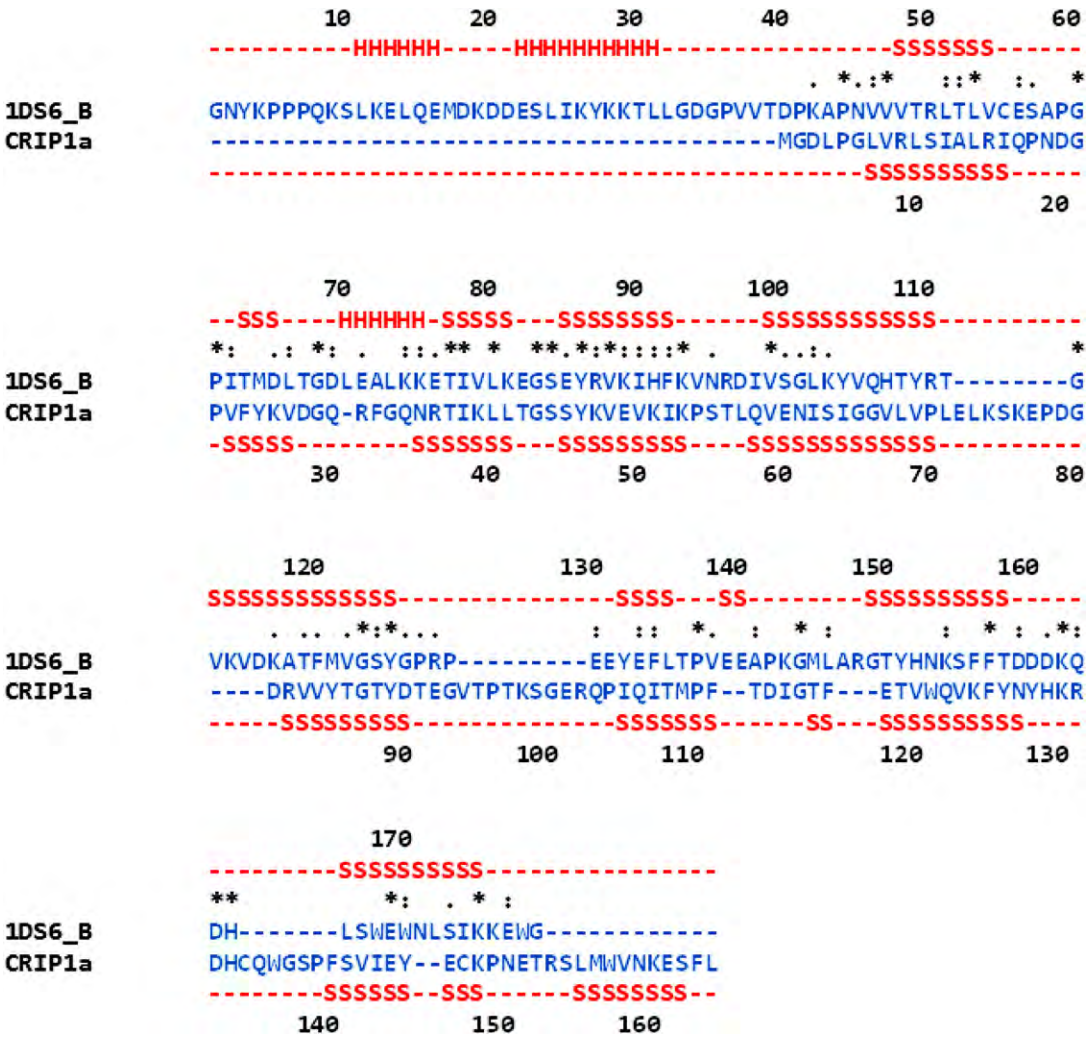


FIGURE 70.1 **CRIP1a and RhoGDI 2 sequence alignment.** RhoGDI 2 (PDB ID 1DS6 chain B) was the best-found scoring template. The RhoGDI 2 secondary structure and the predicted (consensus) secondary structure for CRIP1a are shown in *red*, above and below the sequence alignment, respectively. *S*, β-Sheet; *H*, α-helix; and –, unordered.

to the CRIP1a sequence, which is quite low; however, pDomTHREADER (Lobley et al., 2009) uses the primary sequence of a protein to infer distant relationships to other protein families that are not detectable by simple percentage identity. These relationships often suggest common function, and can often provide templates for the construction of high quality 3D structural models.

While other alignments with higher percentage identity were found, they had low scores, and did not cover as large a fraction of the CRIP1a sequence. For example, 1AOZ (ascorbate oxidase) chain A, and 1AYO (alpha 2-macroglobulin) chain A both have 20% identity with CRIP1a. Their alignment scores, however, were lower than that of RhoGDI 2, and did not provide templates for several of CRIP1a's β-sheets.

Based on the produced alignments, we used MODELLER to generate CRIP1a models, and ranked them according to the DOPE score (Shen & Sali, 2006). The highest scoring model was that based on the RhoGDI 2 template (Fig. 70.2A). The constructed model for CRIP1a follows the predicted secondary structure; however, residues 153–162 were removed, since they exceeded the extents of the template. Thus, to produce a complete model, residues 153–162 were remodeled using the next best scoring template—fibrinogen-binding protein SdrG (PDB ID 1R17) chain B. After energy minimizing and scoring this model, it had the highest score of all generated models (Fig. 70.2B). To ensure no major modeling defects, a Ramachandran plot was generated by MOLPROBITY (Chen et al., 2010) for the model, and was found to be within the acceptable limits (Fig. 70.2C).

The QUARK algorithm for ab initio protein folding and structure prediction (Xu & Zhang, 2012) was used to generate models using replica-exchange Monte Carlo simulation, guided by an atomic-level knowledge-based

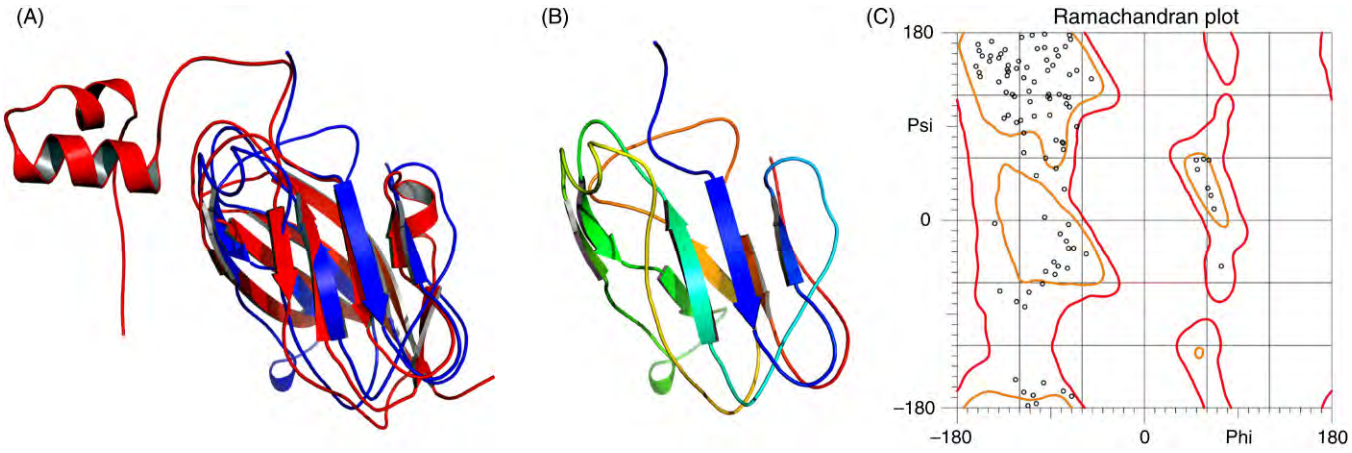


FIGURE 70.2 **CRIP1a protein threading based model.** CRIP1a generated model based on RhoGDI 2 (PDB ID 1DS6 chain B). (A) Superimposition of backbone atoms of CRIP1a model *(blue)* and the RhoGDI 2 crystal structure *(red)*. (B) 3D structure constructed for CRIP1a colored as a rainbow, starting from the N-terminus *(blue)* and ending with the C-terminus *(red)*. (C) Ramachandran plot for the full CRIP1a model and, as can be seen, all of the residues are within the allowed regions of the plot.

force field. Furthermore, hybrid methods combining ab initio with other techniques were used: Bhageerath (incorporating bioinformatics tools) (Jayaram et al., 2006) and Robetta's algorithm (incorporating comparative models of protein domains) (Chivian et al., 2003).

The various models generated by ab initio methods, all of which were β proteins, were evaluated using the DOPE method. The DOPE score of a protein can be viewed as a conformational energy that measures the relative stability of a conformation with respect to other conformations of the same protein. It can be used to choose the best model out of a set of predicted model structures for a particular protein sequence. Because the DOPE energy (Shen & Sali, 2006) is not normalized based on the size of the protein, the absolute score for a protein is not meaningful, but the *relative* energies of different conformations are useful in model evaluation. According to DOPE scoring, models built using ab initio methods were found to have high scores (data not shown); however, none of them had a score better than the model generated using RhoGDI 2 as a template. It is interesting to note that the hybrid bioinformatics/ab initio Bhageerath algorithm generated the best scoring model of this set.

CB_1R C-TERMINUS MODELING

According to Niehaus et al. (2007), the last nine amino acids on the C-terminus of CB_1R are the minimum residues required for CRIP1a binding (Niehaus et al., 2007). PEP-FOLD, which is a de novo approach for prediction of peptide structures from amino acid sequences (Beaufays, Lins, Thomas, & Brasseur, 2012), was used to build the 3D structure of this nine amino acid peptide.

CRIP1A–CB_1R PROTEIN–PROTEIN DOCKING

Protein–protein docking was performed using the HADDOCK (High Ambiguity Driven DOCKing) algorithm (de Vries, van Dijk, & Bonvin, 2010). The site on CRIP1a where the CB_1R C-terminus binds is unknown, so this nine amino acid peptide was docked to all possible sites encompassing amino acids 34–110 of CRIP1a. This range was chosen because these amino acids are common between CRIP1a and CRIP1b, and thus most likely critical for CB_1R binding (Niehaus et al., 2007). Docking results were individually inspected, after which high scoring models were passed into the refinement step.

All docked poses were refined with FireDock (Fast Interaction Refinement in a molecular Docking) (Mashiach, Schneidman-Duhovny, Andrusier, Nussinov, & Wolfson, 2008) algorithm, and then rescored using the HINT force field (Kellogg & Abraham, 2000; Sarkar & Kellogg, 2010) that describes and quantifies all interactions in the biological environment by exploiting the interaction information implicit in $LogP_{o/w}$ (the partition coefficient for 1-octanol/water solute transfer). HINT is known as a "natural" force field because it is based on empirical energetic terms defined by real experiments, and thus encodes interaction types including Coulombic, hydrogen bond, and hydrophobic interactions expected to be found between molecules in the biological environment. It is a free energy force field that includes solvation/desolvation and entropy, in addition to the other enthalpic terms.

The best scoring CRIP1a–CB_1R model according to HINT is shown in Fig. 70.3. The nine amino acids of the CB_1R C-terminus are mostly polar; therefore, polar interactions are the dominant type of interaction between

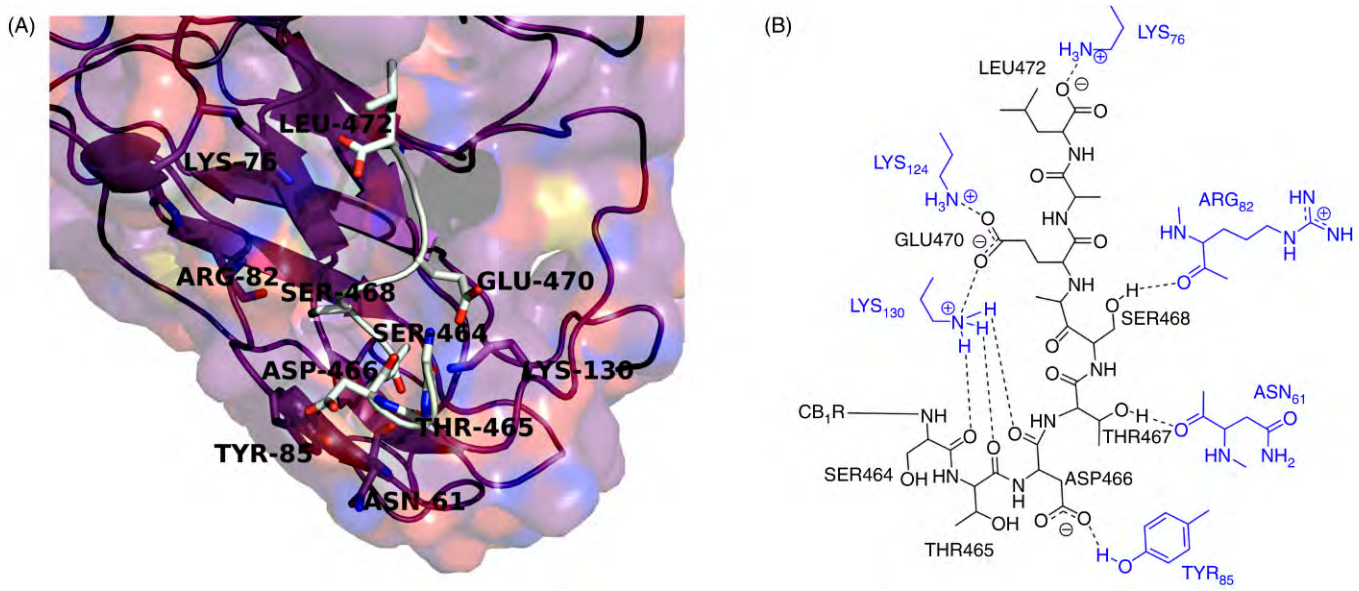


FIGURE 70.3 **Key interactions between the CB_1R and CRIP1a, as predicted by the model.** (A) Model showing CRIP1a *(purple)* bound to C-terminus of CB_1R *(gray)*. (B) 2D representation of the key interactions between CRIP1a *(blue)* and the C-terminus of CB_1R *(black)*.

CRIP1a and CB_1R. Most notably, the model suggests that hydrogen bonds are formed between the CB_1R backbone carbonyl oxygens of Ser464 and Thr465 and Asp466, with the terminal amine in Lys130 from CRIP1a, between one of the CB_1R carboxyl oxygens of Asp466 and the phenolic oxygen of Tyr85 from CRIP1a, and between the Thr467 hydroxyl group from CB_1R and the backbone carbonyl oxygen of Asn61 from CRIP1a. The model suggests that Lys130 from CRIP1a has a quite significant role in the interaction between CRIP1a and CB_1R (Fig. 70.3). In addition, there are some hydrophobic interactions, for example, between Val67 from CRIP1a and Ala471 from CB_1R.

The electrostatic properties of the CRIP1a–CB_1R complex interface was evaluated using Adaptive Poisson–Boltzmann Solver (APBS) tools plug-in developed by Lerner & Carlson from the University of Michigan, Ann Arbor for PyMOL. This tool uses APBS to solve the Poisson–Boltzmann equation (PBE) to assess electrostatic properties (Baker, Sept, Joseph, Holst, & McCammon, 2001). All images were created using the PyMOL Molecular Graphics System, Version 1.2r3pre, from Schrödinger, LLC. As can be seen in Fig. 70.4, the electrostatic complementarity analysis reveals the high complementarity in both CRIP1a and CB_1R interfaces.

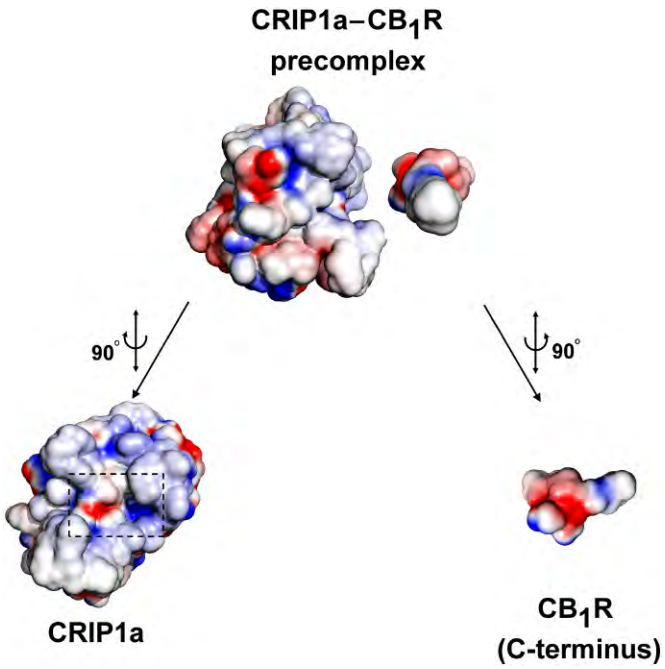


FIGURE 70.4 **Complementarity of electrostatic potentials at the interface of the predicted CRIP1a–CB_1R complex.** In the top middle, CRIP1a and CB_1R C-terminus are aligned in a precomplex position to better show the spatial complementarity of electrostatic potentials of the molecules. Positive potential is shown as *blue*, and negative potential is shown as *red*. Bottom left, CRIP1a is shown; the CB_1R binding site is enclosed in a *back dashed rectangle*. Bottom right, the binding interface of the C-terminus of CB_1R is shown. It can be clearly seen the complementarity of electrostatic potentials at the interface of the complex, as the area of the positive potential *(blue)* on CRIP1a faces the negative potential *(red)* on CB_1R C-terminus, and vice versa.

MOLECULAR DYNAMICS

The CRIP1a–CB_1R complex 3D model was further refined by subjecting it to an all-atom Molecular Dynamics (MD) simulation. MD simulations were carried out

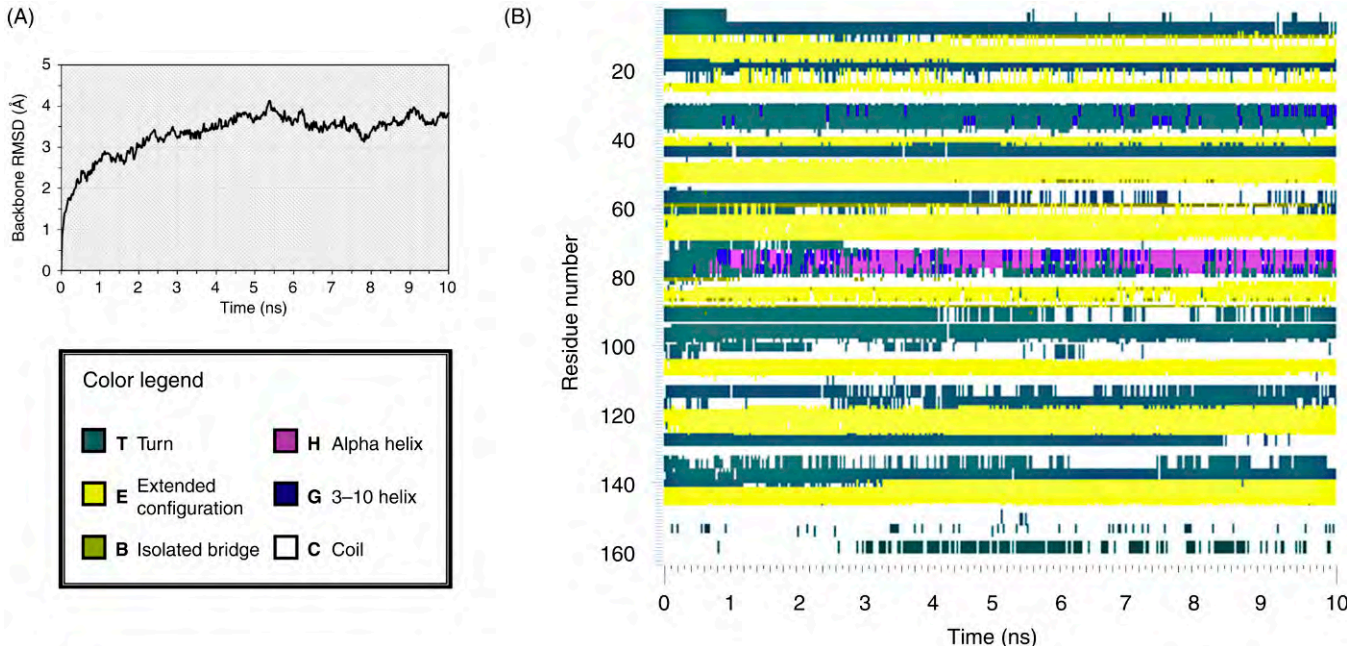


FIGURE 70.5 **Structural analysis of the MD trajectory.** (A) The root mean square deviation of atomic positions (RMSD) plot of the MD trajectory, as can be seen a rapid increase occurs during the first 2 ns, and then the RMSD levels off and becomes stable for the remainder of the simulation. (B) The change in secondary structure of CRIP1a throughout the MD simulation [generated by the Timeline Plugin, Version 2.3. in VMD (Humphrey et al., 1996)].

with the NAMD 2.8 package developed by the Theoretical and Computational Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign (Phillips et al., 2005). CHARMM (Charmm-27) was used as the force field (MacKerell et al., 1998). The initial MD setup was done using the MDWeb's (Hospital et al., 2012) "NAMD FULL MD Setup" workflow. The CRIP1a–CB_1R complex 3D model was first solvated in an equilibrated TIP3P water box with the dimensions of 91.8 Å × 81.8 Å × 73.8 Å, using the center of mass of the complex as the origin. Then Cl^- and Na^+ ions were added to neutralize the system, and appropriate amounts of ions were added up to a concentration of 50 mM. Solvent molecules were first minimized for 500 steps of conjugate gradients minimization method, keeping the protein molecules fixed to allow favorable distribution of water molecules on the complex surface. Subsequently, the system was coupled to a heat bath from 0 to 300 K, and the constraints applied to the solute atoms were gradually decreased, after which the system was allowed to be simulated without restraints for over a period of 10 ps. Finally, a 10 ns molecular dynamics production phase was carried out on the entire system. The analysis of the MD trajectory was done in VMD (Humphrey, Dalke, & Schulten, 1996).

To obtain an estimate of the MD trajectory quality and convergence, the backbone root mean square deviation (RMSD) values relative to the starting structures were plotted as shown in Fig. 70.5A. This analysis shows that, after a rapid increase in RMSD during the first two ns, the trajectory is stabilized with average RMSD values of 3.56 ± 0.21 Å. This suggests that the protein was undergoing modest equilibration in the first two ns of the production phase. The secondary structure of CRIP1a was examined to characterize any instabilities or conformational changes during the MD simulation. Throughout the entirety of the 10 ns simulation, CRIP1a's extended β-sheets were found to be well maintained (Fig. 70.5B). Amino acids 30–35 were alternating between a 3–10 helix, (which matches the secondary structure of the same aligned area in the template, Fig. 70.1) and a turn. Amino acids 73–76, corresponding to a gap in the template in the original sequence alignment (Fig. 70.1), were changed from a turn to an α-helix by the end of the simulation. The rest of the amino acids, which were originally involved in loops, were alternating between pure coils and turns. The binding region of the CB_1R C-terminus was highly maintained throughout the simulation.

As indicated by the root mean square fluctuation (RMSF) calculated over the entire simulation (Fig. 70.6B), the most flexible regions of CRIP1a correspond to the N-terminus (residues 1–5), the A-loop (residues 15–23), the B-loop (residues 68–80), the C-loop (residues 95–100), and the C-terminus (residues 148–160). All these regions correspond to the amino acids that alternate between a pure coil and a turn, with the exception of the B-loop, which changed from a turn to an α-helix. Crystallization

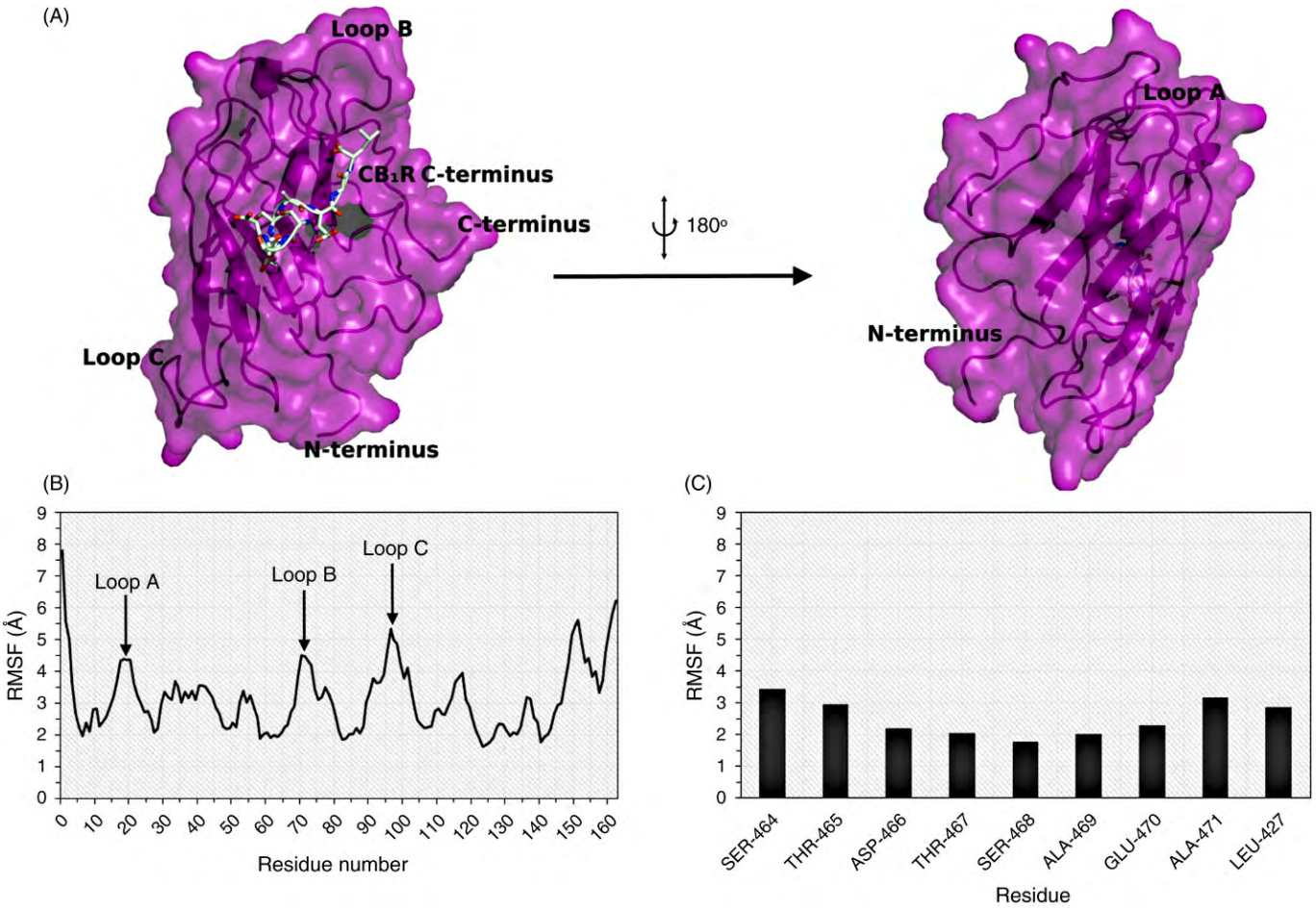


FIGURE 70.6 **The MD simulation results.** (A) The 3D binding mode of CB_1R C-terminus *(gray)* to the CRIP1a protein *(purple)*. (B) Cα-based root mean square fluctuations (RMSFs) calculated over the entire period of 10 ns of the MD production simulation for CRIP1a. (C) Cα-based RMSFs calculated over the entire period of 10 ns of the MD production simulation for the last nine amino acids of the CB_1R C-terminus.

experiments for CRIP1a are currently undergoing, however, it proved to be a difficult task (data not shown). The high flexibility of these regions might be contributing to such difficulties. The RMSF analysis of the nine amino acids CB_1R C-terminus (Fig. 70.6C) showed no significant fluctuations, compared to the whole complex.

Since the trajectory analysis suggested that the protein was undergoing modest equilibration in the first 2 ns of the production phase, as mentioned earlier, it seemed more appropriate to analyze the interactions between CRIP1a and the CB_1R C-terminus in the region between 2 and 10 ns of the trajectory. Among the H-bonds that were observed more than 50% of the time, during the analyzed region of the simulation: CRIP1a-Ser63 and CB_1R-Thr467 sidechains with an average distance of 2.91 ± 0.18 Å, and CRIP1a-Arg82 backbone and CB_1R-Ser468 sidechain with an average distance of 2.87 ± 0.22 Å. In addition, the following salt bridge interactions were observed more than 50% of the time, during the analyzed region of the simulation: CRIP1a-Lys76 sidechain and CB_1R-Leu472 terminal carboxylic group with an average distance of 2.87 ± 0.23 Å, and CRIP1a-Lys130 and CB_1R-Glu470 with an average distance of 2.76 ± 0.17 Å. Finally, hydrophobic interactions were observed more than 50% of the time, during the analyzed region of the simulation for residues Val67, Val69, Val83, Leu163, and Phe162 from CRIP1a, and Ala469, Ala471 and Leu472 from CB_1R.

This CRIP1a–CB_1R interaction model supports the assertion that CRIP1a might be responsible for blocking the coupling of CB_1R to specific $G_{i/o}$ proteins that are responsible for the tonic inhibition of Ca^{2+} channels, but not to other $G_{i/o}$ proteins that could inhibit Ca^{2+} channels, in response to agonist activation (Niehaus et al., 2007). The concept of CB_1R differential G-protein coupling, and the subsequent selective signal transduction mechanisms, was previously discussed by Anavi-Goffer et al. (2007), who found through $G_{i/o}$ protein reconstitution experiments that the combination of CB_1R and $G\alpha_{i3}$(C351G) significantly enhanced the tonic inhibition of Ca^{2+} channels, while CB_1R and $G\alpha_{oA}$(C351G) abolished the tonic inhibition of Ca^{2+} channels (Anavi-Goffer et al., 2007). An earlier study of Nie and

Lewis (2001b) also reinforces the differential coupling concept: they showed that D164N point mutation of CB_1R blocked tonic inhibition of Ca^{2+} channels, whereas agonist-dependent Ca^{2+} channel inhibition was not affected (Nie & Lewis, 2001b). The possibility that CRIP1a blocks the coupling of CB_1R to specific $G_{i/o}$ proteins, without affecting the binding of other $G_{i/o}$ proteins, may provide an explanation for the finding that CRIP1a selectively blocks basal CB_1R modulation of Ca^{2+} channel activity, but not CB_1R agonist-induced modulation of this activity (Niehaus et al., 2007).

In conclusion, previous work has explored the effects of binding CRIP1a to CB_1R; however, this interaction has never been examined at the atomic level, as there are no available X-ray crystal structures for the complex, or either of the interacting proteins. Here, we used three different types of computational techniques, comparative, ab initio, and protein threading, to build 3D models of the CRIP1a protein. The best scoring model was obtained through protein threading, using RhoGDI 2 as a template. RhoGDI 2, another effector with a significant role in G-protein coupled receptor signaling (including CB_1R), is an all β protein of the sandwich fold type, which is the same fold predicted for CRIP1a. Also modeled was the nine amino acid C-terminal end peptide of CB_1R. The peptide was docked as a ligand to the best-scored model of CRIP1a, resulting in mostly favorable polar interactions. The constructed model was then subjected to an all-atom MD simulation for further refinement. The energetics and binding mode analyses suggest that Lys130 of CRIP1a may play a significant role in this interaction. Our model identified potential areas of the CRIP1a that are highly disordered, which is important information for crystallization efforts. In addition, the model may be used to guide the design of future site-directed mutagenesis experiments. Understanding the structures of these proteins and, particularly, their interactions, will form the foundation for understanding mechanisms of CB_1R regulation in the CNS, and may also lead to advances in drug development for the treatment of disorders involving modulation of CB_1R activity.

MINI-DICTIONARY

Concanavalin A-like lectin/glucanases superfamily A family of proteins characterized by having a domain similar to the concanavalin A lectin, which mostly consist of a sandwich of 12–14 β-strands in two sheets with a complex topology.
Homology modeling Constructing an atomic-resolution model of a target protein from its amino acid sequence and a template (an experimental 3D structure of a related homologous protein). Also known as comparative modeling.
Huntington disease A devastating, hereditary, degenerative brain disorder that affects muscle coordination, and leads to cognitive decline and psychiatric problems in mid-adult life. Unfortunately, at present there is no cure.
Palmitoylation site A site on the protein that can undergo a posttranslational modification by binding to palmitate (C16) in this case, which can be reversible or irreversible.
PDZ class I ligand A specific C-terminal sequence recognized by protein interaction modules (PDZ domains), which is a common structural domain of 80–90 amino-acids found in the signaling proteins. PDZ is an acronym combining the first letters of three proteins–post synaptic density protein, drosophila disc large tumor suppressor, and zonula occludens-1 protein, which were the first proteins discovered to have this domain.
Protein threading A method of constructing an atomic-resolution model of a target protein using a template protein that has the same fold. It is used in cases where a closely homologous protein is unavailable. Protein threading is based on the basic observation that the number of different folds in nature is small (approximately 1300).
Root-mean-square deviation (RMSD) It is the measure of the average distance between the atoms of superimposed proteins.
Root-mean-square fluctuation (RMSF) It is a measure of the deviation between the position of an atom and some reference position (a measure of the average atomic mobility).

References

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, *25*, 3389.

Anavi-Goffer, S., Fleischer, D., Hurst, D. P., Lynch, D. L., Barnett-Norris, J., Shi, S., Lewis, D. L., Mukhopadhyay, S., Howlett, A. C., Reggio, P. H., & Abood, M. E. (2007). Helix 8 Leu in the CB1 cannabinoid receptor contributes to selective signal transduction mechanisms. *Journal of Biological Chemistry*, *282*, 25100–25113.

Baker, N. A., Sept, D., Joseph, S., Holst, M. J., & McCammon, J. A. (2001). Electrostatics of nanosystems: application to microtubules and the ribosome. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 10037–10041.

Beaufays, J., Lins, L., Thomas, A., & Brasseur, R. (2012). In silico predictions of 3D structures of linear and cyclic peptides with natural and non-proteinogenic residues. *Journal of Peptide Science*, *18*, 17–24.

Bojnik, E., Turunç, E., Armağan, G., Kanıt, L., Benyhe, S., Yalçın, A., & Borsodi, A. (2012). Changes in the cannabinoid (CB1) receptor expression level and G-protein activation in kainic acid induced seizures. *Epilepsy Research*, *99*, 64–68.

Chen, K., Kurgan, L. A., & Ruan, J. (2008). Prediction of protein structural class using novel evolutionary collocation-based sequence representation. *Journal of Computational Chemistry*, *29*, 1596–1604.

Chen, V. B., Arendall, W. B., Headd, J. J., Keedy, D. A., Immormino, R. M., Kapral, G. J., Murray, L. W., Richardson, J. S., & Richardson, D. C. (2010). MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallographica Section D Biological Crystallography*, *66*, 12–21.

Chivian, D., Kim, D. E., Malmstrom, L., Bradley, P., Robertson, T., Murphy, P., Strauss, C. E. M., Bonneau, R., Rohl, C. A., & Baker, D. (2003). Automated prediction of CASP-5 structures using the Robetta server. *Proteins*, *53*, 524–533.

de Vries, S. J., van Dijk, M., & Bonvin, A. M. (2010). The HADDOCK web server for data-driven biomolecular docking. *Nature Protocols*, *5*, 883–897.

Dovas, A., & Couchman, J. R. (2005). RhoGDI: multiple functions in the regulation of Rho family GTPase activities. *Biochemical Journal*, *390*, 1–9.

Hospital, A., Andrio, P., Fenollosa, C., Cicin-Sain, D., Orozco, M., & Gelpí, J. L. (2012). MDWeb and MDMoby: an integrated web-based platform for molecular dynamics simulations. *Bioinformatics*, *28*, 1278–1279.

Howlett, A. C. (2005). Cannabinoid receptor signaling. In R. G. Pertwee (Ed.), *Cannabinoids. Handbook of experimental pharmacology* (pp. 53–80). New York, NY: Springer.

Howlett, A. C., Blume, L. C., & Dalton, G. D. (2010). CB(1) cannabinoid receptors and their associated proteins. *Current Medicinal Chemistry*, *17*, 1382–1393.

Hu, S. S., Arnold, A., Hutchens, J. M., Radicke, J., Cravatt, B. F., Wager-Miller, J., Mackie, K., & Straiker, A. (2010). Architecture of cannabinoid signaling in mouse retina. *The Journal of Comparative Neurology*, *518*, 3848–3866.

Hudson, B. D., Hébert, T. E., & Kelly, M. E. (2010). Ligand- and heterodimer-directed signaling of the CB(1) cannabinoid receptor. *Molecular Pharmacology*, *77*, 1–9.

Humphrey, W., Dalke, A., & Schulten, K. (1996). VMD—visual molecular dynamics. *Journal of Molecular Graphics*, *14*, 33–38.

Jayaram, B., Bhushan, K., Shenoy, S. R., Narang, P., Bose, S., Agrawal, P., Sahu, D., & Pandey, V. (2006). Bhageerath: an energy based web enabled computer software suite for limiting the search space of tertiary structures of small globular proteins. *Nucleic Acids Research*, *34*, 6195–6204.

Källberg, M., Wang, H., Wang, S., Peng, J., Wang, Z., Lu, H., & Xu, J. (2012). Template-based protein structure modeling using the RaptorX web server. *Nature Protocols*, *7*, 1511–1522.

Kellogg, G. E., & Abraham, D. J. (2000). Hydrophobicity: is LogP(o/w) more than the sum of its parts? *European Journal of Medicinal Chemistry*, *35*, 651–661.

Kurowski, M. A., & Bujnicki, J. M. (2003). GeneSilico protein structure prediction meta-server. *Nucleic Acids Research*, *31*, 3305–3307.

Lee, H. K., Choi, E. B., & Pak, C. S. (2009). The current status and future perspectives of studies of cannabinoid receptor 1 antagonists as anti-obesity agents. *Current Topics in Medicinal Chemistry*, *9*, 482–503.

Lind, G. E., Danielsen, S. A., Ahlquist, T., Merok, M. A., Andresen, K., Skotheim, R. I., Hektoen, M., Rognum, T. O., Meling, G. I., Hoff, G., Bretthauer, M., Thiis-Evensen, E., Nesbakken, A., & Lothe, R. A. (2011). Identification of an epigenetic biomarker panel with high sensitivity and specificity for colorectal cancer and adenomas. *Molecular Cancer*, *10*, 85.

Lobley, A., Sadowski, M. I., & Jones, D. T. (2009). pGenTHREADER and pDomTHREADER: new methods for improved protein fold recognition and superfamily discrimination. *Bioinformatics*, *25*, 1761–1767.

Ludányi, A., Eross, L., Czirják, S., Vajda, J., Halász, P., Watanabe, M., Palkovits, M., Maglóczky, Z., Freund, T. F., & Katona, I. (2008). Downregulation of the CB1 cannabinoid receptor and related molecular elements of the endocannabinoid system in epileptic human hippocampus. *The Journal of Neuroscience*, *28*, 2976–2990.

MacKerell, A. D., Jr., Bashford, D., Bellott, M., Dunbrack, R. L., Jr., Evanseck, J. D., Field, M. J., Fischer, S., Gao, J., Guo, H., Ha, S., Joseph-McCarthy, D., Kuchnir, L., Kuczera, K., Lau, F. T. K., Mattos, C., Michnick, S., Ngo, T., Nguyen, D. T., Prodhom, B., Reiher, W. E., III, Roux, B., Schlenkrich, M., Smith, J. C., Stote, R., Straub, J., Watanabe, M., Wiorkiewicz-Kuczera, J., Yin, D., & Karplus, M. (1998). All-atom empirical potential for molecular modeling and dynamics studies of proteins. *Journal of Physical Chemistry B*, *102*, 3586–3616.

Mashiach, E., Schneidman-Duhovny, D., Andrusier, N., Nussinov, R., & Wolfson, H. J. (2008). FireDock: a web server for fast interaction refinement in molecular docking. *Nucleic Acids Research*, *36*, W229–W232.

Miller, L. K., & Devi, L. A. (2011). The highs and lows of cannabinoid receptor expression in disease: mechanisms and their therapeutic implications. *Pharmacological Reviews*, *63*, 461–470.

Nie, J., & Lewis, D. L. (2001a). The proximal and distal C-terminal tail domains of the CB1 cannabinoid receptor mediate G protein coupling. *Neuroscience*, *107*, 161–167.

Nie, J., & Lewis, D. L. (2001b). Structural domains of the CB1 cannabinoid receptor that contribute to constitutive activity and G-protein sequestration. *Journal of Neuroscience*, *21*, 8758–8764.

Niehaus, J. L., Liu, Y., Wallis, K. T., Egertová, M., Bhartur, S. G., Mukhopadhyay, S., Shi, S., He, H., Selley, D. E., Howlett, A. C., Elphick, M. R., & Lewis, D. L. (2007). CB1 cannabinoid receptor activity is modulated by the cannabinoid receptor interacting protein CRIP 1a. *Molecular Pharmacology*, *72*, 1557–1566.

Oesch, S., & Gertsch, J. (2009). Cannabinoid receptor ligands as potential anticancer agents—high hopes for new therapies? *Journal of Pharmacy and Pharmacology*, *61*, 839–853.

Oster, B., Thorsen, K., Lamy, P., Wojdacz, T. K., Hansen, L. L., Birkenkamp-Demtröder, K., Sørensen, K. D., Laurberg, S., Orntoft, T. F., & Andersen, C. L. (2011). Identification and validation of highly frequent CpG island hypermethylation in colorectal adenomas and carcinomas. *International Journal of Cancer*, *129*, 2855–2866.

Phillips, J. C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., Chipot, C., Skeel, R. D., Kale, L., & Schulten, K. (2005). Scalable molecular dynamics with NAMD. *Journal of Computational Chemistry*, *26*, 1781–1802.

Roy, A., Kucukural, A., & Zhang, Y. (2010). I-TASSER: a unified platform for automated protein structure and function prediction. *Nature Protocols*, *5*, 725–738.

Sarkar, A., & Kellogg, G. E. (2010). Hydrophobicity—shake flasks, protein folding and drug discovery. *Current Topics in Medicinal Chemistry*, *10*, 67–83.

Shen, M. Y., & Sali, A. (2006). Statistical potential for assessment and prediction of protein structures. *Protein Science*, *15*, 2507–2524.

Smith, T. H., Sim-Selley, L. J., & Selley, D. E. (2010). Cannabinoid CB1 receptor-interacting proteins: novel targets for central nervous system drug discovery? *British Journal of Pharmacology*, *160*, 454–466.

Stauffer, B., Wallis, K. T., Wilson, S. P., Egertová, M., Elphick, M. R., Lewis, D. L., & Hardy, L. R. (2011). CRIP1a switches cannabinoid receptor agonist/antagonist-mediated protection from glutamate excitotoxicity. *Neuroscience Letters*, *503*, 224–228.

Thakur, G. A., Tichkule, R., Bajaj, S., & Makriyannis, A. (2009). Latest advances in cannabinoid receptor agonists. *Expert Opinion on Therapeutic Patents*, *19*, 1647–1673.

Turu, G., & Hunyady, L. (2010). Signal transduction of the CB1 cannabinoid receptor. *Journal of Molecular Endocrinology*, *44*, 75–85.

Wu, S., & Zhang, Y. (2007). LOMETS: a local meta-threading-server for protein structure prediction. *Nucleic Acids Research*, *35*, 3375–3382.

Wu, S., & Zhang, Y. (2008). MUSTER: improving protein sequence profile–profile alignments by using multiple sources of structure information. *Proteins*, *72*, 547–556.

Xu, D., & Zhang, Y. (2012). Ab initio protein structure assembly using continuous structure fragments and optimized knowledge-based force field. *Protein*, *80*, 1715–1735.

CHAPTER

71

Synthetic Cannabinoids: a Summary of Selected Phenomena With Respect to Behavioral Pharmacology and Abuse Liability

B.T. Burrows, L.R. Watterson*, J. Egnatios**, M.F. Olive**

***Department of Psychology, Arizona State University, Tempe, AZ, United States**
****School of Medicine, University of California San Diego, La Jolla, CA, United States**

SUMMARY POINTS

- While "Spice" and "K2" are the most known, over 100 different synthetic cannabinoids have been marketed for recreational use, and identified as having Δ^9-tetrahydrocannabinol (THC) like activity.
- Numerous different structural formations have been identified as synthetic cannabinoids, including naphthoylindoles (JWH-018, JWH-073, AM-2201), naphthylmethylindoles (JWH-185), naphthoylpyrroles (JWH-030), naphthylmethylindenes (JWH-176), phenylacetylindoles (JWH-250, RCS-4), cyclohexylphenols (CP47,497), tetrahydrocannabinols (HU-210), and tetramethylcyclopropyl ketone indoles (UR-144, XLR-11).
- The common paradigm for identifying THC-like effects, the cannabinoid tetrad, suggests that many synthetic cannabinoid effects are mediated primarily by the CB_1 receptor. This receptor undergoes downregulation and desensitization through repeated stimuli, but can be recovered within days to weeks of cessation.
- JWH-018, the primary compound of brand names "K2" and "Spice," appears to be significantly more potent, and to have higher affinity for the CB_1 receptor than THC. JWH-018 also appears more potent based on drug discrimination paradigm in rats and rhesus monkeys.
- Tetramethylcyclopropyl ketone indoles such as UR-144 and XLR-11 appear to also be more potent than THC, as assessed in the cannabinoid tetrad paradigm, but equipotent as assessed via drug discrimination testing.
- THC and synthetic cannabinoids produce evidence of cross-tolerance to other cannabinoid molecules.

KEY FACTS OF SYNTHETIC CANNABINOIDS

- Use of synthetic cannabinoids in the general public, through primarily adolescents and young adults, has drastically increased in the last decade.
- Many designer drugs and synthetic cannabinoids are not detected by common toxicology screens, a situation that leaves healthcare providers and law enforcement with limited quantitative tools to confirm use.
- Synthetic cannabinoid use has been linked to acute kidney injury, cardiovascular events and collapse, psychosis, and death.
- Nearly 12% of United States high school seniors admit to having tried a synthetic cannabinoid such as "Spice" or "K2" in the past year.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00082-X
Copyright © 2017 Elsevier Inc. All rights reserved.

- Empirical evidence appears consistent with published literature, suggesting a more significant response to synthetic cannabinoids, with a seemingly higher rate of induced anxiety, dysphoria, agitation, bizarre behavior, and psychosis in patients with prior history of psychiatric disease or mood disorder.
- Parents and healthcare providers are less likely to have knowledge or awareness of synthetic cannabinoids, when compared to classical illicit drugs abused by adolescents.
- SCBs differ not only structurally, but also in their pharmacodynamics, where they display a range of actions including full or partial agonist, antagonist/inverse agonist, and varying affinities at CB_1 and CB_2 receptor sites.

LIST OF ABBREVIATIONS

AM2389	9β-Hydroxy-3-(1-hexyl-cyclobut-1-yl)-hexahydrocannabinol
AM251	*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide
AM356	(R)-(+)-arachidonyl-1′-hydroxy-2′-propylamide
CB_1	Cannabinoid receptor type 1
CB_2	Cannabinoid receptor type 2
CP55,940	5-(1,1-Dimethylheptyl)-2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-phenol
HU-210	1,1-Dimethylheptyl-11-hydroxytetrahydrocannabinol
HU-308	4-[4-(1,1-Dimethylheptyl)-2,6-dimethoxyphenyl]-6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-methanol
JHW-018	(1-Pentyl-1*H*-indol-3-yl)-1-naphthalenylmethanone, also referred to as AM678
MPE	Maximum possible effect
SCB	Synthetic cannabinoid
THC	Δ^9-Tetrahydrocannabinol
WIN 55,212-2	(R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate

INTRODUCTION

Following the isolation and identification of THC as the principal psychoactive component of marijuana, in 1964 (Gaoni & Mechoulam, 1964), and the recognition of cannabis' potential medicinal value, efforts commenced toward development of novel synthetic THC analogues (synthetic cannabinoids, or SCBs) as potential therapeutic agents, as well as pharmacological tools to further study endogenous cannabinoid systems (Watson, Benson, & Joy, 2000). While SCBs have greatly improved our understanding of cannabinoid systems, and led to the development of various pharmacotherapeutics, underground chemists throughout the world have also used the scientific literature to create illicit SCBs intended to be used recreationally as "legal" alternatives to cannabis (Castaneto et al., 2014; Rosenbaum, Carreiro, & Babu, 2012; Seely, Lapoint, Moran, & Fattore, et al., 2012). These more recently developed SCBs have infiltrated international drug markets, and are often sold as herbal marijuana alternatives, herbal incense, or "potpourri" blends under brand names such as "K2," "Spice," "California Spice," "Spike," etc. (Fig. 71.1).

Along with the recent influx of SCBs into drug markets, the number of case reports of psychological impairment and systemic toxicity has increased in parallel (Castaneto et al., 2014; Rosenbaum et al., 2012; Seely et al., 2012; see Fig. 71.2). In addition to their desired euphorigenic effects, SCBs can induce agitation, irritability, anxiety, confusion, psychosis, and impairments in short-term memory, speech, and other cognitive functions. As detailed in examples of case incidents from emergency room visits of self-reported SCB users (Table 71.1), adverse clinical effects of SCB include mydriasis, reddened conjunctivae, nausea, vomiting, hyperventilation, hypertension, tachycardia, angina, diaphoresis, and various hematological abnormalities (de Havenon, Chin, Thomas, & Afra, 2011; Meijer, Russo, & Adhvaryu, 2014; Quan, Haase, & Levitan, 2011; Simmons et al., 2011; Patton et al., 2013).

The SCBs available on drug markets to date come primarily from one of seven different structural classes including naphthoylindoles (JWH-018, JWH-073, AM-2201), naphthylmethylindoles (JWH-185), naphthylpyrroles (JWH-030), naphthylmethylindenes (JWH-176), phenylacetylindoles (JWH-250, RCS-4), cyclohexylphenols (CP47,497), and tetrahydrocannabinols (THC, HU-210). However, newer structural classes such as tetramethylcyclopropyl ketone indoles (UR-144, XLR-11) have emerged as replacements in response to recent legislative actions. The SCBs from these various classes differ not only structurally, but also in their pharmacodynamic effects, where they display a range of actions including full or partial agonism, antagonist/inverse agonist activity, and varying efficacies at CB_1 and CB_2 receptors in the brain and peripheral organs, respectively (Palmer, Thakur, & Makriyannis, 2002; Tai & Fantegrossi, 2014). The potential actions of SCBs on other receptor systems in the brain and periphery, however, are largely unknown.

FIGURE 71.1 **Common packaging of SCB cannabinoid products.** *Source: Reproduced with permission from Penn, Langman, Unold, Shields, & Nichols (2011).*

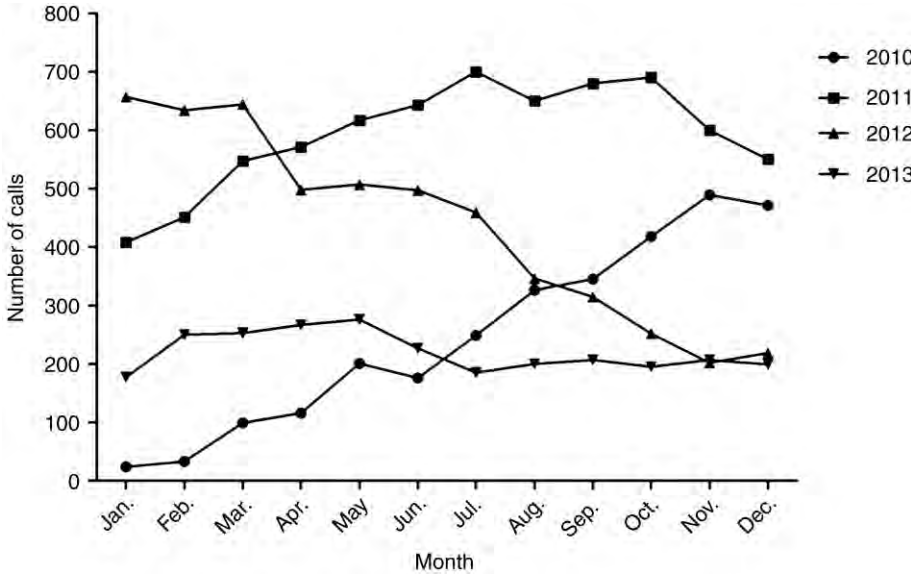


FIGURE 71.2 **Number of calls to US poison control centers regarding SCBs, by month, from 2010 to 2013.** *Source: Reproduced with permission from Castaneto et al. (2014).*

Historically, one of the first SCBs to be developed was WIN 55,212-2 (Fig. 71.3; Huffman, Dai, Martin, & Compton, 1994), an aminoalkylindole cannabinoid that served as a chemical backbone for subsequent development of synthetic indole cannabinoids such as JWH-018 (Wiley, Marusich, & Huffman, 2014b). While THC, WIN 55,212-2, and JWH-018 appear chemically distinct (Fig. 71.3), they possess common moieties and functional groups that have been proposed to interact with cannabinoid receptors at distinct sites on the receptor molecule (Wiley et al., 2014b).

A comprehensive discourse covering all aspects of the chemistry and pharmacology of SCBs is beyond the scope of the present chapter. The reader is therefore directed to several recent and comprehensive reviews for supplemental information (Fattore & Fratta, 2011; Hudson & Ramsey, 2011; Seely et al., 2012; Spaderna, Addy, & D'Souza, 2013; Wiley et al., 2014b; Zawilska & Wojcieszak, 2014; also see Chapter 87). This chapter represents a summary of selected well-characterized and consistent phenomena that have emerged in the literature with respect to the behavioral pharmacology and abuse liability of SCBs. Specifically, these phenomena include: (1) CB_1 receptor mediated characteristic tetrad effects in laboratory animals following exposure to SCBs; (2) discriminative stimulus effects of SCBs relative to THC; and (3) tolerance and cross-tolerance to the effects of SCBs.

CB_1 RECEPTOR MEDIATED CHARACTERISTIC TETRAD EFFECTS IN LABORATORY ANIMALS FOLLOWING EXPOSURE TO SCBs

To date, over 100 SCBs have been detected on international drug markets (United Nations Office on Drugs and Crime, 2014). However, only a handful of these

TABLE 71.1 Emergency Room Case Reports of Adverse Health Effects in Self-Reported SCB Users

Case	Cause for medical evaluation	Clinical presentation	Physical findings	Treatment	Resolution
21-Year old male (Meijer et al., 2014)	Seizure-like activity	Altered mental status, somnolence, confusion, emesis	Sinus tachycardia, tachypnea, minimally reactive dilated pupils, hyperreflexia, leukocytosis, hyperglycemia, negative urine toxicology	Observation, IV 0.9% saline and diphenhydramine	Return to baseline state
27-Year old male (Meijer et al., 2014)	Altered mental status	Emesis, confusion, agitation, combativeness	Sinus tachycardia, tachypnea, leukocytosis, negative urine toxicology	Observation, IV 0.9% saline	Return to baseline state
21-Year old male (Meijer et al., 2014)	Altered mental status	Agitation, emesis	Leukocytosis, hypokalemia, normal pupillary reflexes	Observation, lorazepam, IV 0.9% saline	Return to baseline state
29-Year old male (Meijer et al., 2014)	Panic attack	Feelings of imminent doom, palpitations, shortness of breath, abdominal pain, agitation	Reddened conjunctivae, sinus tachycardia, hyponatremia, slightly elevated leukocyte count, negative urine toxicology	2 Day observation, IV and PO lorazepam every 8 h, CT head scan and blood/urine cultures to rule out other causes	Return to baseline state
26-Year old male (Simmons et al., 2011)	Self-injury	Disorientation, severe agitation	Severe bilateral burns to hands and forearms, 14.5% total body surface area burns from 2nd to 4th degree severity	Emergency irrigation and debridement, amputation of fingers at metacarpo-phalangeal joints	Significant postamputation health impairment
24-Year old male	Generalized tonic-clonic seizure	Suspected postictal confusion, somnolence	No laboratory abnormalities or physical exam findings of significance	MRI, CT scan, CSF analysis, EEG (all negative), observation with IV saline infusion	Return to baseline state
36-Year old male (de Havenon et al., 2011)	Status epilepticus	Unresponsive, seizure activity	Leukocytosis	Intubation, IV lorazepam, etomidate, vecuronium, propofol, levetiracetam, phenytoin. X-ray, urinalysis, blood cultures, CSF analysis, MRI, EEG, CT head scan all negative	Return to baseline state
Grouped case review (Quan et al., 2011)	Altered mental status	Diaphoresis, tremor, anxiety, agitation	Hypertension, sinus tachycardia, mydriasis, leukocytosis	IV saline and benzodiazepine administration	Return to baseline state

This table highlights case reports to illustrate the clinical presentation and acute medical treatment of SCB toxicity in self-reported users. Cases were obtained from the published literature of emergency room reports.
IV, Intravenous; PO, oral.

SCBs have been assessed for their behavioral effects in laboratory animals, and effects reported in humans are largely anecdotal. One of the most common behavioral methods to assess novel SCBs for THC-like effects is termed the "cannabinoid tetrad" (Little, Compton, Johnson, Melvin, & Martin, 1988), and is characterized by a collection of physiological effects including hypothermia, analgesia, catatonia, and locomotor suppression. This collection of physiological and behavioral effects reliably occurs in mice given THC, is largely specific to ligands acting at CB_1 receptors, and is therefore used to predict THC-like effects in humans (Wiley et al., 2014b). As an illustrative example, dose-dependent induction of cannabinoid tetrad behaviors by WIN 55,212-2 is shown in Fig. 71.4 (Wiley et al., 2014b). Overall, the cannabinoid tetrad has been extremely useful in the characterization of biochemical and physiological effects of various ligands on the cannabinoid system, and various recent

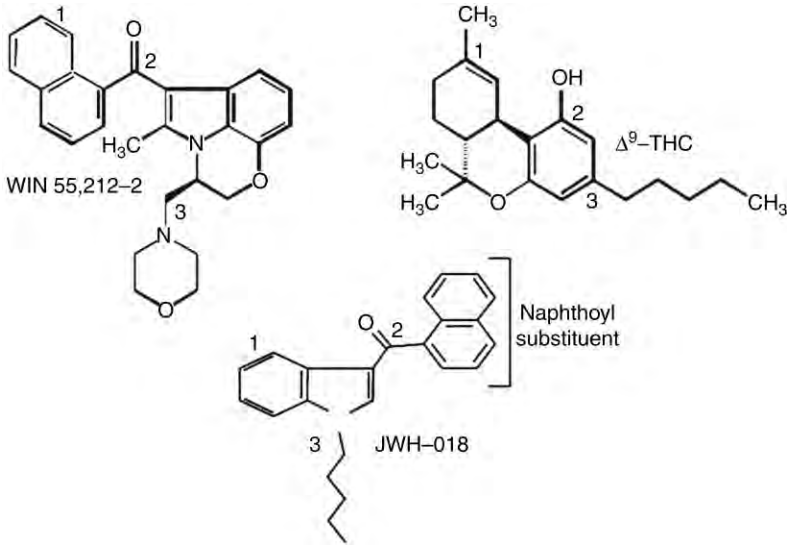


FIGURE 71.3 **Chemical structures of WIN 55,212-2, THC, and JWH-018.** Numerals adjacent to functional groups represent predicted binding sites of the molecule to CB receptors. *Source: Reproduced with permission from Wiley et al. (2014b).*

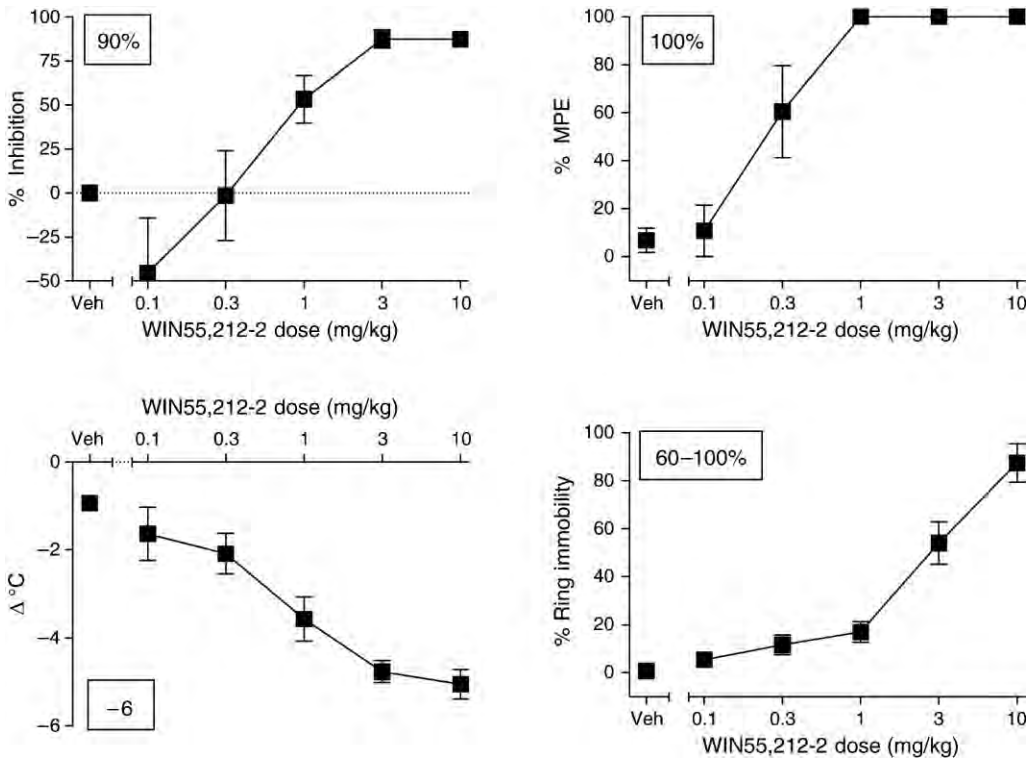


FIGURE 71.4 **Dose-dependent induction of cannabinoid tetrad effects by the SCB WIN 55,212-2 in adult male mice.** *Top left graph*, inhibition of spontaneous locomotor activity. *Top right graph*, antinociception as assessed in the tail flick assay. MPE, maximum possible effect. *Bottom left graph*, rectal temperature. *Bottom right graph*, catalepsy as measured in a ring testing apparatus. *Source: Reproduced with permission from Wiley et al. (2014b).*

studies have shown that some of the commercially available SCBs appear to elicit effects similar to those of THC (Gurney, Scott, Kacinko, Presley, & Logan, 2014; Wiley et al., 2014b).

Early work with the synthetic cannabinoids CP 55,940 and WIN 55,212-2 revealed that these SCBs produce tetrad effects similar to those elicited by THC, but both do so at much lower doses, indicating greater potency (Fan, Compton, Ward, Melvin, & Martin, 1994). JWH-018, one of the earliest SCBs found in "K2/Spice" products (see Chapter 87), has high affinity for CB_1 receptors, and displays full tetrad effects in mice at 2–50 times lower doses than

THC. Tetrad effects also occur in mice exposed to smoke produced from a commercially available "spice" product containing 5.4% JWH-018 (Wiebelhaus et al., 2012). In addition, several structurally related compounds in which the naphthoyl moiety in JWH-018 is replaced with a phenylacetyl group retains CB_1 receptor mediated tetrad effects (Wiley, Marusich, Martin, & Huffman, 2012a; Wiley, Smith, Chen, Martin, & Huffman, 2012b). Not surprisingly, a subset of 1-pentyl-3-phenylacetylindoles, such as JWH-250, has also emerged on drug markets (Rosenbaum et al., 2012; Vardakou, Pistos, & Spiliopoulou, 2010; Wiley et al., 2012a). JWH-073, the alkyl homologue of JWH-018 prominent in "Spice," displays CB_1 mediated tetrad effects in mice, possibly mediated by several of its potential active metabolites (Brents et al., 2012). The bicyclic SCB, CP-47,497, is a potent CB_1 agonist found in commercial preparations, and it also displays potent tetrad-like effects in mice, along with several structurally related bicyclic analogues (Compton, Johnson, Melvin, & Martin, 1992b). HU-210, a highly potent full agonist at CB_1 receptors also causes tetrad-like effects of catalepsy and suppressed locomotion in rats (Bosier et al., 2010). Finally, UR-144 and XLR-11, which are two novel tetramethylcyclopropyl ketone indoles and full CB_1 receptor agonists, have emerged as popular replacements to earlier banned SCBs. In tetrad tests, UR-144 was 3–15 times more potent, and XLR-11 was 4–17 more potent than THC, respectively, across the various tetrad measures. Furthermore, results revealed that UR-144 and XLR-11 are approximately equipotent (Wiley, Marusich, Lefever, Grabenauer, Moore, 2013).

DISCRIMINATIVE STIMULUS EFFECTS OF SCBs, RELATIVE TO THC

Another behavioral method commonly used to assess the THC-like effects of SCBs is drug discrimination. Drug discrimination procedures provide a measure of similarity between the interoceptive cues produced by a novel compound in relation to a drug with known pharmacological properties (Ator & Griffiths, 2003). Drug discrimination has proven extremely useful at predicting marijuana-like effects in humans, as previous research has revealed a high correspondence between THC-like subjective effects in humans and THC-like responding in drug discrimination tests (Ator & Griffiths, 2003). Furthermore, cannabinoids with high CB_1 receptor affinity share discriminative properties with THC, whereas substances from other drug classes such as psychostimulants typically do not support THC-like responding. Therefore, drug discrimination is a pharmacologically selective method for determining the likelihood that novel SCBs will produce THC-like subjective effects in humans (Gold, Balster, Barrett, Britt, & Martin, 1992; Wiley, Huffman, Balster, & Martin, 1995). In drug discrimination procedures, laboratory animals are trained in an operant conditioning apparatus to respond on one lever (eg, right lever) in order to receive a food reinforcer following administration of a drug (Fig. 71.5). Conversely, responding on a different lever (eg, left lever), following administration of saline, results in the presentation of a food reinforcer. Training typically alternates between drug and vehicle sessions until lever-appropriate responding reaches a designated stability criterion (usually ~80%). Thus, interoceptive cues produced by the drug/vehicle serve as discriminative stimuli that guide lever appropriate responding, in order to receive food reinforcement. Following stabilization of responding with the training drug, stimulus generalization tests are conducted after administration of a novel drug, during which both levers are reinforced (Fig. 71.5). A test drug is interpreted as

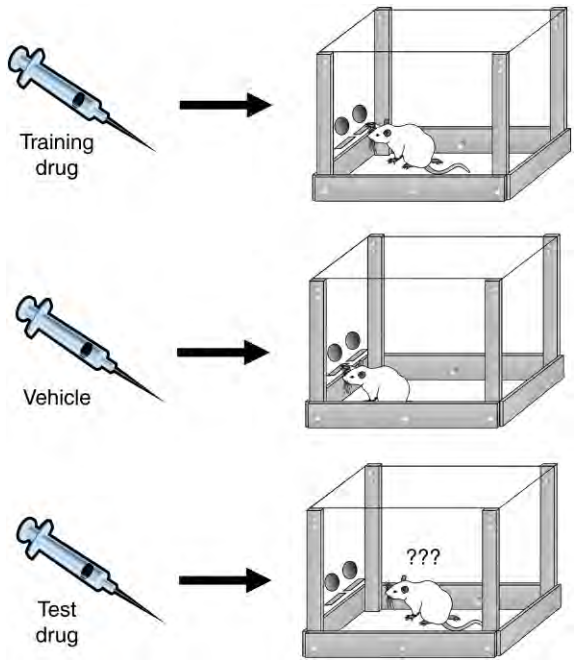


FIGURE 71.5 Drug discrimination procedures. Laboratory rodents are trained in operant conditioning chambers to respond for food reinforcement. During discrimination training, rodents are administered training drugs (eg, THC, top diagram), after which they are placed into the chambers and required to press one of two levers (eg, right lever) in order to receive food reinforcement. Prior to alternate sessions, rodents are administered the corresponding vehicle, and during the ensuing training session, rodents are required to press the opposite lever (eg, left lever, middle diagram) in order to receive food reinforcement. Training alternates between drug and vehicle sessions until lever-appropriate responding reaches a designated stability criterion (~80%). Next, stimulus generalization tests are conducted following administration of a test drug (bottom diagram), during which both levers are reinforced. A test drug (eg, a novel SCB) is interpreted as producing interoceptive effects similar to the training drug (eg, THC) when lever responding occurs more frequently on the training drug-associated lever versus the vehicle-trained lever. When responding following administration of the test drug is ≥80% of that following the training drug, the test drug is interpreted as fully substituting for the training drug.

producing interoceptive effects similar to the training drug when lever responding occurs more frequently on the drug-trained lever versus vehicle-trained lever. Moreover, when responding following administration of the test drug is ≥80% of that following the training drug, the test drug is interpreted as fully substituting for the training drug (Watterson, Watterson, & Olive, 2013).

In one of the first studies of its kind, Gold and colleagues (1992) showed that the SCB CP 55,940, a bicyclic analogue of THC, fully substituted for THC in rats (training dose of THC 3.0 mg/kg, i.p.) and rhesus monkeys (training doses of THC 0.04–0.1 mg/kg, i.m.). In both rats and rhesus monkeys, doses that led to full substitution were much lower than those required for THC, revealing that CP 55,940 was at least 20-fold more potent than THC in eliciting THC-appropriate lever responding (Gold & Balster, 1996). In another set of early studies, full substitution for THC was also found with the full CB_1 agonist WIN 55,212-2 (Compton et al., 1992b; Wiley et al., 1995). In the study by Wiley et al. (1995), the stereoisomers (+)-WIN 55,212-2 and (–)-WIN 55,212-2 displayed differential substitution for THC (3 mg/kg) in rats, such that the (+)-isomer (11.5 μmol/kg) fully substituted for THC, whereas the (–)-isomer was inactive and showed negligible effects at doses up to 57 μmol/kg. Furthermore, the (+)-isomer fully substituted for THC with an ED50 value (0.17 mg/kg) much lower than that produced by THC itself (0.6 mg/kg), revealing increased potency compared to THC (Compton, Gold, Ward, Balster, & Martin, 1992a). In the study by Compton et al. (1992a), WIN 55,212-2 was found to fully substitute in THC trained rhesus monkeys. Interestingly, however, WIN 55,212-2 was less potent than THC in rhesus monkeys than in rats, with ED50 values of 0.1 μmol/kg versus 0.03 μmol/kg for THC (Wiley et al., 1995). With regard to JWH-018 (occasionally referred to as AM678 in earlier studies), drug discrimination in rats revealed full substitution of this compound for both THC and methanandamide, an analog of the endogenous cannabinoid anandamide (Hudson & Ramsey, 2011; Vardakou et al., 2010; Järbe, Li, Vadivel, & Makriyannis, 2010). Furthermore, compared to THC, JWH-018 was approximately 11.3 times as potent as THC in producing full substitution. Following administration of a CB_1 receptor antagonist, a rightward shift in the dose-response curve was observed, indicating CB_1 receptor agonism as the likely mechanism mediating THC-like responding. JWH-018 also fully substituted for methanandamide, and was approximately 57 times more potent. In another study using rhesus monkeys trained to discriminate between THC (0.1 mg/kg, i.v.) and vehicle, or the CB_1 receptor antagonist rimonabant (1 mg/kg, i.v.) and vehicle, JWH-018 was approximately 3.4 times as potent as THC in producing full substitution for THC (Ginsburg & Schulze, 2012). JWH-018 failed to substitute for rimonabant, and coadministration with the CB_1 receptor antagonist rimonabant caused a rightward shift in the dose response curve during THC generalization tests, suggesting surmountable antagonism (Ginsburg & Schulze, 2012). In another recent study (Wiley, Lefever, Cortes & Marusich, 2014a), rats were initially trained to discriminate either JWH-018 (0.3 mg/kg, i.p.) from vehicle, or THC (3.0 mg/kg, i.p.) from vehicle. In THC trained rats, JWH-018 fully substituted for THC at approximately 4–5 times the potency as THC. In JWH-018 trained rats, THC fully substituted for JWH-018, and interestingly, was about 30 times as potent as THC in causing full JWH-018-like responding.

The SBC JWH-073 has also been shown to substitute for THC in a number of studies. In the first (Ginsburg & Schulze, 2012), JWH-073 produced full substitution in rhesus monkeys trained to discriminate THC (3.0 mg/kg, i.p.) from vehicle. While this study also showed that JWH-018 was more potent than THC, JWH-073 was demonstrated to be approximately equipotent to THC, and approximately 4 times less potent than JWH-018 (Ginsburg & Schulze, 2012). In addition, rimonabant caused a rightward shift in the dose response curve, indicting surmountable antagonism and CB_1 receptor-mediated effects. In another study, JWH-073 also fully substituted for THC, yet, despite a much greater affinity for CB_1 receptors, was again shown to be approximately equipotent to THC (Wiley et al., 2014a). Recently, the replacement SCBs XLR-111 and UR-144 have also been shown to fully substitute for THC in mice trained to discriminate THC (5.6 mg/kg, i.p.) from vehicle. XLR-11 was shown to be approximately 1.5 times more potent than both THC and UR-144. UR-144 was found to be approximately equipotent to THC, with both drugs showing full substitution at the 5.6 mg/kg dose (Wiley et al., 2013). Finally, THC substitution was blocked at the 5.6 mg/kg doses by administration of rimonabant (3 mg/kg, i.p.), again suggesting CB_1 mediated responding.

TOLERANCE AND CROSS-TOLERANCE TO THE EFFECTS OF SCBs

With repeated activation by exogenous ligands, CB_1 receptors undergo significant downregulation and desensitization in multiple regions of the brain (Rubino, Vigano, Massi, Spinello, Zagato, 2000). These adaptations are thought to contribute to tolerance to SCB-mediated behavioral effects. However, CB_1 receptor downregulation and desensitization have been shown to recover within days to weeks, following cessation of ligand administration (Hirvonen, Goodwin, Li, Terry, Zoghbi, 2012; Sim-Selley, Schechter, Rorrer, Dalton, Hernandez, 2006), suggesting that long-term neurophysiological changes produced by SCBs may be, at least in part, reversible.

Only a small number of SCBs have been assessed for their ability to lead to tolerance or cross-tolerance to other cannabinoids, most commonly THC. In a 2011 study (McMahon, 2011), rhesus monkeys were initially trained to discriminate THC (0.1 mg/kg, i.v.) from vehicle. Next, the discriminative stimulus effects of THC, WIN 55,212-2, and CP 55,940 were assessed both before and after 1 day of THC treatment. Discriminative stimulus effects of THC were also assessed after 3 and 6 days of repeated treatment. While THC fully substituted for itself, 1 day of THC treatment did not produce significant effects, and repeated treatment with THC for both 3 and 6 days produced a rightward shift in the dose effect curves, revealing tolerance-like effects. Similarly, 1 day of THC treatment did not produce cross-tolerance to WIN 55,212-2 or CP 55,940, although the 3 and 6 day treatment regimens were not tested. However, this study also provided evidence of cross-tolerance of THC to WIN 55,212-2 and CP 55,940.

In a study by Hruba, Ginsburg, and McMahon (2012), rhesus monkeys were initially trained to discriminate THC from vehicle. These investigators observed that CP 55,940, JWH-018 and JWH-073 fully substituted for THC. When tested after 1 and 3 days of repeated treatment with THC, no changes in drug discrimination were observed. However, 14 days of chronic THC treatment (1 mg/kg per day, s.c.) produced significant rightward shifts in the dose-response curve for THC, CP 55,940, JWH-018, and JWH-073 that were 9.2-, 3.6-, 4.3-, and 5.6-fold, respectively. Thus, chronic THC treatment produced cross-tolerance to each of the CB_1 receptor agonist JWH-018 and JWH-073, two of the most commonly abused recreational SCBs.

CONCLUSIONS

The scientific assessment of the behavioral pharmacology and abuse liability of SCBs is still in its infancy, and a great deal of additional work is needed to establish acute and lasting behavioral, psychological, and neurological effects. Of the over 100 SCBs that have appeared on international markets, their effects in humans remain largely anecdotal, and only a handful have been assessed in animal models. Furthermore, continued governmental bans on specific compounds, combined with the multitude of possible structural modifications, will likely lead to the emergence of potentially hundreds of additional SCBs on international markets. Of the SCBs scientifically assessed in animal studies to date, experiments have generally revealed behavioral effects similar to THC, but often with much greater potency and specificity at CB_1 receptors. Thus, the most common recreationally used SCBs, to date, appear to have high abuse potential at least comparable to, and often greater than, cannabis or THC itself. False marketing as safe alternatives to cannabis, ease of procurement, low cost, and failure to produce positive results on traditional drug screens make SCBs attractive to both existing and new drug users, further increasing their potential for abuse. This combination of factors has created a new unique challenge to scientists, educators, and legislators responsible for reducing societal harm that, thus far, has been less than adequate. Given the multitude of SCBs already available on drugs markets, and the hundreds of potential new compounds likely to emerge, it is clear that newer strategies are desperately needed to curb SCB abuse.

MINI-DICTIONARY

Cannabinoid subtype 1 (CB_1) receptor The receptor believed to be most linked to the effects of cannabis and cannabis-mimicking drugs. The CB_1 receptor is a G-protein coupled receptor that is highly expressed in central nervous system tissue.

Downregulation Reduction in the expression and/or function of a particular protein.

Drug discrimination Relating to the ability of test subjects (most commonly rodents or primates) to distinguish between the subjective (interoceptive) effects of drugs, as inferred from operant responding.

Interoceptive effects Internal bodily signals relevant to a designated effect or response to a drug.

Synthetic cannabinoids (SCBs) A collective term referring to research chemicals and "designer" illicit drugs that behave similarly in action and biological effect to THC. Spice and K2 are two of the most popular SCBs.

Tetrad effects A collection of behaviors that appear to be highly related and specific to cannabinoid receptor activity. These effects are primarily suppression of locomotor activity, catalepsy, hypothermia, and analgesia.

Tolerance The ability of a tissue or organism to adapt or attenuate its effects in response to a certain stimuli.

References

Ator, N., & Griffiths, R. (2003). Principles of drug abuse liability assessment in laboratory animals. *Drug and Alcohol Dependence*, *70*, 55–72.

Bosier, B., Sarre, S., Smolders, I., Michotte, Y., Hermans, E., et al. (2010). Revisiting the complex influences of cannabinoids on motor functions unravels pharmacodynamic differences between cannabinoid agonists. *Neuropharmacology*, *59*, 503–510.

Brents, L. K., Gallus-Zawada, A., Radominska-Pandya, A., Vasiljevik, T., Prisinzano, T. E., et al. (2012). Monohydroxylated metabolites of the K2 synthetic cannabinoid JWH-073 retain intermediate to high cannabinoid 1 receptor (CB_1R) affinity and exhibit neutral antagonist to partial agonist activity. *Biochemical Pharmacology*, *83*, 952–961.

Castaneto, M. S., Gorelick, D. A., Desrosiers, N. A., Hartman, R. L., Pirard, S., & Huestis, M. A. (2014). Synthetic cannabinoids: epidemiology, pharmacodynamics, and clinical implications. *Drug and Alcohol Dependence*, *144C*, 12–41.

Compton, D. R., Gold, L. H., Ward, S. J., Balster, R. L., & Martin, B. R. (1992a). Aminoalkylindole analogs: cannabimimetic activity of a class of compounds structurally distinct from delta 9-tetrahydrocannabinol. *Journal of Pharmacology and Experimental Therapeutics*, *263*, 1118–1126.

Compton, D. R., Johnson, M. R., Melvin, L. S., & Martin, B. R. (1992b). Pharmacological profile of a series of bicyclic cannabinoid analogs:

classification as cannabimimetic agents. *Journal of Pharmacology and Experimental Therapeutics*, *260*, 201–209.
de Havenon, A., Chin, B., Thomas, K. C., & Afra, P. (2011). The secret "spice": an undetectable toxic cause of seizure. *Neurohospitalist*, *1*, 182–186.
Fan, F., Compton, D. R., Ward, S., Melvin, L. S., & Martin, B. R. (1994). Development of cross-tolerance between delta 9-tetrahydrocannabinol, CP 55,940 and WIN 55,212. *Journal of Pharmacology and Experimental Therapeutics*, *271*, 1383–1390.
Fattore, L., & Fratta, W. (2011). Beyond THC: the new generation of cannabinoid designer drugs. *Frontiers in Behavioral Neuroscience*, *5*, 1–12.
Gaoni, Y., & Mechoulam, R. (1964). Isolation, structure, and partial synthesis of an active constituent of hashish. *Journal of the American Chemical Society*, *86*, 1646–1647.
Ginsburg, B., & Schulze, D. (2012). JWH-018 and JWH-073: Δ9-tetrahydrocannabinol-like discriminative stimulus effects in monkeys. *Journal of Pharmacology and Experimental Therapeutics*, *340*, 37–45.
Gold, L. H., & Balster, R. L. (1996). Evaluation of the cocaine-like discriminative stimulus effects and reinforcing effects of modafinil. *Psychopharmacology*, *126*, 286–292.
Gold, L., Balster, R., Barrett, R., Britt, D., & Martin, B. (1992). A comparison of the discriminative stimulus properties of delta 9-tetrahydrocannabinol and CP 55,940 in rats and rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics*, *262*, 479–486.
Gurney, S., Scott, K., Kacinko, S. L., Presley, B. C., & Logan, B. K. (2014). Pharmacology, toxicology, and adverse effects of synthetic cannabinoid drugs. *Forensic Science International*, *26*, 53–78.
Hirvonen, J., Goodwin, R. S., Li, C. -T., Terry, G. E., Zoghbi, S. S., et al. (2012). Reversible and regionally selective downregulation of brain cannabinoid CB_1 receptors in chronic daily cannabis smokers. *Molecular Psychiatry*, *7*, 642–649.
Hruba, L., Ginsburg, B. C., & McMahon, L. R. (2012). Apparent inverse relationship between cannabinoid agonist efficacy and tolerance/cross-tolerance produced by Δ9-tetrahydrocannabinol treatment in rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics*, *342*, 843–849.
Hudson, S., & Ramsey, J. (2011). The emergence and analysis of synthetic cannabinoids. *Drug Testing and Analysis*, *3*, 466–478.
Huffman, J. W., Dai, D., Martin, B. R., & Compton, D. R. (1994). Design, synthesis and pharmacology of cannabimimetic indoles. *Bioorganic and Medicinal Chemistry Letters*, *4*, 563–566.
Järbe, T. U. C., Li, C., Vadivel, S. K., & Makriyannis, A. (2010). Discriminative stimulus functions of methanandamide and delta-9-THC in rats: tests with aminoalkylindoles (WIN55,212-2 and AM678) and ethanol. *Psychopharmacology*, *208*, 87–98.
Little, P. J., Compton, D. R., Johnson, M. R., Melvin, L. S., & Martin, B. R. (1988). Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *Journal of Pharmacology and Experimental Therapeutics*, *247*, 1046–1051.
McMahon, L. R. (2011). Chronic Δ9-tetrahydrocannabinol treatment in rhesus monkeys: differential tolerance and cross-tolerance among cannabinoids. *British Journal of Pharmacology*, *162*, 1060–1073.
Meijer, K. A., Russo, R. R., & Adhvaryu, D. V. (2014). Smoking synthetic marijuana leads to self-mutilation requiring bilateral amputations. *Orthopedics*, *37*, e391–e394.
Palmer, S. L., Thakur, G. A., & Makriyannis, A. (2002). Cannabinergic ligands. *Chemistry and Physics of Lipids*, *121*, 3–19.
Patton, A. L., Chimalakonda, K. C., Moran, C. L., McCain, K. R., Radominska-Pandya, A., et al. (2013). K2 toxicity: fatal case of psychiatric complications following AM2201 exposure. *Journal of Forensic Sciences*, *58*, 1676–1680.
Penn, H. J., Langman, L. J., Unold, D., Shields, J., & Nichols, J. H. (2011). Detection of synthetic cannabinoids in herbal incense products. *Clinical Biochemistry*, *44*, 1163–1165.
Quan, D., Haase, D., & Levitan, R. (2011). Legal drugs of abuse. *Emergency Medicine Reports*, *32*, 238–249.
Rosenbaum, C., Carreiro, S., & Babu, K. (2012). Here today, gone tomorrow and back again? A review of herbal marijuana alternatives (K2, Spice), synthetic cathinones (bath salts), kratom, Salvia divinorum, methoxetamine. *Journal of Medical Toxicology*, *8*, 15–22.
Rubino, T., Vigano, D., Massi, P., Spinello, M., Zagato, E., et al. (2000). Chronic delta-9-tetrahydrocannabinol treatment increases cAMP levels and cAMP-dependent protein kinase activity in some rat brain regions. *Neuropharmacology*, *39*, 1331–1336.
Seely, K. A., Lapoint, J., Moran, J. H., & Fattore, L. (2012). Spice drugs are more than harmless herbal blends: a review of the pharmacology and toxicology of synthetic cannabinoids. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *39*, 234–243.
Simmons, J. R., Skinner, C. G., Williams, J., Kang, C. S., Schwartz, M. D., & Wills, B. K. (2011). Intoxication from smoking "spice." *Annals of Emergency Medicine*, *57*, 187–188.
Sim-Selley, L. J., Schechter, N. S., Rorrer, W. K., Dalton, G. D., Hernandez, J., et al. (2006). Prolonged recovery rate of CB_1 receptor adaptation after cessation of long-term cannabinoid administration. *Molecular Pharmacology*, *70*, 986–996.
Spaderna, M., Addy, P. H., & D'Souza, D. C. (2013). Spicing things up: synthetic cannabinoids. *Psychopharmacology*, *228*, 525–540.
Tai, S., & Fantegrossi, W. E. (2014). Synthetic cannabinoids: pharmacology, behavioral effects, and abuse potential. *Current Addiction Reports*, *1*, 129–136.
United Nations Office on Drugs and Crime (2014). World Drug Report 2014. Trends in Organized Crime: *Vol. 3* (11–14).
Vardakou, I., Pistos, C., & Spiliopoulou, C. (2010). Spice drugs as a new trend: mode of action, identification and legislation. *Toxicology Letters*, *197*, 157–162.
Watson, S. J., Benson, J. A., & Joy, J. E. (2000). Marijuana and medicine: assessing the science base. *Archives of General Psychiatry*, *57*, 547–552.
Watterson, L. R., Watterson, E., & Olive, M. F. (2013). Abuse liability of novel "legal high" designer stimulants: evidence from animal models. *Behavioural Pharmacology*, *24*, 341–355.
Wiebelhaus, J. M., Poklis, J. L., Poklis, A., Vann, R. E., Lichtman, et al. (2012). Inhalation exposure to smoke from synthetic "marijuana" produces potent cannabimimetic effects in mice. *Drug and Alcohol Dependence*, *126*, 316–323.
Wiley, J., Huffman, J., Balster, R., & Martin, B. (1995). Pharmacological specificity of the discriminative stimulus effects of delta 9-tetrahydrocannabinol in rhesus monkeys. *Drug and Alcohol Dependence*, *40*, 81–86.
Wiley, J. L., Marusich, J. A., Martin, B. R., & Huffman, J. W. (2012a). 1-Pentyl-3-phenylacetylindoles and JWH-018 share in vivo cannabinoid profiles in mice. *Drug and Alcohol Dependence*, *123*, 148–153.
Wiley, J. L., Smith, V. J., Chen, J., Martin, B. R., & Huffman, J. W. (2012b). Synthesis and pharmacology of 1-alkyl-3-(1-naphthoyl)indoles: steric and electronic effects of 4- and 8-halogenated naphthoyl substituents. *Bioorganic and Medicinal Chemistry Letters*, *20*, 2067–2081.
Wiley, J. L., Marusich, J. A., Lefever, T. W., Grabenauer, M., Moore, K. N., et al. (2013). Cannabinoids in disguise: Δ9-tetrahydrocannabinol-like effects of tetramethylcyclopropyl ketone indoles. *Neuropharmacology*, *75*, 145–154.
Wiley, J. L., Lefever, T. W., Cortes, R. A., & Marusich, J. A. (2014a). Cross-substitution of Δ^9-tetrahydrocannabinol and JWH-018 in drug discrimination in rats. *Pharmacology, Biochemistry, and Behavior*, *124C*, 123–128.
Wiley, J. L., Marusich, J. A., & Huffman, J. W. (2014b). Moving around the molecule: relationship between chemical structure and in vivo activity of synthetic cannabinoids. *Life Sciences*, *97*, 55–63.
Zawilska, J. B., & Wojcieszak, J. (2014). Spice/K2 drugs—more than innocent substitutes for marijuana. *International Journal of Neuropsychopharmacology*, *17*, 509–525.

Page left intentionally blank

CHAPTER

e11

Cannabinoid Signaling in Glioma Cells and Therapeutic implications

A. Ellert-Miklaszewska, I. A. Ciechomska, B. Kaminska

Laboratory of Molecular Neurobiology, Neurobiology Center, Nencki Institute of Experimental Biology, Warsaw, Poland

SUMMARY POINTS

- CB2 receptor is overexpressed in gliomas, and the expression correlates with tumor malignancy grade.
- Ceramide accumulation plays a central role in cannabinoid-induced cell death.
- Inhibition of prosurvival pathways, ER-stress, and autophagy contribute to apoptosis induction in cannabinoid-treated glioma cells.
- Antitumoral activity of cannabinoids in vivo results from inhibition of tumor cell proliferation, blocking of cell invasiveness and angiogenesis, as well as induction of cancer stem cells differentiation.
- Antitumor effectiveness of cannabinoids reported in preclinical studies, and the good safety profile observed in a pilot clinical trial, in patients with malignant human glioblastoma (GBM), encourage to further evaluation of the therapeutic potential of cannabinoids in cancer treatment.

KEY FACTS OF GLIOMA

- Gliomas are the most common primary brain tumors.
- Derived from glial cells or their precursors. Can grossly be divided into astrocytomas (accounting for 60–70% of all gliomas), oligodendrogliomas, and ependymomas, depending which glial cell phenotype they most closely resemble.
- According to the WHO, gliomas are classified from grade I—least aggressive, to grade IV—most aggressive glioblastomas. High-grade gliomas, WHO grade III and IV, account for approximately 70% of all gliomas.
- Tumor cell invasiveness into normal brain tissue is a defined hallmark of gliomas, and accounts for their poor prognosis.
- Difficult to treat—crossing the blood–brain barrier is an important limitation to available drugs.
- The median survival of patients with the malignant glioblastoma is 18 months since diagnosis.

LIST OF ABBREVIATIONS

Δ^9-THC	(−)-*trans*-Δ^9-Tetrahydrocannabinol
2-AG	2-Arachidonoylglycerol
AEA	Anandamide, arachidonoylethanolamide
Akt	Protein kinase B/Akt
ATF4	Activating transcription factor 4
cAMP	Cylic adenosine monophosphate
CB1	Cannabinoid receptor type 1
CB2	Cannabinoid receptor type 2
CHOP	C/EBP-homologous protein
DAG	Diacylglycerol
eIF2α	Eukaryotic translation initiation factor 2α
ER	Endoplasmic reticulum
ERK1/2	Extracellular signal-regulated kinase 1/2
FAAH	Fatty acid amide hydrolase
GBM	Malignant human glioblastoma
IP_3	Inositol 1,4,5-trisphosphate
LPS	Lipopolysacharide

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00076-4
Copyright © 2017 Elsevier Inc. All rights reserved.

MAPK	Mitogen-activated protein kinase
MEK	MAP kinase-ERK kinase
MGL	Monoacylglycerol lipase
mTORC1	Mammalian target of rapamycin, complex 1
NAPE	N-arachidonylphosphatidylethanolamide
PI3K	Phosphatidylinositol 3-kinase
PIP_2	Phosphatidylinositol 4,5-bisphosphate
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
TRB3	Pseudo-kinase tribbles homolog 3
TRPV1	Transient receptor potential cation channel subfamily V member 1, capsaicin or vanilloid receptor
WIN55,212-2	Synthetic cannabinoid

INTRODUCTION

Along with our expanding knowledge on the mechanisms of cannabinoids action, the endocannabinoid system, and the development of synthetic cannabinoids, targeting cannabinoid signaling for therapeutic purposes has inevitably emerged as an interesting area of scientific and clinical investigations. One of the most extensively studied applications of cannabinoids is their potential use as anticancer agents. The pioneer study to show that cannabinoids had antitumor effect was reported by Munson, Harris, Friedman, Dewey, and Carchman (1975). Administration of Δ^9-THC, Δ^8-THC, and cannabinol retarded tumor growth, and prolonged the life of mice bearing Lewis lung adenocarcinoma. Further research in this area was not performed until late 1990s, when an interest in the role of cannabinoids in cancer therapy was renewed. Since then, plant-derived, synthetic, and endogenous cannabinoids have been successfully used to block proliferation and invasive potential of various cancer types (glioma, lymphoma, lung, thyroid, skin, pancreas, breast, and prostate carcinoma) in both in vitro and in vivo models (Guzman, 2003; Pisanti et al., 2009). The research on therapeutic potential of cannabinoids in cancer treatment is the most advanced in gliomas, the most common primary brain tumors.

Signaling pathways and intracellular processes underlying cannabinoid action on glioma cells are reviewed here. A brief overview of the alterations in endocannabinoid system in glial tumors, other pharmacological effects of cannabinoid action in glial tumors, beyond blocking of tumor cells proliferation, and current scientific data relevant to the use of cannabinoids in treatments of glioblastomas are also included.

ENDOCANNABINOID SYSTEM IN HUMAN GLIOMAS

A growing array of data suggest that significant alterations of a balance in the cannabinoid system between the levels of endogenous ligands and their receptors occur along with the malignant transformation in various types of cancer, including brain tumors (Table e11.1). Cannabinoid receptor 1 (CB1) is the predominant receptor for endogenous and exogenous cannabinoids in the central nervous system, expressed on neurons and astrocytes—the major glial cells in the brain. The CB2 receptor is believed to be absent in the healthy brain, its expression has been only detected in microglia-brain macrophages (Stella, 2004; Gong et al., 2006), as well as in a small subpopulation of neurons (Van Sickle et al., 2005; Gong et al., 2006). However, both types of functional cannabinoid receptors were found in primary cultures established cell lines derived from malignant human glioblastoma (GBM) (Galve-Roperh et al., 2000; Sanchez et al., 2001; Howlett et al., 2002). CB2 mRNA and protein levels in tumor tissue extracts were elevated in low-grade gliomas, and even greater in high grade gliomas, in comparison with nontumor tissue controls (Wu et al., 2012). Immunohistochemical analysis of human glioma surgical specimens revealed increased CB2 receptor expression in tumor cells, invading microglia/macrophages and endothelial cells of the tumor blood vessels, as compared to nontumor brain samples (Sanchez et al., 2001; Ellert-Miklaszewska, Grajkowska, Gabrusiewicz, Kaminska, & Konarska, 2007; Schley et al., 2009). An example of CB2 detection in human glioblastoma is presented in (Fig. e11.1) Moreover, CB2 receptor immunoreactivity markedly prevailed over detected CB1 receptor levels in grade IV astrocytomas (Sanchez et al., 2001; Schley et al., 2009). The discrepancies in CB1 receptor expression in glial tumors versus normal tissues were observed by different groups. According to the majority of authors, the levels of CB1 receptor expression in tumor and tumor-associated endothelial cells were not significantly different from the control tissue (Sanchez et al., 2001; Held-Feindt, Dorner, Sahan, Mehdorn, & Mentlein, 2006; Schley et al., 2009).

Increased levels of CB2 are reported in tissues during development, inflammation, injury, and cancer, revealing a critical role for the CB2 receptor in regulating these processes (Howlett et al., 2002). In examined glial tumors, CB2 receptors were usually located in the areas of intense tissue proliferation and invading cells. The enhancement of cannabinoid receptor expression in malignant versus healthy tissues might suggest a possible role of the endocannabinoid system in the tonic suppression of cell divisions and cancer growth. In support of this hypothesis, inhibitors of endocannabinoid transport or degradation (VDM-11 and AA-5-HT) have been shown to inhibit tumor growth and progression in some

TABLE e11.1 Alterations of the Major Components of the Cannabinoid System in Gliomas

Element of the system	Function	Change versus control brain tissue	Detection level	References
Receptors				
CB1	Receptor	0	Protein by IHC	Sanchez et al. (2001)
		0	mRNA	Held-Feindt et al. (2006)
		0	Protein by IHC	Schley et al. (2009)
		↓	Protein by WB	De Jesus et al. (2010)
		↑	mRNA/protein by WB	Wu et al. (2012)
CB2	Receptor	↑(Low-grade) ↑↑ (high-grade)	Protein by IHC	Sanchez et al. (2001)
		0	mRNA	Held-Feindt et al. (2006)
		↑(Low-grade) ↑↑ (high-grade)	Protein by IHC	Ellert-Miklaszewska et al. (2007)
		↑	Protein by IHC	Schley et al. (2009)
		↑	Protein by WB	De Jesus et al. (2010)
		↑(Low-grade) ↑↑ (high-grade)	mRNA/protein by WB	Wu et al. (2012)
Endocannabinoids				
Anandamide (AEA)	Endogenous ligand	↓	Lipid	Maccarrone, Attina, Cartoni, Bari, and Finazzi-Agro (2001)
		↑	Lipid	Petersen et al. (2005)
		↓	Lipid	Wu et al. (2012)
2-AG	Endogenous ligand	0	Lipid	Maccarrone et al. (2001)
		↑	Lipid	Wu et al. (2012)
Enzymes				
FAAH	AEA hydrolysis	↓	Activity	Petersen et al. (2005)
		↓(Low-grade) ↓↓ (high-grade)	mRNA and activity	Wu et al. (2012)
NAPE-PLD	AEA biosynthesis	↓	Activity	Petersen et al. (2005)
		↓	mRNA and activity	Wu et al. (2012)
MGL	2-AG hydrolysis	↓	mRNA and activity	Wu et al. (2012)
DGL-a	2-AG biosynthesis	0	mRNA and activity	Wu et al. (2012)

0, increase; ↓, decrease; ↑, increase; WB, Western-blotting; IHC, immunohistochemistry.

types of cancer, by enhancing the levels of endocannabinoids in the cells (Izzo et al., 2008).

Alterations in the endocannabinoid system in tumors refer also to endogenous ligands, specific enzymes involved in their biosynthesis and degradation, as well as other putative target receptors (Maccarrone et al., 2001; Petersen et al., 2005; Wu et al., 2012). Table e11.1 summarizes the reported levels of the two major endocannabinoids human glioblastoma specimens, compared to nontumor brain tissues. The alterations of the endocannabinoid levels were consistent with the changes in the expressions and activities of the enzymes responsible for their biosynthesis and degradation (Petersen et al., 2005; Wu et al., 2012). Compared to the nontumor tissues, mRNA expression and the enzymatic activity of NAPE-PLD, the enzyme

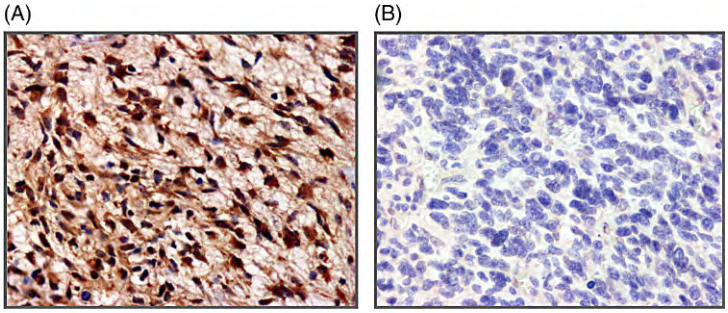


FIGURE e11.1 **Expression of CB2 receptor in human glioblastoma.** (A) Immunohistochemistry was performed using 3,3′-diaminobenzidine (DAB). Cell nuclei were visualized by hematoxylin counterstain. (B) Negative control-specimen incubated with the antibody preabsorbed on the blocking peptide.

responsible for anandamide biosynthesis, decreased in tumor tissues. It was accompanied by the reduction of the anandamide hydrolyzing enzyme FAAH's mRNA expression, and enzymatic activity in low- and high-grade glioma samples (by 30 and 60%, respectively), which might be a result of a negative feedback induced by low levels of the substrate. mRNA expression and activity of MGL, the 2-AG hydrolyzing enzyme, decreased in glioma tissues, compared to that in the nontumor tissues, whereas there was no difference in the expression levels of DGL-a, the 2-AG generating enzyme, between the groups (Wu et al., 2012).

GPR55, an orphan G-protein linked receptor, which can be engaged by cannabinoid-like compounds, appears upregulated in some cancer-derived cell lines, and plays a pivotal role in tumor cells proliferation (Andradas et al., 2011). Higher TRPV1 expression was found in high grade human astrocytomas, as compared to nonneoplastic brains (Stock et al., 2012).

INTRACELLULAR MECHANISMS UNDERLYING ANTIPROLIFERATIVE ACTIVITY OF CANNABINOIDS

Antitumoral Action of Cannabinoids In Vitro

Antiproliferative activity of cannabinoids against glioma cells was first described by Guzman and coworkers (Sanchez, Galve-Roperh, Canova, Brachet, & Guzman, 1998). They showed that Δ^9-THC is able to inhibit growth of rat C6 glioma cells in vitro, and induce programmed cell death with typical features of apoptosis (Sanchez et al., 1998). We reported the induction of apoptosis upon treatment with a mixed CB1/CB2 synthetic agonist WIN 55,212-2 in the same rat glioma cell line (Ellert-Miklaszewska, Kaminska, & Konarska, 2005). Subsequent studies (Gomez del Pulgar, Velasco, Sanchez, Haro, & Guzman, 2002; Salazar et al., 2009) and our own observations show consistently that Δ^9-THC, cannabidiol, endogenous and synthetic cannabinoids, including CB2-selective agonists, effectively decrease cell viability of cultured human glioblastoma cells and tumor-derived primary cultures.

Mechanism of Cannabinoids Proapoptotic Action—Inhibition of Prosurvival Pathways

Several events and signal transduction pathways triggered mostly by stimulation of the CB1 and CB2 receptors have already been described to participate in the cannabinoid-induced apoptosis, a programmed cell death process (Guzman, Sanchez, & Galve-Roperh, 2001; Guzman, 2003). They include inhibition of PKA, superoxide generation, and strong increase in intracellular calcium concentration (Howlett et al., 2002). Sanchez, Velasco, and Guzman (1997) showed that Δ^9-THC was able to partially antagonize the forskolin-induced elevation of intracellular cAMP concentration, but did not affect basal cAMP levels in C6 glioma cells. Glioma C6 cells are characterized by a very low constitutive level of cAMP, and rather increased than decreased concentration of intracellular cAMP may be responsible for inhibition of cell proliferation. It suggest, that cannabinoid receptors may be coupled to inhibition of adenylyl cyclase (AC) in glioma cells, but downregulation of cAMP levels is unlikely to play a role in the induction of apoptosis triggered by cannabinoids in these cells. Involvement of other signaling molecules or adaptor proteins (of still mostly unknown identity) in cannabinoid receptor signaling is a subject of ongoing studies.

The best-characterized mechanism of cannabinoid-induced cell death involves sustained accumulation of proapoptotic sphingolipid ceramide, which modulates signaling pathways crucial in the control of tumor cell growth and survival (Galve-Roperh et al., 2000; Sanchez et al., 2001). Ceramide functions as a physiological signaling molecule, particularly with regard to the control of apoptosis, but also growth arrest, differentiation, cell migration, and adhesion and, as such, has an accepted role in the development of some cancers (Morad & Cabot, 2013). Cannabinoid receptor activation triggers two peaks of ceramide generation in glioma cells (Fig. e11.2) (Galve-Roperh et al., 2000; Sanchez et al., 2001; Gomez del Pulgar et al., 2002). Treatment with Δ^9-THC or another CB1/CB2 receptor agonist produces a rapid release of ceramide via enzymatic hydrolysis of sphingomyelin from the cell membrane. The second ceramide peak is generated within hours or days after receptor activation, and depends on increase of ceramide synthesis de novo via induction of serine palimitoyltransferase, a regulatory enzyme of sphingolipid biosynthesis (Gomez del Pulgar et al., 2002). Selective CB2 receptor agonists, such as JWH133, are supposed to stimulate only the ceramide synthesis process, which is sufficient for turning on the cell death program (Sanchez et al., 2001; Gomez del Pulgar et al., 2002). Thus, enhanced production of ceramide de novo is considered as an important event in cannabinoids-induced apoptosis.

Galve-Roperh et al. (2000) postulated that the increased ceramide levels reported upon cannabinoid challenge led to prolonged activation of Raf-1/MEK/Erk signaling cascade, and thus mediated glioma cell cycle arrest, and cell death. The same authors showed also that pharmacological inhibition of ceramide synthesis de novo prevented the inhibition of protein kinase B (also known as Akt) triggered by cannabinoids (Gomez del Pulgar et al., 2002). Our studies revealed that rather downregulation of Erk activity, together with inhibition of PI3K/Akt pathway, contributed to C6 glioma cell death induced by WIN55,212-2 (Ellert-Miklaszewska

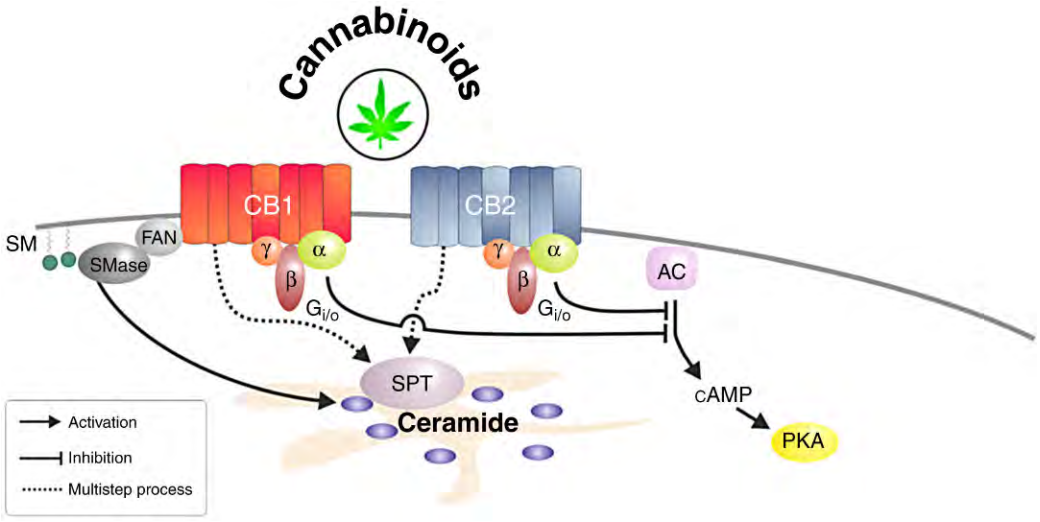


FIGURE e11.2 Ceramide in cannabinoid signaling. Increased ceramide synthesis de novo via induction of serine palmitoyltransferase (SPT) plays a central role in cannabinoid-induced cell death. Signals from CB1 via adaptor protein FAN (factor associated with neutral sphingomyelinase activation) also trigger the ceramide production from sphingomyelin breakdown, catalyzed by neutral sphingomyelinase (SMase). Stimulation of cannabinoids receptors leads to inhibition of adenylyl cyclase, reduction of cAMP levels, and decreased protein kinase A (PKA) activity.

et al., 2005). The serine/threonine protein kinase Akt, activated downstream of phosphoinositide 3-kinase (PI3K), as well as the Ras-activated Raf1/MEK/Erk pathway are widely recognized as key mediators of growth factor-promoted cell survival in gliomas (Kapoor & O'Rourke, 2003). We proposed a mechanism in which the decrease of mitogenic/prosurvival signaling evoked by the synthetic cannabinoid promoted the proapoptotic function of Bad (Fig. e11.3), a proapoptotic member of a Bcl-2 family of proteins. Phosphorylation of Bad by Akt and Erk retains the protein in the cytosol, and directs it for sequestration (Zha, Harada, Yang, Jockel, & Korsmeyer, 1996). Otherwise, Bad forms heterodimers with antiapoptotic proteins, such as Bcl-X_L or Bcl-2, resulting in loss of integrity of the outer mitochondrial membrane. The subsequent release of apoptogenic proteins, including cytochrome c, triggers the executive phase of programmed cell death (Zha et al., 1996). Accordingly, we demonstrated that decreased Bad phosphorylation and collapse of the mitochondrial membrane potential in WIN55,212-2-treated C6 cells preceded activation of apoptotic proteases-caspases (Ellert-Miklaszewska et al., 2005).

Our further studies, as well as some published data, suggest that human glioma cells treated with cannabinoids enter the suicide cell death, using the same mitochondria-dependent (intrinsic) pathway (Carracedo et al., 2006). This mechanism contributes to the induction of apoptosis by cannabinoids in other types of tumor cells (Velasco et al., 2007), as well. However, an involvement of an alternative, death receptor-dependent (extrinsic) pathway in the apoptotic process triggered by these compounds cannot be ruled out.

The Role of ER Stress and Autophagy in Cannabinoid-Induced Cell Death

Proapoptotic and tumor growth-inhibiting activity of cannabinoids relies on the accumulation of de novo–synthesized ceramide, an event that occurs in the ER (endoplasmic reticulum), and eventually leads to execution of cell death via mitochondrial intrinsic pathway. A study by Carracedo et al. (2006) suggested a new link between the two events, alternative to inhibition of prosurvival pathways. They showed that Δ^9-THC treatment of glioma cells leads to upregulation of the stress-regulated protein p8, and ER stress-related downstream targets: ATF-4 (activating transcription factor 4), CHOP (the C/EBP-homologous protein), and TRB3 (tribbles homolog 3) (Fig. e11.4). Selective knockdown of ATF-4 and TRB3 blocked cannabinoid-induced apoptosis in glioma cells. Inhibition of ceramide synthesis de novo prevented Δ^9-THC-induced p8, ATF-4, CHOP, and TRB3 upregulation, as well as ER dilation, indicating that ceramide accumulation is an early event in the cannabinoid-triggered ER stress, and apoptosis in glioma cells (Carracedo et al., 2006). Furthermore, ER stress-evoked stimulation of the p8/TRB3 pathway preceded the inhibition of the Akt/mTOR (mammalian target of rapamycin) axis, considered a key step in the early triggering of autophagy (Fig. e11.4) (Salazar et al., 2009).

Autophagy, is an evolutionarily conserved catabolic process, where a cell self-digests its cytoplasmic contents in newly formed vesicle structures called autophagosomes. Autophagy is activated in response to stress conditions, such as nutrient starvation, the unfolded protein response (ER stress), and hypoxia, both in normal cells

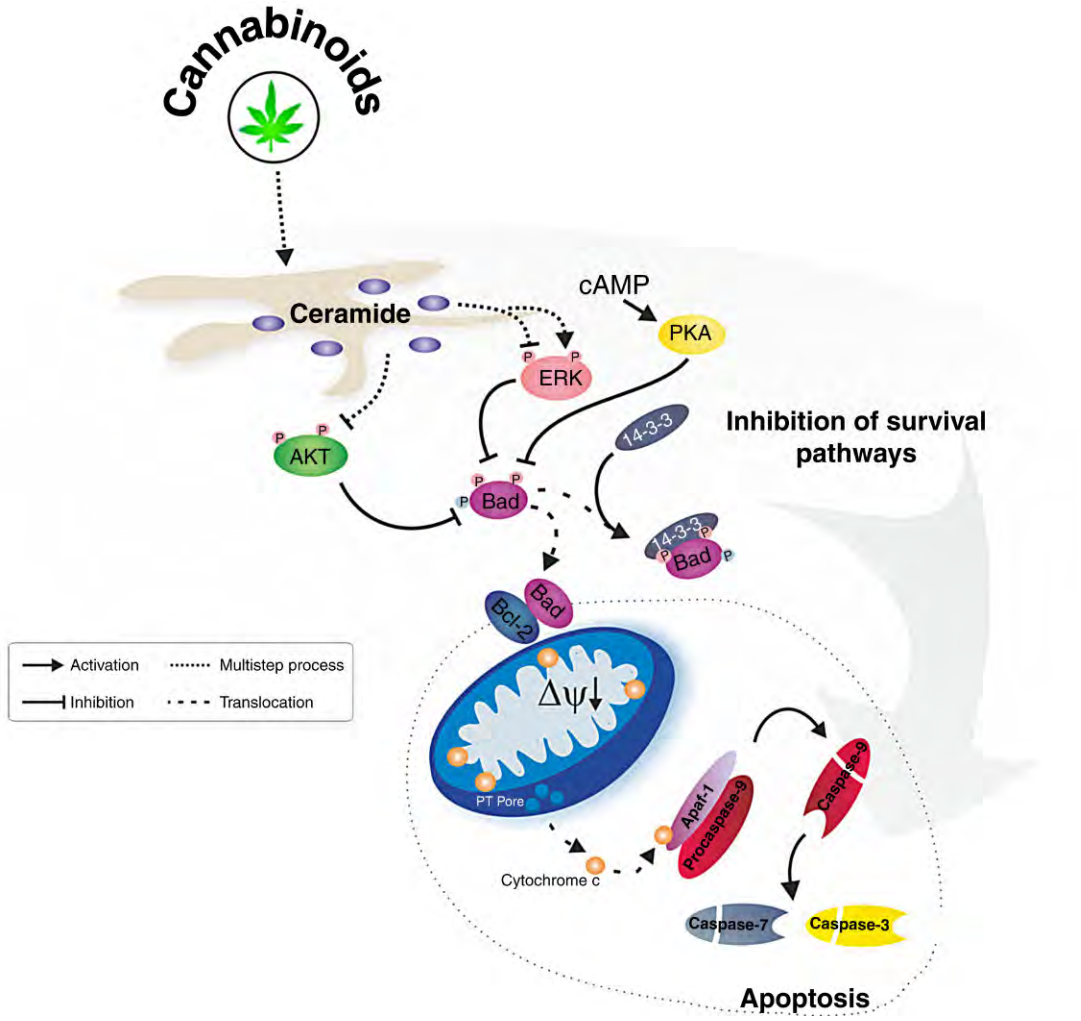


FIGURE e11.3 Inhibition of prosurvival pathways in antitumoral action of cannabinoids. Inhibition Akt and ERK signaling in response to cannabinoids stimulates translocation of Bad to the outer mitochondrial membrane, and its proapoptotic function. Interaction between Bad and Bcl-2 triggers a decrease of the mitochondrial membrane potential (Δψ) and release of proapoptotic factors (such as cytochrome c) to the cytosol, where apoptosis is executed by caspase cascade.

and during cancer progression. Classic autophagy promotes cell survival, but it may also contribute to cell death. Recently it was demonstrated that autophagy, not apoptosis, is induced in glioblastomas by radiation and several chemotherapeutic agents (Aoki et al., 2008).

Δ^9-THC treatment led to formation of autophagosomes in human glioma cells (Salazar et al., 2009). The authors also observed that ER stress occurred earlier than autophagy, which preceded apoptosis in cannabinoid-induced cell death. Administration of Δ^9-THC to mice bearing tumors derived from human astrocytoma cells produced increased TRB3 expression, inhibition of mTOR signaling pathway, occurrence of autophagy markers, and caspase-3 activation. These findings indicate that cannabinoid promotes the autophagy-mediated cell death through stimulation of ER stress, in human glioma cells. The role of autophagy in causing cell death, rather than occurring along with cell death, is still not clear. Pharmacological or genetic inhibition of autophagy, at different levels, prevented Δ^9-THC induced cell death, suggesting that autophagy contributes to cannabinoid antitumoral action in gliomas (Salazar et al., 2009). Of note, cannabinoid treatment also led to autophagy in other type of tumors (Vara et al., 2011).

Actions Beyond CB1/CB2 Receptors

Some cannabinoids activate apoptosis independently of cannabinoid-receptor binding. Cannabidiol antiproliferative action likely involves the induction of oxidative stress through the generation of reactive oxygen species. This effect is inhibited by tocopherol, a potent antioxidant and, interestingly, this was not observed in noncancerous primary glial cells (Massi et al., 2006). Anandamide promotes apoptosis through either the activation of vanilloid receptor (Maccarrone, Lorenzon,

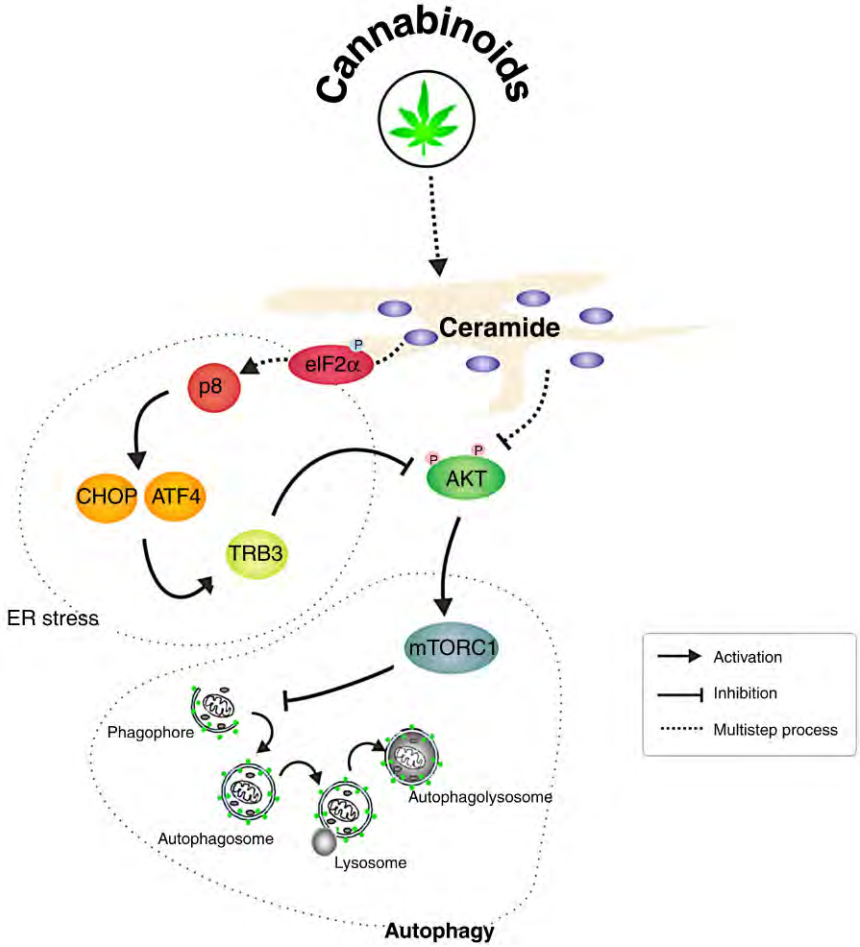


FIGURE e11.4 **Contribution of ER-stress and autophagy to cannabinoids-induced cell death.** Cannabinoid-induced phosphorylation of eIF2α (eukaryotic translation initiation factor 2α) and subsequent upregulation of the stress-regulated protein p8 and ER-stress-related downstream targets: ATF4 (activating transcription factor 4), CHOP (the C/EBP-homologous protein), and TRB3 (tribbles homolog 3) leads to inhibition of Akt, an upstream activator of mTORC1. Decreased activity of Akt/mTORC1 pathway contributes to initiation of autophagy that precedes apoptosis of glioma cell.

Bari, Melino, & Finazzi-Agro, 2000) or the accumulation of the proapoptotic sphingolipid ceramide mediated via CB1 or CB2 receptor activation (Galve-Roperh et al., 2000). All these results suggest that, overall, cannabinoids affect multiple cellular signaling pathways and, thus, have the potential to decrease cancer development. The mechanisms are, however, both cancer- and cannabinoid-specific. The final role of the cannabinoid receptors (CB1 and CB2) versus vanilloid or novel receptors is also likely to be of ongoing importance.

MULTIMODAL ACTION OF CANNABINOIDS AGAINST GLIAL TUMORS

Antitumoral Action of Cannabinoids In Vivo

The antitumor action of cannabinoids, mediated via the CB1 or CB2 cannabinoid receptors, has been also proved in vivo, revealing a significant regression of malignant gliomas in cannabinoid-treated animals (Galve-Roperh et al., 2000; Sanchez et al., 2001; Massi et al., 2004; Duntsch et al., 2006). Local intratumoral administration of Δ^9-THC or WIN55,212-2 (50 μg/day, for 8 days) reduced the size of tumors generated by intracranial inoculation of C6 glioma cells in rats, leading to complete eradication of gliomas, and increased survival in one-third of the treated rats (Galve-Roperh et al., 2000). There has been no recurrence in any of the surviving rats, as monitored periodically by MRI scan. Intratumoral and intraperitoneal administration of cannabidiol (Massi et al., 2004), a CB2-selective agonist such as JWH133 (Sanchez et al., 2001), or a novel cannabinoid such as KM-233 (Duntsch et al., 2006), blocked the proliferation of human astrocytoma cells implanted subcutaneously in the flank of immune-deficient mice. Our preliminary data suggest that systemic cannabinoid administration can effectively hamper intracranial tumor growth in rats (unpublished).

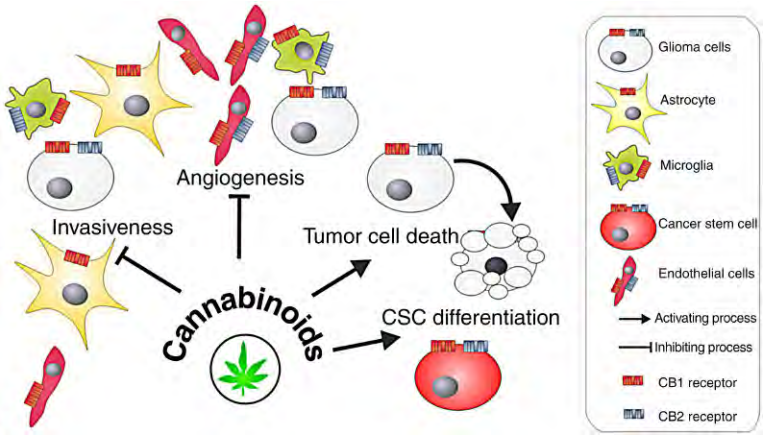


FIGURE e11.5 **Multimodal action of cannabinoids.** Cannabinoids induce tumor cell death, block angiogenesis and invasiveness, crucial for tumor progression, and induce differentiation of cancer stem cells (CSC).

Effects of Cannabinoids on Angiogenesis, Cell Migration, and Invasion

Cannabinoids have displayed a great potency in reducing glioma tumor growth in experimental animal models. Apparently, their effectiveness in vivo is attributed not only to antiproliferative action against tumor cells, but it also points to other cellular targets and additional mechanisms of action. JWH-133 reduced cell viability by 50% in vitro, while in vivo studies showed a 71% decrease in tumor growth after 8 days ($Rag^{-/-}$ mice, 40 μg/day intratumorally). Beyond affecting tumor cell survival, cannabinoids impair tumor angiogenesis, invasiveness, and even gliomagenesis (Fig. e11.5) (Blazquez et al., 2003; Aguado et al., 2007).

Increased demand for oxygen and nutrients supply to proliferating cancer cells makes angiogenesis a critical factor for the progression of solid tumors, and a popular target for oncologic therapies. Local administration of the CB2 selective cannabinoid JWH133 in a mouse flank inoculation model of glioma turned the vascular hyperplasia characteristic of actively growing tumors to a pattern of blood vessels, characterized by small, differentiated, and impermeable capillaries, thus proving antiangiogenic potential of the cannabinoid (Blazquez et al., 2003). In glioma (C6 cells) and astrocytoma xenografts, the overall vascularization of tumors was reduced by 88 and 21%, respectively (50 μg/day intratumorally, 8 or 25 days for gliomas and astrocytomas). This was associated with a reduced expression of proangiogenic factors, such as vascular endothelial growth factor (VEGF), and angiopoietin-2 in cannabinoid treated tumors. Downregulation of the major vascularization factors, including VEGF, was observed following treatment with Δ^9-THC, Met-F-AEA, WIN-55,212-2, and JWH-133, in various types of tumors (Blazquez et al., 2003; Pisanti et al., 2007). More importantly, intratumoral administration of Δ^9-THC to two patients with glioblastoma multiforme (grade IV astrocytoma) decreased VEGF levels and VEGFR-2 activation in the tumors (Blazquez et al., 2004). Antiangiogenic activity of cannabinoids could be partly related to a direct influence of the cannabinoids on endothelial cells migration and survival (Blazquez et al., 2003). Overall, cannabinoids appear to have consistent effects on the vascularization pathway, causing a decrease in tumor vascularization in in vivo models.

Δ^9-THC or JWH133 administration to glioma-bearing mice decreased the activity and expression of matrix metalloproteinase-2 (MMP-2), and tissue inhibitor of metalloproteinase 1 (TIMP-1) (Blazquez et al., 2008a,b). Δ^9-THC inhibited MMP-2 and TIMP-1 expression and cell invasion in cultured glioma cells (Blazquez et al., 2008a,b). MMP-2 is a proteolytic enzyme that allows tissue breakdown and remodeling, during angiogenesis and metastasis, and its upregulation is associated with high progression and poor prognosis of gliomas. Blocking the release of the extracellular matrix degrading enzyme by in vivo administered cannabinoids correlated with decreased tumor volumes (Blazquez et al., 2008a,b). TIMP-1 plays a critical role in the acquisition of migrating and invasive capacities by tumor cells. Decreased TIMP-1 expression was observed in biopsies from patients with recurrent glioblastoma multiforme tumors undergoing a clinical trial of THC efficacy (Blazquez et al., 2008a).

Multiple mechanisms were also suggested for antineoplastic activity of cannabidiol; however, they are unlikely mediated by the cannabinoid receptors. Cannabidiol effectively inhibited glioma cell proliferation, migration, and invasion, and caused a decrease in the expression of a set of proteins specifically involved in growth, invasion, and angiogenesis (Vaccani, Massi, Colombo, Rubino, & Parolaro, 2005; Solinas et al., 2013). Cannabidiol also inhibited HIF-1a, the regulatory subunit of the hypoxia inducible transcription factor, which

is responsible for orchestrating the adaptive transcriptional programs, inducing cell survival, motility, and tumor angiogenesis under hypoxic conditions (Solinas et al., 2013).

Although the identification of the cell of origin of gliomas is still a matter of debate, recent findings support the existence of brain cancer stem cells, responsible for tumor generation and recurrence. Aguado et al. (2007) showed that activation of CB1 and CB2 receptors with HU-210 and JWH133 promoted differentiation of glioma stem-like cells, derived from GBM biopsies and glioma cell lines. Moreover, cannabinoid challenge decreased neurosphere formation and cell proliferation in secondary xenografts, which correlated with the decreased efficiency of cannabinoid-treated glioma stem-like cells to initiate glioma formation in vivo (Aguado et al., 2007). Multimode action of cannabinoids, including inhibition of gliomagenesis, may have important implications for the development of cannabinoid-based therapeutic strategies.

THERAPEUTIC POTENTIAL OF CANNABINOIDS AGAINST HUMAN GLIOMAS

There is emerging evidence that cannabinoids, beyond their application for symptomatic relief, can in fact modify the progression of certain diseases. The efficacy of intratumorally or systemically administered cannabinoids in rat and mouse glioma models (Galve-Roperh et al., 2000; Sanchez et al., 2001; Massi et al., 2004; Duntsch et al., 2006) raised promises of using cannabinoid-based therapies against malignant gliomas in humans.

Safety Profile of Therapeutic Cannabinoids

Based on evidence from in vitro and in vivo studies, cannabinoids appear to have a favorable safety profile, and do not produce the generalized toxic effects as most conventional chemotherapeutic drugs. In contrast to the proapoptotic action of CB1/CB2-activating cannabinoids, such as Δ^9-THC and WIN55,212-2, on transformed cells, treatment of primary cultured astrocytes with these compounds did not trigger ceramide accumulation or induction of ER stress-related genes. Furthermore, cannabinoids promote survival of glial cells and neurons in different models of injury, suggesting that the antiproliferative effect of cannabinoids is selective for brain tumor cells, and viability of normal brain cells remains unaffected, or even favored by cannabinoid challenge (Guzman, 2003).

Apparently negligible expression of CB2 receptor in the brain, and its abundance in high-grade gliomas seems to confer a relative safety of CB2-selective agonists for targeted glioma therapy. Concomitantly, a novel cannabinoid chemotherapeutic exhibiting over 10-times higher affinity for the CB2 versus CB1 receptor produced minimal toxicity to healthy cultured brain slices at micromolar concentrations, required to eradicate U87 glioma cells (Duntsch et al., 2006). Moreover, clinical monitoring of mice with U87-derived subcutaneous tumors proved that intratumoral or systemic administration of the cannabinoid was effective in reducing tumor volume, and produced no general toxic effects. More importantly, animal studies also show a good safety profile of CB1-activating agents. In the studies by Galve-Roperh et al. (2000), Δ^9-THC and WIN55,212-2 were delivered intratumorally for 7 days, leading to partial or complete eradication of the tumors generated by intracranial inoculation of C6 glioma cells. MRI analysis showed no signs of damage related to necrosis, edema, infection, or trauma in tumor-free animals undergoing parallel treatment. Cannabinoid administration to tumor-bearing and control rats induced no substantial modification in behavioral parameters, in food and water intake, or in body weight. No abnormalities in biochemical and hematological parameters, or markers of tissue damage have been revealed during the 7-day delivery period, and for at least 2 months after cannabinoid treatment (Galve-Roperh et al., 2000).

Risks and Constrains

Although the general consensus indicates that cannabinoids have antitumor effects, there are a few studies that have shown contradictory results or duality of cannabinoids effects on tumor cell fate, depending of the dose range used (Guzman, 2003; Pisanti et al., 2009). Some authors questioned whether cannabinoid receptors do constitute relevant molecular targets to treat glioblastoma in humans, as the high concentrations of the currently available cannabinoids required to trigger apoptosis in glioma cells in culture are not pharmacologically relevant (Widmer, Hanemann, & Zajicek, 2008). This emphasizes the need for comprehensive dose-response studies in the future, correlated with analysis of the cannabinoid receptor expression. More importantly, immunosuppressive properties of cannabinoids in vivo, related to CB2 receptor stimulation on immune cells, raise concern about the risk of the antitumor immune response inhibition. Current evidence suggests that the type and stage of the cancer is likely to affect the outcome of cannabinoid exposure.

Results From a Pilot Clinical Study

On the basis of successful preclinical findings, Guzman et al. (2006) conducted a pilot phase I clinical trial, in which nine patients with recurrent glioblastoma

multiforme were administered Δ^9-THC intratumorally, via an infusion catheter placed into the resection cavity (Guzman et al., 2006). The primary endpoint of the study was to determine the safety of intracranial Δ^9-THC administration, in a dose escalation regime from the initial dose of 20–40 µg at day 1, increasing progressively for 2–5 days up to 80–180 µg/day. The cannabinoid treatment did not result in any marked alterations of clinical and laboratory tests in patients. There was no evidence of hemorrhage, edema, or injury over the median treatment duration of 15 days.

The good safety profile observed in this pilot clinical trial, together with remarkable antitumor effects reported in preclinical studies, may set the basis for further evaluation of the therapeutic potential of cannabinoids.

MINI-DICTIONARY

Angiogenesis Formation and maturation of new blood vessels.
Apoptosis A process of programmed cell death, involving specific proteolytic enzymes, called caspases, which leads to fragmentation of cellular content into apoptotic bodies, removed by phagocyting cells.
Autophagy A catabolic process accompanied by the encapsulation of parts of cytoplasm and organelles directed for breakdown and recycling in double-membrane vesicles called autophagosomes, which fuse with lysosomes to trigger degradation.
Cancer stem cell A rare population of cancer generating cells with stem-like properties.
Caspases Cysteine proteases, which play essential roles in apoptosis.
Ceramide A lipid signaling molecule, composed of sphingosine and a long chain fatty acid.
ER stress Cellular stress response related to endoplasmic reticulum.
Immunohistochemistry Imaging technique for detection of proteins in tissue sections.
Signaling pathway A cascade of intracellular events that transforms a signal (ie, growth factor binding to a cell membrane receptor, drug- or UV radiation-induced DNA damage) to cell response (ie, gene expression, cell movement, cell death).
Western blotting Molecular biology technique for detection of proteins bound to nitrocellulose membrane.

References

Aguado, T., Carracedo, A., Julien, B., Velasco, G., Milman, G., Mechoulam, R., Alvarez, L., Guzman, M., & Galve-Roperh, I. (2007). Cannabinoids induce glioma stem-like cell differentiation and inhibit gliomagenesis. *Journal of Biological Chemistry*, *282*, 6854–6862.

Andradas, C., Caffarel, M. M., Perez-Gomez, E., Salazar, M., Lorente, M., Velasco, G., Guzman, M., & Sanchez, C. (2011). The orphan G protein-coupled receptor GPR55 promotes cancer cell proliferation via ERK. *Oncogene*, *30*, 245–252.

Aoki, H., Kondo, Y., Aldape, K., Yamamoto, A., Iwado, E., Yokoyama, T., Hollingsworth, E. F., Kobayashi, R., Hess, K., Shinojima, N., Shingu, T., Tamada, Y., Zhang, L., Conrad, C., Bogler, O., Mills, G., Sawaya, R., & Kondo, S. (2008). Monitoring autophagy in glioblastoma with antibody against isoform B of human microtubule-associated protein 1 light chain 3. *Autophagy*, *4*, 467–475.

Blazquez, C., Carracedo, A., Salazar, M., Lorente, M., Egia, A., Gonzalez-Feria, L., Haro, A., Velasco, G., & Guzman, M. (2008a). Down-regulation of tissue inhibitor of metalloproteinases-1 in gliomas: a new marker of cannabinoid antitumoral activity? *Neuropharmacology*, *54*, 235–243.

Blazquez, C., Casanova, M. L., Planas, A., Del Pulgar, T. G., Villanueva, C., Fernandez-Acenero, M. J., Aragones, J., Huffman, J. W., Jorcano, J. L., & Guzman, M. (2003). Inhibition of tumor angiogenesis by cannabinoids. *FASEB Journal*, *17*, 529–531.

Blazquez, C., Gonzalez-Feria, L., Alvarez, L., Haro, A., Casanova, M. L., & Guzman, M. (2004). Cannabinoids inhibit the vascular endothelial growth factor pathway in gliomas. *Cancer Research*, *64*, 5617–5623.

Blazquez, C., Salazar, M., Carracedo, A., Lorente, M., Egia, A., Gonzalez-Feria, L., Haro, A., Velasco, G., & Guzman, M. (2008b). Cannabinoids inhibit glioma cell invasion by down-regulating matrix metalloproteinase-2 expression. *Cancer Research*, *68*, 1945–1952.

Carracedo, A., Lorente, M., Egia, A., Blazquez, C., Garcia, S., Giroux, V., Malicet, C., Villuendas, R., Gironella, M., Gonzalez-Feria, L., Piris, M. A., Iovanna, J. L., Guzman, M., & Velasco, G. (2006). The stress-regulated protein p8 mediates cannabinoid-induced apoptosis of tumor cells. *Cancer Cell*, *9*, 301–312.

De Jesus, M. L., Hostalot, C., Garibi, J. M., Salles, J., Meana, J. J., & Callado, L. F. (2010). Opposite changes in cannabinoid CB1 and CB2 receptor expression in human gliomas. *Neurochemistry International*, *56*, 829–833.

Duntsch, C., Divi, M. K., Jones, T., Zhou, Q., Krishnamurthy, M., Boehm, P., Wood, G., Sills, A., & Moore, B. M. (2006). Safety and efficacy of a novel cannabinoid chemotherapeutic, KM-233, for the treatment of high-grade glioma. *Journal of Neurooncology*, 143–152.

Ellert-Miklaszewska, A., Grajkowska, W., Gabrusiewicz, K., Kaminska, B., & Konarska, L. (2007). Distinctive pattern of cannabinoid receptor type II (CB2) expression in adult and pediatric brain tumors. *Brain Research*, *1137*, 161–169.

Ellert-Miklaszewska, A., Kaminska, B., & Konarska, L. (2005). Cannabinoids down-regulate PI3K/Akt and Erk signalling pathways and activate proapoptotic function of Bad protein. *Cellular Signalling*, *17*, 25–37.

Galve-Roperh, I., Sanchez, C., Cortes, M. L., del Pulgar, T. G., Izquierdo, M., & Guzman, M. (2000). Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. *Nature Medicine*, *6*, 313–319.

Gomez del Pulgar, T., Velasco, G., Sanchez, C., Haro, A., & Guzman, M. (2002). De novo-synthesized ceramide is involved in cannabinoid-induced apoptosis. *Biochemical Journal*, *363*, 183–188.

Gong, J. P., Onaivi, E. S., Ishiguro, H., Liu, Q. R., Tagliaferro, P. A., Brusco, A., & Uhl, G. R. (2006). Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Research*, *1071*, 10–23.

Guzman, M. (2003). Cannabinoids: potential anticancer agents. *Nature Reviews Cancer*, *3*, 745–755.

Guzman, M., Duarte, M. J., Blazquez, C., Ravina, J., Rosa, M. C., Galve-Roperh, I., Sanchez, C., Velasco, G., & Gonzalez-Feria, L. (2006). A pilot clinical study of Delta9-tetrahydrocannabinol in patients with recurrent glioblastoma multiforme. *British Journal of Cancer*, *95*, 197–203.

Guzman, M., Sanchez, C., & Galve-Roperh, I. (2001). Control of the cell survival/death decision by cannabinoids. *Journal of Molecular Medicine*, *78*, 613–625.

Held-Feindt, J., Dorner, L., Sahan, G., Mehdorn, H. M., & Mentlein, R. (2006). Cannabinoid receptors in human astroglial tumors. *Journal of Neurochemistry*, *98*, 886–893.

Howlett, A. C., Barth, F., Bonner, T. I., Cabral, G., Casellas, P., Devane, W. A., Felder, C. C., Herkenham, M., Mackie, K., Martin, B. R., Mechoulam, R., & Pertwee, R. G. (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacological Reviews*, *54*, 161–202.

Izzo, A. A., Aviello, G., Petrosino, S., Orlando, P., Marsicano, G., Lutz, B., Borrelli, F., Capasso, R., Nigam, S., Capasso, F., Di Marzo, V., & Endocannabinoid Research Group (2008). Increased endocannabinoid levels reduce the development of precancerous lesions in the mouse colon. *Journal of Molecular Medicine (Berlin)*, *86*, 89–98.

Kapoor, G. S., & O'Rourke, D. M. (2003). Receptor tyrosine kinase signaling in gliomagenesis: pathobiology and therapeutic approaches. *Cancer Biology and Therapy*, *2*, 330–342.

Maccarrone, M., Attina, M., Cartoni, A., Bari, M., & Finazzi-Agro, A. (2001). Gas chromatography-mass spectrometry analysis of endogenous cannabinoids in healthy and tumoral human brain and human cells in culture. *Journal of Neurochemistry*, *76*, 594–601.

Maccarrone, M., Lorenzon, T., Bari, M., Melino, G., & Finazzi-Agro, A. (2000). Anandamide induces apoptosis in human cells via vanilloid receptors. Evidence for a protective role of cannabinoid receptors. *Journal of Biological Chemistry*, *275*, 31938–31945.

Massi, P., Vaccani, A., Bianchessi, S., Costa, B., Macchi, P., & Parolaro, D. (2006). The non-psychoactive cannabidiol triggers caspase activation and oxidative stress in human glioma cells. *Cellular and Molecular Life Sciences*, *63*, 2057–2066.

Massi, P., Vaccani, A., Ceruti, S., Colombo, A., Abbracchio, M. P., & Parolaro, D. (2004). Antitumor effects of cannabidiol, a nonpsychoactive cannabinoid, on human glioma cell lines. *Journal of Pharmacology and Experimental Therapeutics*, *308*, 838–845.

Morad, S. A., & Cabot, M. C. (2013). Ceramide-orchestrated signalling in cancer cells. *Nature Reviews Cancer*, *13*, 51–65.

Munson, A. E., Harris, L. S., Friedman, M. A., Dewey, W. L., & Carchman, R. A. (1975). Antineoplastic activity of cannabinoids. *Journal of the National Cancer Institute*, *55*, 597–602.

Petersen, G., Moesgaard, B., Schmid, P. C., Schmid, H. H., Broholm, H., Kosteljanetz, M., & Hansen, H. S. (2005). Endocannabinoid metabolism in human glioblastomas and meningiomas compared to human non-tumour brain tissue. *Journal of Neurochemistry*, *93*, 299–309.

Pisanti, S., Borselli, C., Oliviero, O., Laezza, C., Gazzerro, P., & Bifulco, M. (2007). Antiangiogenic activity of the endocannabinoid anandamide: correlation to its tumor-suppressor efficacy. *Journal of Cell Physiology*, *211*, 495–503.

Pisanti, S., Malfitano, A. M., Grimaldi, C., Santoro, A., Gazzerro, P., Laezza, C., & Bifulco, M. (2009). Use of cannabinoid receptor agonists in cancer therapy as palliative and curative agents. *Best Practice and Research: Clinical Endocrinology and Metabolism*, *23*, 117–131.

Salazar, M., Carracedo, A., Salanueva, I. J., Hernandez-Tiedra, S., Lorente, M., Egia, A., Vazquez, P., Blazquez, C., Torres, S., Garcia, S., Nowak, J., Fimia, G. M., Piacentini, M., Cecconi, F., Pandolfi, P. P., Gonzalez-Feria, L., Iovanna, J. L., Guzman, M., Boya, P., & Velasco, G. (2009). Cannabinoid action induces autophagy-mediated cell death through stimulation of ER stress in human glioma cells. *Journal of Clinical Investigation*, *119*, 1359–1372.

Sanchez, C., de Ceballos, M. L., del Pulgar, T. G., Rueda, D., Corbacho, C., Velasco, G., Galve-Roperh, I., Huffman, J. W., Ramon, S., Cajal, M., & Guzman (2001). Inhibition of glioma growth in vivo by selective activation of the CB(2) cannabinoid receptor. *Cancer Research*, *61*, 5784–5789.

Sanchez, C., Galve-Roperh, I., Canova, C., Brachet, P., & Guzman, M. (1998). Delta9-tetrahydrocannabinol induces apoptosis in C6 glioma cells. *FEBS Letters*, *436*, 6–10.

Sanchez, C., Velasco, G., & Guzman, M. (1997). Delta9-tetrahydrocannabinol stimulates glucose utilization in C6 glioma cells. *Brain Research*, *767*, 64–71.

Schley, M., Stander, S., Kerner, J., Vajkoczy, P., Schupfer, G., Dusch, M., Schmelz, M., & Konrad, C. (2009). Predominant CB2 receptor expression in endothelial cells of glioblastoma in humans. *Brain Research Bulletin*, *79*, 333–337.

Solinas, M., Massi, P., Cinquina, V., Valenti, M., Bolognini, D., Gariboldi, M., Monti, E., Rubino, T., & Parolaro, D. (2013). Cannabidiol, a non-psychoactive cannabinoid compound, inhibits proliferation and invasion in U87-MG and T98G glioma cells through a multitarget effect. *PLoS One*, *8*, e76918.

Stella, N. (2004). Cannabinoid signaling in glial cells. *Glia*, *48*, 267–277.

Stock, K., Kumar, J., Synowitz, M., Petrosino, S., Imperatore, R., Smith, E. S., Wend, P., Purfurst, B., Nuber, U. A., Gurok, U., Matyash, V., Walzlein, J. H., Chirasani, S. R., Dittmar, G., Cravatt, B. F., Momma, S., Lewin, G. R., Ligresti, A., De Petrocellis, L., Cristino, L., Di Marzo, V., Kettenmann, H., & Glass, R. (2012). Neural precursor cells induce cell death of high-grade astrocytomas through stimulation of TRPV1. *Nature Medicine*, *18*, 1232–1238.

Vaccani, A., Massi, P., Colombo, A., Rubino, T., & Parolaro, D. (2005). Cannabidiol inhibits human glioma cell migration through a cannabinoid receptor-independent mechanism. *British Journal of Pharmacology*, *144*, 1032–1036.

Van Sickle, M. D., Duncan, M., Kingsley, P. J., Mouihate, A., Urbani, P., Mackie, K., Stella, N., Makriyannis, A., Piomelli, D., Davison, J. S., Marnett, L. J., Di Marzo, V., Pittman, Q. J., Patel, K. D., & Sharkey, K. A. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science*, *310*, 329–332.

Vara, D., Salazar, M., Olea-Herrero, N., Guzman, M., Velasco, G., & Diaz-Laviada, I. (2011). Anti-tumoral action of cannabinoids on hepatocellular carcinoma: role of AMPK-dependent activation of autophagy. *Cell Death and Differentiation*, *18*, 1099–1111.

Velasco, G., Carracedo, A., Blazquez, C., Lorente, M., Aguado, T., Haro, A., Sanchez, C., Galve-Roperh, I., & Guzman, M. (2007). Cannabinoids and gliomas. *Molecular Neurobiology*, *36*, 60–67.

Widmer, M., Hanemann, C. O., & Zajicek, J. (2008). High concentrations of cannabinoids activate apoptosis in human U373MG glioma cells. *Journal of Neuroscience Research*, *86*, 3212–3220.

Wu, X., Han, L., Zhang, X., Li, L., Jiang, C., Qiu, Y., Huang, R., Xie, B., Lin, Z., Ren, J., & Fu, J. (2012). Alteration of endocannabinoid system in human gliomas. *Journal of Neurochemistry*, *120*, 842–849.

Zha, J., Harada, H., Yang, E., Jockel, J., & Korsmeyer, S. J. (1996). Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X(L). Cell, 87, 619–628.

PART VI

EFFECTS OF SPECIFIC NATURAL AND SYNTHETIC CANNABINOIDS

72 *The role of 5-HT$_{1A}$ receptor, and nausea and vomiting relief by cannabidiol (CBD), cannabidiolic acid (CBDA), and cannabigerol (CBG)* 703

73 *Genetic and molecular aspects of addiction with tetrahydrocannabinol* 713

74 *Effects of Δ^9-tetrahydrocannabinol in human breast cancer* 722

75 *Δ^9-THC and COX-2 signaling* 729

76 *Cannabinoids and the addictive effects of nicotine* 739

77 *Cannabinoid regulation of intraocular pressure: human and animal studies, cellular and molecular targets* 748

78 *Ocular delivery of tetrahydrocannabinol* 760

79 *The role of γ-aminobutyric acid in the interoceptive effects of oral Δ^9-tetrahydrocannabinol in humans* 770

80 *The role of Δ^9-tetrahydrocannabinol in diabetes mellitus* 779

81 *Cannabidiol: an overview of its antipsychotic properties* 787

82 *Cannabidiol for the treatment of epilepsy: an overview of possible mechanisms of action and preclinical and human studies* 795

83 *Cannabidiol and neuroprotection: evidence from preclinical studies* 802

84 *Cannabinoids as potent inhibitors of human CYP1 enzymes* 813

85 *The synthetic analog of Δ^9-tetrahydrocannabinol (THC): nabilone. pharmacology and clinical application* 821

86 *Synthetic cannabinoids in dementia* 828
87 *Synthetic cannabinoid receptor agonists (spice) as new recreational psychoactive substances* 839
88 *Accidents and synthetic cannabinoids in blood of drivers* 848

CHAPTER

72

The Role of 5-HT$_{1A}$ Receptor, and Nausea and Vomiting Relief by Cannabidiol (CBD), Cannabidiolic Acid (CBDA), and Cannabigerol (CBG)

E.M. Rock, L.A. Parker

Department of Psychology and Collaborative Neuroscience Program, University of Guelph, Guelph, ON, Canada

SUMMARY POINTS

- Cannabidiol and cannabidiolic acid reduce acute vomiting, acute nausea, and anticipatory nausea in animal models.
- Cannabidiol and cannabidiolic acid's effects are mediated by indirect agonism of the 5-HT$_{1A}$ receptor.
- Cannabigerol acts as a 5-HT$_{1A}$ receptor antagonist, as it blocks the antinausea and antiemetic effects of cannabidiol
- Cannabidiol and/or cannabidiolic acid may be potential treatments for chemotherapy-induced nausea and vomiting.
- Human clinical trials need to evaluate the efficacy of cannabidiolic acid to reduce chemotherapy-induced nausea and vomiting, especially for the reduction of anticipatory nausea, because there is currently no selective treatment for this symptom.

KEY FACTS OF CANNABIDOL AND CANNABIDIOLIC ACID'S EFFECTS IN ANIMAL MODELS OF NAUSEA AND EMESIS

- Cannabidiol and cannabidiolic acid reduce acute vomiting, acute nausea, and anticipatory nausea in animal models.
- Cannabidiolic acid is much more potent than cannabidiol in these animal models.
- These effects are mediated via the 5-hydroxytryptamine 1A (5-HT$_{1A}$) receptor, as administration of a 5-HT$_{1A}$ antagonist blocks these effects.
- As these phytocannabinoids do not exert their actions via the cannabinoid 1 receptor, these compounds are nonpsychoactive.
- Cannabidiol and/or cannabidiolic acid may be promising therapeutics for cancer patients undergoing chemotherapy.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00083-1
Copyright © 2017 Elsevier Inc. All rights reserved.

KEY FACTS OF CANNABIGEROL'S EFFECTS IN ANIMAL MODELS OF NAUSEA AND EMESIS

- Cannabigerol blocks cannabidiol's antiemetic effects, suggesting that cannabigerol acts as a 5-HT_{1A} receptor antagonist.
- Cannabigerol also blocks cannabidiol's antinausea effects, suggesting that cannabigerol acts as a 5-HT_{1A} receptor antagonist.
- These two phytocannabinoids may be acting in opposition at the 5-HT_{1A} receptor to modulate nausea and vomiting
- This finding has important implications for the potential therapeutic effects of various *Cannabis sativa* strains.
- A *C. sativa* strain with higher cannabidiol relative to cannabigerol may be more effective in reducing nausea and vomiting in human patients.

LIST OF ABBREVIATIONS

AN	Anticipatory nausea
CB_1	Cannabinoid receptor 1
CB_2	Cannabinoid receptor 2
CBD	Cannabidiol
CBDA	Cannabidiolic acid
CBG	Cannabigerol
DRN	Dorsal raphe nucleus
5-HIAA	5-Hydroxyindoleacetic acid
5-HT	Serotonin
5-HT_{1A}	5-Hydroxytryptamine 1A
5-HT_3	5-Hydroxytryptamine 3
LiCl	Lithium chloride
MRN	Median raphe nucleus
NK-1	Neurokinin-1
8-OH-DPAT	8-Hydroxy-2-(di-*n*-propylamino) tetralin
THC	Δ^9-Tetrahydrocannabinol

INTRODUCTION

Cannabis sativa has been used for medicinal purposes for more than 5000 years. Although the psychoactive cannabinoid Δ^9-tetrahydrocannabinol (THC) was isolated in 1964, and extensively studied thereafter, only recently have other cannabinoid compounds been investigated. This chapter reviews recent findings on the potential of the phytocannabinoids cannabidiol, cannabidiolic acid, and cannabigerol, to modulate nausea and vomiting via 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptors. First, we describe chemotherapy-induced nausea and vomiting, the preclinical animal models that are used to evaluate these potential treatments, and the involvement of the 5-HT_{1A} receptors in nausea and vomiting.

NAUSEA AND VOMITING

Emesis (vomiting), the act of eliminating poisonous or toxic contents via expulsion through the mouth, can be observed and quantified objectively. In contrast, nausea, a subjective experience, is less easily defined. It has been characterized as "an unpleasant, but not painful, sensation associated with a heightened awareness of the upper gut, cold sweating and the expectation that vomiting is imminent" (Andrews, Rapeport, & Sanger, 1988). Nausea and vomiting are distressing symptoms associated with pharmacological treatments, such as with anticancer drug therapies.

Chemotherapy-Induced Nausea and Vomiting

Cancer patients undergoing chemotherapy treatment often experience the distressing side effects of acute nausea and vomiting, as well as delayed nausea and vomiting (Fiore & Gralla, 1984). If posttreatment nausea and vomiting are not properly managed, anticipatory nausea (AN) and vomiting—a conditioned response elicited by reexposure to the illness-paired context of the clinic—can result (Morrow, 1982). Please refer to Table 72.1 for a description of the three phases of chemotherapy-induced nausea and vomiting. Although acute vomiting has become relatively well managed since the advent of the 5-hydroxytryptamine 3 (5-HT_3) receptor antagonists such as ondansetron, and the neurokinin-1 (NK-1) receptor antagonists, such as aprepitant (Hickok et al., 2003), delayed nausea and AN are still unmanaged (Hickok et al., 2003). Nausea is often identified as one of the most distressing symptoms experienced by cancer patients undergoing chemotherapy treatment (Aapro, Molassiotis,

TABLE 72.1 Three Phases of Chemotherapy-Induced Nausea and Vomiting

Phase	Description
Acute	Occurring within 24 h after treatment (day 1 of the chemotherapy cycle)
Delayed	Occurring 24 h to 7 days after treatment (days 2–7 of the chemotherapy cycle)
Anticipatory	A conditioned response, whereby patients feel ill upon simply returning (or preparing to return) to the clinic where they have received chemotherapy Occurring when acute and delayed phases are not properly managed

This table describes the three phases of chemotherapy-induced nausea and vomiting experienced by cancer patients, as discussed by Bender et al. (2002).

& Olver, 2005). With some 76% of patients reporting nausea, and up to 43% of patients reporting vomiting after their first treatment cycle (Morrow et al., 1998), the need for effective pharmacotherapies is apparent.

ANIMAL MODELS OF NAUSEA

In order to assess the efficacy of novel pharmacotherapies, reliable preclinical animal models are necessary. While the neural mechanisms of the emetic reflex have been well established using animal models, such as the ferret and the shrew (Hornby, 2001), the central mechanisms regulating nausea are not well understood (Andrews & Horn, 2006). In contrast to vomiting, nausea is subjective, making development of an animal model difficult. Further, we summarize the current selective models (for a comprehensive review refer to Sharkey, Darmani, & Parker, 2014) used to determine the nauseating potential of compounds, and to determine the potential of antinausea agents that can block or reduce nausea.

Conditioned Gaping

Conditioned Gaping to a Nausea-Paired Flavor (Acute Nausea)

Recent evidence suggests that conditioned disgust (gaping) reactions elicited by exposure to a flavor that has been previously paired with an illness-inducing agent, such as lithium chloride (LiCl), is a sensitive and selective model of nausea-induced behavior in rats (Parker, Rana, & Limebeer, 2008). Conditioned gaping reactions (see Fig. 72.1) are only produced by drugs capable of producing vomiting (in emetic species), and antiemetic drugs suppress conditioned gaping (see Parker et al., 2008). Using this model, if a rat is pretreated with a compound, resulting in a reduction of nausea-induced conditioned gaping reactions (relative to vehicle-pretreated controls), then this compound may be a potential therapeutic for reducing acute nausea.

FIGURE 72.1 **Conditioned gaping.** Conditioned gaping in rats, described as a wide, triangular opening of the mouth and jaw, exposing the incisors, is the most sensitive and reliable rodent model of nausea.

Conditioned Gaping to a Nausea-Paired Context (Anticipatory Nausea)

Interestingly, in addition to displaying conditioned gaping reactions to a LiCl-paired flavor, rats also display conditioned gaping reactions to a LiCl-paired context (Limebeer et al., 2008; Rock, Limebeer, Mechoulam, Piomelli, & Parker, 2008). Like the chemotherapy patient who feels nauseous upon returning to the clinic (where they have previously experienced nausea-inducing chemotherapy treatments), these contextually elicited conditioned gaping reactions serve as a rodent model of AN. Indeed, once AN develops in human chemotherapy patients, it is refractive to classic antiemetic treatments such as ondansetron (Hickok et al., 2003). Similarly, pretreatment with ondansetron is ineffective in suppressing conditioned gaping responses elicited by a LiCl-paired context, in rats (Limebeer, Hall, & Parker, 2006; Rock et al., 2014).

The conditioned gaping model is a useful tool for assessing the therapeutic potential of a compound, as a reduction in acute nausea (conditioned gaping to a LiCl-paired flavor) or AN (conditioned gaping to a LiCl-paired context) can be assessed. The work reviewed here employs the conditioned gaping model to assess the antinausea properties of the phytocannabinoids that exert their action via 5-hydroxytryptamine 1A (5-HT_{1A}) receptors.

SEROTONIN$_{1A}$ (5-HYDROXYTRYPTAMINE; 5-HT$_{1A}$) RECEPTORS AND NAUSEA AND VOMITING

5-HT_{1A} Receptors

5-HT_{1A} receptors, coupled to $G_{i/o,}$ are distributed throughout the central nervous system (see Hannon & Hoyer, 2008), with high levels expressed in limbic areas such as the raphe nuclei of the brainstem (Verge et al., 1986). 5-HT_{1A} autoreceptors, found on the cell bodies and dendrites of serotonin (5-HT) containing neurons (Riad et al., 2000) in the rat median raphe nucleus (MRN) and dorsal raphe nucleus (DRN), regulate 5-HT release (Verge et al., 1986) to terminal forebrain regions by decreasing the rate of neuronal firing (see Blier, Pineyro, el Mansari, Bergeron, & de Montigny, 1998). Activation of these somatodendritic 5-HT_{1A} autoreceptors leads to a reduction in neuronal firing (Blier & de Montigny, 1987). Local 5-HT release in the raphe nuclei reduces neuronal firing, producing negative feedback regulation of neurotransmitter release (Adell & Artigas, 1991). Indeed, 5-HT receptor agonists inhibited release of 5-HT from in vitro preparations of rodent brain tissue (Chase, Katz, & Kopin, 1969). In addition, in vivo, 5-HT receptor agonists reduced the electrical activity of serotonergic neurons in the DRN (Aghajanian, 1972). Therefore, stimulation of

somatodendritic 5-HT_{1A} autoreceptors results in a decrease of available 5-HT.

Classic 5-HT_{1A} Receptor Agonists and Antagonists

8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) is a highly selective and centrally active potent ligand (Arvidsson et al., 1981) preferentially binding to the somatodendritic (Hjorth & Magnusson, 1988) 5-HT_{1A} autoreceptor (Middlemiss & Fozard, 1983). 8-OH-DPAT has traditionally been utilized as the standard receptor agonist to evaluate behavioral effects of the 5-HT_{1A} receptor. Indeed, systemically administered 8-OH-DPAT dose-dependently reduces the 5-HT synthesis rate, decreases 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of 5-HT (Sharp, Bramwell, & Grahame-Smith, 1989), and inhibits 5-HT neuronal firing in the DRN (Blier, Steinberg, Chaput, & de Montigny, 1989), ultimately reducing release of 5-HT in the brain (Hjorth et al., 1982).

WAY100135, a 5-HT_{1A} receptor antagonist, has been used to investigate this receptor, however, WAY100135 has since been shown to act at both presynaptic and postsynaptic 5-HT_{1A} receptors (Fletcher et al., 1993). More recently, the highly selective and potent 5-HT_{1A} receptor antagonist WAY100635 has replaced the use of WAY100135 (Forster et al., 1995). These 5-HT_{1A} receptor antagonists effectively block the effects of 8-OH-DPAT on extracellular levels of 5-HT in the rat hippocampus (Assie & Koek, 1996) and, when given alone, WAY100135 but not WAY100635 decreases 5-HT levels, indicating possible partial agonist properties of WAY100135 at somatodendritic 5-HT_{1A} receptors (Assie & Koek, 1996).

Classic 5-HT_{1A} Receptors, and Nausea and Vomiting in Humans

With the advent of the antiemetic 5-hydroxytryptamine 3 (5-HT_3) receptor antagonists, most nausea and vomiting research has focused on the involvement of this receptor, rather than the 5-HT_{1A} receptor, in mediating these effects. Although a great deal of animal literature supports the involvement of the somatodendritic 5-HT_{1A} autoreceptors in the regulation of emesis and nausea, little human literature exists. Chial et al. (2003) reported that administration of the 5-HT_{1A} receptor partial agonist buspirone selectively suppressed self-report nausea scores in the nutrient drink test (an assessment of gastric functioning).

Classic 5-HT_{1A} Receptors and Nausea and Vomiting in Animals

5-HT_{1A} receptor agonists such as 8-OH-DPAT, buspirone, and LY228729 suppress vomiting in emetic species, such as pigeons (Wolff & Leander, 1994, 1995, 1997), *Suncus murinus* (house musk shrew; Javid & Naylor, 2006; Okada, Torii, Saito, & Matsuki, 1994), cats (Lucot & Crampton, 1987a,b, 1989; Lucot, 1990a,b) and dogs (Gupta & Sharma, 2002). In the rat acute nausea model, 8-OH-DPAT reduces conditioned gaping (Limebeer & Parker, 2003; Limebeer, Litt, & Parker, 2009). Please refer to Table 72.2 for a summary of the involvement of 5-HT_{1A} receptor ligands in nausea and vomiting using animal models. This evidence indicates that agonism of the 5-HT_{1A} receptor via classic 5-HT_{1A} ligands reduces toxin-induced nausea and vomiting. The remainder of this chapter will focus on the constituents of the cannabis plant (phytocannabinoids) that exert their antinausea and antiemetic effects via activation of the 5-HT_{1A} receptor.

PHYTOCANNABINOIDS AND NAUSEA AND VOMITING

C. sativa has been used for centuries as a therapeutic for many ailments (Mechoulam, 2005), such as the alleviation of nausea and vomiting. In fact, in the 19th century, *C. sativa* was used in British medical practice to treat nausea and vomiting (O'Shaughnessy, 1843). In the 1970s, young chemotherapy patients anecdotally reported experiencing less (or none) of their nausea and vomiting when they were "high" from smoking cannabis (Sweet, Miller, Weddington, Senay, & Sushelsky, 1981), prompting early clinical trials demonstrating the efficacy of THC, the psychoactive component in cannabis, to reduce chemotherapy-induced nausea and vomiting (Sweet et al., 1981). Due to the unsuccessful control of chemotherapy-induced nausea and vomiting, oncologists began to investigate cannabinoids for their therapeutic potential. Recently, the mechanisms by which cannabinoids reduce chemotherapy-induced nausea and vomiting have been investigated (see Sharkey et al., 2014 for review) using animal models.

Cannabidiol

A major nonpsychoactive component in cannabis, cannabidiol (CBD), was isolated from Lebanese hashish in 1963 by Mechoulam and coworkers (Mechoulam & Hanus, 2002). CBD has a very low affinity (in the micromolar range) for the cannabinoid 1 (CB_1) and cannabinoid 2 (CB_2) receptors (Pertwee, 2008). Although CBD exerts its therapeutic effects via a number of mechanisms, CBD's antiemetic and antinausea effects seem to be mediated by action at the 5-HT_{1A} receptor. For a summary of the antinausea and antiemetic effects of cannabidiol in animal models, please refer to Table 72.3.

TABLE 72.2 Summary of Classic 5-HT_{1A} Receptor Ligand Modulation of Nausea and Vomiting in Animal Models

Compound	Species	Effect	References
8-OH-DPAT	Pigeon	Eliminated ditolyguanidine-induced emesis	Wolff and Leander (1994)
	Suncus murinus	Eliminated motion-induced emesis	Brame and Lucot (2011); Javid and Naylor (2006); Okada et al. (1994)
		Eliminated nicotine-, veratrine-, cisplatin-, or copper sulfate-induced emesis	Brame and Lucot (2011); Okada et al. (1994)
	Cat	Reduced motion-, cisplatin- or xylazine-induced emesis	Lucot and Crampton (1989)
		Eliminated RU 24969-induced emesis	Lucot (1990b)
	Rat	Reduced acute nausea	Limebeer and Parker (2003); Limebeer et al. (2009); Rock et al. (2011, 2012)
Buspirone	*S. murinus*	Eliminated motion-induced emesis	Okada et al. (1994)
	Cat	Reduced motion-, xylazine-induced emesis	Lucot (1990a); Lucot and Crampton (1987b)
		Eliminated cisplatin-induced emesis	Lucot and Crampton (1987a)
	Dog	Reduced cisplatin-induced emesis	Gupta and Sharma (2002)
		Eliminated apomorphine-induced emesis	Gupta and Sharma (2002)
LY228729	Pigeon	Eliminated ditolyguanidine-induced emesis	Wolff and Leander (1994)
		Eliminated cisplatin-, ipecac-, emetine- and *m*-(chlorophenyl)-biguanide-induced emesis	Wolff and Leander (1995)
		Eliminated cyclophosphamide-induced emesis	Wolff and Leander (1997)
SUN8399	*S. murinus*	Eliminated motion-induced emesis Eliminated nicotine-, veratrine-, cisplatin-, copper sulfate-induced emesis	Okada et al. (1994)
Ipsapirone	*S. murinus*	Eliminated motion-induced emesis	Okada et al. (1994)
Tandospirone	*S. murinus*	Eliminated motion-induced emesis	Okada et al. (1994)
Gepirone	*S. murinus*	Eliminated motion-induced emesis	Okada et al. (1994)
WAY100635	*S. murinus*	No effect alone on motion-induced emesis Unable to block 8-OH-DPAT-induced attenuation of motion-induced emesis	Javid and Naylor (2006)

This table summarizes the published effects of classic 5-HT_{1A} receptor ligands in animal models of nausea and emesis.

CBD Reduces Nausea and Vomiting in Human Patients

In a phase II clinical trial, when added to the standard therapy, the oromucosally administered cannabis-based medicine Sativex (containing approximately 50/50 THC and CBD) was effective (57% of Sativex patients vs 22% of placebo patients reporting no delayed emesis, and 71% of Sativex patients vs 22% of placebo patients reporting no delayed emesis) in suppressing delayed nausea and vomiting (Duran et al., 2010). To date, there are no published human clinical trials evaluating the ability of CBD alone to reduce chemotherapy-induced nausea and vomiting.

CBD Reduces Vomiting in Animal Models

In *S. murinus*, CBD produces a biphasic effect, at low doses (5 mg/kg) suppressing, and at higher doses (40 mg/kg) potentiating acute cisplatin-induced vomiting (Kwiatkowska et al., 2004). In addition, acute LiCl-induced vomiting has been suppressed by low doses (5–10 mg/kg) of CBD, while higher doses (20–40 mg/kg) of CBD were found to facilitate (rather than reduce) LiCl-induced vomiting (Parker et al., 2004). Interestingly, CBD is ineffective in reducing motion-induced vomiting (Cluny et al., 2008). Consistent with the low binding affinity of CBD to the CB_1 receptor (Pertwee, 2008), the CB_1 receptor antagonist SR141716 did not block the CBD-induced suppression of vomiting (Parker et al., 2004). WAY100135 effectively blocked the CBD- (5 mg/kg) induced suppression of acute nicotine-, LiCl- and cisplatin- (20 mg/kg, but not 40 mg/kg) induced vomiting in *S. murinus*, indicating a 5-HT_{1A} receptor mediated antiemetic effect (Rock et al., 2012).

CBD Reduces Acute Nausea in Animal Models

In rats, CBD (5 mg/kg) has been shown to reduce conditioned gaping to a LiCl-paired flavor, when

TABLE 72.3 Summary of Cannabidiol's Effects in Animal Models of Nausea and Vomiting

Phytocannabinoid	Animal model	Effect	Mechanism of action	References
Cannabidiol	Emesis in *Suncus murinus*	Reduced cisplatin-induced emesis (5 mg/kg)	5-HT_{1A} receptor	Kwiatkowska, Parker, Burton, and Mechoulam (2004); Rock et al. (2012)
		Potentiated cisplatin-induced emesis (40 mg/kg)	Not evaluated	Kwiatkowska et al. (2004)
		Reduced LiCl-induced emesis (5–10 mg/kg)	5-HT_{1A} receptor	Parker, Kwiatkowska, Burton, and Mechoulam (2004); Rock et al. (2012)
		Potentiated LiCl-induced emesis (20–40 mg/kg)	Not evaluated	Parker et al. (2004)
		Reduced nicotine-induced emesis	5-HT_{1A} receptor	Rock et al. (2012)
		Ineffective for motion-induced emesis	Not evaluated	Cluny, Naylor, Whittle, and Javid (2008)
	Acute nausea in rats	Reduced conditioned gaping	5-HT_{1A} receptor	Rock et al. (2012)
		Enhanced 8-OH-DPAT-induced reduction of conditioned gaping	Not evaluated	Rock et al. (2012)
	Anticipatory nausea in rats	Reduced conditioned gaping	Not evaluated	Rock et al. (2008)

This table summarizes the published effects of cannabidiol (CBD) in animal models of nausea and emesis, as well as whether these effects are mediated by the 5-HT_{1A} receptor.

administered systemically (Rock et al., 2012) or centrally to the DRN (Rock et al., 2012). In addition, at a subthreshold dose (0.5 mg/kg), CBD systemically enhances the antinausea effect of a subthreshold dose (0.005 mg/kg) of 8-OH-DPAT (Rock et al., 2012).

The antinausea effect of CBD has been blocked by WAY100635, when it is systemically or centrally (intra-DRN) administered (Rock et al., 2012). In fact, when WAY100635 was infused outside of the DRN, CBD's antinausea effects were not blocked, indicating that the effect was selective to the DRN. Since the DRN is a site of somatodendritic 5-HT_{1A} autoreceptors, activation of which decreases the firing rate of 5-HT afferents projecting to terminal brain regions (Verge et al., 1985), these results suggest that CBD may be exerting its antinausea effects by reducing the firing rate of 5-HT afferents which project to terminal forebrain areas. CBD enhanced the action of 8-OH-DPAT in vitro (% stimulation of [^{35}S]GTPγS binding above basal) and in vivo (conditioned gaping), without acting as a direct agonist at the 5-HT_{1A} receptor in brainstem preparations (Rock et al., 2012). Therefore, CBD may be acting as an indirect agonist, thereby augmenting endogenous 5-HT activation of the 5-HT_{1A} receptor to reduce toxin-induced acute nausea.

CBD Reduces Anticipatory Nausea in Animal Models

CBD (1 and 5, but not 10 mg/kg) reduced conditioned gaping to a LiCl-paired context (Rock et al., 2008); however, CBD's mechanism of action in AN has not yet been evaluated. As there are currently no specific treatments for AN, this nonpsychoactive component in cannabis could be a promising therapeutic option; however, its limited effective dose range may prove difficult.

Conclusions

It is unfortunate that CBD has not yet been investigated in published human clinical trials with chemotherapy patients, despite its efficacy in reducing acute vomiting, acute nausea, and anticipatory nausea in animal models (via indirect 5-HT_{1A} receptor agonism). Indeed, CBD has also been shown to inhibit paclitaxel-induced neuropathic pain (through 5-HT_{1A} receptors), without diminishing chemotherapeutic efficacy (Ward et al., 2014), and has even been shown to have antitumor action itself (De Petrocellis et al., 2013).

Cannabidiolic Acid

Cannabidiolic acid (CBDA) is the nonpsychoactive carboxylic acidic precursor of CBD. It is present in the fresh cannabis plant, and decarboxylates upon heating, such as when cannabis is smoked or dried. In comparison to CBD, much less work has focused on this acidic precursor. Studies in our laboratory indicate that it is a highly potent antiemetic and antinausea agent. For a summary of the antinausea and antiemetic effects of CBDA in animal models, please refer to Table 72.4.

TABLE 72.4 Summary of Cannabidiolic Acid's Effects in Animal Models of Nausea and Vomiting

Phytocannabinoid	Animal model	Effect	Mechanism of action	References
Cannabidiolic acid	Emesis in *Suncus murinus*	Reduced motion-, LiCl-, or cisplatin-induced emesis	Not evaluated	Bolognini et al. (2013)
	Acute nausea in rats	Reduced conditioned gaping	5-HT_{1A} receptor	Bolognini et al. (2013)
		Enhanced ondansetron- or metoclopramide-induced suppression of conditioned gaping	Not evaluated	Rock and Parker (2013a,b)
	Anticipatory nausea in rats	Reduced conditioned gaping	5-HT_{1A} receptor	Bolognini et al. (2013)
		Enhanced tetrahydrocannabinolic acid-induced suppression of conditioned gaping	5-HT_{1A} receptor and CB_1 receptor	Rock et al. (2014)

This table summarizes the published effects of cannabidiolic acid (CBDA) in animal models of nausea and emesis, as well as whether these effects are mediated by the 5-HT_{1A} receptor.

CBDA Reduces Vomiting in Animal Models

Recent evidence indicates that CBDA (0.1 and/or 0.5 mg/kg, i.p.) potently reduces motion-, LiCl-, and cisplatin-induced vomiting in *S. murinus* (Bolognini et al., 2013). Unfortunately, the 5-HT_{1A} receptor mediation of these effects has not yet been investigated.

CBDA Reduces Acute Nausea in Animal Models

CBDA (0.1 mg/kg) also reduced LiCl-induced acute nausea, an effect that was blocked by WAY100635, but not SR141716 (Bolognini et al., 2013). CBDA also increased the ability of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, to potently stimulate [^{35}S]GTPγS binding to rat brainstem membrane, without activating CB_1 receptors in vitro (Bolognini et al., 2013). CBDA, like CBD, seems to inhibit nausea in rats by enhancing the activation of 5-HT_{1A} receptors. More recently, CBDA has been shown to reduce acute nausea at a dose as low as 0.5 μg/kg (Rock & Parker, 2013a). As well, a subthreshold dose of CBDA (0.1 μg/kg) enhanced the ability of a mildly effective dose of ondansetron (1 μg/kg), or a subthreshold dose of metoclopramide (0.3 mg/kg, a dopamine antagonist) to reduce LiCl-induced acute nausea (Rock & Parker, 2013a,b).

CBDA Reduces Anticipatory Nausea in Animal Models

CBDA suppressed contextually elicited conditioned gaping (at doses as low as 0.001 mg/kg) in the rodent model of AN (Rock et al., 2014), and this effect was blocked by the 5-HT_{1A} receptor antagonist, WAY100635 (Bolognini et al., 2013). In addition, coadministration of subthreshold doses of CBDA (0.1 μg/kg) and tetrahydrocannabinolic acid (the acidic precursor of THC, 5 μg/kg) enhanced the suppression of AN, over that of either agent alone (Rock et al., 2014), indicating that these acidic precursors potently reduce AN. Pretreatment with SR141716 (CB_1 receptor antagonist) or WAY100635 blocked the combined effect of CBDA and THCA, indicating the involvement of both receptors in this effect (Rock et al., 2014).

Conclusions

CBDA suppresses nausea and vomiting in rats, in a 5-HT_{1A} receptor dependent manner, suggesting that CBDA could be developed as a treatment for nausea and vomiting because of its potency. CBDA could be a particularly interesting candidate treatment for AN, as there is no current selective therapeutic available, once AN develops. CBDA is also promising for development as a treatment for acute nausea, because, unlike CBD, it does not potentiate nausea and/or vomiting at high doses. In fact, CBDA is active at much lower doses than CBD, but at high doses CBDA is merely ineffective. As well, CBDA does not modify overall activity level of rats (Bologini et al., 2013; Rock et al., 2014). In addition to its antiemetic and antinausea properties, CBDA (5, 10, 25 mM) inhibits its highly aggressive human breast cancer cell migration, a factor crucial in the prevention of cancer metastasis (Takeda et al., 2012). These results highlight the need for clinical trials with cancer patients undergoing chemotherapy, perhaps especially in breast cancer patients, in order to prevent metastasis.

Cannabigerol

Cannabigerol (CBG), a nonpsychoactive component of cannabis (Gaoni & Mechoulam, 1964) has been shown to act as a 5-HT_{1A} receptor antagonist (Cascio, Gauson, Stevenson, Ross, & Pertwee, 2010), and recent work in our laboratory has confirmed a 5-HT_{1A} mediated effect in our animal models. For a summary of CBG's effects in animal models of nausea and vomiting, please refer to Table 72.5.

CBG Blocks CBD-Induced Antiemetic Effects in Animal Models

CBG (5 mg/kg, i.p.) blocked the CBD- (5 mg/kg, i.p.) induced suppression of LiCl-induced acute vomiting in

TABLE 72.5 Summary of Cannabigerol's Effects in Animal Models of Nausea and Vomiting

Phytocannabinoid	Animal model	Effect	Mechanism of action	References
Cannabigerol	Emesis in *Suncus murinus*	Blocked the CBD-induced reduction of LiCl-induced emesis	5-HT_{1A} receptor	Rock et al. (2011)
	Acute nausea in rats	Blocked the CBD- or 8-OH-DPAT-induced reduction of conditioned gaping (5 or 10 mg/kg)	5-HT_{1A} receptor	Rock et al. (2011)
		Reduced conditioned gaping (1 mg/kg)	Not evaluated	Rock et al. (2011)

This table summarizes the published effects of cannabigerol (CBG) in animal models of nausea and emesis, as well as whether these effects are mediated by the 5-HT_{1A} receptor.

S. murinus, suggesting that CBG exerts its action at the 5-HT_{1A} receptor to block the effect of CBD at this receptor (Rock et al., 2011).

CBG Blocks CBD-Induced Antinausea Effects in Animal Models

CBG (5 or 10 mg/kg, i.p.) blocked the CBD- (5 mg/kg, i.p.) and 8-OH-DPAT- (0.01 mg/kg, s.c.) induced suppression of acute nausea (Rock et al., 2011), again suggesting that CBG is acting on the 5-HT_{1A} receptor to block the 5-HT_{1A} mediated effects of CBD and 8-OH-DPAT. Also of interest is the finding that CBG (1 mg/kg, i.p.) actually suppressed acute nausea on its own (Rock et al., 2011). This result is in line with the in vitro concentration specific effects of CBG, such that low concentrations of CBG stimulate GTPγS binding to mouse brain membranes, but this effect disappears at higher concentrations where CBG acts as a 5-HT_{1A} receptor antagonist (Cascio et al., 2010).

Summary

Interestingly, CBG and CBD, two constituents found in the cannabis plant, both target 5-HT_{1A} receptors, although with opposite actions, to modulate the effects of these receptors on nausea and vomiting. Therefore, the concentration of these opposing cannabinoids in a specific strain of cannabis may be important in its efficacy to reduce chemotherapy-induced nausea and vomiting.

CONCLUSIONS

It is only recently that the therapeutic potential of phytocannabinoids (other than THC) has been investigated. Here we provide evidence that several nonpsychoactive phytocannabinoids (CBD, CBDA, CBG), which act at 5-HT_{1A} receptors, are potential treatment options for chemotherapy-induced nausea and vomiting.

MINI-DICTIONARY

Acute nausea Chemotherapy-induced nausea that occurs within 24 h of the initial chemotherapy administration (see Table 72.1). This condition is modeled in rats with conditioned gaping to a lithium chloride-paired flavor.

Anticipatory nausea A classically conditioned response to the hospital context that becomes associated with the chemotherapy-induced illness experienced by patients (see Table 72.1). This condition is modeled in rats with conditioned gaping to a lithium chloride-paired context.

Cannabidiol (CBD) A nonpsychoactive phytocannabinoid exhibiting a low binding affinity for the CB 1 and 2 receptors. Its antinausea and antiemetic effects are mediated by the 5-HT_{1A} receptor, as the CBD-induced effects can be blocked by a 5-HT_{1A} receptor antagonist.

Cannabidiolic acid (CBDA) The acidic precursor to cannabidiol, found in the fresh *Cannabis sativa* plant. CBDA potently reduces nausea and vomiting through a 5-HT_{1A} receptor mediated mechanism, as the CBDA-induced effects can be blocked by a 5-HT_{1A} receptor antagonist.

Cannabigerol (CBG) A nonpsychoactive phytocannabinoid that acts as a 5-HT_{1A} receptor antagonist, in contrast to CBD. In fact, CBG blocks CBD's antiemetic and antinausea effects.

Conditioned gaping Although rats cannot vomit, they exhibit conditioned disgust reactions (gaping), in response to the delivery into their oral cavity of a flavor that has been previously paired with illness (see Fig. 72.1). This is considered a rodent model of acute nausea. Gaping, described as a wide, triangular opening of the mouth and jaw exposing the incisors, is the most sensitive and reliable rodent model of nausea. Indeed, rats also exhibit gaping in response to a context that has been previously paired with illness—a rodent model of anticipatory nausea.

Emesis Also known as vomiting, it is the body's means of rejecting noxious or toxic substances via the oral cavity. Therefore, antiemetic compounds prevent (or reduce) vomiting.

5-Hydroxytryptamine 1A (5-HT_{1A}) receptors A serotonin (5-HT) receptor subtype binding the endogenous 5-HT neurotransmitter, mediating inhibitory neurotransmission. The 5-HT_{1A} receptors located in the dorsal raphe nucleus are largely somatodendritic (located on the soma and dendrites of serotonergic neurons).

Phytocannabinoid Cannabinoids derived from *C. sativa*.

S. murinus Also known as the house musk shrew, these insectivores vomit in response to toxins such as lithium chloride. Therefore, *S. murinus* are an animal model to assess whether certain compounds can reduce (or eliminate) vomiting, indicating an antiemetic effect.

Acknowledgments

We would like to thank NSERC (Grant #92057) and GW Pharmaceuticals for their support of this work, and in particular GW Pharmaceuticals for supply of several compounds that we have evaluated in the experiments reviewed here.

References

Aapro, M. S., Molassiotis, A., & Olver, I. (2005). Anticipatory nausea and vomiting. *Supportive Care in Cancer*, *13*(2), 117–121.

Adell, A., & Artigas, F. (1991). Differential effects of clomipramine given locally or systemically on extracellular 5-hydroxytryptamine in raphe nuclei and frontal cortex. an in vivo brain microdialysis study. *Naunyn-Schmiedebergs Archives of Pharmacology*, *343*(3), 237–244.

Aghajanian, G. K. (1972). Influence of drugs on the firing of serotonin-containing neurons in brain. *Federation Proceedings*, *31*(1), 91–96.

Andrews, P. L., & Horn, C. C. (2006). Signals for nausea and emesis: implications for models of upper gastrointestinal diseases. *Autonomic Neuroscience: Basic and Clinical*, *125*(1–2), 100–115.

Andrews, P. L., Rapeport, W. G., & Sanger, G. J. (1988). Neuropharmacology of emesis induced by anti-cancer therapy. *Trends in Pharmacological Sciences*, *9*(9), 334–341.

Arvidsson, L. E., Hacksell, U., Nilsson, J. L., Hjorth, S., Carlsson, A., Lindberg, P., et al. (1981). 8-hydroxy-2-(di-*n*-propylamino)tetralin, a new centrally acting 5-hydroxytryptamine receptor agonist. *Journal of Medicinal Chemistry*, *24*(8), 921–923.

Assie, M. B., & Koek, W. (1996). Effects of 5-HT1A receptor antagonists on hippocampal 5-hydroxytryptamine levels: (S)-WAY100135, but not WAY100635, has partial agonist properties. *European Journal of Pharmacology*, *304*(1–3), 15–21.

Bender, C. M., McDaniel, R. W., Murphy-Ende, K., Pickett, M., Rittenberg, C. N., Rogers, M. P., et al. (2002). Chemotherapy-induced nausea and vomiting. *Clinical Journal of Oncology Nursing*, *6*(2), 94–102.

Blier, P., & de Montigny, C. (1987). Modification of 5-HT neuron properties by sustained administration of the 5-HT1A agonist gepirone: electrophysiological studies in the rat brain. *Synapse (New York, NY)*, *1*(5), 470–480.

Blier, P., Pineyro, G., el Mansari, M., Bergeron, R., & de Montigny, C. (1998). Role of somatodendritic 5-HT autoreceptors in modulating 5-HT neurotransmission. *Annals of the New York Academy of Sciences*, *861*, 204–216.

Blier, P., Steinberg, S., Chaput, Y., & de Montigny, C. (1989). Electrophysiological assessment of putative antagonists of 5-hydroxytryptamine receptors: a single-cell study in the rat dorsal raphe nucleus. *Canadian Journal of Physiology and Pharmacology*, *67*(2), 98–105.

Bolognini, D., Rock, E. M., Cluny, N. L., Cascio, M. G., Limebeer, C. L., Duncan, M., et al. (2013). Cannabidiolic acid prevents vomiting in *Suncus murinus* and nausea-induced behaviour in rats by enhancing 5-HT1A receptor activation. *British Journal of Pharmacology*, *168*(6), 1456–1470.

Brame, R. E., & Lucot, J. B. (2011). Guamanian *Suncus murinus* responsiveness to emetic stimuli and the antiemetic effects of 8-OH-DPAT. *Pharmacology, Biochemistry and Behavior*, *99*(3), 381–384.

Cascio, M. G., Gauson, L. A., Stevenson, L. A., Ross, R. A., & Pertwee, R. G. (2010). Evidence that the plant cannabinoid cannabigerol is a highly potent alpha2-adrenoceptor agonist and moderately potent 5HT1A receptor antagonist. *British Journal of Pharmacology*, *159*(1), 129–141.

Chase, T. N., Katz, R. I., & Kopin, I. J. (1969). Release of [3H]serotonin from brain slices. *Journal of Neurochemistry*, *16*(4), 607–615.

Chial, H. J., Camilleri, M., Burton, D., Thomforde, G., Olden, K. W., & Stephens, D. (2003). Selective effects of serotonergic psychoactive agents on gastrointestinal functions in health. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *284*(1), G130–G137.

Cluny, N. L., Naylor, R. J., Whittle, B. A., & Javid, F. A. (2008). The effects of cannabidiol and tetrahydrocannabinol on motion-induced emesis in *Suncus murinus*. *Basic and Clinical Pharmacology and Toxicology*, *103*(2), 150–156.

De Petrocellis, L., Ligresti, A., Schiano Moriello, A., Iappelli, M., Verde, R., Stott, C. G., et al. (2013). Non-THC cannabinoids inhibit prostate carcinoma growth in vitro and in vivo: pro-apoptotic effects and underlying mechanisms. *British Journal of Pharmacology*, *168*(1), 79–102.

Duran, M., Perez, E., Abanades, S., Vidal, X., Saura, C., Majem, M., et al. (2010). Preliminary efficacy and safety of an oromucosal standardized cannabis extract in chemotherapy-induced nausea and vomiting. *British Journal of Clinical Pharmacology*, *70*(5), 656–663.

Fiore, J. J., & Gralla, R. J. (1984). Pharmacologic treatment of chemotherapy-induced nausea and vomiting. *Cancer Investigation*, *2*(5), 351–361.

Fletcher, A., Bill, D. J., Bill, S. J., Cliffe, I. A., Dover, G. M., Forster, E. A., et al. (1993). WAY100135: A novel, selective antagonist at presynaptic and postsynaptic 5-HT1A receptors. *European Journal of Pharmacology*, *237*(2–3), 283–291.

Forster, E. A., Cliffe, I. A., Bill, D. J., Dover, G. M., Jones, D., Reilly, Y., et al. (1995). A pharmacological profile of the selective silent 5-HT1A receptor antagonist, WAY-100635. *European Journal of Pharmacology*, *281*(1), 81–88.

Gaoni, Y., & Mechoulam, R. (1964). The structure and synthesis of cannabigerol, a new hashish constituent. *Proceedings of the Chemical Society*, *1964*, 82–182.

Gupta, Y. K., & Sharma, S. S. (2002). Involvement of 5-HT1A and 5-HT2 receptor in cisplatin induced emesis in dogs. *Indian Journal of Physiology and Pharmacology*, *46*(4), 463–467.

Hannon, J., & Hoyer, D. (2008). Molecular biology of 5-HT receptors. *Behavioural Brain Research*, *195*(1), 198–213.

Hickok, J. T., Roscoe, J. A., Morrow, G. R., King, D. K., Atkins, J. N., & Fitch, T. R. (2003). Nausea and emesis remain significant problems of chemotherapy despite prophylaxis with 5-hydroxytryptamine-3 antiemetics: a University of Rochester James P. Wilmot Cancer Center community clinical oncology program study of 360 cancer patients treated in the community. *Cancer*, *97*(11), 2880–2886.

Hjorth, S., Carlsson, A., Lindberg, P., Sanchez, D., Wikström, H., Arvidsson, L., et al. (1982). 8-hydroxy-2-(di-*n*-propylamino)tetralin, 8-OH-DPAT, a potent and selective simplified ergot congener with central 5-HT-receptor stimulating activity. *Journal of Neural Transmission*, *55*(3), 169–188.

Hjorth, S., & Magnusson, T. (1988). The 5-HT 1A receptor agonist, 8-OH-DPAT, preferentially activates cell body 5-HT autoreceptors in rat brain in vivo. *Naunyn-Schmiedebergs Archives of Pharmacology*, *338*(5), 463–471.

Hornby, P. J. (2001). Central neurocircuitry associated with emesis. *The American Journal of Medicine*, *111*(Suppl. 8A), 106S–112S.

Javid, F. A., & Naylor, R. J. (2006). The effect of the 5-HT1A receptor agonist, 8-OH-DPAT, on motion-induced emesis in *Suncus murinus*. *Pharmacology, Biochemistry and Behavior*, *85*(4), 820–826.

Kwiatkowska, M., Parker, L. A., Burton, P., & Mechoulam, R. (2004). A comparative analysis of the potential of cannabinoids and ondansetron to suppress cisplatin-induced emesis in the *Suncus murinus* (house musk shrew). *Psychopharmacology*, *174*(2), 254–259.

Limebeer, C. L., Hall, G., & Parker, L. A. (2006). Exposure to a lithium-paired context elicits gaping in rats: a model of anticipatory nausea. *Physiology and Behavior*, *88*(4–5), 398–403.

Limebeer, C. L., Krohn, J. P., Cross-Mellor, S., Litt, D. E., Ossenkopp, K. P., & Parker, L. A. (2008). Exposure to a context previously associated with nausea elicits conditioned gaping in rats: a model of anticipatory nausea. *Behavioural Brain Research*, *187*(1), 33–40.

Limebeer, C. L., Litt, D. E., & Parker, L. A. (2009). Effect of 5-HT3 antagonists and a 5-HT(1A) agonist on fluoxetine-induced conditioned gaping reactions in rats. *Psychopharmacology*, *203*(4), 763–770.

Limebeer, C. L., & Parker, L. A. (2003). The 5-HT1A agonist 8-OH-DPAT dose-dependently interferes with the establishment and the expression of lithium-induced conditioned rejection reactions in rats. *Psychopharmacology*, *166*(2), 120–126.

Lucot, J. B. (1990a). Effects of serotonin antagonists on motion sickness and its suppression by 8-OH-DPAT in cats. *Pharmacology, Biochemistry and Behavior*, *37*(2), 283–287.

Lucot, J. B. (1990b). RU 24969-induced emesis in the cat: 5-HT1 sites other than 5-HT1A, 5-HT1B or 5-HT1C implicated. *European Journal of Pharmacology*, *180*(2–3), 193–199.

Lucot, J. B., & Crampton, G. H. (1987a). Buspirone blocks cisplatin-induced emesis in cats. *Journal of Clinical Pharmacology*, *27*(10), 817–818.

Lucot, J. B., & Crampton, G. H. (1987b). Buspirone blocks motion sickness and xylazine-induced emesis in the cat. *Aviation, Space and Environmental Medicine*, *58*(10), 989–991.

Lucot, J. B., & Crampton, G. H. (1989). 8-OH-DPAT suppresses vomiting in the cat elicited by motion, cisplatin or xylazine. *Pharmacology, Biochemistry and Behavior*, *33*(3), 627–631.

Mechoulam, R. (2005). Plant cannabinoids: a neglected pharmacological treasure trove. *British Journal of Pharmacology*, *146*(7), 913–915.

Mechoulam, R., & Hanus, L. (2002). Cannabidiol: an overview of some chemical and pharmacological aspects. Part I: chemical aspects. *Chemistry and Physics of Lipids*, *121*(1–2), 35–43.

Middlemiss, D. N., & Fozard, J. R. (1983). 8-hydroxy-2-(di-*n*-propylamino)-tetralin discriminates between subtypes of the 5-HT1 recognition site. *European Journal of Pharmacology*, *90*(1), 151–153.

Morrow, G. R. (1982). Prevalence and correlates of anticipatory nausea and vomiting in chemotherapy patients. *Journal of the National Cancer Institute*, *68*(4), 585–588.

Morrow, G. R., Roscoe, J. A., Hynes, H. E., Flynn, P. J., Pierce, H. I., & Burish, T. (1998). Progress in reducing anticipatory nausea and vomiting: a study of community practice. *Supportive Care in Cancer: Official Journal of the Multinational Association of Supportive Care in Cancer*, *6*(1), 46–50.

O'Shaughnessy, W. B. (1843). On the preparations of the Indian hemp, or gunjah: *Cannabis indica*, their effects on the animal system in health, and their utility in the treatment of tetanus and other convulsive diseases. *Provincial Medical Journal and Retrospect of the Medical Sciences*, *5*, 363–369.

Okada, F., Torii, Y., Saito, H., & Matsuki, N. (1994). Antiemetic effects of serotonergic 5-HT1A-receptor agonists in *Suncus murinus*. *Japanese Journal of Pharmacology*, *64*(2), 109–114.

Parker, L. A., Kwiatkowska, M., Burton, P., & Mechoulam, R. (2004). Effect of cannabinoids on lithium-induced vomiting in the *Suncus murinus* (house musk shrew). *Psychopharmacology*, *171*(2), 156–161.

Parker, L. A., Rana, S. A., & Limebeer, C. L. (2008). Conditioned nausea in rats: assessment by conditioned disgust reactions, rather than conditioned taste avoidance. *Canadian Journal of Experimental Psychology*, *62*(3), 198–209.

Pertwee, R. G. (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: Delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. *British Journal of Pharmacology*, *153*(2), 199–215.

Riad, M., Garcia, S., Watkins, K. C., Jodoin, N., Doucet, E., Langlois, X., et al. (2000). Somatodendritic localization of 5-HT1A and preterminal axonal localization of 5-HT1B serotonin receptors in adult rat brain. *Journal of Comparative Neurology*, *417*(2), 181–194.

Rock, E. M., Bolognini, D., Limebeer, C. L., Cascio, M. G., Anavi-Goffer, S., Fletcher, P. J., et al. (2012). Cannabidiol, a non-psychotropic component of cannabis, attenuates vomiting and nausea-like behaviour via indirect agonism of 5-HT(1A) somatodendritic autoreceptors in the dorsal raphe nucleus. *British Journal of Pharmacology*, *165*(8), 2620–2634.

Rock, E. M., Goodwin, J. M., Limebeer, C. L., Breuer, A., Pertwee, R. G., Mechoulam, R., et al. (2011). Interaction between non-psychotropic cannabinoids in marihuana: effect of cannabigerol (CBG) on the anti-nausea or anti-emetic effects of cannabidiol (CBD) in rats and shrews. *Psychopharmacology*, *215*(3), 505–512.

Rock, E. M., Limebeer, C. L., Mechoulam, R., Piomelli, D., & Parker, L. A. (2008). The effect of cannabidiol and URB597 on conditioned gaping (a model of nausea) elicited by a lithium-paired context in the rat. *Psychopharmacology*, *196*(3), 389–395.

Rock, E. M., Limebeer, C. L., Navaratnam, R., Sticht, M. A., Bonner, N., Engeland, K., et al. (2014). A comparison of cannabidiolic acid with other treatments for anticipatory nausea using a rat model of contextually elicited conditioned gaping. *Psychopharmacology*, *231*, 3207–3215.

Rock, E. M., & Parker, L. A. (2013a). Effect of low doses of cannabidiolic acid and ondansetron on LiCl-induced conditioned gaping (a model of nausea-induced behaviour) in rats. *British Journal of Pharmacology*, *169*(3), 685–692.

Rock, E. M., & Parker, L. A. (2013b). Suppression of lithium chloride-induced conditioned gaping (a model of nausea-induced behaviour) in rats (using the taste reactivity test) with metoclopramide is enhanced by cannabidiolic acid. *Pharmacology, Biochemistry and Behavior*, *111*, 84–89.

Sharkey, K. A., Darmani, N. A., & Parker, L. A. (2014). Regulation of nausea and vomiting by cannabinoids and the endocannabinoid system. *European Journal of Pharmacology*, *722*, 134–146.

Sharp, T., Bramwell, S. R., & Grahame-Smith, D. G. (1989). 5-HT1 agonists reduce 5-hydroxytryptamine release in rat hippocampus in vivo as determined by brain microdialysis. *British Journal of Pharmacology*, *96*(2), 283–290.

Sweet, D. L., Miller, N. J., Weddington, W., Senay, E., & Sushelsky, L. (1981). Delta 9-tetrahydrocannabinol as an antiemetic for patients receiving cancer chemotherapy. A pilot study. *Journal of Clinical Pharmacology*, *21*(8–9 Suppl.), 70S–75S.

Takeda, S., Okajima, S., Miyoshi, H., Yoshida, K., Okamoto, Y., Okada, T., et al. (2012). Cannabidiolic acid, a major cannabinoid in fiber-type cannabis, is an inhibitor of MDA-MB-231 breast cancer cell migration. *Toxicology Letters*, *214*(3), 314–319.

Verge, D., Daval, G., Marcinkiewicz, M., Patey, A., el Mestikawy, S., Gozlan, H., et al. (1986). Quantitative autoradiography of multiple 5-HT1 receptor subtypes in the brain of control or 5,7-dihydroxytryptamine-treated rats. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *6*(12), 3474–3482.

Verge, D., Daval, G., Patey, A., Gozlan, H., el Mestikawy, S., & Hamon, M. (1985). Presynaptic 5-HT autoreceptors on serotonergic cell bodies and/or dendrites but not terminals are of the 5-HT1A subtype. *European Journal of Pharmacology*, *113*(3), 463–464.

Ward, S. J., McAllister, S. D., Kawamura, R., Murase, R., Neelakantan, H., & Walker, E. A. (2014). Cannabidiol inhibits paclitaxel-induced neuropathic pain through 5-HT(1A) receptors without diminishing nervous system function or chemotherapy efficacy. *British Journal of Pharmacology*, *171*(3), 636–645.

Wolff, M. C., & Leander, J. D. (1994). Antiemetic effects of 5-HT1A agonists in the pigeon. *Pharmacology, Biochemistry, and Behavior*, *49*(2), 385–391.

Wolff, M. C., & Leander, J. D. (1995). Comparison of the antiemetic effects of a 5-HT1A agonist, LY228729, and 5-HT3 antagonists in the pigeon. *Pharmacology, Biochemistry and Behavior*, *52*(3), 571–575.

Wolff, M. C., & Leander, J. D. (1997). Effects of a 5-HT1A receptor agonist on acute and delayed cyclophosphamide-induced vomiting. *European Journal of Pharmacology*, *340*(2–3), 217–220.

CHAPTER

73

Genetic and Molecular Aspects of Addiction with Tetrahydrocannabinol[1]

*T. Janus**, *A. Machoy-Mokrzyńska***, *K. Borowiak**

**Department of Clinical and Forensic Toxicology, Pomeranian Medical University, Szczecin, Poland
**Department of Pharmacology, Pomeranian Medical University, Szczecin, Poland

SUMMARY POINTS

- Marijuana and hashish are currently the most frequently self-administered "soft narcotics," worldwide.
- The discovery of specific THC receptors (CB1 and CB2) was a decisive point in the research of the biological activity of their compounds.
- The biological activity of cannabinoids is closely connected with specific receptor-agonist interactions.
- The activity of a "reward system" of CNS, stimulation of dopaminergic pathway of neurotransmission, and receptors response are believed to be crucial for dependence development.
- This mechanism of dependence development is believed to be a universal process for most addictive substances.
- Chronic exposure to THC can influence the stimulation of genetically-related changes connected with stimulation of long-term response genes.
- Addiction is a complex disease that is multifactorial and polygenic, and does not follow a Mendelian pattern of gene expression.
- No single gene has been identified that would predispose individuals to develop addictive behaviors.

KEY FACTS OF THC ADDICTION DEVELOPMENT

- Addiction with THC and other cannabinoids develops according to general mechanisms, similar to the mechanisms typical for other drugs of abuse.
- The dopaminergic system, as well as negative reinforcement, play a critical role in addiction with cannabinoids.
- Molecular mechanisms of addiction are closely connected with cannabinoids receptors expression, signaling cascades, synaptic plasticity, and changes in gene expression.
- In spite of the differences in chemical structure of newly designed synthetic cannabinoids, they act through cannabinoid receptors, but involving different neuronal pathways.

LIST OF ABBREVIATIONS

BDNF	Brain-derived neurotrophic factor
cAMP	Cyclic adenosine monophosphate
CB1	Cannabinoid receptor 1
CB2	Cannabinoid receptor 2
CNR 1	Cannabinoid receptor 1 gene
CNR 2	Cannabinoid receptor 2 gene
CNS	Central nervous system
CREB	c-AMP response element binding protein

[1]Genetic links to dependence, expression, and polymorphism of involved genes, neurotransmission and signaling cascade, brain plasticity, THC chemical structure and CB receptors response.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00084-3
Copyright © 2017 Elsevier Inc. All rights reserved.

DNA	Dezoxyribonucleic acid
ERK	Extracellular signal-regulated kinase
ΔFosB	Δ Fos family protein
GABA	Gamma-aminobutyric acid
LTD	Long-term depression
LTP	Long-term potentiation
MAPK	Mitogen-activated protein kinase
mRNA	Messenger ribonucleic acid
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	*N*-methyl-D-aspartate
RNA	Ribonucleic acid
SNP	Single gene polymorphism
TrkB receptor	Neurotrophic tyrosine kinase receptor type 2
THC	Δ^9-Tetrahydrocannabinol

INTRODUCTION

Cannabis sativa derivatives (such as Δ^9-tetrahydrocannabinol, THC) are currently the most frequently self-administered "soft narcotics" worldwide. Despite common opinion, regular use of THC results in complete physical and psychological addiction, and in a number of somatic disorders. THC, like other psychoactive substances, leads to a chronic and recurrent central nervous system (CNS) disease, complex in terms of its etiology, molecular mechanisms, clinical course, and treatment. This phenomenon is thought to be influenced by several concurrently acting genetic, molecular, psychological, and social factors, but the molecular aspects of this process are not fully known yet.

Gene expression and genetic polymorphism may influence the risk of dependence development, the clinical course of the disease, as well as the frequency of intake of the drug of abuse. Therefore, the polymorphism and expression of dopaminergic neurotransmission genes and brain-derived neurotrophic factor become adequate candidates for the examination of the correlation between genetic factors and the development of addiction.

Regardless of genetic component, participation in the development of dependence is variously assessed and continually discussed. We must be aware that CNS disorders, as well as addiction, are polygene qualified and, therefore they are not inherited according to Mendelian pattern. The methodology (heterogeneity of estimated population), techniques, and conclusions regarding molecular examination of addiction were postulated by numerous scientists and practitioners. This could explain the differences and often contradictory results obtained by researchers.

The development of cannabinoid addiction impacts several neurobiological processes. To understand this problem better, it is necessary to analyze how THC acts during an initial exposure, and after repetitive exposure. This problem was described in detail by Nestler (2001). Over the last two decades, cannabinoid investigation has made enormous progress. Recent research has focused on mechanisms by which cannabinoids bring on alterations in brain functioning. The changes caused by cannabinoids in brain neuronal circuits, among which are neurotransmitters receptors, signaling cascades and gene expression are intensively investigated. Owing to the fact that genetic variability may increase the risk of addictive behaviors, exposure to THC may result in neuroadaptation—for example, an addicted state. This adaptation occurs at the cellular, molecular, or genetic level, and is associated with synaptic plasticity and altered gene expression. A significant breakthrough has been the discovery of an endogenous cannabinoid system which plays a major role in neuromodulation.

Currently, apart from natural THC residues, a pivotal problem appears to be brought by a number of newly designed, synthetic THC derivatives. Due to the phenomena of their receptor-related activity, regardless of distinctly different chemical structures, this problem is extensively discussed in the subsequent section of this chapter.

THE ROLE OF THE DOPAMINERGIC SYSTEM IN THE DEVELOPMENT OF THC ADDICTION

There is a variety of drugs of abuse presenting a diverse chemical and pharmacological profile. These substances act on different neurobiological systems, and undergo different metabolic pathways. However, each one of them appears to exert its initial effects on the mesocorticolimbic dopaminergic pathway, despite their chemical diversity and individual molecular targets, a matter that was emphasized by Nestler (2005).

Dopamine is a messenger involved in many functions that are essential for the survival of the organism. Berke and Hyman (2000) conclude in their study that the main behaviors affected by dopamine include movement, cognition, pleasure, and motivation, as well as learning and memorization. Dopamine is an essential element in identifying natural rewards for the organism, and it seems that all substances of abuse increase the release of this neurotransmitter in specific areas of the brain, including cannabinoids. Hyman, Malenka, and Nestler (2005) show that dopamine is directly implicated in the positive reinforcement of drug addiction, and that it plays a major role in motivation. The development of addiction recruits different sources of reinforcement, different neuroadaptive mechanisms, and different neurochemical changes to deregulate the brain reward system. This

problem was a main topic in studies performed by Koob and Le Moal (2001). Several behavioral studies have used different animal models to clarify the consequences of chronic exposure to cannabinoids. There is considerable evidence that an increase in extracellular dopamine concentration in the nucleus accumbens may be observed following acute and chronic administration of THC. It was also demonstrated that THC releases dopamine along the reward pathway, and definitely increases dopamine activity in the ventral tegmental area. The repeated use of psychoactive substances is thought to arise from neurochemical actions causing the positive reinforcing effects of a drug. Nevertheless, the transition from occasional drug use to drug addiction requires an additional source of reinforcement, and the reduction of the negative emotional state resulting from the repeated exposure. Since the majority of drugs of abuse have been found to cause a negative emotional state during acute abstinence, the combination of the positive reinforcing effects of the psychoactive substances with the reduction of the negative emotional states of drug abstinence provides a powerful motivational force for compulsive drug taking, which is characteristic for addiction. Drug discontinuation is accompanied by electrophysiological and biochemical changes resulting from decrease of dopamine function. The increased reward threshold during abstinence from psychoactive substances intake has been observed in the course of the withdrawal from THC. Due to the complex nature of drug addiction, it seems necessary to analyze two different types of brain responses to addictive substances: neuroadaptation, and synaptic plasticity. Neuroadaptation is the brain's response to repeated psychoactive drugs administration, and exhibits as a ground for tolerance, sensitization, and withdrawal syndrome. The following subsequent exposures to the addictive substances can lead to the various adaptations in key neural circuits, and may play a significant role in the development and maintenance of addiction. Studies carried out by Lüscher and Malenka (2011) showed that these long-lasting changes are associated with reward circuitry via a mechanism known as synaptic plasticity, which is the dynamic adjustment of synaptic efficacy occurring in response to environmental or internal stimuli. The best known forms of synaptic plasticity are described as long-term potentiation (LTP) and long-term depression (LTD), and are involved in adaptive learning and memory. The previously mentioned mechanisms of synaptic plasticity could lead to the changes in synaptic strength in several brain regions, and reorganization of neural circuitry by altering gene and protein expression in neurons that receive diverse signals as effect of LTP or LTD. LTP is the enhancement of synaptic strength that results from synchronous firing of connecting neurons, whereas LTD is the weakening of synaptic strength. Synaptic transmission is a dynamic process, and can be modified by several factors, for example, *Cannabis* administration. THC increases mesolimbic dopamine transmission in the nucleus accumbens, probably via a μ-opioid receptor-mediated mechanism because μ-receptor antagonists prevent the THC-induced dopamine increase in the brain mesolimbic area, as proved by Tanda, Pontieri, and Di Chiara (1997). THC also inhibits release of glutamate and GABA in the striatum, and causes long-term forms of synaptic plasticity.

MOLECULAR MECHANISMS OF ADDICTION

A central role of endocannabinoid physiological control system in the regulation of a variety of constitutional processes, as well as in the regulation of the rewarding effects of abused substances, is well evidenced. The endocannabinoid system appears to play a role as a modulator of dopaminergic activity, therefore it participates in the primary rewarding effects of THC, and acts as retrograde messenger to inhibit classical transmitters such dopamine, serotonin, GABA, glutamate, acetylcholine, or norepinephrine, as described by Onaivi (2007). This system comprises endogenous cannabinoids, the metabolic enzymes responsible for the formation and degradation of endocannabinoids, and the cannabinoid receptors. The endocannabinoids are endogenous lipid-signaling molecules, which are generated in the cell membrane from phospholipids precursors, and possess cannabimimetic properties, since they bind and activate cannabinoid receptor subtypes. The best-studied endocannabinoids are anandamide and 2-arachidonylglycerol. These molecules are involved in the action of a variety of addictive substances' mechanisms. Both endogenous and exogenous cannabinoids (like THC) produce their effects through the activation of two different receptors identified as G protein-coupled cannabinoid receptors. The first subtype of cannabinoid receptors was sequenced, cloned, and named CB_1, and is encoded by the *CNR1* gene. These receptors are highly expressed in brain, and mediate most of central effects of THC. The second subtype, abbreviated CB_2, initially was believed to be peripheral, and that it did not mediate any central effect of THC. This cannabinoid receptor is both positively and negatively coupled to MAPK and cAMP pathways, as revealed by Pertwee (2010). CB_2 receptors are encoded by the *CNR2* gene located on chromosome 1p36. CB_2 receptors are also present at low levels in several areas of the brain, and are expressed in the CNS on microglia, astrocytes, and subpopulations of neurons, which was the aim of the study carried out by Roche and Finn (2010). It is important to stress that the activation of metabotropic receptors can produce effects which can

persist for a considerable period. For example, the effect of protein kinase A action is protein phosphorylation (ie, transcription factors) that regulates gene expression. The expression of CB_1 cannabinoid receptors in the brain and peripheral tissues is well studied, but the data connected with brain neuronal expression of CB_2 receptors are controversial. It seems that CB_1 and CB_2 receptors work independently, but also cooperate in different neuronal populations to regulate a number of physiological activities influenced by THC use. MAPK/ERK cascade seems to be crucial for neuronal plasticity, and is activated by cannabinoids. Some experiments performed on animal models reveal that acute administration of THC induces a progressive and transient activation (via phosphorylation) of MAPK/ERK cascade in the dorsal striatum and the nucleus accumbens. It is also shown that ERK inhibition blocks THC-induced rewarding properties. The data strongly support that ERK activation in the striatum is critically involved in long-term neuronal adaptive responses underlying THC-induced long-term behaviors.

Koob, Sanna, and Bloom (1998) suggested that the study of transcriptional mechanisms of addiction is based on the hypothesis that regulation of gene expression is an important mechanism by which chronic exposure to addictive substances causes long-lasting changes in the brain. There are a number of mechanisms by which drugs of abuse are able to regulate gene expression, including activation and suppression of transcription factors, epigenetic mechanisms, and induction of noncoding RNAs. Transcription factors are proteins that bind to specific response elements in the promoter regions of target genes, and regulate those genes' expression. A number of transcription factors have been identified for their potential role in addiction because their expression and activation is regulated in the mesocorticolimbic pathway at the time of exposure to drugs of abuse. Prominent examples include: ΔFosB, CREB, and NFκB. ΔFosB is one such transcription factor that has received particular attention due to its unusual stability; it is a truncated splice variant of the *FosB* gene, and it shares homology with other Fos family members. ΔFosB accumulates in the striatum, following chronic drug administration, and its expression persists for several weeks after the last drug exposure. Due to its ability to increase the incentive motivational properties of drugs of abuse, this transcription factor is called a "molecular switch" which facilitates the transition to addiction. CREB is expressed at a high level in the brain, and is believed to have an effect as a negative modulator of drug reward. NFκB controls transcription of DNA, and belongs to the category of "rapid-acting" primary transcription factors. NFκB seems to be a factor regulating addiction-induced plasticity, and is implicated in processes of memory.

THE MOLECULAR BASIS OF LONG-TERM BRAIN PLASTICITY

The central topic in cellular and molecular biology is the issue of gene expression, and involves studies on the mechanisms of transcription initiation, elongation, and termination. Numerous studies analyzed phenomena of primary transcripts by RNA polymerases, the processing of primary transcripts to produce mature mRNAs, intercompartmental transport of mRNAs to sites of translation, translation of mRNA by polyribosomes, and, finally, the processing and decay of the protein products. There are a number of mechanisms by which drugs of abuse are able to regulate gene expression, including activation and suppression of transcription factors, epigenetic mechanisms, and induction of noncoding RNAs. Some of them were described by Madsen, Brown, and Lawrence (2012).

Regulation of gene expression leads to relatively stable changes within neurons. Repeated exposure to a drug of abuse eventually causes changes in nuclear function, and alters rates of transcription of particular target genes by causing repeated perturbation of intracellular signal transduction pathways. Modified gene expression leads to altered activity of the neurons in which those changes occur. Ultimately, these circumstances can cause changes in the neural circuits in which the neurons operate. Investigations of long-term changes in brain structure and function that accompany chronic exposure to abused drugs suggest that alterations in gene regulation contribute substantially to the addictive phenotype.

Recent findings indicate that there are specific mechanisms which regulate posttranslational modification of gene expression, as well. This enables the control of heritable changes to phenotypes that are not dependent on changes in the genetic code. Epigenetics has a number of definitions, but in neuroscience it is commonly defined as changes in gene expression that occur through modulation of chromatin which are not brought about by changes in the underlying DNA sequence. So-called epigenetic modifications occur beyond remodeling of chromatin structure, incorporating the effect of environmental stimuli. Consequently, enhanced or suppressed transcription of genes to proteins is influenced by the accumulation of posttranslational modifications to histones, or changes to DNA, independent of the DNA sequence. These modifications limit access of transcription factors, and thus result in altered gene expression. Data published by Robison and Nestler (2011) suggested that knowledge regarding the function of epigenetics in addictive behaviors is incomplete, and is focused on alterations in the accessibility of genes within their native chromatin structure induced by histone tail modifications and DNA methylation, and the regulation of gene expression by noncoding RNAs. Methylation in promoter regions inhibits the ability

of transcription factors to interact with the gene. DNA methylation is crucial in synaptic plasticity, learning, and memory formation. Addictive substances induce specific changes to histones which can be heavily influenced by acute and chronic exposure, and histone methylation may play a critical role in the process of neuroadaptation. The regulation of gene expression by noncoding RNAs determines a number of cellular processes by influencing inhibition or activation of gene expression in processes including regulation of the cell cycling, differentiation signal transmission, apoptosis, synaptic plasticity, and response to DNA damage. Heterogeneous morphology of dendritic spines is thought to be the morphological basis for synaptic plasticity associated with learning and memory formation. Indeed, dendritic spines' structure is regulated by molecular mechanisms that are fine-tuned and adjusted according to the level and direction of synaptic activity, development, specific brain region, and different experimental behavioral conditions, as revealed in studies performed by Sala, Cambianica, and Rossi (2008). Drugs of abuse can induce presynaptic and postsynaptic plasticity, and cause alterations in neuronal signaling and synaptic rearrangement resulting from modification in synaptic structures and effecting signal transduction, and in the level of receptor expression. Among the many neuroadaptations, most important are the alterations in cell morphology, including changes in dendritic spines. Changes to the density and morphology of dendritic spines represent a commonly reported neuroadaptation, following exposure to a drug of abuse, or during withdrawal. It appears as a significant increase in the density and branching of dendritic spines of medium spiny neurons. Neurotrophic factors also play an important role in drug-induced neuroadaptative changes. They regulate survival, development, and function of the nervous tissue, and act via appropriate receptors and activation of various signaling pathways in the target cells.

GENETIC LINKS TO DEPENDENCE

Addiction is a complex disease that is multifactorial and polygenic, and does not follow a Mendelian pattern of gene expression. No single gene has been identified which predisposes individuals to develop addictive behaviors. However, genetic background appears to influence addiction development, with mutations in certain genes believed to increase an individual's vulnerability to addictive behaviors. So far, 1500 genes have been linked to an addiction phenotype in humans. Duncan (2012) suggested that the best studied effects in the genetic sequence occur at the level of change to a single nucleotide in a gene (single gene polymorphism—SNP). There is strong evidence that SNPs have the potential to influence addictive behaviors, but the relationship between SNPs and drug addiction is not fully understood. Understanding this relationship is complicated by the possibility of gene splicing. As proteins are not derived from continual sequences in the genes, splicing occurs when different exons of RNA are reconstituted in different ways, thus producing different isoforms of the same protein. Patterns of gene expression are regulated by transcription factors which are transiently expressed to act locally in order to regulate gene expression in the cell in which they were initially produced. Transcription factors are proteins which bind to a specific DNA sequence to regulate the transcription of DNA to mRNA, often acting to enhance or decrease the activity of enzymes such as RNA polymerase, or molecules which regulate the acetylation of histones.

To elucidate the genetic aspects of drug dependence, it is important to note that there is increasingly more evidence that genetics and alterations in gene expression have long-term consequences on addictive behaviors. Changes to gene structure via SNPs may increase an individual's vulnerability to becoming addicted, if they are exposed to psychoactive substances. There is also a greater understanding of the significance of epigenetic mechanisms and the noncoding RNA in mediating addiction, and altering the transcription and translation of DNA and RNA.

SIGNIFICANCE OF RECENT FINDINGS OF MOLECULAR AND GENETIC EFFECTS OF CHRONIC CANNABINOID ADMINISTRATION, AND PROSPECTS OF NEW STRATEGIES TO TREAT CANNABINOID DEPENDENCE

Recent developments in cellular and molecular neurobiology focused on the mechanisms by which cannabinoids produce persistent alterations in brain functioning, and facilitate the development of drug dependence. Neuronal circuits and their elements undergo significant adaptations in response to exposure to cannabinoids. Fratta and Fattore (2013) suggest that THC acts not only via presynaptic CB_1 receptors to inhibit release of GABA and glutamate, but also causes long-term forms of synaptic plasticity by modulation of the number and strength of connections between the neurons. Uncoupling and downregulation of brain CB_1 receptors, due to prolonged THC exposure, result in the development of cannabinoid addiction and tolerance. Neuroadaptive changes in various brain regions are reported in studies on animal models, and in humans. Decreased mRNA expression in limbic area, and a reversible and regionally selective downregulation of cortical CB_1 receptors are observed in the brains of chronic cannabis smokers. Signaling cascades activated by THC are involved in neuronal plasticity, and it is thought that different neuronal circuits

might be involved in the early and late phases of cannabinoid addiction. Chronic THC exposure affects induction of immediate early genes, and also increases the expression of brain-derived neurotrophic factor (BDNF), a key regulator of the mesolimbic dopamine pathway that was described by Ninan (2011). BDNF potentiates dopamine release in the nucleus accumbens through activation of TrkB receptors on dopaminergic nerve terminals. BDNF-TrkB signaling is involved in transcription, translation, and trafficking of proteins during various phases of synaptic development, and has been implicated in several forms of synaptic plasticity. The latest data published by Biskupska et al. (2013) indicate the importance of the relationship between the Val66Met BDNF gene polymorphism, and dependence of psychoactive agent. The expression of BDNF is partially regulated by the transcription factor CREB, a central integrator of signaling from a number of extracellular stimuli that influence neuronal plasticity. A number of gene modifications occur in brain during THC abstinence. They are mostly related to three molecular networks, such as the glutamate/corticoids and CREB/ERK networks, as well as the network involving transcription factor regulating addiction induced plasticity, such as NFκB.

Recently, the growing field of epigenetics highlighted some of the mechanisms by which drugs of abuse regulate the transcriptional potential of cells in order to initiate lasting changes in gene expression. Increasing evidence implicates various mechanisms of gene regulation in the lasting changes that THC induces in the brain. Only through an integrated approach that establishes casual links between the molecular, cellular, and behavioral levels, as well as genetic aspects, it may be possible to understand the complex nature of the phenomenon of cannabis abuse. Nemeroff and Vale (2005) presented their opinion that recent technological advances, including the completion of the sequencing of the human genome, chromosome mapping, and several neurobiological findings, offer the hope of identifying the genetic basis of addiction, and new approaches to addiction therapy.

THE STRUCTURES AND BIOLOGICAL ACTIVITIES OF THC AND ITS ANALOGS—OWN CONSIDERATIONS

The biological activity of cannabinoids is closely connected to specific receptor–agonist interactions. The localization of CB1 and CB2 receptors is known and

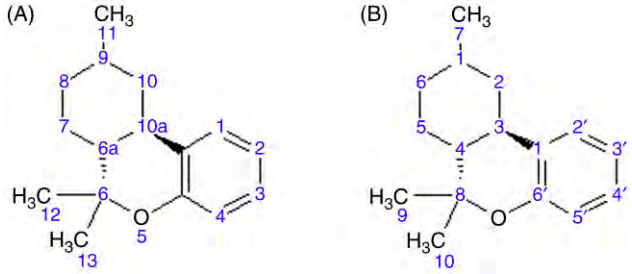


FIGURE 73.1 **Chemical structure of cannabinoles.** (A) Dibenzopyran numbering; (B) monoterpenoid numbering. *Source: The structures prepared in ACD/Labs ChemSketch software.*

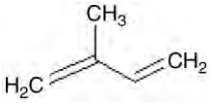


FIGURE 73.2 **The chemical structure of isoterpen.** *Source: The structure prepared in ACD/Labs ChemSketch software.*

widely described in the literature. All the narcotic properties of THC can be easily explained by the fact that CB1 receptors are localized in brain regions responsible for emotions and feelings, among other physiological functions. However, the chemical variety of THC-like compounds has not been fully discussed yet. Newly designed synthetic molecules, revealed nearly every month, indicate that, in spite of their chemical composition being different from natural cannabinomimetics, their internal activity against CB receptors is even better than the original compounds. To understand the chemical variety better, it is necessary to study the chemical structures of these molecules in detail. Cannabinoles belong to a specific class of compounds whose core fragment is dibenzopyran (Fig. 73.1A).

On the other hand, the core moiety can be also classified as monoterpenoid derivate (Fig. 73.1B); however, the first classification seems to better reflect the chemical properties of cannabinoles. In fact, THC is not a typical terpen composed of several five-carbon isoprene units (Fig. 73.2), but it possesses only one isoprene fragment without isolated double bond system, composed of at least two of the same units typically found in terpens (Fig. 73.3).

Moreover, specific benzene coupled double-bond system, included in the molecules, influence the three-dimensional shape of cannabinoles, while electron distribution in terpens is known to form regular

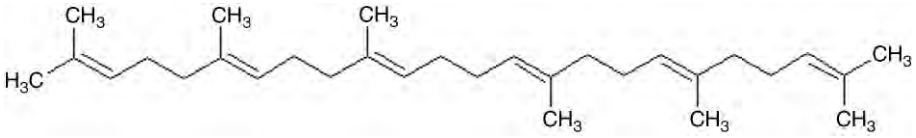


FIGURE 73.3 **Squalene—typical terpen structure.** *Source: The structure prepared in ACD/Labs ChemSketch software.*

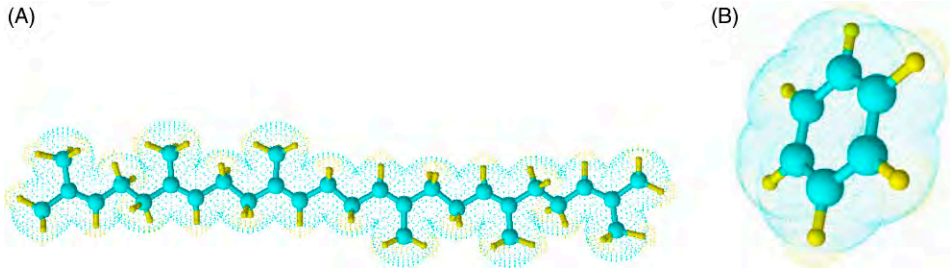


FIGURE 73.4 **The three-dimensional shape of squalene (A) and benzene (B) molecules.** *Source: The structures prepared in ACD/Labs 3D Viewer software.*

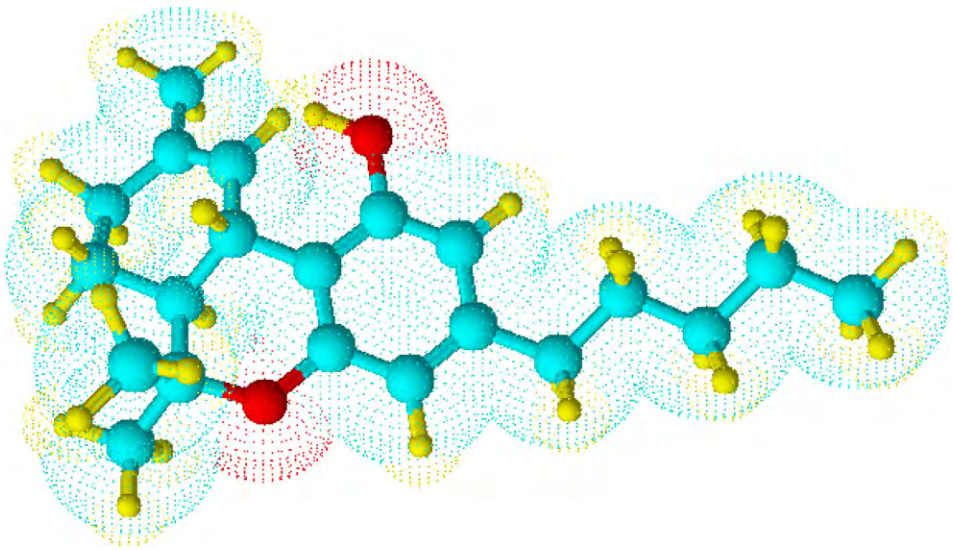


FIGURE 73.5 **The three-dimensional shape of THC structure.** *Source: The structure prepared in ACD/Labs 3D Viewer software.*

round-shaped spaces, whereas substituted benzene structure is nearly flat (Fig. 73.4).

The presence of the benzene ring in cannabinoles results in the specific shape of the molecule, and may have considerable influence on its biological activity, that is, receptor interactions. Another characteristic feature of cannabinoles is the presence of oxygen ether bridges coupling the benzene ring (sp2 hybridization), with carbon (sp3 hybridization) bounding two methyl groups. This connection of flat benzene rings with space-distributed aliphatic chains of nonaromatic properties influences additionally the very characteristic shape and properties of the molecule (Fig. 73.5).

Depending on the name of the core used as the basic cannabinoles' moiety, there are different rules for naming the structures of cannabinoles (Fig. 73.1). Regardless of the core name, cannabinoles include two stereoisomeric carbons (positions 6a, 10a), and thus appear as four optical stereoisomers, all indicating narcotic properties. Apart from their optical activity, cannabinoles form a number of geometrical isomers resulting mainly from the localization of double bond in cyclic hexane (positions 7, 8, 9, 10). The change in localization of this double bond is of key importance for narcotic properties because only when situated in position 9 it forms the most active THC. The localization of the penthyl aliphatic chain is limited only to position 3 in most cannabinoles exhibiting of biological activity, and this fragment of the molecule, besides providing characteristic shape, is responsible for increased lipophilic properties, rather than increased narcotic properties.

While analyzing the molecular composition of cannabinoles, it is clear that all of them are hydrophobic and do not have any heteroatom which may be easily metabolized to more easily water soluble derivatives. Even though there is oxygen present in the ethyl bridge, the ethers used will not undergo biological first phase reactions such as, for example, nitrogen including compounds. Instead, THC is metabolized through oxidization of methyl group (position 11) but the ether bridge remains unchanged (Fig. 73.6). This process incorporates many multistage enzymatic reactions, and it results in long time for THC elimination.

The comparison of psychoactive cannabinoles' molecular properties with other CB receptors agonists provides very interesting findings. The molecular structure of anandamide or 2-arachidonoglycerol, known to be a physiological, endogenous agonist of CB receptors,

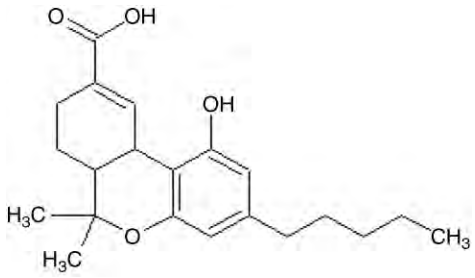


FIGURE 73.6 **The structure of 11-nor-9-carboxy-THC.** *Source: The structure prepared in ACD/Labs ChemSketch software.*

belongs to a completely different chemical group than THC, like many THC functional analogs (Fig. 73.7).

Anandamide is composed of a long aliphatic chain with coupled double-bond system, ending with second order amide bound with aliphatic alcohol, and it forms structures of no optical activity. The core of anandamide is more likely to be similar to isoterpens than THC. The part common to both THC and anandamide is the aliphatic chain of butane that ends anandamide, and is coupled to the benzene ring of THC. Anandamide does not include oxygen, having instead nitrogen; it is of aliphatic structure, without aromatic chemical properties, and it poses aliphatic alcohol hydroxyl group, not phenol, as in the case of THC. All these differences in chemical composition make important differences for these two molecules. It is clear that the key for the narcotic properties of THC is localization of double bond in position 9, since the other natural tetrahydrocannabinol, Δ^8-THC, does not have narcotic action. On the other hand, the presence of aliphatic butane chain being common for CB receptors interacting compounds suggests some hard importance of this substructure, especially compared to newly designed CB agonists belonging to the general cannabinoids group.

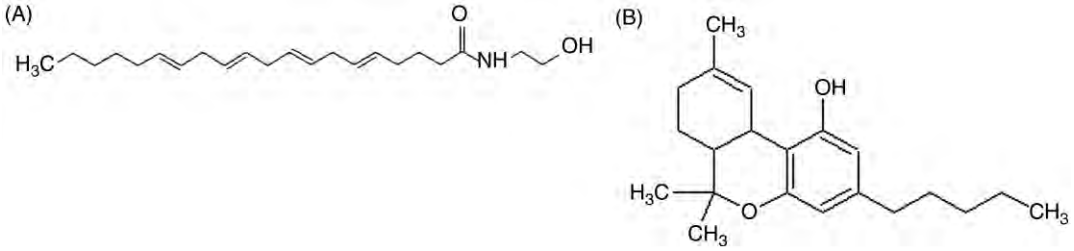


FIGURE 73.7 **The chemical structure of anandamide (A) and THC (B).** *Source: The structures prepared in ACD/Labs ChemSketch software.*

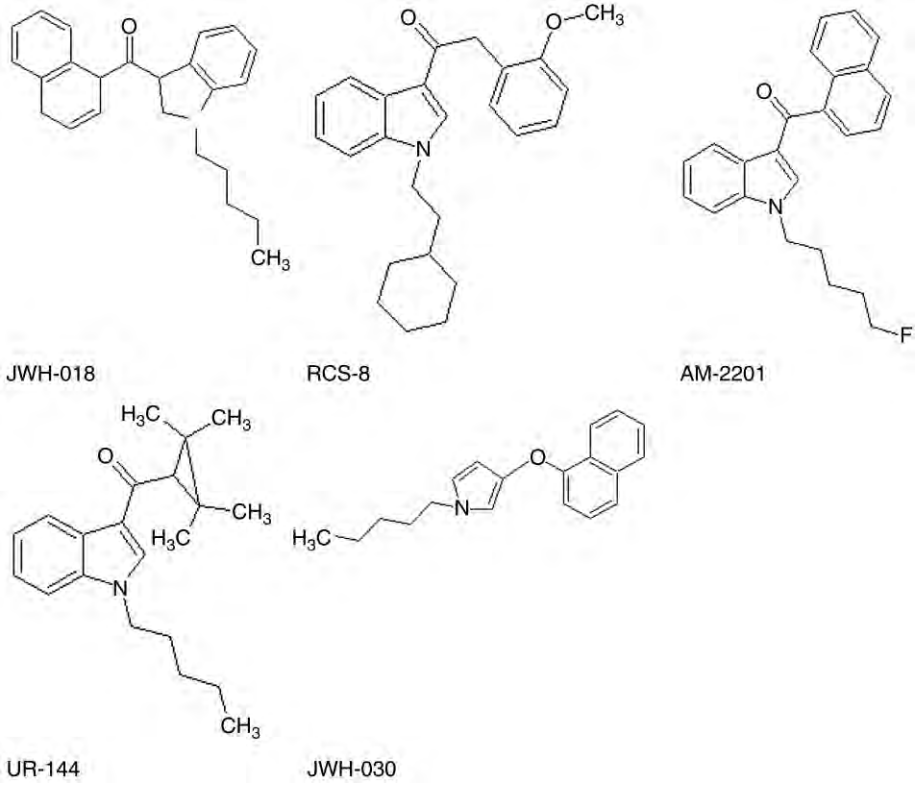


FIGURE 73.8 **Newly designed synthetic cannabinoids classes.** *Source: The structures prepared in ACD/Labs ChemSketch software.*

All the numerous newly designed synthetic cannabinoids indicate more chemical variety, both against anandamide and THC. All the compounds so far known can be classified into five main groups (Fig. 73.8):

- Naphtoylindoles (JWH-018, JWH-073),
- Phenylacethylindoles (RCS-8, JWH-250),
- Benzoylindoles (AM-2201, AM-1241),
- Cyclopropanoylindoles (UR-144, A-834,735),
- Naphthoylpyrroles (JWH-030, JWH-147).

We can easily see indole structure to be common for four of five newly designed cannabinomimetics, and the same is valid for the four carbon aliphatic chains attached to nitrogen, which we pointed out in typical cannabinoles. All the structures of newly designed compounds contain nitrogen, similar to anandamide, but here it is implemented typically into the cyclic system of the indole. The chemical properties of the molecules with an indole system (cyclic third order amine coupled to aromatic ring) are considerably different from amide properties such as anandamide, therefore it is hard to find chemical similarity between these five molecules, tetrahydrocannabinoles, and anandamide. Indole structure is more likely to be found in serotoninergic agents (the base core of serotonin), and its presence may result in nonselective, cannabinomimetic action of these compounds. In fact, the symptoms observed after intake of these substances are not only connected with stimulation of endocannabinoid system, but also to serotoninergic and GABAergic systems. Moreover, narcotic symptoms following their consumption are described as more emphatogenic. It is not clear if increased activity of these compounds results from stronger CB receptors' interaction, or rather multidirectional way of action involving more than one CNS pathway. Other characteristic feature of these newly designed cannabinoids that results from different chemical composition is their water solubility that stands in full contrast with THC, which is completely hydrophobic. This physical property is very important in respect of the way of administration (not only smoking, but could be sniffed or eaten) but it may also have a large influence on biological half-life time, rate of brain blood barrier crossover and, finally, elimination time from the body. The nitrogen present in all these molecules is more easily metabolized and eliminated through the urinary system.

References

Berke, J. D., & Hyman, S. E. (2000). Addiction, dopamine, and the molecular mechanisms of memory. *Neuron*, *25*, 515–532.

Biskupska, J., Borowiak, K. S., Karlin-Grazewicz, K., Janus, T., Waloszczyk, P., Potocka-Banas, B., Machoy-Mokrzynska, A., Ossowski, A., & Ciechanowicz, A. (2013). Estimation of BDNF gene polymorphism and predisposition to dependence development for selected psychoactive compounds: genetic aspects of addiction with the selected drugs, amphetamine, tetrahydrocannabinol and opiates. *Human and Experimental Toxicology*, *32*(3), 236–240.

Duncan, J. R. (2012). Current perspectives on the neurobiology of drug addiction: a focus on genetics and factors regulating gene expression. *International Scholarly Research Network (ISRN) Neurology*, *2012*, 972607.

Fratta, W., & Fattore, L. (2013). Molecular mechanisms of cannabinoid addiction. *Current Opinion in Neurobiology*, *23*, 1–6.

Hyman, S. E., Malenka, R. C., & Nestler, E. J. (2005). Neural mechanisms of addiction: the role of reward-related learning and memory. *Annual Review of Neuroscience*, *29*, 565–598.

Koob, G. F., & Le Moal, M. (2001). Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology*, *24*(2), 97–129.

Koob, G. F., Sanna, P. P., & Bloom, F. E. (1998). Neuroscience of addiction. *Neuron*, *21*, 467–476.

Lüscher, C., & Malenka, R. C. (2011). Drug-evoked synaptic plasticity in addiction: from molecular changes to circuit remodeling. *Neuron*, *69*, 650–663.

Madsen, H. B., Brown, R. M., & Lawrence, A. J. (2012). Neuroplasticity in addiction cellular and transcriptional perspectives. *Frontiers in Molecular Neuroscience*, *5*, 99.

Nemeroff, C. B., & Vale, W. W. (2005). The neurobiology of depression: inroads to treatment and new drug discovery. *Journal of Clinical Psychiatry*, *66*(Suppl. 7), 5–13.

Nestler, E. J. (2001). Molecular basis of long-term plasticity underlying addiction. *Nature Reviews Neuroscience*, *2*, 119–128.

Nestler, E. J. (2005). Is there a common molecular pathway for addiction? *Nature Neuroscience*, *8*(11), 1445–1449.

Ninan, I. (2011). Synaptic regulation of affective behaviors, role of BDNF. *Neuropharmacology*, *76*, 681–695.

Onaivi, E. S. (2007). An endocannabinoid hypothesis of drug reward. *Cannabinoids*, *2*(3), 22–26.

Pertwee, R. G. (2010). Cannabinoid receptor ligands. *Tocris Reviews*, *27*, 1–16.

Robison, A. J., & Nestler, E. J. (2011). Transcriptional and epigenetic mechanisms of addiction. *Nature Reviews Neuroscience*, *12*, 625–637.

Roche, M., & Finn, D. P. (2010). Brain CB2 receptors: implications for neuropsychiatric disorders. *Pharmaceuticals*, *3*, 2517–2553.

Sala, C., Cambianica, I., & Rossi, F. (2008). Molecular mechanisms of dendritic spine development and maintenance. *Acta Neurobiologiae Experimentalis*, *68*, 289–304.

Tanda, G., Pontieri, F. E., & Di Chiara, G. (1997). Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. *Science*, *276*, 2048–2050.

CHAPTER

74

Effects of Δ^9-Tetrahydrocannabinol in Human Breast Cancer

S. Takeda[*], *E. Ikeda*[**], *H. Okazaki*[†], *K. Watanabe*[‡], *H. Aramaki*[**,†]

*Laboratory of Xenobiotic Metabolism and Environmental Toxicology, Faculty of Pharmaceutical Sciences, Hiroshima International University (HIU), Kure, Hiroshima, Japan
**Department of Molecular Biology, Daiichi University of Pharmacy, Fukuoka, Japan
†Drug Innovation Research Center, Daiichi University of Pharmacy, Fukuoka, Japan
‡Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa, Japan

SUMMARY POINTS

- Δ^9-Tetrahydrocannabinol (Δ^9-THC) is currently used medicinally (ie, dronabinol, a synthetic form of Δ^9-THC) as well as recreationally (drug abuse).
- Δ^9-THC is not yet used as an anticancer agent in patients.
- There are conflicting findings regarding the effects of Δ^9-THC on the proliferation of cancer cells (protumor effects); (1) depending on the concentrations, cannabinoids either inhibit or stimulate the proliferation of cancer cells (Bifulco et al., 2006; Velasco et al., 2012), and (2) the large majority of studies published to date have yet to demonstrate that cannabinoids exert anticancer effects on breast, as well as on many other types of cancer (Bifulco et al., 2006; Velasco et al., 2012).
- Concerning the inconsistencies described earlier, our group and other researchers have suggested that Δ^9-THC can stimulate the proliferation of breast cancer cells under conditions in which the expression of cannabinoid receptors is very low or undetectable (McKallip et al., 2005; Takeda et al., 2008a, 2009; Watanabe et al., 2005), although it is currently difficult to directly compare the expression levels of CB receptors.
- The mechanism(s) responsible for Δ^9-THC evoked estrogen receptor (ERα)-positive MCF-7 cell proliferation observed in the absence of cannabinoid (CB) receptors remains unknown; we hypothesized that cyclooxygenase-2 (COX-2)/prostaglandin E_2 (PGE_2) pathways, which are suppressed by CB receptors, are activated under 17β-estradiol (E2)-deprived conditions (E2–) due to the downregulation of the receptors that are positively modulated by E2/ERα, and this results in the Δ^9-THC stimulation of MCF-7 cell growth (Fig. 74.4).
- Under E2-rich conditions (E2+), (1) upregulated CB receptors suppress COX-2 pathways, (2) Δ^9-THC activates CB receptors, and (3) simultaneously increased ERβ inhibits MCF-7 cell growth by suppressing E2/ERα (Figs. 74.5 and 74.6).
- Further studies including in vivo experiments are needed to confirm this hypothesis.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00085-5
Copyright © 2017 Elsevier Inc. All rights reserved.

KEY FACTS OF BREAST CANCER AND ESTROGEN RECEPTORS

- The vast majority of human breast cancers originate from epithelial cells.
- Epithelial human breast cancer cell lines such as MCF-7 and MDA-MB-231 cells are frequently used in the experiments to analyze molecular mechanisms of breast cancer growth.
- Approximately 60% of all breast cancer patients have 17β-estradiol (E2)-dependent breast cancer that expresses estrogen receptor α (ERα), and requires E2 for tumor growth. MCF-7 breast cancer cell growth is sensitive to treatments with E2 due to its stimulation (as a ligand) of ERα. On the contrary, MDA-MB-231 cells are known as ERα-negative.
- In addition to ERα, research is now focusing on ERβ, the second type of ER discovered in 1996. The expression levels of ERβ were found to be the highest in normal mammary tissue and decreased with the development of breast tumors.
- Although the physiological role of ERβ in breast cancer cells has not been examined in as much detail as ERα, accumulating evidence suggests that ERβ can act as an inhibitory molecule for ERα activity via the heterodimerization of ERα and ERβ.

LIST OF ABBREVIATIONS

CB	Cannabinoid
CHIP	Chromatin immunoprecipitation
COX-2	Cyclooxygenase-2
E2	17β-Estradiol
ER	Estrogen receptor
ERE	E2-responsive element
FAAH	Fatty acid amide hydrolase
HBCC	Human breast cancer cell
PGE_2	Prostaglandin E_2
Δ^9-THC	Δ^9-Tetrahydrocannabinol

INTRODUCTION

Δ^9-Tetrahydrocannabinol (Δ^9-THC) (Fig. 74.1), a major cannabinoid component of marijuana, is currently used not only recreationally (drug abuse), but also medicinally (ie, dronabinol) for the treatment of pain and nausea in cancer patients undergoing chemotherapy, in the United States and other countries. At least two cannabinoid (CB) receptor subtypes have been discovered and cloned: CB1 and CB2 receptors. Both CB receptor subtypes are members of the G-protein coupled receptor family. Δ^9-THC and anandamide (Fig. 74.2) have been identified as nonselective agonists for CB1 and CB2 receptors (Guindon & Hohmann, 2011; Pertwee, 2008), and Δ^9-THC has been shown to exert a wide variety of biological effects by mimicking the actions of endogenous substances, referred to as endocannabinoids (ie, anandamide) (Guindon & Hohmann, 2011; Pisanti & Bifulco, 2009). The expression of CB1 and CB2 receptors has been detected in many types of cancer cells (Sarfaraz, Adhami, Syed, Afaq, & Mukhtar, 2008; Velasco, Sánchez, & Guzmán, 2012). Previous studies reported that Δ^9-THC exhibited anticancer activities (eg, antiangiogenic, antimetastatic, and antiproliferative effects) in various types of cancers such as breast, glioma, lymphoma, lung, pancreas, prostate, skin, and thyroid cancers, using both in vitro and in vivo systems (Bifulco, Laezza, Pisanti, & Gazzerro, 2006; Velasco et al., 2012); however, Δ^9-THC may also stimulate the proliferation of cancer cells such as breast cancer (MCF-7 cells), glioblastoma (U373-MG), lung carcinoma (NCI-H292), and astrocytomas (Cudaback, Marrs, Moeller, & Stella, 2010; Hart, Fischer, & Ullrich, 2004; Watanabe et al., 2005; Takeda et al., 2008a; Takeda, Yamamoto, & Watanabe, 2009). Furthermore, there have been conflicting findings regarding the roles of endocannabinoids in cancer cell growth (Sánchez, Sánchez, Ruiz-Llorente, & Díaz-Laviada, 2003).

When studies focused on the effects of Δ^9-THC on epithelial human breast cancer cell (HBCC) lines such as MCF-7 and MDA-MB-231 cells, these cells were found to be resistant to Δ^9-THC induced cytotoxicity (McKallip, Nagarkatti, & Nagarkatti, 2005). These findings indicated that the sensitivity of breast cancer cells to Δ^9-THC may be related to the expression levels of CB1 and CB2 receptors, and that exposure to Δ^9-THC may enhance the growth rate of cancer cells with very low to the absent expression of cannabinoid receptors. We also demonstrated that exposure to Δ^9-THC stimulated the proliferation of MCF-7 cells, when CB receptors were not detected at the mRNA level (Watanabe et al., 2005; Takeda et al., 2008a, 2009). A previous study reported that the expression of CB receptors in MCF-7 cells could be influenced by the culture

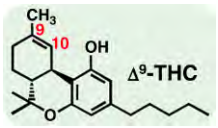


FIGURE 74.1 **Chemical structure of Δ^9-THC.** Numbers in the structure correspond to the dibenzopyran numbering system.

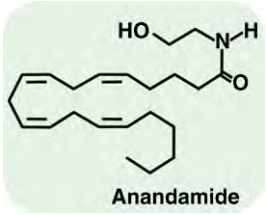


FIGURE 74.2 **Chemical structure of anandamide.**

conditions (Melck et al., 2000). Since Δ^9-THC itself, but not marijuana smoking, was shown to have no carcinogenic properties, as demonstrated by the Ames test (Glatt et al., 1979; Hall & MacPhee, 2002), Δ^9-THC may "accelerate" (possibly as a tumor promoter) the proliferation of cells after they are malignantly transformed. CB receptor expression levels have generally been implicated as a fundamental factor for the growth inhibitory effects by cannabinoids, including endocannabinoids (Guindon & Hohmann, 2011; Pertwee, 2008). Cannabinoids, including Δ^9-THC, can induce cell-cycle arrest and/or apoptosis in different human cancer cell lines, through CB receptors, but are less efficient in their nontransformed cell counterparts, in which the expression of these receptors is very low and/or undetectable (Pisanti & Bifulco, 2009). If this is a general in vivo phenomenon, Δ^9-THC may be able to provide some advantage(s) over currently used antitumor agents. Although Δ^9-THC is not currently used as an anticancer agent in patients, it has been approved for, and is used to reduce the side effects associated with cancer chemotherapy, such as nausea and vomiting (Watson, Benson, & Joy, 1999).

In this chapter, we will summarize the findings of studies that focused on the biological effects of Δ^9-THC on breast cancer, and provide an insight into mechanisms responsible for the modulation of Δ^9-THC sensitivity in breast cancer cells.

INTERPLAY BETWEEN Δ^9-THC AND CANNABINOID RECEPTORS IN BREAST CANCER CELL GROWTH

Studies that investigated the effects of cannabinoids on tumor biology were performed using HBCC lines such as MCF-7 (cell type classification: luminal) and MDA-MB-231 (cell type classification: mesenchymal) cells. MCF-7 cells are an HBCC line that is utilized for the in vitro screening of antiproliferative agents. The majority of studies published to date suggest that Δ^9-THC may abrogate the proliferation of different types of cancer cells and, coupled with the induction of cell death (ie, apoptosis, necrosis, and autophagy) (Velasco et al., 2012), the responsiveness of MCF-7 cells to Δ^9-THC is very complex: (1) sensitive (decrease in the growth rate), (2) resistant, or (3) sensitive (increase in the growth rate) (Ligresti et al., 2006; McKallip et al., 2005; Takeda et al., 2008a, 2009; Watanabe et al., 2005). McKallip et al. (2005) and our group previously reported that Δ^9-THC-mediated stimulating effects on the proliferation of MCF-7 cells were specifically observed when the expression levels of CB1 and CB2 receptors in these cells were very low or undetectable (McKallip et al., 2005; Watanabe et al., 2005; Takeda et al., 2008a, 2009). Details of these inconsistencies have not yet been definitively resolved. Wang et al. (2008) reported that CB1 receptor expression was silenced in human colorectal cancer through DNA methylation of the CB1 promoter, and the loss of CB1 accelerated intestinal tumor growth in vivo (Wang et al., 2008). The estrogenic induction of CB1 in human colon cancer cell lines has been reported, and the CB1 receptor is suggested to be an estrogen (17β-estradiol, E2)-responsive gene in DLD-1, HT-29, and SW620 human colon cancer cells (Notarnicola et al., 2008; Proto et al., 2012). Furthermore, E2 regulated the CB2 receptor by recruiting a putative E2-responsive element (ERE) in CB2 encoding for the gene (Rossi et al., 2013). Thus, since the regulation of the CB1/CB2 receptors by E2 is also true for MCF-7 breast cancer cells, these findings suggest that the biological fate of Δ^9-THC for MCF-7 cell growth can be affected by E2 levels. In support of these findings, (1) Δ^9-THC was able to stimulate the proliferation of MCF-7 cells (Watanabe et al., 2005; Takeda et al., 2008a, 2009) cultured in the phenol-red-free media containing 5% dextran-coated charcoal-treated serum ("E2-deprived medium;" in particular for a long time) (Lewis, Osipo, Meeke, & Jordan, 2005; Martin, Farmer, Johnston, Ali, & Dowsett, 2005; Yue et al., 2002), and (2) although fatty acid amide hydrolase (known as FAAH), which breaks down anandamide, was detected, the expression of the CB1/2 receptors was not detectable at mRNA levels in the cells (Watanabe et al., 2005; Takeda et al., 2008a, 2009). The physiological levels of E2 (10 pM–1 nM)-stimulated proliferation of MCF-7 cells precultured in E2-deprived medium (ie, long-term E2 deprivation) was completely antagonized by Δ^9-THC (Takeda et al., 2009). This interactive phenomenon between E2 and Δ^9-THC, for the proliferation of MCF-7 cells, has been demonstrated previously (von Bueren, Schlumpf, & Lichtensteiger, 2008) (see Section "Interplay Between Δ^9-THC and Estrogen Signaling in Breast Cancer Cell Growth").

By performing a chromatin immunoprecipitation (CHIP) assay, Bifulco and coworkers demonstrated that estrogen receptor α (ERα), but not ERβ, associated with the CB1 promoter region containing one full ERE and two ERE half-sites in human colon cancer SW620 cells treated with 100 nM E2 (Proto et al., 2012). Since colon cancer SW620 cells express high levels of both CB1 and ERα, it may be reasonable to expect the ERα-mediated regulation of CB1 in the presence of E2, a ligand for ERα. In accordance with this, E2 was shown to induce the expression of CB1 in human colon cancer cell lines (Notarnicola et al., 2008). Our preliminary study also demonstrated the E2-mediated regulation of CB receptors in ERα-positive MCF-7 cells (Takeda et al., unpublished observations).

INTERPLAY BETWEEN Δ^9-THC AND ESTROGEN SIGNALING IN BREAST CANCER CELL GROWTH

Approximately 60% of all breast cancer patients have E2-dependent breast cancer that expresses ERα, and requires E2 for tumor growth. MCF-7 breast cancer cell

growth is sensitive to treatments with E2 due to its stimulation of ERα. As described in Section "Interplay Between Δ9-THC and Cannabinoid Receptors in Breast Cancer Cell Growth," an interaction may exist between Δ9-THC and E2 in order to regulate the proliferation of MCF-7 cells. The proliferation of MCF-7 cells precultured under E2-deprived conditions was more likely to be stimulated by the addition of E2 with an EC_{50} value of approximately 10 pM (Fokialakis et al., 2004; Takeda et al., 2009). In these deprived conditions (ie, long-term E2 deprivation), MCF-7 cells endogenously produced very low levels of E2 (pmol concentration; 0.26 pM/10^6 cells) of E2 (Miki et al., 2007), and Δ9-THC could stimulate the proliferation of MCF-7 cells. The stimulating effects of Δ9-THC were further enhanced in a concentration-dependent manner by the addition of two types of aromatase (cytochrome P450 19A1) inhibitors (aminoglutethimide and α-naphthoflavone) (Takeda et al., 2009). Aromatase has been identified as the enzyme that produces intracellular E2 through the metabolism of testosterone, while MCF-7 cells express functional aromatase. In support of the phenomenon described earlier, Δ9-THC-induced proliferation was blocked by a cotreatment with testosterone, although the physiological concentrations of testosterone (1–100 nM), which can be converted into E2, stimulated the MCF-7 cell growth (Takeda et al., 2009). Thus, these findings strongly implied that Δ9-THC may be able to interact with the very low levels of E2 produced by cells, resulting in the abrogation of the E2-stimulated proliferation of MCF-7 cells when simultaneously treated; however, Δ9-THC stimulation was counteracted by E2 in these cells (Fig. 74.3). The mechanism by which Δ9-THC stimulates the proliferation of MCF-7 cells in a low E2 milieu currently remains unknown. We are now investigating this phenomenon; until now, it has been suggested that cyclooxygenase-2 (COX-2) and its metabolite prostaglandin E_2 (PGE_2), which can inhibit apoptosis signals, as well as

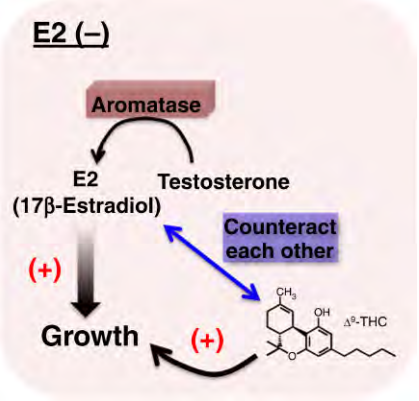


FIGURE 74.3 A model suggesting that 17β-estradiol (E2) or Δ9-THC can stimulate the growth of MCF-7 cells under E2-deprived conditions (Takeda et al., 2009). However, the respective stimulation effects are counteracted when they are administered simultaneously. E2 is produced from testosterone by aromatase (CYP19A1).

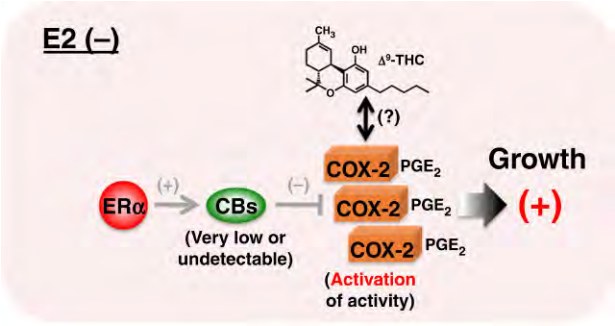


FIGURE 74.4 A hypothetical model for the interaction between Δ9-THC and estrogen signaling pathways in ERα-positive MCF-7 human breast cancer cells. The following hypothetical model was made together with previous findings. Under 17β-estradiol (E2)-deprived conditions (indicated as ER–), CB1/CB2 receptors regulated by ERα are downregulated, which, consequently, activates cyclooxygenase-2 (COX-2)/prostaglandin E_2 (PGE_2) signaling pathways, followed by the Δ9-THC stimulation of breast cancer cell growth (Notarnicola et al., 2008; Proto et al., 2012; Takeda et al., 2009). However, there is currently no evidence to suggest how Δ9-THC utilizes COX-2/PGE_2 to induce cell proliferation (indicated as "?") (see also Fig. 74.6).

positively enhance the tumorigenic potential of cancers (Tsujii & DuBois, 1995), may be involved in Δ9-THC-induced cell proliferation (Takeda et al., 2009) (Fig. 74.3). Because CB2 receptor agonism has been suggested to inhibit the COX-2/PGE_2 axis in breast cancer cells (Qamri et al., 2009), COX-2/PGE_2 signaling pathway(s) may manifest under E2-depleted conditions, and participate in the Δ9-THC stimulation of MCF-7 growth (Fig. 74.4) (see Fig. 74.6). No inhibitory/stimulation effects of Δ9-THC have yet been confirmed on the activity/expression of COX-2 (Takeda et al., 2008b, 2009).

We next investigated the effects of Δ9-THC on the proliferation of MCF-7 cells precultured for 24 h in E2-deprived medium (ie, short-term E2 deprivation). The results obtained revealed that Δ9-THC itself was able to "suppress" the proliferation of MCF-7 cells in a concentration-dependent manner, and that the antiproliferative effects of Δ9-THC were positively enhanced by the exogenous addition of E2 (100 pM) (the IC_{50} values of Δ9-THC alone and Δ9-THC + E2: 34.5 vs 10.4 μM, respectively) (Takeda et al., 2013). Taken together, these findings suggested that the sensitivity of MCF-7 cells to Δ9-THC may be affected by the levels of E2 supplied by breast cancer cells themselves, or by its exogenous addition.

INTERPLAY BETWEEN Δ9-THC AND ESTROGEN SIGNALING IN BREAST CANCER CELL GROWTH: POSSIBLE INVOLVEMENT OF ERβ

To comprehensively understand the mechanisms underlying the proliferation of breast cancer cells, in addition to ERα, research is now focusing on ERβ, the second type of ER, discovered in 1996 (Kuiper, Enmark,

Pelto-Huikko, Nilsson, & Gustafsson, 1996). Previous studies reported that approximately 60–70% of breast tumors express ERβ, and most tumors simultaneously express both ERα and ERβ (Dotzlaw, Leygue, Watson, & Murphy, 1997; Fuqua et al., 2003). Furthermore, the expression levels of ERβ were found to be the highest in normal mammary tissue, and decreased with the development of breast tumors (Roger et al., 2001; Zhao et al., 2003). Although the physiological role of ERβ in breast cancer cells has not been examined in as much detail as ERα, accumulating evidence suggests that ERβ can act as an inhibitory molecule for ERα activity through the heterodimerization of ERα and ERβ (Hall & McDonnell, 1999; Pettersson, Delaunay, & Gustafsson, 2000; Powell & Xu, 2008; Zhao et al., 2007), and that ERβ itself acts as a tumor suppressor with both antiproliferative and antimigration activities (Lazennec, Bresson, Lucas, Chauveau, & Vignon, 2001; Paruthiyil et al., 2004). In addition, the antagonistic effects of ERβ on E2/ERα-mediated transcription have been explained by the altered recruitment of c-Fos/c-Jun transcription factors composed of activator protein 1 (AP-1) to estrogen-positive promoters (Matthews et al., 2006). Thus, ERβ itself, or the expression of ERβ in ERα-positive breast cancer, may be associated with better clinical outcomes in women.

Based on the experimental findings that Δ9-THC abrogated the E2-mediated proliferation of ERα-positive MCF-7 cells (Takeda et al., 2009; von Bueren et al., 2008), we first focused on the effects of Δ9-THC on ERα activity. Accumulating evidence has demonstrated that Δ9-THC exhibits "no" binding/activation potential to ERα (Okey & Bondy, 1978; Ruh, Taylor, Howlett, & Welshons, 1997; Sauer, Rifka, Hawks, Cutler, & Loriaux, 1983), and also that aromatase activity is insensitive to Δ9-THC (Lee, Oh, Lee, & Chung, 2005); however, the antiestrogenic activity/property of Δ9-THC has so far been demonstrated in both in vitro and in vivo experiments (Paria, Kapur, & Dey, 1992; Smith & Asch, 1984; Takeda et al., 2009, 2013; von Bueren et al., 2008). Thus, a definitive answer regarding the Δ9-THC disruption of E2 signaling in ERα-positive breast cancer cells has not yet been obtained. To clarify the properties of Δ9-THC acting as an antiestrogen for E2/ERα-mediated transcription, we performed a DNA microarray analysis of ERα-positive MCF-7 cells cultured under short-term E2-deprived conditions, in the presence of Δ9-THC. The upregulation of ERβ (9.6-fold) was significantly higher than that in vehicle-treated groups, whereas ERα levels were not essentially affected by the cannabinoid, which demonstrated that Δ9-THC disrupted the balanced expression between ERα and ERβ by upregulating ERβ, the basal expression of which was very low due to the DNA methylation of CpG sites on the promoter region in mammary carcinoma and MCF-7 breast cancer cells (Rody et al., 2005; Zhao et al., 2007). The Δ9-THC stimulation

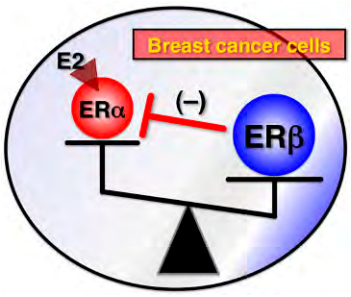


FIGURE 74.5 **A model suggesting that Δ9-THC can abrogate 17β-estradiol (E2)/ERα signaling by upregulating ERβ in MCF-7 human breast cancer cells (see also Takeda et al., 2013).**

of ERβ was also demonstrated by western immunoblot analysis. We next determined whether upregulated ERβ affected the expression of the E2/ERα-regulated target gene (Lin et al., 2007), CDC2, a key player in cell cycle regulation; the expression level of CDC2 was reduced when ERβ was upregulated by the Δ9-THC treatment. We also demonstrated that Ki-67, a general proliferation marker upregulated by E2 in breast cancer, was downregulated by Δ9-THC in a similar manner to that of CDC. Furthermore, the overexpression of ERβ resulted in the further inhibition of E2/ERα-mediated transcription over that with the Δ9-THC alone system, which indicated that ERβ was a likely target of the antiestrogenic actions of Δ9-THC on E2/ERα activity, possibly coupled with Δ9-THC-mediated antiproliferative effects (see Section "Interplay Between Δ9-THC and Estrogen Signaling in Breast Cancer Cell Growth") (Figs. 74.5 and 74.6). It should be noted that Δ9-THC could not bind to the ligand

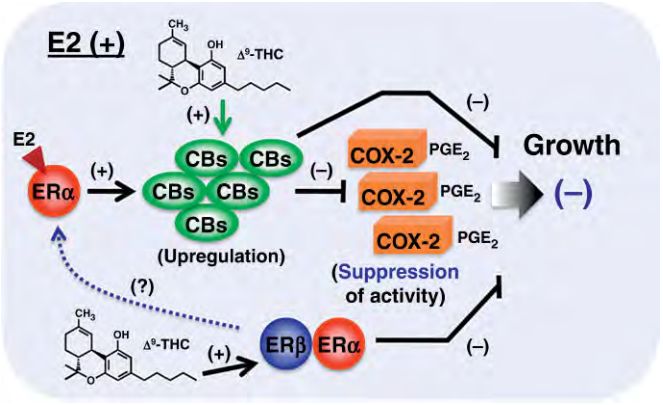


FIGURE 74.6 **A hypothetical model for the interaction between Δ9-THC and estrogen signaling pathways in ERα-positive MCF-7 human breast cancer cells.** Under 17β-estradiol (E2)-rich conditions (indicated as E2+), the basal expression of CB1/CB2 receptors is stimulated by E2/ERα coupled with the suppression of cyclooxygenase-2 (COX-2) activity (Qamri et al., 2009). The activation of CB1/CB2 receptors by Δ9-THC then inhibits breast cancer cell growth. In addition, concomitantly upregulated ERβ inhibits breast cancer cell growth through the abrogation of E2/ERα (Takeda et al., 2013). However, there is currently no evidence for the effects of ERβ on E2/ERα involved in the expression of CB receptors (indicated as "?").

(E2) binding domain of ERβ, as was also the case for ERα (Ruh et al., 1997; Takeda et al., 2013). In ERα-negative MDA-MB-231 cells, Δ^9-THC did not evoke antiproliferative effects on ERα-deficient cells at 48 h, in the presence of E2 (100 pM); however, the expression of ERβ was clearly induced (Takeda et al., 2013). Taken together, these findings suggested that Δ^9-THC may require ERα for its antiproliferative effects on MCF-7 breast cancer cells under short-term E2-rich conditions. We are now investigating the mechanism(s) underlying the Δ^9-THC-mediated upregulation of ERβ by focusing on the effects of Δ^9-THC on the promoter DNA methylation status of ERβ.

MINI-DICTIONARY

Antiestrogens Compounds with the potential to block the production or utilization of E2, or directly (antagonism of E2 for ERα) or indirectly (upregulation of ERβ, eg, Δ^9-THC) inhibit the effects of E2 on ERα.

Aromatase (CYP19A1) An enzyme that converts testosterone (an androgen) into E2 (an estrogen); thus, its inhibitor is used in the treatment of estrogen receptor (ER)-positive breast cancer.

Cannabinoid (CB) receptors Two different cannabinoid receptors (ie, CB1 and CB2) have been identified and cloned; both types are members of the G-protein coupled receptor (GPCR) family.

Cannabinoids Originally defined as a group of C_{21} compounds uniquely produced by *C. sativa*. Approximately 70 different cannabinoids have been identified to date. Δ^9-THC is a naturally occurring "phyto"cannabinoid. (cf. "endo"cannabinoids, produced and released in the body).

Chromatin immunoprecipitation (CHIP) assay Used to determine the relationship between protein(s) with a specific DNA region, including promoter regions in vivo.

Cyclooxygenase-2 (COX-2) Highly expressed in cells with inflammation and in cancer cells; it is known to be involved in the aggressive phenotype of tumors (eg, survival, migration etc.).

17β-Estradiol (E2) Having two hydroxyl groups in its molecular structure, and the most potent form of mammalian estrogenic steroids (see: Estrogen receptor β).

Estrogen receptor β (ERβ) Discovered as a second type of estrogen receptor in 1996; its physiological function has not yet been fully elucidated, but it is being recognized as a tumor suppressor through its own and/or the inhibition of ERα activity. ERβ has weaker transcriptional activity than ERα in response to E2.

MCF-7 cell line A human breast cancer cell line expressing ERα (ER-positive), which was established in 1973 at the Michigan Cancer Foundation [Soule H. D., Vazguez J., Long A., Albert S., & Brennan M. (1973). A human cell line from a pleural effusion derived from a breast carcinoma. *Journal of the National Cancer Institute*, *51*, 1409–1413]. (cf. MDA-MB-231 cell line, a highly aggressive human breast cancer cell line with an ER-negative phenotype).

Δ^9-Tetrahydrocannabinol (Δ^9-THC) A major component of marijuana that is used both recreationally (drug abuse) and medicinally (ie, dronabinol, a synthetic form of Δ^9-THC) (see: cannabinoids)

Acknowledgments

This study was supported in part by a Grant-in-Aid for Scientific Research (C) [25460182, (to S.T.)] from the Japan Society for the Promotion of Science (JSPS) KAKENHI. The author wishes to thank students at the Department of Molecular Biology, Daiichi University of Pharmacy, for their technical contributions.

References

Bifulco, M., Laezza, C., Pisanti, S., & Gazzerro, P. (2006). Cannabinoids and cancer: pros and cons of an antitumour strategy. *British Journal of Pharmacology*, *148*, 123–135.

Cudaback, E., Marrs, W., Moeller, T., & Stella, N. (2010). The expression level of CB_1 and CB_2 receptors determines their efficacy at inducing apoptosis in astrocytomas. *PLoS One*, *5*, e8702.

Dotzlaw, H., Leygue, E., Watson, P. H., & Murphy, L. C. (1997). Expression of estrogen receptor-β in human breast tumors. *Journal of Clinical Endocrinology and Metabolism*, *82*, 2371–2374.

Fokialakis, N., Lambrinidis, G., Mitsiou, D. J., Aligiannis, N., Mitakou, S., Skaltsounis, A. L., Pratsinis, H., Mikros, E., & Alexis, M. N. (2004). A new class of phytoestrogens; evaluation of the estrogenic activity of deoxybenzoins. *Chemistry and Biology*, *11*, 397–406.

Fuqua, S. A., Schiff, R., Parra, I., Moore, J. T., Mohsin, S. K., Osborne, C. K., Clark, G. M., & Allred, D. C. (2003). Estrogen receptor β protein in human breast cancer: correlation with clinical tumor parameters. *Cancer Research*, *63*, 2434–2439.

Glatt, H., Ohlsson, A., Agurell, S., & Oesch, F. (1979). D^1-Tetrahydrocannabinol and 1a, 2a-epoxyhexahydrocannabinol: mutagenicity investigation in the Ames test. *Mutation Research*, *66*, 329–335.

Guindon, J., & Hohmann, A. G. (2011). The endocannabinoid system and cancer: therapeutic implication. *British Journal of Pharmacology*, *163*, 1447–1463.

Hall, W., & MacPhee, D. (2002). Cannabis use and cancer. *Addiction*, *97*, 243–247.

Hall, J. M., & McDonnell, D. P. (1999). The estrogen receptor β-isoform (ERβ) of the human estrogen receptor modulates ERα transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens. *Endocrinology*, *140*, 5566–5578.

Hart, S., Fischer, O. M., & Ullrich, A. (2004). Cannabinoids induce cancer cell proliferation via tumor necrosis factor α-converting enzyme (TACE/ADAM17)-mediated transactivation of the epidermal growth factor receptor. *Cancer Research*, *64*, 1943–1950.

Kuiper, G. G., Enmark, E., Pelto-Huikko, M., Nilsson, S., & Gustafsson, J. A. (1996). Cloning of a novel receptor expressed in rat prostate and ovary. *Proceedings of the National Academy of Sciences of the United States of America*, *93*, 5925–5930.

Lazennec, G., Bresson, D., Lucas, A., Chauveau, C., & Vignon, F. (2001). ERβ inhibits proliferation and invasion of breast cancer cells. *Endocrinology*, *142*, 4120–4130.

Lee, S. Y., Oh, S. M., Lee, S. K., & Chung, K. H. (2005). Antiestrogenic effects of marijuana smoke condensate and cannabinoid compounds. *Archives of Pharmacal Research*, *28*, 1365–1375.

Lewis, J. S., Osipo, C., Meeke, K., & Jordan, V. C. (2005). Estrogen-induced apoptosis in a breast cancer model resistant to long-term estrogen withdrawal. *Journal of Steroid Biochemistry and Molecular Biology*, *94*, 131–141.

Ligresti, A., Moriello, A. S., Starowicz, K., Matias, I., Pisanti, S., De Petrocellis, L., Laezza, C., Portella, G., Bifulco, M., & Di Marzo, V. (2006). Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. *Journal of Pharmacology and Experimental Therapeutics*, *318*, 1375–1387.

Lin, C. Y., Ström, A., Li Kong, S., Kietz, S., Thomsen, J. S., Tee, J. B., Vega, V. B., Miller, L. D., Smeds, J., Bergh, J., Gustafsson, J. A., & Liu, E. T. (2007). Inhibitory effects of estrogen receptor beta on specific hormone-responsive gene expression and association with disease outcome in primary breast cancer. *Breast Cancer Research*, *9*, R25.

Martin, L. A., Farmer, I., Johnston, S. R., Ali, S., & Dowsett, M. (2005). Elevated ERK1/ERK2/estrogen receptor cross-talk enhances

estrogen-mediated signaling during long-term estrogen deprivation. *Endocrine-Related Cancer*, *12*(Suppl. 1), S75–S84.

Matthews, J., Wihlén, B., Tujague, M., Wan, J., Ström, A., & Gustafsson, J. A. (2006). Estrogen receptor (ER) β modulates ERα-mediated transcriptional activation by altering the recruitment of c-Fos and c-Jun to estrogen-responsive promoters. *Molecular Endocrinology*, *20*, 534–543.

McKallip, R. J., Nagarkatti, M., & Nagarkatti, P. S. (2005). Δ-9-Tetrahydrocannabinol enhances breast cancer growth and metastasis by suppression of the antitumor immune response. *Journal of Immunology*, *174*, 3281–3289.

Melck, D., De Petrocellis, L., Orlando, P., Bisogno, T., Laezza, C., Bifulco, M., & Di Marzo, V. (2000). Suppression of nerve growth factor Trk receptors and prolactin receptors by endocannabinoids leads to inhibition of human breast and prostate cancer cell proliferation. *Endocrinology*, *141*, 118–126.

Miki, Y., Suzuki, T., Tazawa, C., Yamaguchi, Y., Kitada, K., Honma, S., Moriya, T., Hirakawa, H., Evans, D. B., Hayashi, S., Ohuchi, N., & Sasano, H. (2007). *Cancer Research*, *67*, 3945–3954.

Notarnicola, M., Messa, C., Orlando, A., Bifulco, M., Laezza, C., Gazzerro, P., & Caruso, M. G. (2008). *Scandinavian Journal of Gastroenterology*, *43*, 66–72.

Okey, A. B., & Bondy, G. P. (1978). Δ^9-Tetrahydrocannabinol and 17β-estradiol bind to different macromolecules in estrogen target tissues. *Science*, *200*, 312–314.

Paria, B. C., Kapur, S., & Dey, S. K. (1992). Effects of 9-ene-tetrahydrocannabinol on uterine estrogenicity in the mouse. *Journal of Steroid Biochemistry and Molecular Biology*, *42*, 713–719.

Paruthiyil, S., Parmar, H., Kerekatte, V., Cunha, G. R., Firestone, G. L., & Leitman, D. C. (2004). Estrogen receptor β inhibits human breast cancer cell proliferation and tumor formation by causing a G_2 cell cycle arrest. *Cancer Research*, *64*, 423–428.

Pertwee, R. G. (2008). The diverse CB_1 and CB_2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and Δ^9-tetrahydrocannabivarin. *British Journal of Pharmacology*, *153*, 199–215.

Pettersson, K., Delaunay, F., & Gustafsson, J. A. (2000). Estrogen receptor β acts as a dominant regulator of estrogen signaling. *Oncogene*, *19*, 4970–4978.

Pisanti, S., & Bifulco, M. (2009). Endocannabinoid system modulation in cancer biology and therapy. *Pharmacological Research*, *60*, 107–116.

Powell, E., & Xu, W. (2008). Intermolecular interactions identify ligand-selective activity of estrogen receptor α/β dimers. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 19012–19017.

Proto, M. C., Gazzerro, P., Di Croce, L., Santoro, A., Malfitano, A. M., Pisanti, S., Laezza, C., & Bifulco, M. (2012). Interaction of endocannabinoid system and steroid hormones in the control of colon cancer cell growth. *Journal of Cellular Physiology*, *227*, 250–258.

Qamri, Z., Preet, A., Nasser, M. W., Bass, C. E., Leone, G., Barsky, S. H., & Ganju, R. K. (2009). Synthetic cannabinoid receptor agonists inhibit tumor growth and metastasis of breast cancer. *Molecular Cancer Therapeutics*, *8*, 3117–3129.

Rody, A., Holtrich, U., Solbach, C., Kourtis, K., von Minckwitz, G., Engels, K., Kissler, S., Gätje, R., Karn, T., & Kaufmann, M. (2005). Methylation of estrogen receptor β promoter correlates with loss of ER-β expression in mammary carcinoma and is an early indication marker in premalignant lesions. *Endocrine-Related Cancer*, *12*, 903–916.

Roger, P., Sahla, M. E., Mäkelä, S., Gustafsson, J. A., Baldet, P., & Rochefort, H. (2001). Decreased expression of estrogen receptor β protein in proliferative preinvasive mammary tumors. *Cancer Research*, *61*, 2537–2541.

Rossi, F., Bellini, G., Luongo, L., Mancusi, S., Torella, M., Tortora, C., Manzo, I., Guida, F., Nobili, B., de Novellis, V., & Maione, S. (2013). The 17-β-oestradiol inhibits osteoclast activity by increasing the cannabinoid CB2 receptor expression. *Pharmacological Research*, *68*, 7–15.

Ruh, M. F., Taylor, J. A., Howlett, A. C., & Welshons, W. V. (1997). Failure of cannabinoid compounds to stimulate estrogen receptors. *Biochemical Pharmacology*, *53*, 35–41.

Sánchez, M. G., Sánchez, A. M., Ruiz-Llorente, L., & Díaz-Laviada, I. (2003). Enhancement of androgen receptor expression induced by (R)-methanandamide in prostate LNCaP cells. *FEBS Letters*, *555*, 561–566.

Sarfaraz, S., Adhami, V. M., Syed, D. N., Afaq, F., & Mukhtar, H. (2008). Cannabinoids for cancer treatment: progress and promise. *Cancer Research*, *68*, 339–342.

Sauer, M. A., Rifka, S. M., Hawks, R. L., Cutler, G. B., Jr., & Loriaux, D. L. (1983). Marijuana: interaction with the estrogen receptor. *Journal of Pharmacology and Experimental Therapeutics*, *224*, 404–407.

Smith, C. G., & Asch, R. H. (1984). Acute, short-term, and chronic effects of marijuana on the female primate reproductive function. *NIDA Research Monograph*, *44*, 82–96.

Takeda, S., Yamaori, S., Motoya, E., Matsunaga, T., Kimura, T., Yamamoto, I., & Watanabe, K. (2008a). Δ^9-Tetrahydrocannabinol enhances MCF-7 cell proliferation via cannabinoid receptor-independent signaling. *Toxicology*, *245*, 141–146.

Takeda, S., Misawa, K., Yamamoto, I., & Watanabe, K. (2008b). Cannabidiolic acid as a selective cyclooxygenase-2 inhibitory component in cannabis. *Drug Metabolism and Disposition*, *36*, 1917–1921.

Takeda, S., Yamamoto, I., & Watanabe, K. (2009). Modulation of Δ^9-tetrahydrocannabinol-induced MCF-7 breast cancer cell growth by cyclooxygenase and aromatase. *Toxicology*, *259*, 25–32.

Takeda, S., Yoshida, K., Nishimura, H., Harada, M., Okajima, S., Miyoshi, H., Okamoto, Y., Amamoto, T., Watanabe, K., Omiecinski, C. J., & Aramaki, H. (2013). Δ^9-Tetrahydrocannabinol disrupts estrogen-signaling through up-regulation of estrogen receptor β (ERβ). *Chemical Research in Toxicology*, *26*, 1073–1079.

Tsujii, M., & DuBois, R. N. (1995). Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell*, *83*, 493–501.

Velasco, G., Sánchez, C., & Guzmán, M. (2012). Towards the use of cannabinoids as antitumour agents. *Nature Reviews Cancer*, *12*, 436–444.

von Bueren, A., Schlumpf, M., & Lichtensteiger, W. (2008). Delta(9)-tetrahydrocannabinol inhibits 17β-estradiol-induced proliferation and fails to activate androgen and estrogen receptors in MCF7 human breast cancer cells. *Anticancer Research*, *28*, 85–89.

Wang, D., Wang, H., Ning, W., Backlund, M. G., Dey, S. K., & DuBois, R. N. (2008). Loss of cannabinoid receptor 1 accelerates intestinal tumor growth. *Cancer Research*, *68*, 6468–6476.

Watanabe, K., Motoya, E., Matsuzawa, N., Funahashi, T., Kimura, T., Matsunaga, T., Arizono, K., & Yamamoto, I. (2005). Marijuana extracts possess the effects like the endocrine disrupting chemicals. *Toxicology*, *206*, 471–478.

Watson, S. J., Benson, J. A., Jr., & Joy, J. E. (1999). Marijuana and medicine: assessing the science base: a summary of the 1999 Institute of Medicine report. *Archives of General Psychiatry*, *57*, 547–552.

Yue, W., Wang, J. P., Conaway, M., Masamura, S., Li, Y., & Santen, R. J. (2002). Activation of the MAPK pathway enhances sensitivity of MCF-7 breast cancer cells to the mitogenic effect of estradiol. *Endocrinology*, *143*, 3221–3229.

Zhao, C., Lam, E. W., Sunters, A., Enmark, E., De Bella, M. T., Coombes, R. C., Gustafsson, J. A., & Dahlman-Wright, K. (2003). Expression of estrogen receptor β isoforms in normal breast epithelial cells and breast cancer: regulation by methylation. *Oncogene*, *22*, 7600–7606.

Zhao, C., Matthews, J., Tujague, M., Wan, J., Ström, A., Toresson, G., Lam, E. W., Cheng, G., Gustafsson, J. A., & Dahlman-Wright, K. (2007). Estrogen receptor β2 negatively regulates the transactivation of estrogen receptor α in human breast cancer cells. *Cancer Research*, *67*, 3955–3962.

CHAPTER

75

Δ^9-THC and COX-2 Signaling

J. Zhang, C. Chen

Neuroscience Center of Excellence, School of Medicine, Louisiana State University Health Sciences Center, New Orleans, LA, United States

SUMMARY POINTS

- This chapter briefly describes the role of cyclooxygenase-2 (COX-2) in THC-induced synaptic and cognitive impairments, and other behavioral changes.
- COX-2 is a constitutively expressed and inducible enzyme converting arachidonic acid to prostaglandins.
- THC includes CB1 receptor-dependent increases in activity and expression of COX-2 in the brain.
- THC-increased COX-2 expression is mediated through CB1 receptor-coupled G-protein βγ subunits and downstream NF-κB signaling pathway.
- Pharmacological or genetic inhibition of COX-2 prevents disruptive effects of repeated THC exposure on synaptic structure and function, spatial learning, and memory.
- Medical use of marijuana would be broadened by concurrent COX-2 inhibition, which eliminates the major adverse effects of THC, while retaining cannabinoid beneficial effects.

KEY FACTS OF CANNABIS-INDUCED MAJOR ADVERSE EFFECTS

- Repeated or heavy use of marijuana induces cannabis-related disorders, including adverse physical, mental, emotional, and behavioral changes.
- Major intoxication of cannabis brings impairments in synaptic function, learning, and memory.
- Repeated use of marijuana induces tolerance, dependence, and possibly addiction.
- Abuse of marijuana is often associated with taking other addictive substances.

KEY FACTS OF POTENTIAL MEDICAL CANNABIS USE

- Chronic pain
- Epilepsy
- Inflammatory and neurodegenerative diseases (eg, multiple sclerosis, Alzheimer disease)
- Weight loss and poor appetite
- Nausea
- Cancer
- Sleep disorders
- Traumatic brain injury

LIST OF ABBREVIATIONS

AA	Arachidonic acid
2-AG	2-Arachidonoylglycerol
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
CB1R	Cannabinoid receptor 1
COX-2	Cyclooxygenase-2
CRD	Cannabis-related disorders
ERK	Extracellular signal-regulated kinase
FAAH	Fatty acid amide hydrolase
G-protein	GTP-binding protein
IL-1β	Interleukin-1β
LPS	Lipopolysaccharides
LTP	Long-term potentiation
MGL	Monoacylglycerol lipase
NF-κB	Nuclear factor-kappa B

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00086-7
Copyright © 2017 Elsevier Inc. All rights reserved.

NMDA	*N*-methyl-D-aspartic acid
NSAIDs	Nonsteroidal antiinflammatory drugs
p38 MAPK	p38 mitogen-activated protein kinase
PGD_2	Prostaglandin D_2
PGE_2	Prostaglandin E_2
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PGI_2	Prostacyclin
PLA_2	Phospholipase A_2
PLC	Phospholipase C
PTGS	Prostaglandin-endoperoxide synthase
THC	Δ^9-Tetrahydrocannabinol
TNFα	Tumor necrosis factor α
TXA_2	Thromboxanes

INTRODUCTION

Marijuana, a mixture of the leaves and flowering tops of the *Cannabis sativa* plant, has been used by humans for a few thousand years, as a recreational or medicinal drug. The chemical ingredients of cannabis were unknown until the 1960s, when a research group led by Professor Raphael Mechoulam identified Δ^9-tetrahydrocannabinol (THC) as the major psychoactive ingredient in marijuana (Gaoni & Mechoulam, 1971; Mechoulam & Gaoni, 1965). Even though humans have been using marijuana or cannabis as medical treatments for a wide array of intractable medical conditions (Robson, 2001; Russo, 2007), its neuropsychological adverse effects greatly limit the medicinal use of cannabis (Carlini, 2004). Marijuana is also the most commonly used illicit drug of abuse in the United States. The increasing spread of marijuana use, especially among middle and high school students, has heightened societal awareness of the risks associated with this drug. The use of marijuana is also associated with taking other addictive substances such as alcohol, cocaine, morphine, or nicotine (Maldonado, Valverde, & Berrendero, 2006). Use of marijuana can produce adverse physical, mental, emotional, and cognitive changes, which are termed cannabis-related disorders (CRD). Like other abused drugs, repeated administration of marijuana induces tolerance, dependence, and addiction (Bass & Martin, 2000; Gonzalez, Cebeira, & Fernandez-Ruiz, 2005; Lichtman & Martin, 2005; Lupica, Riegel, & Hoffman, 2004). Neuropsychological and memory deficits induced by short-term or long-term THC exposure are the major effects of intoxication with cannabinoids, confirmed both in humans and laboratory animals (Bolla, Brown, Eldreth, Tate, & Cadet, 2002; Chen et al., 2013; Hampson & Deadwyler, 1999; Han et al., 2012; Heishman, Arasteh, & Stitzer, 1997; Lichtman & Martin, 1996; Messinis, Kyprianidou, Malefaki, & Papathanasopoulos, 2006; Niyuhire, Varvel, Martin, & Lichtman, 2007; Pope, Gruber, Hudson, Huestis, & Yurgelun-Todd, 2002; Puighermanal et al., 2009; Solowij & Battisti, 2008; Solowij et al., 2002). For a long time, it was not clear how cannabinoids induce these unwanted side effects, and what signaling molecules mediate these effects. Given the extent of the use of cannabis in the general population as a recreational drug, and in clinic as medical treatments, it is imperative to understand the molecular mechanisms contributing to the major side effects produced by cannabinoid exposure. In this chapter, we will primarily discuss the recent progress toward our understanding of signaling molecules that mediate the cannabinoid-induced undesirable side effects.

PGE_2 IS A SIGNALING MOLECULE IN THC-INDUCED BEHAVIORAL RESPONSES

Hypomotility, analgesia, hypothermia, and catalepsy are physiological and behavioral responses to cannabinoids, and are termed "tetrad effects." However, little is known about signaling molecules that mediate these responses. Earlier studies show that the cataleptic effect induced by oral or intraperitoneal administration of THC in mice was blocked by aspirin or indomethacin, which are nonsteroidal antiinflammatory drugs (NSAIDs), and was restored by exogenous application of prostaglandin E_2 (PGE_2), but not $PGF_{2\alpha}$ or PGE_1 (Fairbairn & Pickens, 1979). However, aspirin failed to abolish the cataleptic effect of chlorpromazine (CPZ), a dopamine antagonist. This study provides evidence that the THC-elicited cataleptic response is associated with PGE_2, while the CPZ-induced catalepsy is related to different signaling mediator(s). This assumption was supported by another study where immunization of mice with a thyroglobulin-PGE_2 conjugate eliminates the cataleptic response of THC (Burstein, Hull, Hunter, & Shilstone, 1989). In particular, it was observed that plasma levels of PGE_2 and prostacyclin (PGI_2) were significantly elevated after oral administration of THC, and also blocked by indomethacin. Elevation of plasma PGE_2 by THC was confirmed in human subjects who smoked marijuana, and the elevation was also shown to be inhibited by pretreatment with indomethacin (Perez-Reyes, Burstein, White, McDonald, & Hicks, 1991). Importantly, PGE_2 levels are also elevated in the brains of rats and mice exposed to THC, and this elevation can be antagonized by NSAIDs (Bhattacharya, 1986; Hunter, Audette, & Burstein, 1991). In addition, it has been reported that THC-induced neurotoxicity in cultured hippocampal neurons was prevented by indomethacin or aspirin (Chan, Hinds, Impey, & Storm, 1998). These earlier studies provide information that cannabinoids trigger formation of PGE_2, resulting in an elevation of PGE_2 levels in the plasma and brain (Fig. 75.1). Particularly, THC-

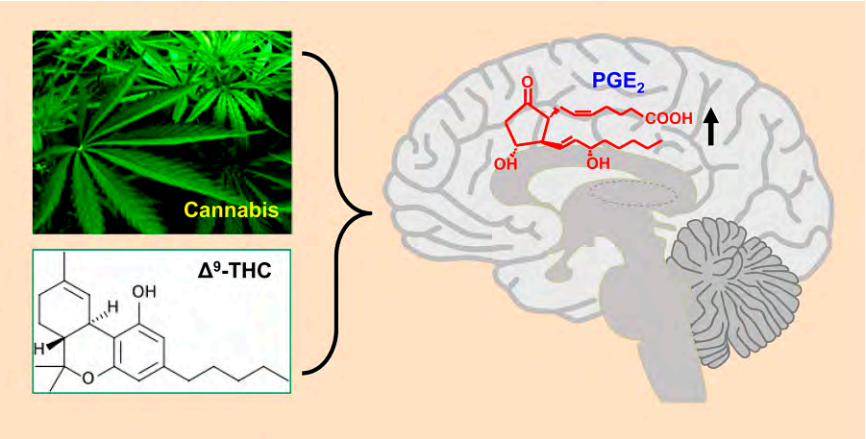


FIGURE 75.1 **Cannabis or Δ^9-tetrahydrocannabinol (THC) exposure increases synthesis of prostaglandin E_2 (PGE_2) in the brain.**

induced cataleptic effects and behavioral suppression in animals, and marijuana smoking-induced "high" and heart rate acceleration in humans are attenuated by NSAIDs (Burstein, Hull, Hunter, & Latham, 1988; Fairbairn & Pickens, 1979; Perez-Reyes et al., 1991; Yamaguchi, Shoyama, Watanabe, & Yamamoto, 2001), suggesting that PGE_2 is likely a signaling mediator for these behavioral responses to cannabinoids.

THC-ELEVATED PGE_2 IS DERIVED FROM THE COX-2 PATHWAY

THC or marijuana elevates the plasma and brain levels of PGE_2 both in animals and humans, and the elevation of PGE_2 is blocked by aspirin or indomethacin. Together, this suggests that cyclooxygenase-1 and -2 (COX-1 and COX-2) are likely involved in cannabinoid-induced responses. As shown in Fig. 75.2, COX-1 and COX-2 are the rate-limiting enzymes that catalyze the first two steps converting arachidonic acid (AA) to prostanoids (Sang & Chen, 2006; Smith, Urade, & Jakobsson, 2011; Vane, Bakhle, & Botting, 1998). While COX-1 is constitutively expressed everywhere in our body, and responsible for the production of prostanoids needed for complex integrative physiological functions, COX-2 is an inducible isoform expressed in all the tissues, and is responsible for injury and inflammation (Sang & Chen, 2006; Vane et al., 1998). Interestingly, COX-2 is also constitutively expressed in only a few organs, including the central nervous system (CNS), the kidney, and the testis, indicating the importance of COX-2 in these tissues. In the CNS, constitutively-expressed COX-2 is restricted to the cortex, hippocampus, hypothalamus, and spinal cord (Vane et al., 1998), suggesting that two forms of COX-2 are present in these regions of the CNS.

Commonly used NSAIDs, such as ibuprofen, aspirin, indomethacin and naproxen are nonselective COX-1 and COX-2 inhibitors. Also, as illustrated in Fig. 75.2, both COX-1 and COX-2 are capable of converting AA into prostanoids, including prostaglandins (PGD_2, PGE_2, $PGF_{2\alpha}$), prostacyclin (PGI_2), and thromboxane (TX_{A2}). This suggests that PGE_2 stimulated by cannabinoids may be derived from COX-1 and/or COX-2. It has been documented that COX-1 and COX-2 exhibit their preference in synthesizing these prostanoids. For instance, COX-1 is primarily responsible for synthesizing PGD_2, $PGF_{2\alpha}$, and TX_{A2}, whereas COX-2 is predominant in making PGE_2 and PGI_2 (Brock, McNish, & Peters-Golden, 1999; Sang, Zhang, Marcheselli, Bazan, & Chen, 2005). Therefore, PGE_2 produced by marijuana or THC is likely derived from the COX-2 pathway. This speculation has been confirmed by a recent study where THC-induced increase in PGE_2 in mouse hippocampal tissue is blocked by Celebrex (Celecoxib), a selective COX-2 inhibitor (Chen et al., 2013). Moreover, THC failed to increase hippocampal PGE_2 in COX-2 knockout animals. These results indicate that THC-triggered release of PGE_2 is through the COX-2 pathway. This conclusion was further supported by the fact that THC induces time- and dose-dependent increase of COX-2 expression, but not COX-1, in the mouse brain (Chen et al., 2013). In addition, this study also reveals that COX-2 expression triggered by THC was more pronounced in an astroglial cell-enriched culture than that in a relatively pure neuronal culture, suggesting that THC-induced released PGE_2 occurs largely from astroglial cells. The results generated from this study provided the compelling evidence that marijuana- or THC-triggered generation of PGE_2 is derived from the COX-2 pathway. This compelling evidence fills a conceptual gap on origination of PGE_2 induced by cannabinoids.

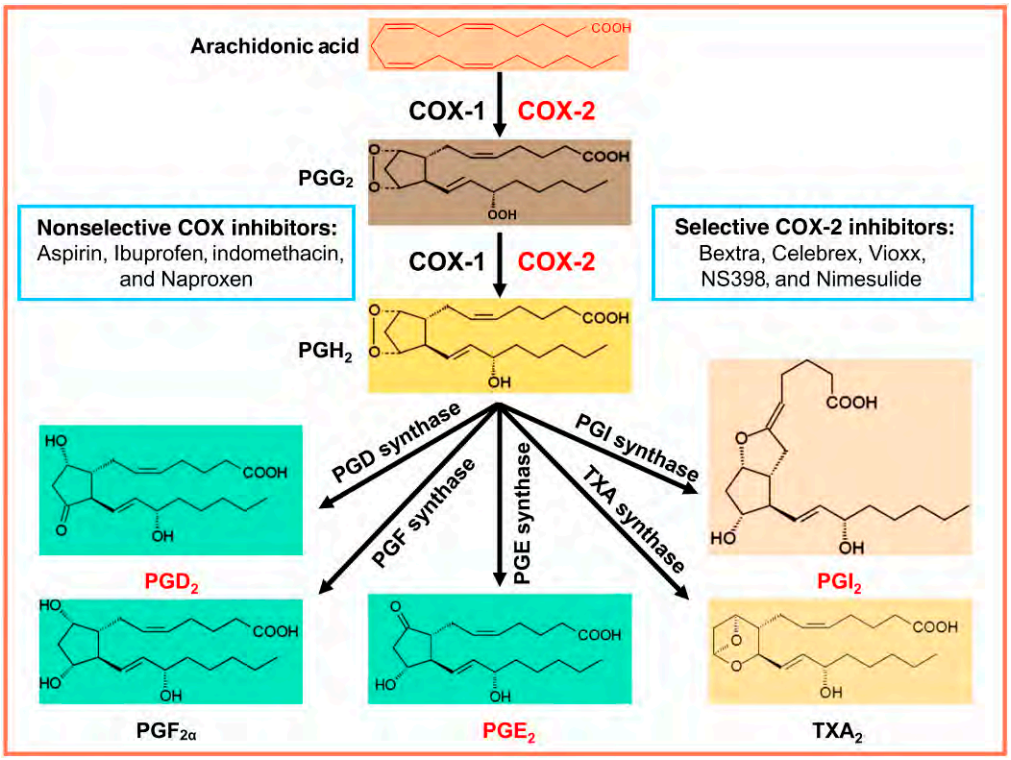


FIGURE 75.2 **Biosynthesis of prostanoids.** Prostanoids consist of prostaglandins (PGD_2, PGE_2, and $PGF_{2\alpha}$), thromboxane (TXA_2), and prostacyclin (PGI_2). Prostanoids are synthesized from the precursor arachidonic acid (AA), derived from membrane phospholipids catalyzed by phospholipase C (PLC) and phospholipase A_2 (PLA_2). AA is converted to intermediate PGG_2, which is rapidly converted to unstable PGH_2 by prostaglandin-endoperoxide synthase (PTGS), also called cyclooxygenases, including COX-1 and COX-2. PGH_2 is then immediately transformed to a series of biologically active PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and TXA_2 through specific isomerases, including PGD, PGE, PGF, PGI, and TXA synthases.

COX-2 INDUCTION BY THC IS MEDIATED VIA THE CB1 RECEPTOR, AND ITS COUPLED Gβγ SUBUNITS

Two types of cannabinoid receptors (CB1R and CB2R) have been cloned and identified. It is clear now that neuropsychological and behavioral responses to cannabinoids are primarily mediated via CB1R, which is predominantly expressed in the CNS. For instance, marijuana- or THC-induced memory impairments, and cannabinoid tolerance and dependence, are mediated by CB1R (Gonzalez et al., 2005; Hampson & Deadwyler, 1999; Han et al., 2012; Lichtman & Martin, 1996; Niyuhire et al., 2007; Puighermanal et al., 2009). As discussed earlier, COX-2 derived PGE_2 is an important signaling mediator in mediating some of the behavioral responses to cannabinoids, suggesting that THC-induced increase in COX-2 expression may be mediated by CB1R. Indeed, Chen et al. (2013) reported that THC-increased expression of COX-2 in the brain is diminished by rimonabant (SR141716), a selective CB1R antagonist, and in mice lacking CB1R (Chen et al., 2013), suggesting that THC-induced increases in COX-2 activity and expression is mediated by CB1R.

It has been reported previously that the endogenous cannabinoid 2-arachidonoylglycerol (2-AG) suppresses COX-2 expression in response to proinflammatory and excitotoxic insults, both in vitro and in vivo, via a CB1R-dependent mechanism (Zhang & Chen, 2008). This brought up a question of why the same CB1R exhibits different, even opposite, effects on COX-2 expression upon binding of the exogenous cannabinoid THC or the endocannabinoid 2-AG to the receptors. CB1R is a pertussis toxin (PTX)-sensitive GTP-binding protein-coupled receptor. Activation of CB1R by agonists results in a dissociation of Gβγ subunits from the GTP-bound Gαi subunit. It is possible that differential regulation of COX-2 expression by CB1R results from the functioning of Gαi or Gβγ. To validate this assumption, Chen et al. (2013) designed elegant experiments in which they used several approaches, including overexpressing, silencing, or rescuing silenced Gαi or Gβγ, and found that COX-2 induction by THC is mediated via Gβγ subunits (Fig. 75.3), while 2-AG suppression of elevated COX-2 expression in response to proinflammatory stimuli is mediated via Gαi subunit (Fig. 75.4). Interestingly, disruption of Gαi does not affect the COX-2 induction effect of THC, while disruption of Gβγ does not change

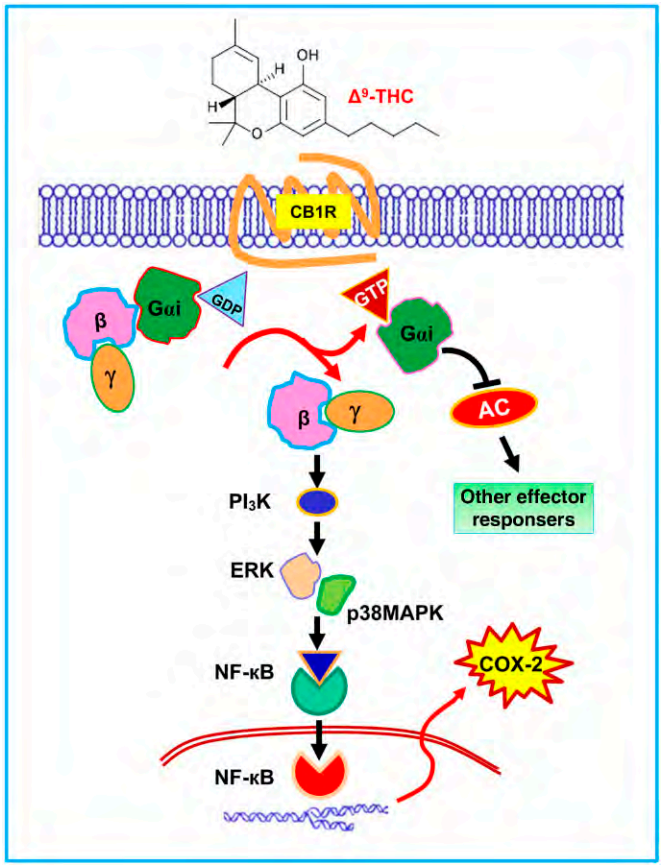


FIGURE 75.3 **Δ⁹-THC stimulates COX-2 induction via CB1R-coupled G-protein βγ subunits.** THC binds to and activates CB1R, which leads to dissociating CB1R-coupled G-protein βγ subunits from the GTP-bound Gαi subunit. Gβγ subunits induce phosphorylation of Akt, ERK/p38MAPK, and NF-κB, resulting in transcription of the COX-2 gene (ptgs2). GTP-bound α subunit may induce other effector responses.

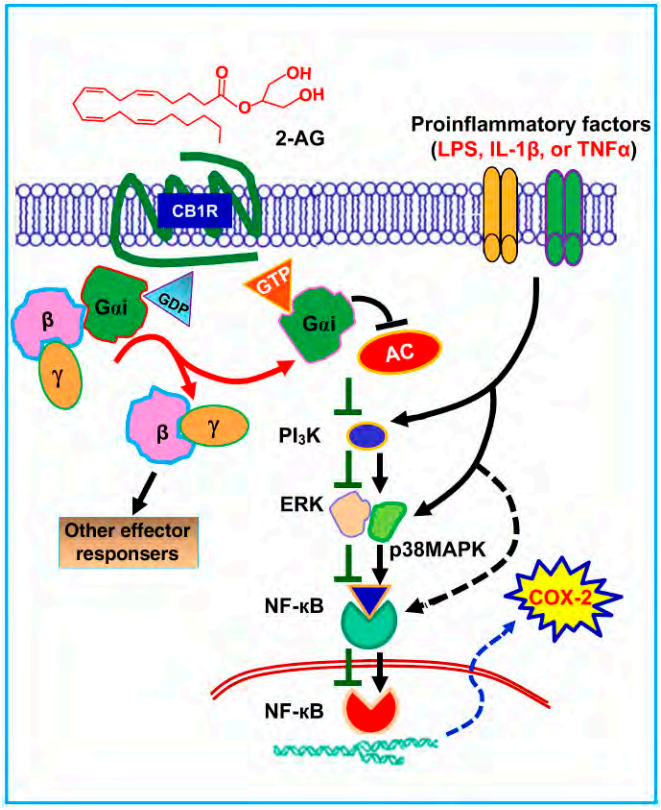


FIGURE 75.4 **The endocannabinoid 2-arachidonoylglycerol (2-AG) suppresses COX-2 overexpression induced by proinflammatory factors via CB1R-coupled G-protein αi subunit.** Proinflammatory factors interleukin-1β (IL-1β), tumor necrosis factor α (TNFα) or lipopolysaccharides (LPS) increase COX-2 expression through phosphorylation of PI_3K, ERK/p38MAPK, and NF-κB. 2-AG binds to and activates G-protein coupled CB1R, resulting in a dissociation of the Gαi subunit from Gβγ subunits. GTP-bound Gαi subunit inhibits proinflammatory stimulus-induced phosphorylation of PI_3K, ERK/p38MAPK, and NF-κB, thereby suppressing COX-2 expression.

the ability of 2-AG to resolve excessive COX-2 expression induced by a proinflammatory assault. Thus, it is evident that THC-induced COX-2 activation and expression are mediated through CB1R-coupled Gβγ subunits (Fig. 75.3).

THC-STIMULATED COX-2 EXPRESSION IS VIA ACTIVATION OF NF-κB

Nuclear factor kappa B (NF-κB) is an important transcription factor that regulates the genes involved in inflammation, including COX-2. It is possible that THC-elevated COX-2 expression is transcriptionally regulated by NF-κB. This speculation is supported by the fact that THC increases phosphorylation of NF-κB, and the binding activity of NF-κB p65 in the promoter region of the COX-2 gene, and that elevation of COX-2 expression induced by THC is reduced by inhibition of NF-κB activity (Chen et al., 2013). The signaling mediators of THC-stimulated COX-2 expression between Gβγ subunits and NF-κB are likely Akt, ERK, and p38MAPK. This is largely due to that fact that THC induces phosphorylation of Akt, ERK, and p38MAPK both in vitro and in vivo. The data from the experiments with silencing and rescuing strategies confirm that this is the case. Knockdown of Gβγ prevents THC-induced phosphorylation of Akt, ERK, p38MAPK, and NF-κB, and the effects of Gβγ loss of function on phosphorylation of these signaling molecules are diminished when Gβγ is rescued from silencing (Chen et al., 2013). Therefore, the COX-2 induction effect by THC is likely mediated through CB1R-coupled Gβγ subunits, and the downstream Akt, ERK, p38MAPK, and NF-κB signaling pathway (Fig. 75.3).

THC RAISES EXTRACELLULAR GLUTAMATE THROUGH COX-2 DERIVED PGE_2

Previous studies demonstrated that the cannabinoid THC and the synthetic cannabinoid WIN55,212-2 (WIN) elevate extracellular glutamate levels both in vitro and in vivo, via a CB1R-dependent mechanism (Ferraro et al., 2001; Tomasini et al., 2002). Prenatal exposure to WIN increases glutamate uptake in the front cerebral cortex of rats (Castaldo et al., 2007), indicating a long-lasting increase in the release of glutamate, after exposure to cannabinoids. The increased basal release probability of glutamatergic synaptic transmission has been observed in the cerebellum, after repeated injections of THC (Tonini et al., 2006). Increased extracellular glutamate levels following cannabinoid exposure are likely associated with the increased release of glutamate from presynaptic terminals and astroglial cells, or reduced uptake of glutamate by glutamate transporters (Chen et al., 2013; Fan, Yang, Zhang, & Chen, 2010; Ferraro et al., 2001; Han et al., 2012; Hoffman, Oz, Yang, Lichtman, & Lupica, 2007; Navarrete & Araque, 2010; Suarez et al., 2004b; Tomasini et al., 2002; Tonini et al., 2006). Expression of glutamate transporters is significantly reduced after repeated exposure to THC (Chen et al., 2013). The reduced expression of glutamate transporters will lead to a decrease in uptake of glutamate, resulting in accumulation of glutamate in an extracellular space. It has also been demonstrated previously that PGE_2 stimulates or facilitates both synaptic and astrocytic release of glutamate (Bezzi et al., 1998; Chen, Magee, & Bazan, 2002; Dave et al., 2010; Chen & Bazan, 2005; Sang et al., 2005; Sanzgiri, Araque, & Haydon, 1999). In particular, astroglial cells may be the primary source for COX-2 derived PGE_2, because THC-induced increase in COX-2 expression is more pronounced in astroglial-enriched culture than in relatively pure neuronal culture (Chen et al., 2013). Therefore, accumulated glutamate in extracellular compartments, after THC exposure, is associated with increased synthesis of PGE_2, which facilitates release of glutamate from synapses, and exocytosis of glutamate from astrocytes.

THC EXPOSURE-IMPAIRED SYNAPTIC PLASTICITY AND COGNITIVE FUNCTION ARE ASSOCIATED WITH REDUCED FUNCTIONAL GLUTAMATE RECEPTOR SUBUNITS, AND DENDRITIC SPINES

Long-term potentiation (LTP) at excitatory synapses is a long-lasting synaptic strength of glutamatergic transmission, and has been long recognized as a cellular model of learning and memory. Repeated THC exposure impairs hippocampal LTP via a CB1R-dependent mechanism (Chen et al., 2013; Fan et al., 2010; Hoffman et al., 2007), which may underlie marijuana- or THC-caused memory impairments. Synaptic and memory impairments by cannabis or THC are apparently associated with alterations in glutamatergic synaptic transmission, and functional expression of glutamate receptor subunits (Fan et al., 2010; Han et al., 2012; Hoffman et al., 2007; Suarez et al., 2004a; Tonini et al., 2006). Recent studies show that cannabinoid exposure leads to downregulation, internalization, and endocytosis of glutamate receptor subunits (Chen et al., 2013; Fan et al., 2010; Han et al., 2012). For instance, pre- and perinatal THC exposure downregulates the AMPA glutamate receptor subunits GluA1 and GluA2/3 in the rat cerebellum (Suarez et al., 2004a). Repeated exposure to THC in young adult mice significantly reduces total and surface expressions of AMPA glutamate receptor subunit GluA1, and NMDA receptor subunits GluN2A and GluN2B in the hippocampus, while the amount of these subunits in intracellular compartment are increased, suggesting that chronic cannabinoid exposure not only reduces expression of glutamate receptor subunits, but also changes receptor trafficking (Chen et al., 2013; Fan et al., 2010). While we still lack clearly defined evidence of how cannabinoid exposure decreases glutamate receptor expression and function, the reduced expression and trafficking of glutamate receptor subunits by THC are likely associated with elevated extracellular glutamate levels stimulated by COX-2 derived PGE_2 (Fig. 75.5). This is supported by the fact that pharmacological or genetic inhibition of COX-2 prevents alterations in expression of glutamate receptor subunits induced by chronic treatment with THC (Chen et al., 2013). Repeated cannabinoid exposure induces persistent elevation of COX-2 expression and PGE_2 formation, which facilitates glutamate release and increases extracellular glutamate accumulation, leading to downregulation and internalization of glutamate receptor subunits. One recent study provides evidence that this is the case by showing that COX-2 expression is steadily elevated in the hippocampus from mice that received THC for 7 days (Chen et al., 2013). This information suggests that reduced functional expression of glutamate receptor subunits, upon repeated THC exposure, is associated with sustained elevation of COX-2, and its derived PGE_2, which stimulates glutamate release. This may be a homeostatic mechanism in response to elevated extracellular glutamate levels.

Dendritic spines, tiny protrusions on dendrites, are a specialized structure where synapses are localized. It is clear now that the density and geometry of dendritic spines are critical for functioning of glutamatergic synaptic transmission and plasticity (Nimchinsky, Sabatini, & Svoboda, 2002). Recent evidence shows that adolescent chronic treatment with THC results in

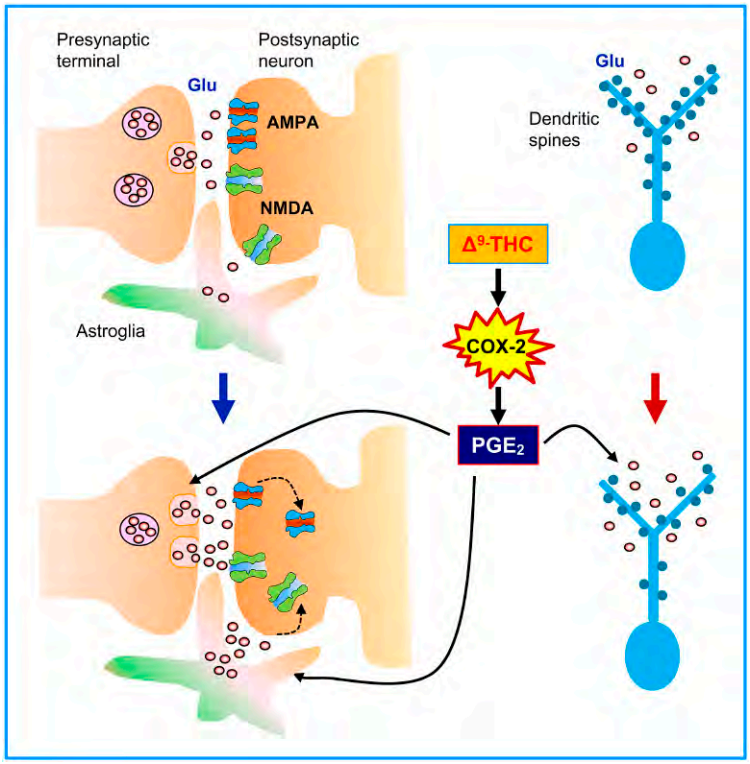


FIGURE 75.5 **Δ^9-THC exposure alters expression and trafficking of glutamate receptor subunits and density of dendritic spines.** COX-2 derived PGE_2 stimulated by THC facilitates release of excitatory neurotransmitter glutamate from presynaptic terminals, and exocytosis of glutamate from astroglial cells. Also, THC-induced suppression of glutamate transporters reduces uptake of glutamate from extracellular space. Accumulated extracellular glutamate causes downregulation, internalization, or endocytosis of AMPA and NMDA glutamate receptor subunits, and decreases density of dendritic spines and PSD-95.

reduced density of dendritic spines, and it lowers the length and number of dendrites in the hippocampus (Rubino et al., 2009). This suggests that changes in dendritic morphology, including size, density, and shape, are important in synaptic and cognitive impairments, following chronic THC exposure. A recent study by Chen et al. (2013) provides convincing evidence that density of dendritic spines is reduced in hippocampal CA1 pyramidal neurons in animals repeatedly exposed to THC. In particular, THC significantly reduces density of mushroom spines, where functional AMPA and NMDA receptors are expressed. Reduced postsynaptic density protein 95 (PSD-95), a postsynaptic marker, in hippocampal neurons of THC-exposed animals further confirms the decreased density of dendritic spines. The changes in dendritic spine density and PSD-95 by THC are blunted in COX-2 inhibitor Celebrex-treated animals, or in COX-2 knockout mice. While reduced expression of functional AMPA and NMDA glutamate receptor subunits, and density of dendritic spines after repeated exposure to THC, may underlie cannabis-caused memory impairments, these deficits in synaptic and cognitive function induced by THC are likely mediated via COX-2 signaling. This assumption is supported by the experimental results showing that impairments in fear memory, spatial learning, and memory induced by chronic THC exposure are prevented by inhibition of COX-2 with selective COX-2 inhibitors, or in mice deficient in COX-2 (Chen et al., 2013). Moreover, it has been demonstrated that a single injection of THC impairs synaptic and cognitive function. For example, a single in vivo exposure to THC has been demonstrated to be sufficient to induce changes in synaptic plasticity, both in the hippocampus and nucleus accumbens, and deficits in working memory (Han et al., 2012; Mato et al., 2004; Puighermanal et al., 2009). This also has been confirmed in a study by Chen et al. (2013), which provides evidence that long-term depression (LTD) of glutamatergic synaptic transmission in the hippocampus is impaired by a single injection of THC, and this effect is blocked by COX-2 inhibition. In addition, cataleptic and locomotion suppressive effects of THC are reduced by COX-2 inhibition. These experimental data suggest that COX-2 is involved in synaptic and memory impairments, as well as behavioral changes induced by both single and repeated exposure to THC. Apparently, marijuana or THC-caused synaptic and memory impairments are likely mediated through CB1R-coupled G protein βγ subunits, and their downstream PI3K, ERK/p38MAPK, NF-κB, and COX-2 signaling pathway (Fig. 75.6).

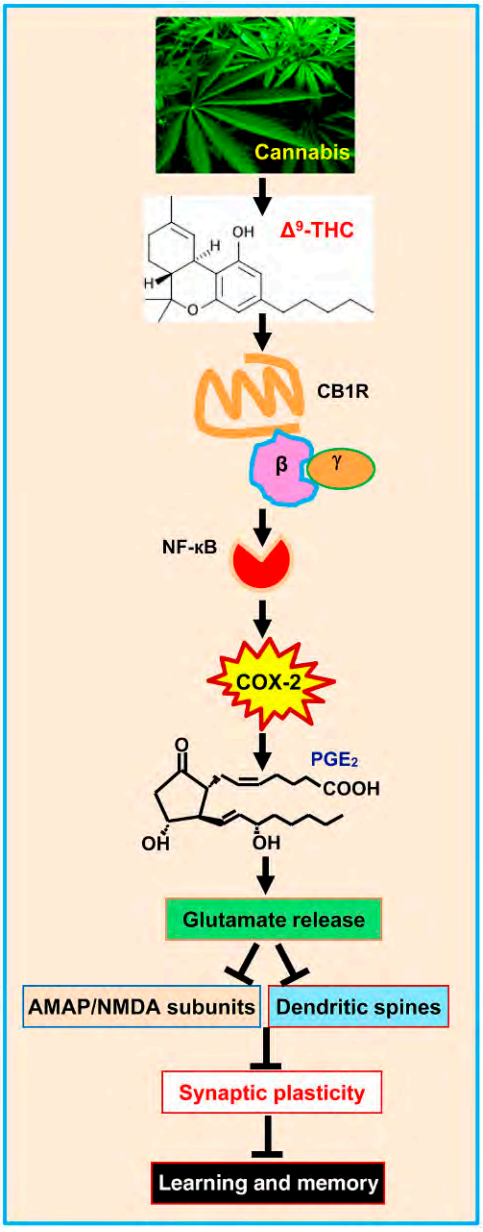


FIGURE 75.6 **Summary of cannabis exposure-impaired synaptic plasticity and memory.** Exposure to THC causes synaptic and cognitive impairments through CB1R-coupled Gβγ, NF-κB, and COX-2 signaling pathway. COX-2 derived PGE_2 increases extracellular glutamate accumulation, which decreases functional glutamate receptors and dendritic spine density. Alterations in structure and function of synapses impair synaptic plasticity, learning, and memory.

CONCLUSIONS

As stated previously, cannabis or marijuana has been used for thousands of years, as a recreational drug and for medical treatments. Heavy use of marijuana is known to induce cannabis-related disorders (CRD), including physical, neuropsychological, and cognitive changes. However, there are no FDA-approved medications available to prevent or treat CRD. Also, undesirable side effects of cannabinoids greatly limit the medical use of cannabis or THC (Marinol/Dronabinol, FDA-approved cannabinoid medicine). If COX-2 is a critical signaling mediator in THC-induced unwanted effects, inhibition of COX-2 with a COX-2 inhibitor may alleviate symptoms of CRD. In addition, concurrent inhibition of COX-2 would broaden the applicability of medical marijuana. In particular, selective COX-2 inhibitors, such as Celebrex, are currently used in clinical applications, and over-the-counter NSAIDs, such as ibuprofen and aspirin, are nonselective COX inhibitors. Thus, a combination of THC with a COX-2 inhibitor may provide a novel therapeutic intervention for preventing or treating some intractable medical conditions. Chen et al. (2013) provides initial evidence that THC is capable of reducing β-amyloid, a neuropathological hallmark of Alzheimer disease (AD), and neurodegeneration in an animal model of AD, showing that these beneficial effects are preserved when COX-2 is concurrently inhibited. This suggests that a cocktail intervention of THC and Celebrex may form a novel therapy for preventing and treating AD, or halting disease progression. Nevertheless, we still have a lot of unknowns about many other beneficial effects or therapeutic applications of cannabis or THC. Therefore, it is imperative to enhance marijuana research, particularly since legalization of marijuana use is currently spreading throughout the United States, and around the world.

MINI-DICTIONARY

Alzheimer disease (AD) A neurodegenerative disease characterized by deposition of β-amyloid, neuroinflammation, synaptic dysfunction, neurodegeneration, loss of memory, and dementia.
CB1 receptor A G-protein coupled cannabinoid type 1 receptor that mediates cannabinoid-induced biological responses.
Cyclooxygenase-2 (COX-2) An inducible enzyme that converts arachidonic acid to prostaglandins.
Long-term potentiation (LTP) A form of long-lasting synaptic strength.
Nonsteroidal antiinflammatory drugs (NSAIDs) Over-the-counter nonselective cyclooxygenase inhibitors.
Synapse A point of contact between nerve cells for transmitting chemical or electrical signals from one cell to another (cell-to-cell communication).
Δ9-Tetrahydrocannabinol (THC) The major psychoactive ingredient of cannabis.

Acknowledgments

This work was supported in part by National Institutes of Health grants R01NS054886 and R01NS076815.

References

Bass, C. E., & Martin, B. R. (2000). Time course for the induction and maintenance of tolerance to Delta(9)-tetrahydrocannabinol in mice. *Drug and Alcohol Dependence*, *60*(2), 113–119.

Bezzi, P., Carmignoto, G., Pasti, L., Vesce, S., Rossi, D., Rizzini, B. L., …, Volterra, A. (1998). Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. *Nature*, *391*(6664), 281–285.

Bhattacharya, S. K. (1986). Delta-9-tetrahydrocannabinol (THC) increases brain prostaglandins in the rat. *Psychopharmacology (Berlin)*, *90*(4), 499–502.

Bolla, K. I., Brown, K., Eldreth, D., Tate, K., & Cadet, J. L. (2002). Dose-related neurocognitive effects of marijuana use. *Neurology*, *59*(9), 1337–1343.

Brock, T. G., McNish, R. W., & Peters-Golden, M. (1999). Arachidonic acid is preferentially metabolized by cyclooxygenase-2 to prostacyclin and prostaglandin E2. *Journal of Biological Chemistry*, *274*(17), 11660–11666.

Burstein, S. H., Hull, K., Hunter, S. A., & Latham, V. (1988). Cannabinoids and pain responses: a possible role for prostaglandins. *FASEB Journal*, *2*(14), 3022–3026.

Burstein, S. H., Hull, K., Hunter, S. A., & Shilstone, J. (1989). Immunization against prostaglandins reduces delta 1-tetrahydrocannabinol-induced catalepsy in mice. *Molecular Pharmacology*, *35*(1), 6–9.

Carlini, E. A. (2004). The good and the bad effects of (−) trans-delta-9-tetrahydrocannabinol (Delta 9-THC) on humans. *Toxicon*, *44*(4), 461–467.

Castaldo, P., Magi, S., Gaetani, S., Cassano, T., Ferraro, L., Antonelli, T., …, Cuomo, V. (2007). Prenatal exposure to the cannabinoid receptor agonist WIN 55,212-2 increases glutamate uptake through overexpression of GLT1 and EAAC1 glutamate transporter subtypes in rat frontal cerebral cortex. *Neuropharmacology*, *53*(3), 369–378.

Chan, G. C., Hinds, T. R., Impey, S., & Storm, D. R. (1998). Hippocampal neurotoxicity of Delta9-tetrahydrocannabinol. *Journal of Neuroscience*, *18*(14), 5322–5332.

Chen, C., & Bazan, N. G. (2005). Endogenous PGE(2) regulates membrane excitability and synaptic transmission in hippocampal CA1 pyramidal neurons. *Journal of Neurophysiology*, *93*(2), 929–941.

Chen, C., Magee, J. C., & Bazan, N. G. (2002). Cyclooxygenase-2 regulates prostaglandin E-2 signaling in hippocampal long-term synaptic plasticity. *Journal of Neurophysiology*, *87*(6), 2851–2857.

Chen, R., Zhang, J., Fan, N., Teng, Z. Q., Wu, Y., Yang, H., …, Chen, C. (2013). Delta9-THC-caused synaptic and memory impairments are mediated through COX-2 signaling. *Cell*, *155*(5), 1154–1165.

Dave, K. A., Platel, J. C., Huang, F., Tian, D., Stamboulian-Platel, S., & Bordey, A. (2010). Prostaglandin E2 induces glutamate release from subventricular zone astrocytes. *Neuron Glia Biology*, *6*(3), 201–207.

Fairbairn, J. W., & Pickens, J. T. (1979). The oral activity of delta'-tetrahydrocannabinol and its dependence on prostaglandin E2. *British Journal of Pharmacology*, *67*(3), 379–385.

Fan, N., Yang, H., Zhang, J., & Chen, C. (2010). Reduced expression of glutamate receptors and phosphorylation of CREB are responsible for in vivo Delta9-THC exposure-impaired hippocampal synaptic plasticity. *Journal of Neurochemistry*, *112*(3), 691–702.

Ferraro, L., Tomasini, M. C., Gessa, G. L., Bebe, B. W., Tanganelli, S., & Antonelli, T. (2001). The cannabinoid receptor agonist WIN 55,212-2 regulates glutamate transmission in rat cerebral cortex: an in vivo and in vitro study. *Cerebral Cortex*, *11*(8), 728–733.

Gaoni, Y., & Mechoulam, R. (1971). The isolation and structure of delta-1-tetrahydrocannabinol and other neutral cannabinoids from hashish. *Journal of the American Chemical Society*, *93*(1), 217–224.

Gonzalez, S., Cebeira, M., & Fernandez-Ruiz, J. (2005). Cannabinoid tolerance and dependence: a review of studies in laboratory animals. *Pharmacology Biochemistry and Behavior*, *81*(2), 300–318.

Hampson, R. E., & Deadwyler, S. A. (1999). Cannabinoids, hippocampal function and memory. *Life Sciences*, *65*(6–7), 715–723.

Han, J., Kesner, P., Metna-Laurent, M., Duan, T., Xu, L., Georges, F., …, Zhang, X. (2012). Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. *Cell*, *148*(5), 1039–1050.

Heishman, S. J., Arasteh, K., & Stitzer, M. L. (1997). Comparative effects of alcohol and marijuana on mood, memory, and performance. *Pharmacology Biochemistry and Behavior*, *58*(1), 93–101.

Hoffman, A. F., Oz, M., Yang, R., Lichtman, A. H., & Lupica, C. R. (2007). Opposing actions of chronic Delta9-tetrahydrocannabinol and cannabinoid antagonists on hippocampal long-term potentiation. *Learning and Memory*, *14*(1–2), 63–74.

Hunter, S. A., Audette, C. A., & Burstein, S. (1991). Elevation of brain prostaglandin E2 levels in rodents by delta 1-tetrahydrocannabinol. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *43*(3), 185–190.

Lichtman, A. H., & Martin, B. R. (1996). Delta 9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. *Psychopharmacology (Berlin)*, *126*(2), 125–131.

Lichtman, A. H., & Martin, B. R. (2005). Cannabinoid tolerance and dependence. *Handbook of Experimental Pharmacology*, *168*, 691–717.

Lupica, C. R., Riegel, A. C., & Hoffman, A. F. (2004). Marijuana and cannabinoid regulation of brain reward circuits. *British Journal of Pharmacology*, *143*(2), 227–234.

Maldonado, R., Valverde, O., & Berrendero, F. (2006). Involvement of the endocannabinoid system in drug addiction. *Trends in Neuroscience*, *29*(4), 225–232.

Mato, S., Chevaleyre, V., Robbe, D., Pazos, A., Castillo, P. E., & Manzoni, O. J. (2004). A single in-vivo exposure to delta 9THC blocks endocannabinoid-mediated synaptic plasticity. *Nature Neuroscience*, *7*(6), 585–586.

Mechoulam, R., & Gaoni, Y. (1965). A total synthesis of Dl-Delta-1-tetrahydrocannabinol, the active constituent of hashish. *Journal of the American Chemical Society*, *87*, 3273–3275.

Messinis, L., Kyprianidou, A., Malefaki, S., & Papathanasopoulos, P. (2006). Neuropsychological deficits in long-term frequent cannabis users. *Neurology*, *66*(5), 737–739.

Navarrete, M., & Araque, A. (2010). Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. *Neuron*, *68*(1), 113–126.

Nimchinsky, E. A., Sabatini, B. L., & Svoboda, K. (2002). Structure and function of dendritic spines. *Annual Review of Physiology*, *64*, 313–353.

Niyuhire, F., Varvel, S. A., Martin, B. R., & Lichtman, A. H. (2007). Exposure to marijuana smoke impairs memory retrieval in mice. *Journal of Pharmacology and Experimental Therapeutics*, *322*(3), 1067–1075.

Perez-Reyes, M., Burstein, S. H., White, W. R., McDonald, S. A., & Hicks, R. E. (1991). Antagonism of marihuana effects by indomethacin in humans. *Life Science*, *48*(6), 507–515.

Pope, H. G., Jr., Gruber, A. J., Hudson, J. I., Huestis, M. A., & Yurgelun-Todd, D. (2002). Cognitive measures in long-term cannabis users. *Journal of Clinical Pharmacology*, *42*(11 Suppl.), 41S–47S.

Puighermanal, E., Marsicano, G., Busquets-Garcia, A., Lutz, B., Maldonado, R., & Ozaita, A. (2009). Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling. *Nature Neuroscience*, *12*(9), 1152–1158.

Robson, P. (2001). Therapeutic aspects of cannabis and cannabinoids. *British Journal of Psychiatry*, *178*, 107–115.

Rubino, T., Realini, N., Braida, D., Guidi, S., Capurro, V., Vigano, D., …, Parolaro, D. (2009). Changes in hippocampal morphology and neuroplasticity induced by adolescent THC treatment are associated with cognitive impairment in adulthood. *Hippocampus*, *19*(8), 763–772.

Russo, E. B. (2007). History of cannabis and its preparations in saga, science, and sobriquet. *Chemistry and Biodiversity*, *4*(8), 1614–1648.

Sang, N., & Chen, C. (2006). Lipid signaling and synaptic plasticity. *Neuroscientist*, *12*(5), 425–434.

Sang, N., Zhang, J., Marcheselli, V., Bazan, N. G., & Chen, C. (2005). Postsynaptically synthesized prostaglandin E_2 (PGE_2) modulates hippocampal synaptic transmission via a presynaptic PGE2 EP2 receptor. *Journal of Neuroscience*, *25*(43), 9858–9870.

Sanzgiri, R. P., Araque, A., & Haydon, P. G. (1999). Prostaglandin E(2) stimulates glutamate receptor-dependent astrocyte neuromodulation in cultured hippocampal cells. *Journal of Neurobiology*, *41*(2), 221–229.

Smith, W. L., Urade, Y., & Jakobsson, P. J. (2011). Enzymes of the cyclooxygenase pathways of prostanoid biosynthesis. *Chemical Reviews*, *111*(10), 5821–5865.

Solowij, N., & Battisti, R. (2008). The chronic effects of cannabis on memory in humans: a review. *Current Drug Abuse Reviews*, *1*(1), 81–98.

Solowij, N., Stephens, R. S., Roffman, R. A., Babor, T., Kadden, R., Miller, M., …, Marijuana Treatment Project Research Group. (2002). Cognitive functioning of long-term heavy cannabis users seeking treatment. *JAMA*, *287*(9), 1123–1131.

Suarez, I., Bodega, G., Fernandez-Ruiz, J., Ramos, J. A., Rubio, M., & Fernandez, B. (2004a). Down-regulation of the AMPA glutamate receptor subunits GluR1 and GluR2/3 in the rat cerebellum following pre- and perinatal delta9-tetrahydrocannabinol exposure. *Cerebellum*, *3*(2), 66–74.

Suarez, I., Bodega, G., Rubio, M., Fernandez-Ruiz, J. J., Ramos, J. A., & Fernandez, B. (2004b). Prenatal cannabinoid exposure down-regulates glutamate transporter expressions (GLAST and EAAC1) in the rat cerebellum. *Developmental Neuroscience*, *26*(1), 45–53.

Tomasini, M. C., Ferraro, L., Bebe, B. W., Tanganelli, S., Cassano, T., Cuomo, V., & Antonelli, T. (2002). Delta(9)-tetrahydrocannabinol increases endogenous extracellular glutamate levels in primary cultures of rat cerebral cortex neurons: involvement of CB(1) receptors. *Journal of Neuroscience Research*, *68*(4), 449–453.

Tonini, R., Ciardo, S., Cerovic, M., Rubino, T., Parolaro, D., Mazzanti, M., & Zippel, R. (2006). ERK-dependent modulation of cerebellar synaptic plasticity after chronic Delta9-tetrahydrocannabinol exposure. *Journal of Neuroscience*, *26*(21), 5810–5818.

Vane, J. R., Bakhle, Y. S., & Botting, R. M. (1998). Cyclooxygenases 1 and 2. *Annual Review of Pharmacology and Toxicology*, *38*, 97–120.

Yamaguchi, T., Shoyama, Y., Watanabe, S., & Yamamoto, T. (2001). Behavioral suppression induced by cannabinoids is due to activation of the arachidonic acid cascade in rats. *Brain Research*, *889*(1–2), 149–154.

Zhang, J., & Chen, C. (2008). Endocannabinoid 2-arachidonoylglycerol protects neurons by limiting COX-2 elevation. *Journal of Biological Chemistry*, *283*(33), 22601–22611.

CHAPTER

76

Cannabinoids and the Addictive Effects of Nicotine

L.V. Panlilio, S.R. Goldberg

Preclinical Pharmacology Section, Behavioral Neuroscience Branch, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD, United States

SUMMARY POINTS

- THC and nicotine (in the forms of cannabis and tobacco, respectively) are commonly used together.
- These drugs affect reward-related brain circuits that partially overlap and interact.
- These interactions could be responsible for promoting addiction in co-users of THC and nicotine.
- Interaction between the endogenous cannabinoid and nicotinic acetylcholine systems might also provide new avenues for the treatment of addiction.
- In animal models of drug abuse, simultaneous administration of THC and nicotine can enhance the rewarding effects of nicotine.
- A history of THC exposure can also increase nicotine reward making rats more likely to start taking nicotine and to become highly persistent in seeking nicotine.
- Cannabinoid antagonists, which bind to cannabinoid receptors but do not produce THC-like effects, decrease nicotine use in humans and animals, but are unsuitable as medications due to adverse side effects.
- Drugs that activate peroxisome proliferator-activated receptors have beneficial effects in animal models of nicotine reward and relapse, and some of these drugs are already in use to treat lipid disorders in humans.
- Drugs that reduce the effects of acetylcholine at alpha-7 nicotinic receptors block THC reward and relapse to THC seeking in nonhuman primates.

KEY FACTS OF BEHAVIORAL MODELS OF NICOTINE REWARD

- Drug self-administration and conditioned place-preference are the main models of nicotine reward.
- Drug self-administration—in which the animal is allowed to take nicotine by pressing a lever—provides the clearest evidence of rewarding effects, has high face validity, and is the most homologous animal model of human drug use.
- Since smoke and the bitter taste of most drugs are aversive to animals, self-administered nicotine is delivered through an intravenous catheter.
- Conditioned place-preference—in which nicotine is given in a distinctive environment, and the animal is later tested to see if it prefers to be in that environment—is a less direct measure of reward, but is widely used because it is easier to perform; usually the drug is injected intraperitoneally or subcutaneously once per day, so catheter implantation and maintenance are not required.
- Conditioned place-preference is well-suited to making comparisons between groups, for example, when irreversible treatments such as genetic manipulations are being studied.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00087-9
2017 Published by Elsevier Inc.

- Drug self-administration is well-suited for making within-subject comparisons, such as varying the dose of the self-administered drug, or a treatment drug.
- With both conditioned place-preference and drug self-administration, relapse can be modeled by discontinuing delivery of the drug until the learned drug-seeking behavior ceases, then presenting triggers that reinstate the behavior. Reinstatement is induced in animals by the same kinds of triggers that induce relapse in humans: reexposure to the drug, reexposure to environmental cues previously associated with the drug, and exposure to stress.

LIST OF ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
CB_1	Type-1 cannabinoid receptor
FAAH	Fatty acid amide hydrolase
OEA	Oleoylethanolamine
PEA	Palmitoylethanolamide
PPARs	Peroxisome proliferator-activated receptors
THC	Δ^9-Tetrahydrocannabinol
URB597	A FAAH inhibitor
WIN55,212-2	A synthetic cannabinoid agonist

INTRODUCTION

Cannabis and tobacco are commonly used together. Besides sharing similar routes of administration, these drugs have certain effects that overlap and interact in the brain, and their simultaneous or sequential use might increase the likelihood of developing addiction. Information about interactions between these drugs comes from two general sources, human research and animal research. Human research (Agrawal, Budney, & Lynskey, 2012; Meier & Hatsukami, 2016) is clearly most relevant to human drug use. However, animal models of drug use also have distinct advantages, such as the ability to maintain a higher level of control over experimental variables, and to use neuroscience techniques that are not feasible in humans. The present article will focus on information obtained with animals, using procedures that model processes relevant to cannabinoid drugs and nicotine addiction in humans.

It is well-known that the main psychoactive constituents of cannabis and tobacco are Δ^9-tetrahydrocannabinol (THC) and nicotine, respectively. The abuse-related effects of THC are mediated by its actions at type-1 cannabinoid receptors (CB_1) in the brain, while nicotine's effects are mediated by its actions at nicotinic acetylcholine receptors. Cannabinoid receptors normally bind endogenous substances, including anandamide and 2-arachidonoylglycerol (2-AG), as part of the endocannabinoid system. Nicotinic receptors normally bind endogenous acetylcholine. Most of the research to be described here has used purified forms of nicotine and THC, or synthetic drugs that act on the same brain systems as these drugs.

Like all addictive drugs, THC and nicotine have rewarding effects in the brain that lead the user to seek out and use the drug again in the future. Rewarding effects are believed to depend largely on increased activity of neurons that release the neurotransmitter dopamine in the nucleus accumbens, part of the reward system of the brain. Most addiction-related studies of how THC and nicotine interact in the brain have involved looking at this reward system by measuring dopamine levels, the density and functionality of cannabinoid and dopamine receptors, and the firing of dopamine cells.

The following sections will describe findings from animal research relevant to tobacco addiction, including evidence that simultaneous use of THC and nicotine enhances the rewarding effects of nicotine, and that THC exposure has lasting effects that increase the rewarding effects of nicotine taken at a later date. On a more positive note, we will also describe findings suggesting that pharmacological manipulations of the endogenous cannabinoid system might have beneficial effects that promote abstinence from tobacco, and vice versa: that pharmacological manipulations of the endogenous nicotinic system might promote abstinence from cannabis.

SIMULTANEOUS THC AND NICOTINE USE

Many simultaneous users report that they combine tobacco and cannabis to enhance the effects of cannabis. In addition, there are probably many simultaneous users who take each drug for its unique effects. While users can become dependent on cannabis (as defined by the *Diagnostic and Statistical Manual, 4th edition*), tobacco is generally considered more addictive, and complications stemming from its use are the eventual cause of death in a substantial percentage of smokers. So, it could be highly beneficial to determine whether (and how) combinations of THC and nicotine interact to increase the likelihood or severity of nicotine addiction.

In an extensive early study in rats, (Pryor, Larsen, Husain, & Braude, 1978) it was found that—unlike cocaine and amphetamine, which reversed the motor depressant effects of THC—a subthreshold dose of nicotine combined with THC essentially abolished spontaneous locomotor activity. This depression was specific to exploratory behavior, since nicotine did not increase the effect of THC on a conditioned avoidance response. The interactive effect was also persistent; the combination of nicotine and THC

continued to suppress spontaneous locomotion, even after tolerance had developed to the effects of THC alone.

More recently, combinations of THC and nicotine have been tested in rodent models of drug reward. In mice, Valjent, Mitchell, Besson, Caboche, and Maldonado (2002) found that combining subthreshold doses of these drugs had a rewarding effect in a conditioned place-preference procedure (Fig. 76.1). Scherma et al. (2012) observed a similar potentiation of conditioned place-preference when they combined subthreshold doses of THC and nicotine in rats, and they found that THC potentiated the motor-depressant effects of a low dose of nicotine. Scherma et al. (2012) also found that THC did not alter the place-preference produced by a higher dose of nicotine, possibly due to a ceiling effect. Valjent et al. (2002) found that the combination of THC and nicotine had a potentiating effect on neuronal activity on several brain areas highly innervated by dopamine inputs, although the combined effect was approximately additive in the nucleus accumbens shell. In the same study, nicotine also potentiated the anxiolytic effect of a low dose of THC in two models of anxiety, and it enhanced three of the classic effects of high doses of THC: hypothermia, antinociception, and hypolocomotion. The fact that most of these effects involve potentiation rather than simple additive effects of THC and nicotine suggests the cannabinoid and cholinergic systems interact in functional, meaningful ways.

Unfortunately, these few studies represent the extent of animal research into the reward-related effects of simultaneous THC and nicotine administration. This is likely due to the fact that, compared to opioids and psychomotor stimulants, the rewarding effects of cannabinoids and nicotine are difficult to study in rodents. In particular, self-administration of THC in rodents is not robust, and conditioned place-preference occurs only within a narrow range of doses. Rodents might be particularly sensitive to aversive effects of THC, such as anxiety, which counteract or mask rewarding effects. Robust models of THC self-administration have been developed in nonhuman primates, but they have yet to be applied in this area.

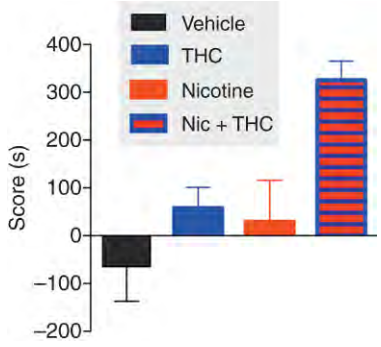


FIGURE 76.1 Rats expressed a preference for a place previously associated with a combination of a low dose of THC and a low dose of nicotine ("Nic + THC"), but not for a place associated with only vehicle, THC, or nicotine. Positive score (mean ± s.e.m.) indicates how much more time rats chose to spend in the treatment-associated compartment, compared to a control compartment. *Source: Adapted from Valjent et al. (2002). Used with permission.*

LASTING EFFECTS OF THC EXPOSURE

It has long been recognized that drug use tends to follow a certain progression over time, with alcohol and cannabis use initiated prior to use of drugs such as cocaine and heroin. This phenomenon is known as the gateway effect. There is also accumulating evidence for a reverse gateway effect, in which cannabis use develops before tobacco use, and increases the likelihood of tobacco addiction.

There has been much debate about the causes of gateway effects and, for many reasons, it is hard to draw strong conclusions about causality in human populations. However, animal models of drug abuse are well-suited for studying at least one factor that might contribute to gateway phenomena: drug exposure, per se. The animal's environment can be carefully controlled, and social influences such as use by conspecifics are not confounded with pharmacologic effects. The animals' drug history can also be precisely manipulated, for example by exposing them to THC during the perinatal or adolescent stage. Due to the difficulty of studying the rewarding effects of THC in rodents, gateway studies in rodents have focused on the ability of prior THC exposure to alter the rewarding effects of other drugs. These studies have primarily involved opioid self-administration, with only a few investigating cocaine, amphetamine, or nicotine self-administration.

The endocannabinoid system modulates dopamine signaling in brain circuits involved in reward, motor control, motivation, emotion, and cognition (Rodriguez de Fonseca et al., 2005). Exposure to THC produces not only immediate effects on the dopamine system, but also longer-lasting changes. In rats, exposure to THC can alter the expression and sensitivity of dopamine receptors after repeated prenatal (DiNieri et al., 2011) or perinatal exposure (Moreno, Trigo, Escuredo, Rodríguez de Fonseca, & Navarro, 2003), and even after a single exposure in adulthood (Gorriti et al., 2005). Perinatal exposure to THC can alter the activity of dopamine cells in reward-related brain areas during adulthood (González et al., 2003; Singh, McGregor, & Mallet, 2006). Subchronic exposure to THC (21 days) induced neurochemical and functional supersensitivity of dopamine receptors in the nucleus accumbens that were present 1 day after THC treatment, and only partially reversed 1 week later (Ginovart et al., 2012). Kolb, Gorny, Limebeer, and Parker

(2006) found that 12 days of exposure to low or moderate doses of THC (0.5–4 mg/kg) increased both the length of dendrites and the number of dendritic branches in the nucleus accumbens shell and medial prefrontal cortex, measured 40 days after THC exposure. After a single exposure to a synthetic CB_1-receptor agonist, adolescent rats showed increased dopamine-receptor density, but adult rats only showed such an effect after 14 days of exposure, suggesting that the adult rat brain has compensatory mechanisms not present in adolescents.

To study the addiction-related behavioral effects of THC exposure, a series of studies has been conducted, using an exposure regimen in which rats are given two intraperitoneal injections of THC per day, for 3 consecutive days, with the dose escalating over days from 2 to 8 mg/kg per injection; the last THC injection is given at least a week before behavioral or neurophysiological tests are begun (Cadoni, Pisanu, Solinas, Acquas, & Di Chiara, 2001; Cadoni, Simola, Espa, Fenu, & Di Chiara, 2013; Cadoni, Valentini, & Di Chiara, 2008; Panlilio, Solinas, Matthews, & Goldberg, 2007; Panlilio, Zanettini, Barnes, Solinas, & Goldberg, 2013; Solinas, Panlilio, & Goldberg, 2004). This treatment models a period of regular cannabis use, with escalating intake to compensate for the development of tolerance. Compared to control rats given only vehicle injections during the exposure period, rats that were exposed to THC under this regimen showed reduced dopamine transmission in the nucleus accumbens shell when they were given morphine (Cadoni et al., 2008), and higher levels of spontaneous locomotor activity when given morphine (Cadoni et al., 2001, 2008) or heroin (Panlilio et al., 2007). When allowed to self-administer heroin starting 1 week after the last THC injection, THC-exposed rats took more heroin than controls when only a single response was required for each injection of heroin (Solinas et al., 2004). However, when the price of heroin was raised by increasing the number of responses required for each injection, THC-exposed rats did not differ from controls suggesting that THC exposure did not increase the reward value of heroin. Rather, the higher rate of intake under the low-price condition might be explained in terms of THC-exposed rats being more active than controls after receiving heroin, allowing them to take heroin more rapidly, with only brief pauses between injections.

Another series of studies has focused on the effects of light, occasional exposure to THC, with rats given intraperitoneal injections of THC every third day during adolescence (postnatal days 28–49), at a dose of 1.5 mg/kg per injection (Ellgren, Spano, & Hurd, 2007; Szutorisz et al., 2014; Tomasiewicz et al., 2012). In these studies, THC exposure produced increases in opioid self-administration similar to those observed by Solinas et al. (2004), and long-lasting changes in the endogenous opioid system were also revealed, such as elevated expression of the precursor gene of the endogenous opioid, enkephalin (Tomasiewicz et al., 2012). It has even been shown that parental exposure can produce addiction-relevant physiological and behavioral changes in offspring conceived after THC was no longer in the system (Szutorisz et al., 2014). There has also been one study of the effects of THC exposure on the later self-administration of amphetamine and the dopamine agonist apomorphine (Cortright, Lorrain, Beeler, Tang, & Vezina, 2011); amphetamine self-administration was unaffected, but apomorphine self-administration was slightly increased, and postsynaptic dopamine receptor signaling was increased in the nucleus accumbens.

Extending the findings that Solinas et al. (2004) obtained with heroin self-administration, Panlilio et al. (2007) studied the effects of 3-day escalating THC exposure on subsequent cocaine self-administration. Rats exposed to THC in young adulthood did not differ from vehicle-exposed control rats when only one response was required for each cocaine injection. However, THC exposed rats responded less than controls when the response requirement was increased, indicating that prior THC exposure actually decreased the reward value of cocaine. Since THC exposure is known to alter the effects of morphine and heroin on spontaneous locomotor activity, THC-exposed rats were also tested to determine whether similar effects would occur with cocaine. Although they did not differ significantly from controls, in the overall level of locomotor activity when given cocaine, THC-exposed rats showed a different spatial pattern of activity, avoiding the center of the open field. This behavior is considered an indication of anxiety, and further testing with another animal model of anxiety also indicated that cocaine induced higher levels of anxiety in THC-exposed rats than in controls. Together, these findings suggest that THC exposure might have exacerbated the anxiety-inducing effect of cocaine, making it less valuable as a reward. THC exposure might have had a similar effect on anxiety induced by yohimbine, in a study by Stopponi et al. (2014) in which stress-induced reinstatement of heroin seeking was increased in THC-exposed rats.

To our knowledge, there has only been one study investigating the effects of previous THC exposure on the reward-related effects of nicotine (Panlilio et al., 2013), and one study investigating effects in the opposite direction, with exposure to nicotine prior to testing with cannabinoid-receptor CB_1 agonists. Werling, Reed, Wade, and Izenwasser (2009) exposed rats to nicotine or vehicle, and later tested their sensitivity to cannabinoids by administering THC or a synthetic CB_1 agonist. Previous exposure to nicotine led to enhanced locomotor-decreasing effects of cannabinoid agonists in adolescent male rats, but not in adult male rats. Cannabinoid-receptor density was increased in the prelimbic prefrontal cortex and

ventral tegmental area of nicotine-exposed adolescent male rats, compared with controls. This suggests that adolescent nicotine self-administration might increase sensitivity to cannabinoids, in line with the traditional gateway effect in which tobacco use seems to promote cannabis use.

Panlilio et al. (2013) examined a reverse gateway hypothesis, that cannabis exposure might facilitate subsequent formation of tobacco addiction. This study extended the series of experiments in which the 3-day escalating THC exposure regimen was given prior to allowing rats to self-administer heroin or cocaine. In the earlier studies, about 80% of rats acquired the heroin or cocaine self-administration response, regardless of whether they were THC-exposed or vehicle-exposed. In contrast, THC exposure increased the percentage of rats that acquired the nicotine self-administration response, from 65% in the vehicle-exposed group to 94% in the THC-exposed group (Fig. 76.2). This finding is consistent with cocaine and heroin generally having more powerful rewarding effects than nicotine, but with the rewarding effects of nicotine being increased by a history of THC exposure. This interpretation was further supported by the finding that, when the price of nicotine was increased, THC-exposed rats placed a higher value on nicotine than control rats did, a phenomenon that did not occur with either heroin (which was equally valued by both groups) or cocaine (which was valued less by THC-exposed groups). These findings suggest that humans who progress from cannabis use to tobacco addiction might do so, at least in part, because of pharmacological effects of THC exposure that specifically enhance the addictive potential of nicotine. This contribution of prior THC exposure to reverse gateway effects would be in addition to any pharmacological enhancement produced by simultaneous use of tobacco and cannabis (which has yet to be modeled with nicotine self-administration in animals), and also in addition to any nonpharmacological factors such as peer pressure, experience with smoking as a route of administration, or individual disposition toward addiction in general.

POSSIBLE AVENUES TO THE DEVELOPMENT OF TREATMENTS FOR ADDICTION

Epidemiological and animal-model studies suggest that co-use of cannabis and tobacco has the potential to lead to or intensify addiction, probably due to interactions between reward-related circuits in the cannabinoid and nicotinic systems of the brain. However, this interactivity might also provide the basis for pharmacological treatments that help induce and maintain abstinence from tobacco.

Some of the strongest evidence for interactions between the cannabinoid and nicotinic systems comes from studies in which cannabinoid receptors are genetically deleted or are blocked with CB_1 antagonist drugs, such as rimonabant. For example, in rats, CB_1-antagonists decrease nicotine self-administration and nicotine-induced dopamine elevations in the nucleus accumbens (Cohen, Perrault, Voltz, Steinberg, & Soubrie, 2002; Shoaib, 2008). Cannabinoid antagonists also have protective effects in reinstatement models of relapse, decreasing the effects of reexposure to nicotine or nicotine-associated cues (Cohen, Perrault, Griebel, & Soubrie, 2005; De Vries, de Vries, Janssen, & Schoffelmeer, 2005; Shoaib, 2008). When given to rats during the acquisition phase, rimonabant prevented the development of preference for a place associated with nicotine, and when given only before a test session, rimonabant prevented the expression of such a preference; however, expression was only blocked when

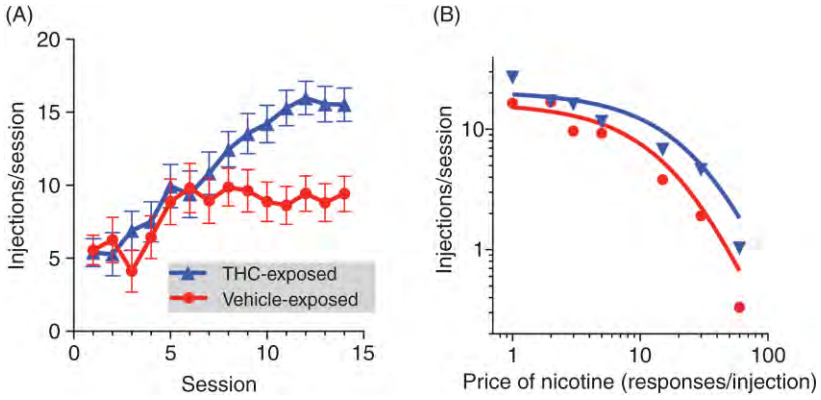


FIGURE 76.2 **Rats were exposed to THC or vehicle for 3 days, 8 days before being allowed to self-administer nicotine.** (A) THC-exposed rats acquired the self-administration response more readily, and took larger amounts of nicotine. (B) When the price of nicotine (responses per injection) was raised, THC-exposed rats took nicotine at higher prices than vehicle-exposed rats, indicating that nicotine was more valued by THC-exposed rats. *Source: Adapted from Panlilio et al. (2013). Used with permission.*

the test was performed 24 h after the last conditioning session, and was ineffective when rats were tested 3 or 12 weeks later (Forget, Hamon, & Thiebot, 2005).

Cossu et al. (2001) found that CB_1-receptor knockout mice would self-administer nicotine, cocaine, or amphetamine, but not morphine. In contrast, CB_1 knockout mice and wild-type mice given rimonabant did not develop nicotine-induced conditioned place-preference (Castane et al., 2002; Merritt, Martin, Walters, Lichtman, & Damaj, 2008). This difference between nicotine self-administration and nicotine-induced conditioned place-preference might be due to the cannabinoid system being more relevant to the rewarding effects of nicotine-associated cues (which provide the basis for conditioned place-preference and cue-induced reinstatement), as opposed to the direct reinforcing effects of nicotine (which are more prominent in self-administration procedures).

When tested in humans, blockade of cannabinoid receptors by rimonabant showed efficacy for the treatment of tobacco smoking, but was also found to produce depression-like side effects, leading to the drug being withdrawn from the market in Europe, and never receiving Food and Drug Administration approval in the United States (Le Foll, Gorelick, & Goldberg, 2009). The adverse effects of rimonabant might be due to the fact that it not only blocks the cannabinoid receptor from being activated by endogenous cannabinoids, such as anandamide and 2-AG, but also has inverse-agonist effects that are opposite to those of the endogenous cannabinoids. It remains to be seen whether cannabinoid antagonists that lack inverse-agonist effects would be better tolerated, and whether they would be useful as a treatment for tobacco addiction. However, there are two other strategies for manipulating the endocannabinoid system that also might provide effective treatment for tobacco addiction, without rimonabant-like side effects: inhibition of fatty acid amide hydrolase (FAAH), and direct activation of peroxisome proliferator-activated receptors (PPARs).

FAAH is the main enzyme responsible for inactivation of the endogenous cannabinoid CB_1 agonist anandamide, and the related fatty acid amides, OEA and PEA, which act at PPARs. Thus, pharmacological inhibition or genetic deletion of FAAH increases endogenous levels of anandamide, OEA, and PEA, enhancing and prolonging their effects when they are naturally released. Despite the abuse potential of CB_1 agonists such as THC, and the fact that FAAH inhibition increases levels of anandamide, at least one FAAH-inhibiting drug, URB597, has been found to be devoid of rewarding effects in animals (Justinova et al., 2008). But, surprisingly, FAAH inhibition counteracts the rewarding effects of nicotine in rats and monkeys. For example, URB597 prevented acquisition and reinstatement of nicotine self-administration and conditioned place-preference in rats (Fig. 76.3), and it reduced nicotine-induced elevations

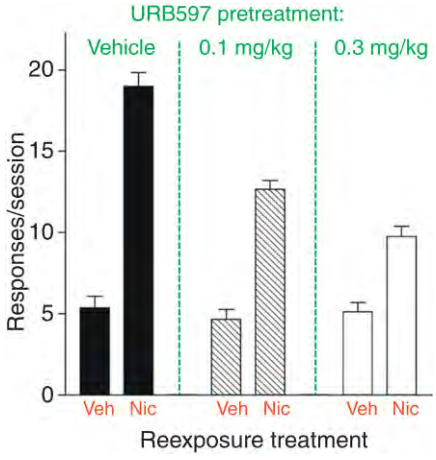


FIGURE 76.3 **In rats that had learned to self-administer intravenous nicotine, abstinence was imposed by discontinuing nicotine delivery.** Then, rats were reexposed to nicotine (or vehicle) by intraperitoneal injection. Nicotine reexposure reinstated the nicotine-seeking response, but pretreatment with the FAAH inhibitor URB597 blocked this relapse-like effect, with a higher dose of URB597 having the strongest blocking effect. *Source: Adapted from Scherma et al. (2008). Used with permission.*

of dopamine in the nucleus accumbens shell (Scherma et al., 2008). However, these effects of FAAH inhibition appear to be species-dependent, with FAAH knockout mice and wild-type mice treated with URB597 showing the opposite effect: enhanced nicotine-induced conditioned place-preference (Merritt et al., 2008).

The potentially beneficial effects of FAAH inhibition on nicotine reward appear to be due to its ability to increase levels of both anandamide and the endogenous PPAR ligands, OEA and PEA. Selectively increasing endogenous anandamide levels blocks the effects of nicotine on nucleus-accumbens dopamine, and also blocks the development and reinstatement of nicotine-induced conditioned place-preference (Scherma et al., 2012). Using electrophysiology techniques, Melis et al. (2008) found that some of the effects of URB597 on nicotine-induced firing of dopamine cells in the ventral tegmental area (which projects to the nucleus accumbens) were mediated by cannabinoid receptors, and others by PPARs. The paradox—in which systemic injection of one CB_1 agonist (THC) enhances nicotine's effects, but increasing endogenous levels of another CB_1 agonist (anandamide) blocks nicotine's effects—might be due to endogenous anandamide affecting CB_1 receptors only in specific brain areas, as opposed to exogenous THC affecting them globally, throughout the brain.

Subsequent experiments with behavioral models of nicotine reward, in vivo microdialysis, and electrophysiology suggest that selective PPAR agonists (with no activity at CB_1 receptors) could be useful for the treatment of tobacco smoking. Mascia et al. (2011) found that the alpha-type PPAR agonist WY14643 decreased: (1) nicotine

self-administration and nicotine-induced reinstatement in rats and monkeys (Fig. 76.4); (2) nicotine-induced excitation of dopamine neurons in the ventral tegmental area of rats; and (3) nicotine-induced dopamine elevations in the nucleus accumbens shell of rats (Fig. 76.5). Similar effects were produced by another PPAR-alpha agonist, clofibrate, a representative of the fibrate class of medications used to treat lipid disorders in humans (Fig. 76.6) (Panlilio et al., 2012). In monkeys, clofibrate not only decreased ongoing nicotine self-administration, but also decreased reinstatement induced by reexposure to either nicotine or nicotine-associated cues; these findings suggest that fibrate medications already approved for other purposes could be useful for both inducing and maintaining abstinence from tobacco.

In comparison with tobacco, cannabis is less addictive, and less of a public health problem. Nonetheless, there are substantial numbers of people who meet DSM criteria for cannabis dependence, many of whom seek treatment, and there are no approved pharmacotherapies for this purpose. However, just as it has led to new approaches to the treatment of tobacco addiction, research on interactivity between the endogenous cannabinoid and nicotinic systems has led to identification of a pharmacological strategy for treating cannabis dependence.

Alpha-7 nicotinic receptors are localized in reward-related brain areas, and control glutamate release, which in turn, controls dopamine release. In rats, Solinas et al. (2007) found that blocking alpha-7 nicotinic receptors with methyllycaconitine decreased self-administration of the cannabinoid-receptor synthetic agonist WIN55,212-2, and that it also completely prevented THC from elevating dopamine in the nucleus accumbens shell. While these findings suggest that manipulation of nicotinic receptors might decrease the abuse-related effects of cannabis, unfortunately, direct agonists of apha-7 receptors such as methyllycaconitine are unsuitable as medications, due to side effects such as cognitive impairment.

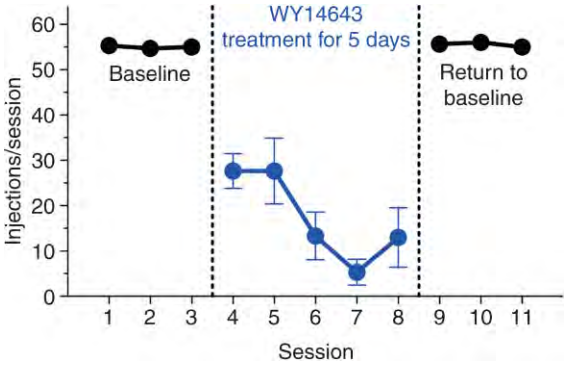


FIGURE 76.4 Monkeys with a long history of nicotine self-administration substantially decreased their nicotine intake when treated for 5 days with the PPAR-alpha agonist WY14643. *Source: Adapted from Mascia et al. (2011). Used with permission.*

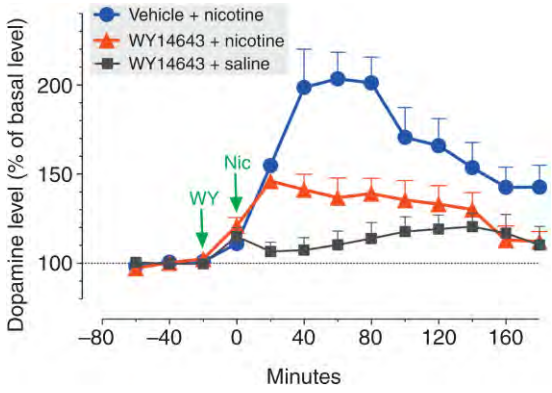


FIGURE 76.5 Intraperitoneal nicotine treatment increased dopamine levels in the nucleus accumbens shell of rats that were pretreated with vehicle ("Vehicle + Nicotine"), but nicotine's effect was substantially reduced by pretreatment with the PPAR-alpha agonist WY14643 ("WY14643 + Nicotine"). WY14643 by itself had little or no effect on dopamine levels ("WY14643 + Saline"). *Green arrows* indicate time of pretreatment ("WY") and treatment ("Nic") injection. *Source: Adapted from Mascia et al. (2011). Used with permission.*

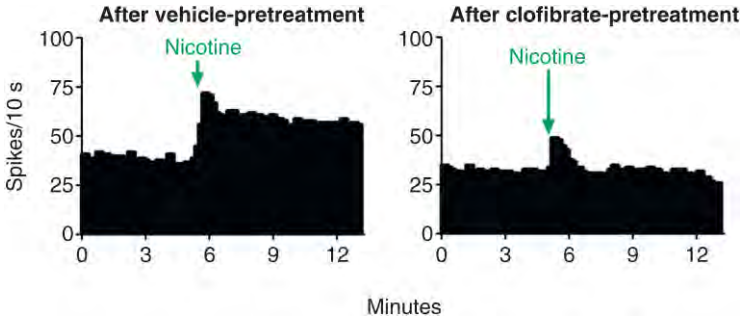


FIGURE 76.6 In anesthetized rats, intravenous nicotine increased the firing of ventral tegmental area dopamine neurons that project to the nucleus accumbens shell (A), but pretreatment with the PPAR-alpha agonist clofibrate blocked this effect (B). Each panel shows the firing rate of a single neuron over time. *Source: Adapted from Panlilio et al. (2012). Used with permission.*

A potentially safer means of blocking alpha-7 receptors in the brain is by enhancing endogenous levels of kynurenic acid, a negative allosteric modulator of alpha-7 receptors. Negative allosteric modulation of alpha-7 receptors does not directly affect neuronal signaling, but diminishes the effects of nicotine or endogenous acetylcholine when they bind to the receptor. Justinova et al. (2013) found that enhancing endogenous levels of kynurenic acid has potentially beneficial effects in rodent and nonhuman-primate models of cannabis reward and reward-related effects, including: (1) preventing THC and WIN55,212-2 from elevating dopamine levels in the nucleus accumbens and ventral tegmental area of rats; (2) decreasing WIN55,212-2 self-administration and WIN55,212-2-induced reinstatement in rats; (3) decreasing THC self-administration in monkeys; and (4) decreasing reinstatement induced by reexposure to THC or THC-associated cues in monkeys. All of these effects were shown to be due to negative allosteric modulation of alpha-7 receptors, since the effects were reversed by coadministration of positive allosteric modulators of alpha-7 receptors. This study also provided some evidence for the safety of this treatment strategy by showing that the same treatments that blocked cannabinoid reward did not induce nonspecific depressant effects or cognitive deficits in working memory. These findings suggest that manipulations of the nicotinic system might eventually be used to treat cannabis dependence and, perhaps, dual cannabis-tobacco dependence.

MINI-DICTIONARY

CB_1 Receptor subtype that binds THC and endogenous cannabinoids (including anandamide), and modulates their psychoactive effects.
CB_1 knockout mice Mice genetically engineered to have no CB_1 receptors.
Conditioned place-preference A model of drug seeking that measures rodents' preference for a place previously associated with a drug effect.
Drug reward Drug reward occurs when a specific behavior that causes a drug to be delivered and this increases the likelihood that the behavior will continue to occur in the future. Environmental cues associated with drugs can also acquire rewarding effects, such as in the conditioned-place preference procedure.
Drug self-administration A model of drug reward that allows animals to take a drug, usually by pressing a lever that produces a small intravenous injection.
Endocannabinoid system The brain circuitry that involves CB_1 and CB_2 receptors, anandamide, and other endogenous chemicals that bind to these receptors, and also anandamide-related chemicals such as OEA and PEA that bind to PPARs.
Fatty acid amide hydrolase The main enzyme responsible for deactivating anandamide, OEA, PEA, and related chemicals.
Nucleus accumbens Brain area considered to be important in the rewarding effects of drugs, natural substances such as food, and events such as sexual activity. One of the main sources of input to this area is dopamine-releasing neurons originating in the ventral tegmental area.
Peroxisome proliferator-activated receptors PPARs have a range of functions, including regulation of feeding and metabolism. Alpha-type PPARs negatively modulate beta-2 nicotinic receptors. Due to their structural and functional properties, endogenous PPAR ligands such as OEA and PEA are considered part of the extended endocannabinoid family.
Reinstatement A model of relapse in which drug seeking is established, then diminished by discontinuing drug availability, and finally tested by exposing the animal to triggers such as stress, reexposure to the drug, or reexposure to drug-associated environmental cues.

Acknowledgments

Preparation of the manuscript was supported by the Intramural Research Program of the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services.

References

Agrawal, A., Budney, A. J., & Lynskey, M. T. (2012). The co-occurring use and misuse of cannabis and tobacco: a review. *Addiction*, *107*(7), 1221–1233.
Cadoni, C., Pisanu, A., Solinas, M., Acquas, E., & Di Chiara, G. (2001). Behavioural sensitization after repeated exposure to Delta 9-tetrahydrocannabinol and cross-sensitization with morphine. *Psychopharmacology (Berlin)*, *158*(3), 259–266.
Cadoni, C., Simola, N., Espa, E., Fenu, S., & Di Chiara, G. (2013). Strain dependence of adolescent Cannabis influence on heroin reward and mesolimbic dopamine transmission in adult Lewis and Fischer 344 rats. *Addiction Biology*, *20*, 132–142.
Cadoni, C., Valentini, V., & Di Chiara, G. (2008). Behavioral sensitization to delta 9-tetrahydrocannabinol and cross-sensitization with morphine: differential changes in accumbal shell and core dopamine transmission. *Journal of Neurochemistry*, *106*(4), 1586–1593.
Castane, A., Valjent, E., Ledent, C., Parmentier, M., Maldonado, R., & Valverde, O. (2002). Lack of CB1 cannabinoid receptors modifies nicotine behavioural responses, but not nicotine abstinence. *Neuropharmacology*, *43*(5), 857–867.
Cohen, C., Perrault, G., Griebel, G., & Soubrie, P. (2005). Nicotine-associated cues maintain nicotine-seeking behavior in rats several weeks after nicotine withdrawal: reversal by the cannabinoid (CB1) receptor antagonist, rimonabant (SR 141716). *Neuropsychopharmacology*, *30*(1), 145–155.
Cohen, C., Perrault, G., Voltz, C., Steinberg, R., & Soubrie, P. (2002). SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. *Behavioral Pharmacology*, *13*(5–6), 451–463.
Cortright, J. J., Lorrain, D. S., Beeler, J. A., Tang, W. J., & Vezina, P. (2011). Previous exposure to delta9-tetrahydrocannibinol enhances locomotor responding to but not self-administration of amphetamine. *Journal of Pharmacology and Experimental Therapeutics*, *337*(3), 724–733.
Cossu, G., Ledent, C., Fattore, L., Imperato, A., Bohme, G. A., Parmentier, M., & Fratta, W. (2001). Cannabinoid CB1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse. *Behavioural Brain Research*, *118*(1), 61–65.
De Vries, T. J., de Vries, W., Janssen, M. C., & Schoffelmeer, A. N. (2005). Suppression of conditioned nicotine and sucrose seeking by the cannabinoid-1 receptor antagonist SR 141716A. *Behavioural Brain Research*, *161*(1), 164–168.
DiNieri, J.A., Wang, X., Szutorisz, H., Spano, S.M., Kaur, J., Casaccia, P., …, Hurd, Y.L. (2011). Maternal cannabis use alters ventral striatal dopamine D2 gene regulation in the offspring. *Biological Psychiatry*, *70*(8), 763–769.

Ellgren, M., Spano, S. M., & Hurd, Y. L. (2007). Adolescent cannabis exposure alters opiate intake and opioid limbic neuronal populations in adult rats. *Neuropsychopharmacology*, *32*(3), 607–615.

Forget, B., Hamon, M., & Thiebot, M. H. (2005). Cannabinoid CB1 receptors are involved in motivational effects of nicotine in rats. *Psychopharmacology (Berlin)*, *181*(4), 722–734.

Ginovart, N., Tournier, B. B., Moulin-Sallanon, M., Steimer, T., Ibanez, V., & Millet, P. (2012). Chronic Delta(9)-tetrahydrocannabinol exposure induces a sensitization of dopamine D(2)/(3) receptors in the mesoaccumbens and nigrostriatal systems. *Neuropsychopharmacology*, *37*(11), 2355–2367.

González, B., de Miguel, R., Martín, S., Pérez-Rosado, A., Romero, J., García-Lecumberri, C., …, Ambrosio, E. (2003). Effects of perinatal exposure to Δ9-tetrahydrocannabinol on operant morphine-reinforced behavior. *Pharmacology Biochemistry and Behavior*, *75*(3), 577–584.

Gorriti, M. A., Ferrer, B., del Arco, I., Bermudez-Silva, F. J., de Diego, Y., Fernandez-Espejo, E., …, Rodriguez de Fonseca, F. (2005). Acute delta9-tetrahydrocannabinol exposure facilitates quinpirole-induced hyperlocomotion. *Pharmacology Biochemistry and Behavior*, *81*(1), 71–77.

Justinova, Z., Mangieri, R.A., Bortolato, M., Chefer, S.I., Mukhin, A.G., Clapper, J.R., …, Goldberg, S.R. (2008). Fatty acid amide hydrolase inhibition heightens anandamide signaling without producing reinforcing effects in primates. *Biological Psychiatry*, *64*(11), 930–937.

Justinova, Z., Mascia, P., Wu, H.Q., Secci, M.E., Redhi, G.H., Panlilio, L.V., …, Goldberg, S.R. (2013). Reducing cannabinoid abuse and preventing relapse by enhancing endogenous brain levels of kynurenic acid. *Nature Neuroscience*, *16*(11), 1652–1661.

Kolb, B., Gorny, G., Limebeer, C. L., & Parker, L. A. (2006). Chronic treatment with Delta-9-tetrahydrocannabinol alters the structure of neurons in the nucleus accumbens shell and medial prefrontal cortex of rats. *Synapse*, *60*(6), 429–436.

Le Foll, B., Gorelick, D. A., & Goldberg, S. R. (2009). The future of endocannabinoid-oriented clinical research after CB1 antagonists. *Psychopharmacology (Berlin)*, *205*(1), 171–174.

Mascia, P., Pistis, M., Justinova, Z., Panlilio, L.V., Luchicchi, A., Lecca, S., …, Goldberg, S.R. (2011). Blockade of nicotine reward and reinstatement by activation of alpha-type peroxisome proliferator-activated receptors. *Biological Psychiatry*, *69*(7), 633–641.

Meier, E., & Hatsukami, D. K. (2016). A review of the additive health risk of cannabis and tobacco co-use. *Drug and Alcohol Dependence*, pii: S0376-8716(16)30210-1. doi: 10.1016/j.drugalcdep.2016.07.013.

Melis, M., Pillolla, G., Luchicchi, A., Muntoni, A. L., Yasar, S., Goldberg, S. R., & Pistis, M. (2008). Endogenous fatty acid ethanolamides suppress nicotine-induced activation of mesolimbic dopamine neurons through nuclear receptors. *Journal of Neuroscience*, *28*(51), 13985–13994.

Merritt, L. L., Martin, B. R., Walters, C., Lichtman, A. H., & Damaj, M. I. (2008). The endogenous cannabinoid system modulates nicotine reward and dependence. *Journal of Pharmacology and Experimental Therapeutics*, *326*(2), 483–492.

Moreno, M., Trigo, J. M., Escuredo, L., Rodríguez de Fonseca, F., & Navarro, M. (2003). Perinatal exposure to Δ9-tetrahydrocannabinol increases presynaptic dopamine D2 receptor sensitivity: a behavioral study in rats. *Pharmacology Biochemistry and Behavior*, *75*(3), 565–575.

Panlilio, L.V., Justinova, Z., Mascia, P., Pistis, M., Luchicchi, A., Lecca, S., …, Goldberg, S.R. (2012). Novel use of a lipid-lowering fibrate medication to prevent nicotine reward and relapse: preclinical findings. *Neuropsychopharmacology*, *37*(8), 1838–1847.

Panlilio, L. V., Solinas, M., Matthews, S. A., & Goldberg, S. R. (2007). Previous exposure to THC alters the reinforcing efficacy and anxiety-related effects of cocaine in rats. *Neuropsychopharmacology*, *32*(3), 646–657.

Panlilio, L. V., Zanettini, C., Barnes, C., Solinas, M., & Goldberg, S. R. (2013). Prior exposure to THC increases the addictive effects of nicotine in rats. *Neuropsychopharmacology*, *38*(7), 1198–1208.

Pryor, G. T., Larsen, F. F., Husain, S., & Braude, M. C. (1978). Interactions of delta9-tetrahydrocannabinol with d-amphetamine, cocaine, and nicotine in rats. *Pharmacology. Biochemistry and Behavior*, *8*(3), 295–318.

Rodriguez de Fonseca, F., Del Arco, I., Bermudez-Silva, F. J., Bilbao, A., Cippitelli, A., & Navarro, M. (2005). The endocannabinoid system: physiology and pharmacology. *Alcohol and Alcoholism*, *40*(1), 2–14.

Scherma, M., Fadda, P., Le Foll, B., Forget, B., Fratta, W., Goldberg, S. R., & Tanda, G. (2008). The endocannabinoid system: a new molecular target for the treatment of tobacco addiction. *CNS and Neurological Disorders. Drug Targets*, *7*(5), 468–481.

Scherma, M., Justinova, Z., Zanettini, C., Panlilio, L.V., Mascia, P., Fadda, P., …, Goldberg, S.R. (2012). The anandamide transport inhibitor AM404 reduces the rewarding effects of nicotine and nicotine-induced dopamine elevations in the nucleus accumbens shell in rats. *British Journal of Pharmacology*, *165*(8), 2539–2548.

Shoaib, M. (2008). The cannabinoid antagonist AM251 attenuates nicotine self-administration and nicotine-seeking behaviour in rats. *Neuropharmacology*, *54*(2), 438–444.

Singh, M. E., McGregor, I. S., & Mallet, P. E. (2006). Perinatal exposure to delta(9)-tetrahydrocannabinol alters heroin-induced place conditioning and fos-immunoreactivity. *Neuropsychopharmacology*, *31*(1), 58–69.

Solinas, M., Panlilio, L. V., & Goldberg, S. R. (2004). Exposure to delta-9-tetrahydrocannabinol (THC) increases subsequent heroin taking but not heroin's reinforcing efficacy: a self-administration study in rats. *Neuropsychopharmacology*, *29*(7), 1301–1311.

Solinas, M., Scherma, M., Fattore, L., Stroik, J., Wertheim, C., Tanda, G., …, Goldberg, S.R. (2007). Nicotinic alpha 7 receptors as a new target for treatment of cannabis abuse. *Journal of Neuroscience*, *27*(21), 5615–5620.

Stopponi, S., Soverchia, L., Ubaldi, M., Cippitelli, A., Serpelloni, G., & Ciccocioppo, R. (2014). Chronic THC during adolescence increases the vulnerability to stress-induced relapse to heroin seeking in adult rats. *European Neuropsychopharmacology*, *24*(7), 1037–1045.

Szutorisz, H., DiNieri, J.A., Sweet, E., Egervari, G., Michaelides, M., Carter, J.M., …, Hurd, Y.L. (2014). Parental THC exposure leads to compulsive heroin-seeking and altered striatal synaptic plasticity in the subsequent generation. *Neuropsychopharmacology*, *39*(6), 1315–1323.

Tomasiewicz, H. C., Jacobs, M. M., Wilkinson, M. B., Wilson, S. P., Nestler, E. J., & Hurd, Y. L. (2012). Proenkephalin mediates the enduring effects of adolescent cannabis exposure associated with adult opiate vulnerability. *Biological Psychiatry*, *72*(10), 803–810.

Valjent, E., Mitchell, J. M., Besson, M. J., Caboche, J., & Maldonado, R. (2002). Behavioural and biochemical evidence for interactions between Delta 9-tetrahydrocannabinol and nicotine. *British Journal of Pharmacology*, *135*(2), 564–578.

Werling, L. L., Reed, S. C., Wade, D., & Izenwasser, S. (2009). Chronic nicotine alters cannabinoid-mediated locomotor activity and receptor density in periadolescent but not adult male rats. *International Journal of Developmental Neuroscience*, *27*(3), 263–269.

CHAPTER

77

Cannabinoid Regulation of Intraocular Pressure: Human and Animal Studies, Cellular and Molecular Targets

A. Aloway, A. Kumar, A.S. Laun, Z.H. Song

Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY, United States

SUMMARY POINTS

- All three classes of cannabinoids, including phytocannabinoids, synthetic cannabinoids, and endocannabinoids, have been demonstrated to lower IOP in human subjects, as well as in animal models.
- The molecular targets mediating the IOP-lowering effects cannabinoids include CB1 and CB2 cannabinoid receptors, non-CB1/CB2 cannabinoid receptors, endocannabinoid metabolizing enzymes, and transporters.
- These molecular targets are found in the regions of the eye that are important for IOP regulation, including ciliary epithelium, trabecular meshwork, and ciliary muscle.
- Cannabinoids exhibit their IOP-lowering effects either through reducing aqueous humor production, or by enhancing aqueous humor outflow through conventional or uveoscleral routes.
- Cannabinoids represent a class of promising therapeutic agents for the treatment of glaucoma.

KEY FACTS INTRAOCULAR PRESSURE

- Intraocular pressure is the pressure within the eye.
- Intraocular pressure is determined by the production and drainage of aqueous humor.
- Aqueous humor is produced by nonpigmented epithelial cells of the ciliary body in the eye, and is drained by either the trabecular meshwork pathway, or the uveoscleral pathway.
- Elevated intraocular pressure is a risk factor for the development of glaucoma.
- Drugs can lead to a change in intraocular pressure by altering aqueous humor production or outflow.

LIST OF ABBREVIATIONS

AEA	Arachidonylethanolamide
2-AG	2-Arachidonylglycerol
CB1, CB2	Cannabinoid receptors type 1 and 2
IOP	Intraocular pressure
PEA	Palmitoylethanolamide
Δ^9-THC	Δ^9-Tetrahydrocannabinol
TM	Trabecular meshwork

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00088-0
Copyright © 2017 Elsevier Inc. All rights reserved.

GLAUCOMA AND INTRAOCULAR PRESSURE

Glaucoma is the second leading cause of human blindness affecting as many as two million people in the United States (Rocha-Sousa et al., 2013; Toris, 2010; Weinreb, Aung, & Medeiros, 2014). Increased intraocular pressure (IOP) is the primary risk factor for developing glaucoma. Current medical intervention for glaucoma slows the progression of the disease by lowering the IOP (Rocha-Sousa et al., 2013; Toris, 2010; Weinreb et al., 2014).

IOP is determined by the rate of secretion of aqueous humor into the posterior chamber of the eye, and the rate of aqueous humor outflow (Rocha-Sousa et al., 2013; Toris, 2010; Weinreb et al., 2014). Aqueous humor is produced and secreted by nonpigmented epithelium of the ciliary body. Aqueous humor then flows from the posterior to the anterior chamber through the pupil, exiting the anterior chamber by the conventional or nonconventional pathways. In the conventional pathway, aqueous humor passes through the trabecular meshwork (TM) and exits into Schlemm's canal, where it eventually empties into veins under the conjunctiva. The nonconventional, uveoscleral pathway involves the diffusion of aqueous humor through intercellular spaces of the ciliary muscle fibers. All three aspects of aqueous humor dynamics, including secretion, conventional, and nonconventional outflow processes provide potential pharmacological targets for controlling IOP (Rocha-Sousa et al., 2013; Toris, 2010; Weinreb et al., 2014).

CURRENT TREATMENT OF GLAUCOMA

Glaucoma is clinically treated by surgical means or noninvasive pharmacotherapy (Rocha-Sousa et al., 2013; Toris, 2010; Weinreb et al., 2014). Surgical treatments reduce IOP by trabeculectomy which removes a section of the TM to facilitate draining of aqueous humor. Current pharmacotherapy of glaucoma is comprised of five categories of IOP-lowering drugs: β blockers, α agonists, cholinergic agonists, carbonic anhydrase inhibitors, and prostaglandin analogs. These treatments reduce IOP by decreasing the rate of aqueous humor production, or increasing the rate of aqueous humor outflow through the nonconventional, uveoscleral pathway (Rocha-Sousa et al., 2013; Toris, 2010; Weinreb et al., 2014).

The currently available IOP-lowering drugs are able to reduce IOP through varied mechanisms, but shortcomings still remain: suitable drugs that target the conventional, TM pathway to enhance the rate of outflow remain unavailable. In addition, currently available IOP-lowering drugs frequently result in undesirable side effects. This necessitates newer antiglaucoma drugs targeting the TM pathway, and with fewer side effects (Rocha-Sousa et al., 2013; Toris, 2010; Weinreb et al., 2014).

CANNABINOIDS AND INTRAOCULAR PRESSURE

Marijuana smoking was first reported to lower IOP by Hepler and Frank (1971). From then on, the IOP-lowering effects of many cannabinoids, including phytocannabinoids, synthetic cannabinoids, and endocannabinoids, have been explored.

Phytocannabinoids

Δ^9-Tetrahydrocannabinol

The most prominent phytocannabinoid is Δ^9-tetrahydrocannabinol (Δ^9-THC), well known for its psychoactive properties (Howlett et al., 2002; Pertwee, 1999; Pertwee et al., 2010). Δ^9-THC is a heterotricyclic compound found in marijuana that interacts with both CB1 and CB2 cannabinoid receptors. The following studies provide evidence of the abilities of Δ^9-THC to reduce IOP (Table 77.1).

HUMANS

Many human trials have been performed to examine the role of Δ^9-THC in reduction of IOP.

An early study of two humans receiving intravenous (i.v.) administration of Δ^9-THC showed a reduction in IOP (Purnell & Gregg, 1975).

Furthermore, in a study of 8 normotensive individuals, 8 hypotensive patients, and 16 patients diagnosed with open angle glaucoma, IOP was measured after inhalation of Δ^9-THC, and significant reduction of IOP was noted, lasting for 4 h (Crawford & Merritt, 1979).

In another study, nine glaucoma patients receiving current glaucoma treatment added a regimen of inhaled or oral tablet of Δ^9-THC. These patients achieved their goal reduction in IOP (Flach, 2002).

In a more recent study, utilizing six patients with open angle glaucoma, the effect of sublingual administration of Δ^9-THC was compared with that of a placebo. Δ^9-THC was shown to significantly reduce IOP in study participants, from an average of 27.3 to 23.5 mmHg (Tomida et al., 2006).

In another study, after an oral dose of Δ^9-THC, normotensive patients showed significant reductions in IOP, from 13.2 to 11.8 mmHg (Plange et al., 2007).

TABLE 77.1 Effect of Phytocannabinoids on Intraocular Pressure

Drug	Study	Species	Route of administration	Intraocular pressure change
Δ^9-THC	Purnell and Gregg (1975)	Human	Intravenous	Decrease
	Crawford and Merritt (1979)	Human	Inhalation	Decrease
	Flach (2002)	Human	Inhalation/oral	Decrease/decrease
	Tomida et al. (2006)	Human	Sublingual	Decrease
	Plange et al. (2007)	Human	Oral	Decrease
	Colasanti, Powell, and Craig (1984c)	Cat	Topical	Decrease
	Colasanti (1990)	Cat	Topical	Decrease
	Elsohly, Harland, Murphy, Wirth, and Waller (1981)	Rabbit	Intravenous	Decrease
	Green, Symonds, Oliver, and Elijah (1982)	Rabbit	Intravenous	Decrease
	Liu and Dacus (1987)	Rabbit	Cerebral bolus/intravenous	No change/decrease
	Szczesniak, Kelly, Whynot, Shek, and Hung (2006)	Rat	Intratracheal/intraperitoneal	Decrease/no change
	Crandall et al. (2007)	Rat	Intravenous	Decrease
	Fischer, Ward, and Hendrix (2013)	Dog	Topical	Decrease
	Green et al. (1982)	Monkey	Intravenous	No change
CBD	Elsohly et al. (1981)	Rabbit	Intravenous	No change
	Green et al. (1982)	Rabbit	Intravenous	No change
	Tomida et al. (2006)	Human	Sublingual	No change
	Colasanti, Brown, and Craig (1984a)	Cat	Topical	Decrease
Δ^8-THC	Elsohly et al. (1981)	Rabbit	Intravenous	Decrease
	Green et al. (1982)	Rabbit	Intravenous	No change
	Liu and Dacus (1987)	Rabbit	Intravenous	No change
	Muchtar, Almog, Torracca, Saettone, and Benita (1992)	Cat	Topical emulsion	Decrease
CBG	Green et al. (1982)	Rabbit	Intravenous	No change
	Green et al. (1982)	Monkey	Intravenous	No change
	Colasanti et al. (1984a)	Cat	Topical	Decrease
CBN	Elsohly et al. (1981)	Rabbit	Intravenous	Decrease
	Green et al. (1982)	Rabbit	Intravenous	Decrease
	Colasanti et al. (1984a)	Cat	Topical	Decrease

Δ^9-THC, Δ^9-Tetrahydrocannabinol; CBD, cannabidiol; Δ^8-THC, Δ^8-tetrahydrocannabinol; CBG, cannabigerol; CBN, cannabinol.

CATS

In cats, a comparison of acute delivery and chronic delivery of Δ^9-THC provided different outcomes. Acute delivery by ophthalmic drops to cat eyes provided little reduction in IOP, whereas chronic delivery resulted in significant reduction in IOP (Colasanti, 1990; Colasanti et al., 1984c). Δ^9-THC had no effect on the rate of formation of aqueous humor, but produced a two- to three-fold increase in aqueous outflow facility (Colasanti, 1990).

RABBITS

Several studies have shown that i.v. administration of Δ^9-THC reduces IOP in rabbits (Elsohly et al., 1981; Green et al., 1982; Liu & Dacus, 1987). However, direct perfusion of Δ^9-THC to albino rabbit cerebral ventricles did not change IOP, but intravenous injection did decrease IOP (Liu & Dacus, 1987). These data indicate that the IOP-lowering effects of Δ^9-THC do not originate in the central nervous system (CNS).

RATS

An intratracheal (i.t.) administration of a liposome-entrapped Δ^9-THC has been shown to reduce IOP in rats (Szczesniak et al., 2006). In another study, in a rat model of glaucoma, i.v. administration of Δ^9-THC led to a reduction of elevated IOP in operated eye, without affecting the contralateral control eyes (Crandall et al., 2007).

DOGS

Topical application of 2% Δ^9-THC ophthalmic solution resulted in moderate reduction of mean IOP in clinically normal dogs (Fischer et al., 2013).

MONKEYS

Intravenous administration of Δ^9-THC did not change IOP or total outflow facility in the rhesus monkey (Green et al., 1982). This indicates that the IOP-lowering effect of Δ^9-THC is species specific.

In summary, Δ^9-THC has been shown in several species to reduce IOP. However, species differences may account for the wide differences in drug efficacy. In addition, route of administration plays an important role in achieving IOP-lowering effects of Δ^9-THC.

Cannabidiol

Cannabidiol (CBD), a non-CB1/CB2 phytocannabinoid, has been studied for its ability to reduce IOP. Intravenous administration of CBD in normotensive rabbits was unable to reduce IOP (Elsohly et al., 1981; Green et al., 1982). Additionally, a comparison of the effects of CBD and Δ^9-THC sublingual application in humans with open angle glaucoma showed that CBD is unable to reduce IOP (Tomida et al., 2006). However, in another study, CBD delivered topically to cat eye had a hypotensive effect, without ocular toxicity or neurotoxicity (Colasanti et al., 1984a). Thus, CBD is able to reduce IOP in cats, but not in rabbits or humans.

Δ^8-Tetrahydrocannabinol Analog

Natural Δ^8-tetrahydrocannabinol (Δ^8-THC) also has affinities to cannabinoid receptors similar to those of Δ^9-THC, and behaves as partial agonist for CB1 receptors (Howlett et al., 2002; Pertwee, 1999; Pertwee et al., 2010). A study has demonstrated the ability of Δ^8-THC to reduce IOP by i.v. administration to rabbits (Elsohly et al., 1981). Additionally, another study has shown that administration of a submicron emulsion preparation of Δ^8-THC to normotensive rabbit eyes led to a decrease in IOP (Muchtar et al., 1992).

In contrast, two studies have failed to show the IOP-lowering effects of Δ^8-THC. Green et al. (1982) have shown that i.v. administration of Δ^8-THC to normotensive rabbits does not produce a reduction of IOP (Green et al., 1982). In addition, intravenous administration of Δ^8-THC did not reduce IOP (Liu & Dacus, 1987).

Cannabigerol

Cannabigerol (CBG) binds CB1 and CB2, but functions as a competitive antagonist for the CB1. This compound also functions as an agonist for the α2 adrenoceptor (Cascio, Gauson, Stevenson, Ross, & Pertwee, 2010). Green et al. (1982) showed that i.v. administration of CBG to normotensive rabbits or monkeys does not lower IOP. However, in a later study, topical administration of CBG to normotensive cats showed a reduction in IOP by 7 mmHg. This effect was sustained with chronic use, and with minor systemic side effects (Colasanti, Craig, & Allara, 1984b). The CBG-induced reduction of IOP was enhanced when given with Δ^9-THC, with little CNS side effects (Colasanti, 1990).

Cannabinol

Cannabinol (CBN) has greater affinity for CB2 than CB1 (Howlett et al., 2002; Pertwee, 1999; Pertwee et al., 2010). In rabbits, i.v. administration of CBN resulted in a reduction in IOP (Elsohly et al., 1981; Green et al., 1982). Further examination of topical CBN administration to cat eyes has been shown to reduce IOP, and the greatest reduction in pressure was noted with chronic daily administration (Colasanti, Craig, & Allara, 1984b).

Synthetic Cannabinoids

Classical

NABILONE

Oral administration of nabilone to glaucoma patients resulted in a reduction in IOP; in addition, topical administration of nabilone to normotensive rabbits resulted in a reduction in IOP (Newell, Stark, Jay, & Schanzlin, 1979; Table 77.2).

HU-211

HU-211 is the enantiomer of HU-210. HU-211 differs from other cannbinoids since it works through the NMDA receptor, and has low affinity for CB1/CB2 (Howlett et al., 2002; Pertwee, 1999; Pertwee et al., 2010). Topical application of an ophthalmic emulsion of HU-211 resulted in a reduction of IOP in normotensive rabbits, sustained for 6 h (Naveh et al., 2000). In another study, intravenous administration of HU-211 to rabbits resulted in a decrease in IOP without significant changes in blood pressure or heart rate (Beilin et al., 2000).

BW146Y

A final example of classical synthetic cannabinoid agonist is BW146Y. In a pilot study of ocular hypertensive patients, an oral dose of BW146Y was found to lower IOP without affecting the orthostatic blood pressure, or producing neurological side effects (Tiedeman et al., 1981).

TABLE 77.2 Effect of Synthetic Cannabinoids on Intraocular Pressure

Drug	Study	Species	Route of administration	Intraocular pressure change
CLASSICAL				
Nabilone	Newell et al. (1979)	Human	Oral	Decrease
	Newell et al. (1979)	Rabbit	Topical	Decrease
HU-211	Naveh, Weissman, Muchtar, Benita, and Mechoulam (2000)	Rabbit	Topical emulsion	Decrease
	Beilin, Neumann, Belkin, Green, and Bar-Ilan (2000)	Rabbit	Intravenous	Decrease
BW146Y	Tiedeman et al. (1981)	Human	Oral	Decrease
JWH-133	Laine, Jarvinen, and Jarvinen (2003)	Rabbit	Topical	No change
NONCLASSICAL BICYCLIC				
CP-55,940	Laine et al. (2003)	Rabbit	Topical	Decrease
	Pate, Jarvinen, Urtti, Mahadevan, and Jarvinen (1998)	Rabbit	Topical	Decrease
Abn-CBD	Szczesniak, Maor, Robertson, Hung, and Kelly (2011)	Rat	Intraperitoneal/topical	Decrease/decrease
AMINOALKYLINDOLE				
WIN55212-2	Hodges, Reggio, and Green (1997)	Rabbit	Intravenous	No change
	Song and Slowey (2000)	Rabbit	Topical	Decrease
	Porcella, Maxia, Gessa, and Pani (2001)	Human	Topical	Decrease
	Chien, Wang, Mittag, and Podos (2003)	Monkey	Topical	Decrease
	Hosseini et al. (2006)	Rat	Topical	Decrease
	Oltmanns et al. (2008)	Rat	Topical	Decrease

Abn-CBD, Abnormal cannabidiol.

Nonclassical

CP-55,940

Nonclassical, bicyclic cannabinoids are represented by the prototype compound, CP-55,940. This compound behaves as a full agonist for both CB1 and CB2 (Howlett et al., 2002; Pertwee, 1999; Pertwee et al., 2010). Topical application of aqueous solution of CP-55,940 to normotensive rabbits resulted in reduced IOP, in at least two studies (Laine et al., 2003; Pate et al., 1998).

ABNORMAL CANNABIDIOL

Abnormal cannabidiol (abn-CBD) is a synthetic regioisomer of cannabidiol. When abn-CBD was given by intraperitoneal injection or topically in rat, IOP was reduced (Szczesniak et al., 2011).

Aminoalkylindoles

WIN55212-2

RABBITS WIN55212-2 displays high affinity for both CB1 and CB2, with moderate selectivity for CB2, and exhibits high intrinsic activity at both CB1 and CB2 (Howlett et al., 2002; Pertwee, 1999; Pertwee et al., 2010). In an initial study, systemic intravenous administration of WIN55212-2 in normotensive rabbits resulted in no significant change in IOP (Hodges et al., 1997). However, in a later study, topical application of WIN55212-2 to rabbits significantly reduced IOP in treated eyes, in a dose-dependent manner (Song & Slowey, 2000). Thus, the route of administration in rabbit seems to be important for the ability of WIN55212-2 to reduce IOP.

HUMANS Topical application of WIN55212-2 decreased IOP in human glaucoma cases resistant to conventional therapies. This demonstrates the potential for WIN55212-2 for treating glaucoma patients who have exhausted other methods of treatment (Porcella et al., 2001).

MONKEYS Topical administration of WIN55121-2 reduced IOP in both normal and glaucomatous monkey eyes. The reduction in IOP was sustained for 5 days with daily administration of the WIN55212-2 compound (Chien et al., 2003).

RATS Topical application of WIN-55212-2 in rat also led to reduction in IOP. The reduction in IOP was noted without cardiovascular toxic effects (Hosseini et al., 2006; Oltmanns et al., 2008).

TABLE 77.3 Effect of Endocannabinoids, Enzyme Inhibitors, and Transport Inhibitors on Intraocular Pressure

Drug	Study	Species	Route of administration	Intraocular pressure change
ENDOCANNABINOID				
AEA	Pate et al. (1998)	Rabbit	Topical	Decrease
	Mikawa et al. (1997)	Rabbit	Topical	Decrease
	Pate et al. (1996)	Rabbit	Topical	Decrease
	Pate et al. (1995)	Rabbit	Topical	Decrease
	Laine et al. (2002b)	Rabbit	Topical	Decrease
2-AG	Laine et al. (2002a)	Rabbit	Topical	Decrease
PEA	Gagliano et al. (2011)	Human	Topical	Decrease
	Pescosolido, Librando, Puzzono, and Nebbioso (2011)	Human	Oral	Decrease
ENZYME INHIBITOR				
PMSF	Laine et al. (2002b)	Rabbit	Topical	Prolongs decrease by AEA
TRANSPORT INHIBITOR				
AM404	Laine et al. (2001)	Rabbit	Topical	Decrease
Olvanil	Laine et al. (2001)	Rabbit	Topical	Decrease

AEA, Arachidonylethanolamide; 2-AG, 2-arachidonylglycerol; PEA, palmitoylethanolamide; PMSF, phenylmethylsulfonyl fluoride.

Endocannabinoids

Arachidonylethanolamide

Arachidonylethanolamide (AEA) was the first compound to be identified as an endocannbinoid ligand, and behaves as a partial agonist at both CB1 and CB2 (Howlett et al., 2002; Pertwee, 1999; Pertwee et al., 2010). In several studies, topical administration of AEA in normotensive rabbits caused an IOP reduction in the treated eyes (Laine, Jarvinen, Pate, Urtti, & Jarvinen, 2002b; Mikawa et al., 1997; Pate, Jarvinen, Urtti, Jarho, & Jarvinen, 1995; Pate et al., 1996, 1998) (Table 77.3).

2-Arachidonylglycerol

2-Arachidonylglycerol (2-AG) is an endogenous cannabinoid agonist for both CB1 and CB2 (Howlett et al., 2002; Pertwee, 1999; Pertwee et al., 2010). Topical application of 2-AG in rabbits reduced IOP (Laine, Jarvinen, Mechoulam, Breuer, & Jarvinen, 2002a).

Palmitoylethanolamide

Palmitoylethanolamide (PEA) is an endogenous fatty acid amide related to AEA, and was found to reduce IOP, in recent clinical studies of glaucoma patients. Utilizing 42 glaucoma patients, oral administration of PEA resulted in a 3.2 mmHg drop in IOP. This drop was not accompanied by a change in heart rate or systemic side effects (Gagliano et al., 2011; Pescosolido et al., 2011).

MOLECULAR TARGETS FOR THE IOP-LOWERING EFFECTS OF CANNABINOIDS

To determine the involvements of molecular targets in the IOP-lowering effects of cannabinoids, several approaches have been used. For example, receptor involvement has been examined by using receptor selective antagonists. In addition, partial knockdown (through RNA interference) and total knockout of gene expression have been used to confirm the role of particular molecular targets in mediating the IOP-lowering effects of cannabinoids.

CB1

The expression of CB1 in the eye has been established in several studies. Initially, CB1 mRNA was detected in rat eyes, with the greatest concentration of mRNA found in the ciliary body, retina, and choroid (Porcella, Casellas, Gessa, & Pani, 1998). In a second study, using human eye tissues, immunohistochemistry staining for CB1 showed strong expression in the ciliary body and TM, with moderate signals within the ciliary muscle and vasculature (Straiker, Maguire, Mackie, & Lindsey, 1999). In addition, Stamer et al. (2001) confirmed the expression of both CB1 mRNA and proteins in the ciliary body and TM of human eye tissues.

The involvement of CB1 in the IOP-lowering effects of cannabinoids has been demonstrated using selective CB1 antagonist SR141716A, in combination with a variety of

nonselective CB1 agonists, including phytocannabinoid Δ^9-THC, nonclassical, bicyclic cannabinoid CP-55,940, aminoalkylindole prototypic ligand WIN55212-2, and endocannabinoids AEA and 2-AG.

Intertracheal (i.t.) administration of a liposome-entrapped Δ^9-THC led to a significant decrease in IOP. This decrease in IOP was blocked by pretreatment with CB1 antagonist SR 141716A (Szczesniak et al., 2006).

Topical administration of the nonclassical cannabinoid CP-55,940 caused a dose-dependent reduction in IOP in normotensive rabbits. Further experiments demonstrated that the reduction in IOP caused by CP-55,940 can be blocked by administration of CB1 antagonist SR 141716A (Laine et al., 2003; Pate et al., 1998).

In normotensive rabbits, topical application of WIN55212-2 significantly reduced IOP, and this reduction was partially blocked by SR141716A (Song & Slowey, 2000). In rats, similar findings were observed (Oltmanns et al., 2008).

Normotensive rabbits treated with AEA and 2-AG had reduced IOP, and AEA- or 2-AG-induced IOP reduction was blocked by CB1 antagonists SR141716A or AM251 (Laine et al., 2002a; Pate et al., 1998).

These studies have emphasized the prominent role of CB1 receptors involved in the regulation of IOP.

CB2

Because of low expression levels of CB2 in eye tissues, doubt initially arose regarding the role of CB2 in maintaining IOP (Porcella et al., 1998). However, more recent studies have established the presence of CB2 receptor in the eye. Using porcine eye tissues, immunohistochemistry staining showed the presence of CB2 in the TM. Furthermore, Western blot confirmed the expression of CB2 in cultured porcine TM cells (Zhong, Geng, Njie, Feng, & Song, 2005).

An initial study conducted by the Jarvinen group showed that topical application of the CB2 agonist JWH-133 to normotensive rabbits had no effect on IOP; they concluded that CB2 is not involved in regulating IOP (Laine et al., 2003). However, recent studies conducted in our lab have shown that CB2 plays an important role in regulating aqueous humor outflow. Stimulation of CB2 receptor by JWH015, a selective CB2 agonist, resulted in an enhancement of aqueous humor flow. The increase in aqueous humor flow was completely blocked by the pretreatment of CB2 antagonist, SR144528 (Zhong et al., 2005).

Further studies are needed to establish a clearer role for the CB2 receptor in regulating IOP.

Non-CB1/CB2 Cannabinoid Receptors/Targets

The presence of non-CB1/CB2 receptors/targets that interact with a variety of cannabinoids has been established recently (Pertwee et al., 2010). Additionally, several of these receptors/targets have been shown to be expressed in the eye. For example, using immunohistochemistry, GPR18 protein was detected prominently in the ciliary epithelium, corneal epithelium, and in the TM (Caldwell et al., 2013). In addition, using Western blot analysis, the presence of GPR55 and PPARα has been demonstrated in the porcine TM model (Kumar, Qiao, Kumar, & Song, 2012).

In an initial IOP study, systemic administration of a nonpsychotropic cannabinoid, abnormal cannabidiol (abn-CBD) decreased IOP in the normotensive Brown Norway rat eye, independent of CB1 or CB2 activation (Szczesniak et al., 2011). Further studies showed that topical administration of GPR18 ligands, abn-CBD and *n*-arachidonyl glycine, caused a reduction of IOP in normotensive mice. In addition, the IOP-lowering effects of abn-CBD and *n*-arachidonyl glycine are independent of CB1, CB2, and GPR55, because these two GPR18 ligands were able to lower IOP in mice whose CB1, CB2, or GPR55 were knocked out (Caldwell et al., 2013).

Furthermore, non-CB1/CB2 receptors have been shown to influence aqueous humor outflow in animal models. Treatment with abn-CBD resulted in a dose-dependent increase in aqueous humor outflow through the TM. This increase in outflow was lost with pretreatment of O-1918, a selective non-CB1/CB2 receptor antagonist. Pretreatment with CB1 antagonist SR141716A or the CB2 antagonist SR144528 had no effect on the increase in humoral outflow induced by abn-CBD (Qiao, Kumar, Kumar, & Song, 2012).

Additionally, PEA caused an enhancement of aqueous humor outflow in perfused porcine eye anterior segments. This PEA-induced increase in aqueous humor outflow was partially blocked when PPARα or GPR55 receptors were knocked down by introducing to the perfusion system small hairpin RNA (shRNA) against PPARα or GPR55 (Kumar et al., 2012).

Enzyme Inhibitors

Enzyme inhibition can be used to prolong the effects of endocannabinoids in the reduction of IOP. Enzyme inhibition works indirectly to regulate IOP: by inhibiting degradation enzymes, endocannabinoids are no longer degraded, and free to work on receptors.

Phenylmethylsulfonyl fluoride (PMSF) is a nonspecific inhibitor of fatty acid amide hydrolase (FAAH), which degrades AEA. When rabbits were pretreated with PMSF, researchers noted an immediate reduction in IOP, if AEA was also administered. In contrast, rabbits not treated with the enzyme inhibitor showed a biphasic response of initial increase in IOP, followed by a decrease in IOP (Laine et al., 2002b).

Using porcine TM tissues, studies conducted in our lab have shown that FAAH inhibitor URB597 inhibited the

degradation of AEA. Administration of URB597 in the porcine organ model prolonged the outflow-enhancing effects of AEA (Njie, Qiao, Xiao, Wang, & Song, 2008b).

Additionally, monoacylgylcerol lipase (MGL) inhibitor LY2183240 inhibited the breakdown of 2-AG. The outflow-enhancing effects of 2-AG was prolonged by the coadministration of LY2183240 (Njie, He, Qiao, & Song, 2008a).

Transporter Inhibitors

Transporters work to reuptake endocannabinoids after their release (Pazos, Nunez, Benito, Tolon, & Romero, 2005). By inhibiting the transporters, it is possible to potentiate the IOP-lowering effects of endocannabinoids.

For example, AEA transport inhibitors AM404 and olvanil, administered unilaterally onto rabbit eyes, dramatically reduced IOP in the eyes that received these ligands. Hence, this study demonstrated the role of transporter inhibitors in regulation of IOP (Laine et al., 2001).

Association of CB1 Receptor With β-Adrenergic Receptor

To determine which receptors are essential for IOP reduction, Hudson, Beazley, Szczesniak, Straiker, and Kelly (2011) administered β-adrenergic agonists, β-adrenergic antagonists, and cannabinoid agonists to knockout mice for three receptors: CB1, CB2, and β-adrenergic receptor (βAD). Reduction in IOP was noted in CB2 knockout mice, suggesting that ligand binding to CB2 was not essential for IOP reduction. However, administration of these ligands in either βAD or CB1 deficient mice led to no reduction in IOP. Further examination showed that desensitization of βAD or depletion of catecholamines prevented reduction in IOP. Finally, immunohistochemistry staining revealed colocalization of CB1 with adrenergic neurons. Taken together, these data imply that CB1 receptors associate with β-adrenergic to reduce IOP (Hudson et al., 2011).

CELLULAR MECHANISMS OF CANNABINOID-INDUCED REDUCTION OF IOP

There are three main mechanisms to decrease IOP: to decrease aqueous humor production in the ciliary epithelium, to increase aqueous humor outflow through the conventional, TM pathway, and to increase aqueous humor outflow by the nonconventional, uveoscleral pathway in the ciliary muscle. These three mechanisms are controlled by multiple signaling pathways which can be affected by cannabinoids.

Ciliary Epithelium

Aqueous humor is produced and secreted by the ciliary epithelium. Therefore, interactions between cannabinoids and the ciliary epithelium is a mechanism by which IOP can be controlled. Ciliary epithelium expresses high levels of CB1, as shown by immunohistochemistry (Straiker et al., 1999).

Morphological changes in the ciliary epithelium have been observed after exposure to marijuana. Specifically, monkey ciliary epithelium experienced swelling, followed by a reduction in IOP after intravitreal injection of soluble marijuana extracts (McDonald, Cheeks, Slagle, & Green, 1991).

Further mechanistic studies demonstrated that cannabinoid-induced IOP reduction may involve prostaglandins and metalloproteinase produced by nonpigmented ciliary epithelium. Treatment of human nonpigmented ciliary epithelium with R(+)-methanandamide, a CB1 agonist, resulted an increase in cyclooxygenase-2 (COX-2) mRNA expression. Increased COX-2 expression was followed by increased phosphorylation of p38 and p42/44 mitogen-activated protein kinase (MAPK), two key signaling molecules in metalloproteinase (MMP) regulation. Correspondingly, mRNA and protein expression of MMP-1, MMP-3, MMP-9, and tissue inhibitor of metalloproteinase 1 (TIMP-1) was increased; therefore a potential mechanism contributing to the IOP-lowering effects of cannabinoids may be the stimulation of COX-2 and MMP expression in nonpigmented ciliary epithelium (Rosch, Ramer, Brune, & Hinz, 2006).

Trabecular Meshwork

The TM is located at the base of the cornea, near the ciliary body. This tissue regulates the aqueous humor outflow in the anterior chamber. Within this tissue, there are a variety of receptors/targets for cannabinoid ligands, including the CB1, CB2, as well as non-CB1/CB2 cannabinoid receptors (Caldwell et al., 2013; Kumar et al., 2012; Qiao et al., 2012; Stamer et al., 2001; Straiker et al., 1999; Zhong et al., 2005). This section reviews the signaling pathways of these receptors in the TM, and their relevance to the regulation of aqueous humor outflow.

cAMP

Previously, it has been shown that cAMP is an important regulator of aqueous humor outflow through the TM pathway. Usually, CB1/CB2 receptors are G_i-protein receptors, and there is a decrease in cAMP when these receptors are activated. However, in TM cells, CP-55,940 has been shown to cause a two- to three-fold increase in cAMP, as a result of CB1 activation (Stamer et al., 2001).

Ions and Ion Channels

Relaxation of TM is thought to result in a reduction in IOP. Stimulation of human TM strips with CP-55,940 or WIN55212-2 caused a dose-dependent relaxation of TM that is blocked by AM251. In addition, cannabinoids opened high-conductance Ca(2+)-activated K(+) channels, and this effect may contribute to the relaxation of TM, thus modulating aqueous humor outflow (Stumpff et al., 2005).

Further studies showed the ability of cannabinoids to modulate intracellular calcium in TM. Stimulation of human TM cells with WIN55212-2 or CP-55,940 yielded an increase in intracellular Ca^{2+} release. However, the mechanism of Ca^{2+} release was ligand-dependent. WIN55212-2-induced increase in $[Ca^{2+}]_i$ was insensitive to PTX, and is therefore independent of $G_{i/o}$ coupling. In contrast, the $[Ca^{2+}]_i$ increase mediated by CP-55,940 was PTX-sensitive, indicative of CB1- $G_{i/o}$ coupling (McIntosh, Hudson, Yegorova, Jollimore, & Kelly, 2007).

P42/44 MAP Kinase

P42/44 MAP kinase is an important regulator of aqueous humor outflow through the TM pathway.

Various cannabinoid ligands have been shown to activate P42/44 MAP kinase in the TM. Furthermore, P42/44 MAP kinase has been shown to be important for the outflow-enhancing effects of a variety of cannabinoid agonists, including CB1 selective ligand Noladin ether, CB2 selective agonist JWH015, endocannabinoids AEA and 2-AG, and non-CB1/CB2 cannabinoid ligand abn-CBD and PEA (Kumar et al., 2012; McIntosh et al., 2007; Njie et al., 2008a,b; Qiao et al., 2012; Zhong et al., 2005).

Ciliary Muscle

Nonconventional, uveoscleral outflow pathway represents another mechanism that might mediate the IOP-lowering actions of cannabinoids. Immunohistochemstry experiments have demonstrated the presence of CB1 in the human ciliary muscle (Straiker et al., 1999). To our knowledge, there are no published studies on the effects of cannabinoids on the uveoscleral pathway. However, cannabinoids have been shown to cause ciliary muscle contraction, which may lead to an enhanced aqueous humor outflow through the conventional pathway.

Exposure of bovine ciliary muscle to CP-55,940 and AEA caused a dose-dependent contraction of ciliary muscle that is prevented by SR141716A. Further investigation showed that the CP55,940- and AEA-induced contraction is dependent on the $G_{i/o}$-protein, and phospholipase C (Lograno & Romano, 2004).

Stimulation of ciliary muscle by AEA in presence of muscarinic agonist carbachol increased contractility in a greater manner than AEA alone. This showed a possibility of using muscarinic agonists in concert with cannabinoids to reduce IOP (Romano & Lograno, 2013).

In conclusion, modulation of the ciliary muscle by cannabinoids maybe an important mechanism for the IOP-lowering effects.

PROBLEMS AND FUTURE DIRECTIONS

Problems

Cannabinoids represent a promising way of reducing IOP. However, as with all emerging therapeutics, cannabinoid treatment of ocular hypertension is associated with several problems that must be remedied.

First of all, administration of cannabinoids is associated with systemic side effects, such as psychoactivity and cardiovascular problems (Purnell & Gregg, 1975). These systemic side effects interfere with patient compliance (Flach, 2002). Systemic side effects can be minimized through effective ocular drug delivery.

A second problem is the short duration of action of cannabinoids. The duration of action for topical administration of Δ^9-THC in humans is 2–4 h before a return to hypertensive ocular pressure (Plange et al., 2007). This poses a treatment problem, as hypertensive conditions last for the full day.

Finding new, innovative ways to solve these problems is of utmost importance for treating ocular hypertension with cannabinoids.

Solutions and Future Directions

Non-CB1 Cannabinoid Ligands

Systemic side effects such as psychoactivity and cardiovascular problems can be remedied by utilizing cannabinoids that do not target the CB1. For example, topical administration of HU-211 in rabbit caused a reduction in IOP without the cardiac side effect of increased heart rate (Naveh et al., 2000). Unlike Δ^9-THC and HU-210, which act on the CB1 and CB2, HU-211 blocks the NMDA receptor (Pertwee et al., 2010). Therefore, HU-211 should not have the psychoactive and cardiac side effects mediated by the CB1.

Sustained Drug Delivery

Nanotechnology poses a possible way to overcome the short duration of action problem of cannabinoids. Nanomedicine utilizes particles that are between 1 and 100 nm in size, and exhibit distinct physical properties. Because of these properties, nanoparticles are able to protect and transport complex molecules, and prevent degradation. Nanoparticles can be composed of many types of materials and forms, including liposomes, hydrogels, and dendrimers. These higher order structures

tightly regulate drug delivery, and allow for target delivery of therapy to desired tissues.

Nanotechnology has been illustrated to improve the delivery of glaucoma treatments. For example, a study has been conducted in rabbits using uniform Egg-phosphatidylcholine (EggPC) liposomes loaded with latanoprost for ocular delivery. These liposomes loaded with latanoprost were found to be stable, and a single injection to rabbit eye lowered IOP for at least 90 days. Therefore, nanoparticles can be potentially used to deliver cannabinoids directly to targets, and to not only reduce systemic side effects, but also increase efficacy and durations of actions (Kim et al., 2014).

In summary, future directions for lowering IOP with cannabinoids include non-CB1 ligands, and sustained drug delivery.

MINI-DICTIONARY

Aqueous humor A gelatinous fluid that provides nutrients for avascular tissues in the eye. Secretion and outflow of aqueous humor regulate intraocular pressure.
Ciliary epithelium Located in the posterior chamber of the eye. Generates and secretes aqueous humor.
Ciliary muscle Composed of smooth muscle, it is part of the uveoscleral pathway.
Conventional outflow pathway Aqueous humor flows through the trabecular meshwork, and then empties into Schlemm's canal.
Glaucoma An eye disease begins with peripheral vision loss, and eventually total vision loss. Risk factors include genetic predisposition and elevated intraocular pressure.
Intraocular pressure The pressure within the eye. Many antiglaucoma medications work to lower intraocular pressure.
Nonconventional/uveoscleral outflow pathway Aqueous humor flows through the ciliary muscle and passes over the suprachoroidal space, then exiting through the sclera.
Ocular hypertension A condition in which the intraocular pressure is higher than normal. It is one of the most important risk factors for glaucoma.
Trabecular meshwork Located in the anterior portion of the eye, it is a component of the conventional outflow pathway.

References

Beilin, M., Neumann, R., Belkin, M., Green, K., & Bar-Ilan, A. (2000). Pharmacology of the intraocular pressure (IOP) lowering effect of systemic dexanabinol (HU-211), a non-psychotropic cannabinoid. *Journal of Ocular Pharmacology and Therapeutics*, *16*(3), 217–230.

Caldwell, M. D., Hu, S. S., Viswanathan, S., Bradshaw, H., Kelly, M. E., & Straiker, A. (2013). A GPR18-based signalling system regulates IOP in murine eye. *British Journal of Pharmacology*, *169*(4), 834–843.

Cascio, M. G., Gauson, L. A., Stevenson, L. A., Ross, R. A., & Pertwee, R. G. (2010). Evidence that the plant cannabinoid cannabigerol is a highly potent alpha2-adrenoceptor agonist and moderately potent 5HT1A receptor antagonist. *British Journal of Pharmacology*, *159*(1), 129–141.

Chien, F. Y., Wang, R. F., Mittag, T. W., & Podos, S. M. (2003). Effect of WIN 55212-2, a cannabinoid receptor agonist, on aqueous humor dynamics in monkeys. *Archives of Ophthalmology*, *121*(1), 87–90.

Colasanti, B. K. (1990). A comparison of the ocular and central effects of delta 9-tetrahydrocannabinol and cannabigerol. *Journal of Ocular Pharmacology*, *6*(4), 259–269.

Colasanti, B. K., Brown, R. E., & Craig, C. R. (1984a). Ocular hypotension, ocular toxicity, and neurotoxicity in response to marihuana extract and cannabidiol. *General Pharmacology*, *15*(6), 479–484.

Colasanti, B. K., Craig, C. R., & Allara, R. D. (1984b). Intraocular pressure, ocular toxicity and neurotoxicity after administration of cannabinol or cannabigerol. *Experimental Eye Research*, *39*(3), 251–259.

Colasanti, B. K., Powell, S. R., & Craig, C. R. (1984c). Intraocular pressure, ocular toxicity and neurotoxicity after administration of delta 9-tetrahydrocannabinol or cannabichromene. *Experimental Eye Research*, *38*(1), 63–71.

Crandall, J., Matragoon, S., Khalifa, Y. M., Borlongan, C., Tsai, N. T., Caldwell, R. B., & Liou, G. I. (2007). Neuroprotective and intraocular pressure-lowering effects of (-)Delta9-tetrahydrocannabinol in a rat model of glaucoma. *Ophthalmic Research*, *39*(2), 69–75.

Crawford, W. J., & Merritt, J. C. (1979). Effects of tetrahydrocannabinol on arterial and intraocular hypertension. *International Journal of Clinical Pharmacology and Biopharmaceutics*, *17*(5), 191–196.

Elsohly, M. A., Harland, E., Murphy, J. C., Wirth, P., & Waller, C. W. (1981). Cannabinoids in glaucoma: a primary screening procedure. *Journal of Clinical Pharmacology*, *21*(Suppl. 8–9), 472S–478S.

Fischer, K. M., Ward, D. A., & Hendrix, D. V. (2013). Effects of a topically applied 2% delta-9-tetrahydrocannabinol ophthalmic solution on intraocular pressure and aqueous humor flow rate in clinically normal dogs. *American Journal of Veterinary Research*, *74*(2), 275–280.

Flach, A. J. (2002). Delta-9-tetrahydrocannabinol (THC) in the treatment of end-stage open-angle glaucoma. *Transactions of the American Ophthalmology Society*, *100*, 215–222, discussion 222–214.

Gagliano, C., Ortisi, E., Pulvirenti, L., Reibaldi, M., Scollo, D., Amato, R., …, Longo, A. (2011). Ocular hypotensive effect of oral palmitoylethanolamide: a clinical trial. *Investigative Ophthalmology and Visual Science*, *52*(9), 6096–6100.

Green, K., Symonds, C. M., Oliver, N. W., & Elijah, R. D. (1982). Intraocular pressure following systemic administration of cannabinoids. *Current Eye Research*, *2*(4), 247–253.

Hepler, R. S., & Frank, I. R. (1971). Marihuana smoking and intraocular pressure. *Journal of the American Medical Association*, *217*(10), 1392.

Hodges, L. C., Reggio, P. H., & Green, K. (1997). Evidence against cannabinoid receptor involvement in intraocular pressure effects of cannabinoids in rabbits. *Ophthalmic Research*, *29*(1), 1–5.

Hosseini, A., Lattanzio, F. A., Williams, P. B., Tibbs, D., Samudre, S. S., & Allen, R. C. (2006). Chronic topical administration of WIN-55-212-2 maintains a reduction in IOP in a rat glaucoma model without adverse effects. *Experimental Eye Research*, *82*(5), 753–759.

Howlett, A. C., Barth, F., Bonner, T. I., Cabral, G., Casellas, P., Devane, W. A., …, Pertwee, R. G. (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacological Reviews*, *542*, 161–202.

Hudson, B. D., Beazley, M., Szczesniak, A. M., Straiker, A., & Kelly, M. E. (2011). Indirect sympatholytic actions at beta-adrenoceptors account for the ocular hypotensive actions of cannabinoid receptor agonists. *Journal of Pharmacology and Experimental Therapeutics*, *339*(3), 757–767.

Kim, N. J., Harris, A., Gerber, A., Tobe, L. A., Amireskandari, A., Huck, A., & Siesky, B. (2014). Nanotechnology and glaucoma: a review of the potential implications of glaucoma nanomedicine. *British Journal of Ophthalmology*, *98*(4), 427–431.

Kumar, A., Qiao, Z., Kumar, P., & Song, Z. H. (2012). Effects of palmitoylethanolamide on aqueous humor outflow. *Investigative Ophthalmology and Visual Science*, *53*(8), 4416–4425.

Laine, K., Jarvinen, K., & Jarvinen, T. (2003). Topically administered CB(2)-receptor agonist, JWH-133, does not decrease intraocular pressure (IOP) in normotensive rabbits. *Life Sciences*, *72*(7), 837–842.

Laine, K., Jarvinen, K., Mechoulam, R., Breuer, A., & Jarvinen, T. (2002a). Comparison of the enzymatic stability and intraocular pressure effects of 2-arachidonylglycerol and noladin ether, a novel putative endocannabinoid. *Investigative Ophthalmology and Visual Science*, *43*(10), 3216–3222.

Laine, K., Jarvinen, K., Pate, D. W., Urtti, A., & Jarvinen, T. (2002b). Effect of the enzyme inhibitor, phenylmethylsulfonyl fluoride, on the IOP profiles of topical anandamides. *Investigative Ophthalmology and Visual Science*, *43*(2), 393–397.

Laine, K., Jarvinen, T., Savinainen, J., Laitinen, J. T., Pate, D. W., & Jarvinen, K. (2001). Effects of topical anandamide-transport inhibitors, AM404 and olvanil, on intraocular pressure in normotensive rabbits. *Pharmaceutical Research*, *18*(4), 494–499.

Liu, J. H., & Dacus, A. C. (1987). Central nervous system and peripheral mechanisms in ocular hypotensive effect of cannabinoids. *Archives of Ophthalmology*, *105*(2), 245–248.

Lograno, M. D., & Romano, M. R. (2004). Cannabinoid agonists induce contractile responses through Gi/o-dependent activation of phospholipase C in the bovine ciliary muscle. *European Journal of Pharmacology*, *494*(1), 55–62.

McDonald, T. F., Cheeks, L., Slagle, T., & Green, K. (1991). Marijuana-derived material-induced changes in monkey ciliary processes differ from those in rabbit ciliary processes. *Current Eye Research*, *10*(4), 305–312.

McIntosh, B. T., Hudson, B., Yegorova, S., Jollimore, C. A., & Kelly, M. E. (2007). Agonist-dependent cannabinoid receptor signalling in human trabecular meshwork cells. *British Journal of Pharmacology*, *152*(7), 1111–1120.

Mikawa, Y., Matsuda, S., Kanagawa, T., Tajika, T., Ueda, N., & Mimura, Y. (1997). Ocular activity of topically administered anandamide in the rabbit. *Japanese Journal of Ophthalmology*, *41*(4), 217–220.

Muchtar, S., Almog, S., Torracca, M. T., Saettone, M. F., & Benita, S. (1992). A submicron emulsion as ocular vehicle for delta-8-tetrahydrocannabinol: effect on intraocular pressure in rabbits. *Ophthalmic Research*, *24*(3), 142–149.

Naveh, N., Weissman, C., Muchtar, S., Benita, S., & Mechoulam, R. (2000). A submicron emulsion of HU-211, a synthetic cannabinoid, reduces intraocular pressure in rabbits. *Graefe's Archive for Clinical and Experimental Ophthalmology*, *238*(4), 334–338.

Newell, F. W., Stark, P., Jay, W. M., & Schanzlin, D. J. (1979). Nabilone: a pressure-reducing synthetic benzopyran in open-angle glaucoma. *Ophthalmology*, *86*(1), 156–160.

Njie, Y. F., He, F., Qiao, Z., & Song, Z. H. (2008a). Aqueous humor outflow effects of 2-arachidonylglycerol. *Experimental Eye Research*, *87*(2), 106–114.

Njie, Y. F., Qiao, Z., Xiao, Z., Wang, W., & Song, Z. H. (2008b). *N*-arachidonylethanolamide-induced increase in aqueous humor outflow facility. *Investigative Ophthalmology and Visual Science*, *49*(10), 4528–4534.

Oltmanns, M. H., Samudre, S. S., Castillo, I. G., Hosseini, A., Lichtman, A. H., Allen, R. C., …, Williams, P. B. (2008). Topical WIN55212-2 alleviates intraocular hypertension in rats through a CB1 receptor mediated mechanism of action. *Journal of Ocular Pharmacology and Therapeutics*, *24*(1), 104–115.

Pate, D. W., Jarvinen, K., Urtti, A., Jarho, P., Fich, M., Mahadevan, V., & Jarvinen, T. (1996). Effects of topical anandamides on intraocular pressure in normotensive rabbits. *Life Sciences*, *58*(21), 1849–1860.

Pate, D. W., Jarvinen, K., Urtti, A., Jarho, P., & Jarvinen, T. (1995). Ophthalmic arachidonylethanolamide decreases intraocular pressure in normotensive rabbits. *Current Eye Research*, *14*(9), 791–797.

Pate, D. W., Jarvinen, K., Urtti, A., Mahadevan, V., & Jarvinen, T. (1998). Effect of the CB1 receptor antagonist, SR141716A, on cannabinoid-induced ocular hypotension in normotensive rabbits. *Life Sciences*, *63*(24), 2181–2188.

Pazos, M. R., Nunez, E., Benito, C., Tolon, R. M., & Romero, J. (2005). Functional neuroanatomy of the endocannabinoid system. *Pharmacology Biochemistry and Behavior*, *81*(2), 239–247.

Pertwee, R. G. (1999). Pharmacology of cannabinoid receptor ligands. *Current Medicinal Chemistry*, *6*(8), 635–664.

Pertwee, R. G., Howlett, A. C., Abood, M. E., Alexander, S. P., Di Marzo, V., Elphick, M. R., …, Ross, R. A. (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB(1) and CB(2). *Pharmacological Reviews*, *62*(4), 588–631.

Pescosolido, N., Librando, A., Puzzono, M., & Nebbioso, M. (2011). Palmitoylethanolamide effects on intraocular pressure after Nd:YAG laser iridotomy: an experimental clinical study. *Journal of Ocular Pharmacology and Therapeutics*, *27*, 629–635.

Plange, N., Arend, K. O., Kaup, M., Doehmen, B., Adams, H., Hendricks, S., …, Remky, A. (2007). Dronabinol and retinal hemodynamics in humans. *American Journal of Ophthalmology*, *143*(1), 173–174.

Porcella, A., Casellas, P., Gessa, G. L., & Pani, L. (1998). Cannabinoid receptor CB1 mRNA is highly expressed in the rat ciliary body: implications for the antiglaucoma properties of marihuana. *Brain Research Molecular Brain Research*, *58*(1–2), 240–245.

Porcella, A., Maxia, C., Gessa, G. L., & Pani, L. (2001). The synthetic cannabinoid WIN55212-2 decreases the intraocular pressure in human glaucoma resistant to conventional therapies. *European Journal of Neuroscience*, *13*(2), 409–412.

Purnell, W. D., & Gregg, J. M. (1975). Delta(9)-tetrahydrocannabinol, euphoria and intraocular pressure in man. *Annals of Ophthalmology*, *7*(7), 921–923.

Qiao, Z., Kumar, A., Kumar, P., & Song, Z. -H. (2012). Involvement of a non-CB1/CB2 cannabinoid receptor in the aqueous humor outflow-enhancing effects of abnormal-cannabidiol. *Experimental Eye Research*, *100*(0), 59–64.

Rocha-Sousa, A., Rodrigues-Araujo, J., Gouveia, P., Barbosa-Breda, J., Azevedo-Pinto, S., Pereira-Silva, P., & Leite-Moreira, A. (2013). New therapeutic targets for intraocular pressure lowering. *ISRN Ophthalmology*, *2013*, 261386.

Romano, M. R., & Lograno, M. D. (2013). Signaling cross-talk between cannabinoid and muscarinic systems actives Rho-kinase and increases the contractile responses of the bovine ciliary muscle. *European Journal of Pharmacology*, *702*(1–3), 174–179.

Rosch, S., Ramer, R., Brune, K., & Hinz, B. (2006). R(+)-methanandamide and other cannabinoids induce the expression of cyclooxygenase-2 and matrix metalloproteinases in human nonpigmented ciliary epithelial cells. *Journal of Pharmacology and Experimental Therapeutics*, *316*(3), 1219–1228.

Song, Z. H., & Slowey, C. A. (2000). Involvement of cannabinoid receptors in the intraocular pressure-lowering effects of WIN55212-2. *Journal of Pharmacology and Experimental Therapeutics*, *292*(1), 136–139.

Stamer, W. D., Golightly, S. F., Hosohata, Y., Ryan, E. P., Porter, A. C., Varga, E., …, Yamamura, H. I. (2001). Cannabinoid CB(1) receptor expression, activation and detection of endogenous ligand in trabecular meshwork and ciliary process tissues. *European Journal of Pharmacology*, *431*(3), 277–286.

Straiker, A. J., Maguire, G., Mackie, K., & Lindsey, J. (1999). Localization of cannabinoid CB1 receptors in the human anterior eye and retina. *Investigative Ophthalmology and Visual Science*, *40*(10), 2442–2448.

Stumpff, F., Boxberger, M., Krauss, A., Rosenthal, R., Meissner, S., Choritz, L., …, Thieme, H. (2005). Stimulation of cannabinoid (CB1) and prostanoid (EP2) receptors opens BKCa channels and relaxes ocular trabecular meshwork. *Experimental Eye Research*, *80*(5), 697–708.

Szczesniak, A. M., Kelly, M. E., Whynot, S., Shek, P. N., & Hung, O. (2006). Ocular hypotensive effects of an intratracheally delivered liposomal delta9-tetrahydrocannabinol preparation in rats. *Journal of Ocular Pharmacology and Therapeutics*, *22*(3), 160–167.

Szczesniak, A. M., Maor, Y., Robertson, H., Hung, O., & Kelly, M. E. (2011). Nonpsychotropic cannabinoids, abnormal cannabidiol and canabigerol-dimethyl heptyl, act at novel cannabinoid receptors to reduce intraocular pressure. *Journal of Ocular Pharmacology and Therapeutics*, *27*(5), 427–435.

Tiedeman, J. S., Shields, M. B., Weber, P. A., Crow, J. W., Cocchetto, D. M., Harris, W. A., & Howes, J. F. (1981). Effect of synthetic cannabinoids on elevated intraocular pressure. *Ophthalmology*, *88*(3), 270–277.

Tomida, I., Azuara-Blanco, A., House, H., Flint, M., Pertwee, R. G., & Robson, P. J. (2006). Effect of sublingual application of cannabinoids on intraocular pressure: a pilot study. *Journal of Glaucoma*, *15*(5), 349–353.

Toris, C. B. (2010). Pharmacotherapies for glaucoma. *Current Molecular Medicine*, *10*(9), 824–840.

Weinreb, R. N., Aung, T., & Medeiros, F. A. (2014). The pathophysiology and treatment of glaucoma: a review. *Journal of the American Medical Association*, *311*(18), 1901–1911.

Zhong, L., Geng, L., Njie, Y., Feng, W., & Song, Z. H. (2005). CB2 cannabinoid receptors in trabecular meshwork cells mediate JWH015-induced enhancement of aqueous humor outflow facility. *Investigative Ophthalmology and Visual Science*, *46*(6), 1988–1992.

CHAPTER

78

Ocular Delivery of Tetrahydrocannabinol

G.R. Adelli, P. Bhagav*, M.A. Repka*, W. Gul**, M.A. ElSohly**, S. Majumdar**

***Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, Oxford, MS, United States**

****ElSohly Laboratories Inc., Oxford, MS, United States**

SUMMARY POINTS

- This chapter focuses on ocular drug delivery of tetrahydrocannabinol (THC).
- THC is a key constituent of the cannabis plant, and has been shown to have beneficial effects in glaucoma and other ocular disorders.
- Numerous studies have shown that THC reduces intraocular pressure (IOP) in glaucoma, and also has neuroprotective actions.
- Ocular delivery of THC encounters several challenges: those associated with anatomical and physiological barriers of the eye, and also the physicochemical properties of THC such as solubility, lipophilicity, and stability.
- Various approaches to improve the physicochemical characteristics, and hence ocular delivery, are being explored.

KEY FACTS OF GLAUCOMA

- Glaucoma, a leading cause of irreversible blindness worldwide, characterized by damage to the optic nerve leading to blindness, manifests as rise in intraocular pressure (IOP).
- Though the exact cause is not known, elevated IOP for a prolonged period is suggested to be a risk factor behind optic nerve damage and vision loss.
- Glaucoma is categorized into open angle glaucoma and closed angle glaucoma, depending on the iridocorneal angle.
- Open angle glaucoma is mostly asymptomatic, progresses slowly, with gradual damage to the optic nerve, and, if undetected, it leads to irreversible blindness which continues even if the intraocular pressure is normal with conventional glaucoma drugs; meanwhile, closed angle glaucoma develops rapidly and produces symptoms such as eye pain and, if untreated, results in irreversible blindness.
- Current treatment is based on reducing the elevated intraocular pressure by either increasing the aqueous humor outflow, via trabecular and uveoscleral outflow and/or by decreasing the production of aqueous humor.

KEY FACTS OF PRODRUGS

- Prodrugs are inactive, bioreversible derivatives of active drug molecules that, upon enzymatic and/or chemical modifications, release the active parent drug in the body, which exhibits pharmacological effect in the target organ/tissue.
- It is estimated that, currently, about 10% of worldwide marketed drugs can be classified as prodrugs.
- The basic aim of designing a prodrug is to mask the undesirable properties of the parent drugs molecule, such as poor solubility, poor bioavailability, due to poor physical, chemical or biological stability, low target selectivity, undesirable taste, or pain/local irritation on administration, presystemic metabolism, etc.
- Esters are the most commonly employed prodrugs, as numerous catalytic esterases are present in the body,

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00089-2
Copyright © 2017 Elsevier Inc. All rights reserved.

and actively hydrolyze simple esters to the respective active parent molecules.
- With species variation, there exists a difference in the amounts and types of enzymes (esterases) which results in varied substrate specificities and rates of hydrolysis, which generates a lot of variations in preclinical animal model studies and clinical studies.

LIST OF ABBREVIATIONS

BRB	Blood retinal barrier
CB1	Cannabinoid receptor 1
CB2	Cannabinoid receptor 2
CBD	Cannabidiol
CNS	Central nervous system
EDTA	Ethylenediaminetetraacetic acid
HPMC	Hydroxypropyl methylcellulose
IOP	Intraocular pressure
LMO	Light mineral oil
mm	Millimeter
NMDA	*N*-methyl-D-aspartate receptor
PEG	Polyethylene glycol
RGC	Retinal ganglion cell
Δ^8-THC	Δ^8-Tetrahydrocannabinol
THC	Δ^9-Tetrahydrocannabinol
THC-HS	Δ^9-Tetrahydrocannabinol hemisuccinate
THC-HG	Δ^9-Tetrahydrocannabinol hemiglutarate
VEGF	Vascular endothelial growth factor

INTRODUCTION

Schlicker, Timm, and Gothert (1996) discovered the existence of cannabinoid (CB) receptors in the ocular tissues, stimulating investigations into the role of these receptors in the eye. Straiker, Maguire, Mackie, and Lindsey (1999) observed that CB1 receptors are more prevalent in the human ciliary epithelium, corneal epithelium, and endothelium, trabecular meshwork, Schlemm's canal, ciliary muscle, and ciliary blood vessels. The human retina was also found to express CB1 receptors (Straiker et al., 1999).

Unlike CB1 receptors, CB2 receptor mRNA has been reported to be undetectable in the human retina (Porcella, Maxia, Gessa, & Pani, 2000), although its expression in the rat retina has been demonstrated (Lu, Straiker, & Maguire, 2000). The trabecular meshwork cells have also been shown to have functional CB2 receptors (He and Song, 2007).

The localized distribution of cannabinoid receptors in the eye strongly suggests the influence of endogenous cannabinoids on aqueous humor formation, and trabecular and uveoscleral outflow. CB1 and CB2 receptors are also thought to play an important role in visual function, and in protection against glutamate toxicity in glaucoma (Lu et al., 2000).

THERAPEUTIC POTENTIAL OF THE CANNABINOIDS IN OCULAR DISORDERS

In view of their broad spectrum of biological activity, cannabinoids hold tremendous therapeutic potential in various ocular diseases. The following sections discuss the potential utility of the cannabinoids in the management and treatment of various ocular disorders.

Glaucoma

Lowering of Intraocular Pressure

Glaucoma is defined as an ocular neuropathy with progressive and irreversible loss of vision, and is the second largest cause of blindness worldwide (Cook & Foster, 2012). Elevated IOP has been identified as an important risk factor in the pathogenesis of the disease which leads to degeneration of retinal ganglion cell (RGC) axons. Current therapy targets a reduction in IOP to prevent progression of the disease.

Following the initial observation that marijuana smoking reduced IOP (Hepler & Frank, 1971), multiple studies confirmed the IOP-lowering properties of cannabinoids (Pertwee, 1999). Initially, it was assumed that the effect of the cannabinoids was mediated through the central nervous system (CNS). Several studies, however, presented strong evidence that the IOP lowering is not only via CNS, but could also be associated with CB receptors in the eye (Colasanti, Craig, & Allara, 1984a). In one study it was observed that the IOP lowering effect of the cannabinoids was not related to arterial blood pressure reduction (Green, 1979), a sign of lack of CNS involvement. In another study, Δ^9-tetrahydrocannabinol (THC) reduced ciliary processes, and dilated the ocular blood vessels via adrenergic mechanism (Green & Pederson, 1973). A calcium channel influx inhibition was also observed to reduce norepinephrine release in the ciliary bodies, thus reducing aqueous humor production (Sugrue, 1997). A vasodilatory action of cannabinoids on the ocular blood vessels has also been reported (Porcella, Casellas, Gessa, & Pani, 1998), predominantly in anterior uvea, thus improving the aqueous humor uveoscleral outflow. A prostaglandin-mediated pathway has also been suggested (Green, Kearse, & McIntyre, 2001). Thus, it appears that the cannabinoids can potentially lower IOP through multiple mechanisms which could set it apart from the currently available antiglaucoma medicaments.

Retinal Neuroprotection

RGC cell death as a result of glaucoma-mediated apoptosis is the major implication of elevated IOP for prolonged periods of time (Weinreb & Levin, 1999). *N*-methyl-D-aspartate (NMDA) receptor activation appears to be one of the several pathways that result in apoptotic cell death. Activation of NMDA receptors increases the influx of calcium into the cells, resulting in the generation of free radicals. Recent studies have reported the neuroprotective properties of cannabinoids. THC, the major component of marijuana, inhibits the release of glutamic acid by increasing potassium and decreasing calcium permeability, while HU-211 (a synthetic cannabinoid) inhibits glutamate (NMDA) receptors (Howlett et al., 2002; Jin, Mao, Goldsmith, & Greenberg, 2000) through their action on CB1 presynaptic receptors. In an optic nerve axotomy study, wherein calibrated crush injury was induced in rats, HU-211 administered intraperitoneally showed neuroprotective actions (Yoles, Belkin, & Schwartz, 1996). Cannabinoids such as THC, HU-211, and cannabidiol (CBD), also demonstrated antioxidant properties that are not mediated by CB1 receptors suggesting free radical scavenging activity of the cannabinoids (Marsicano, Moosmann, Hermann, Lutz, & Behl, 2002).

Cannabinoids have also been reported to possess vasorelaxant activity, increasing the blood flow to the ocular tissues. An endogenous cannabinoid, 2-arachidonoylglycerol, was shown to reduce endothalin-1 induced calcium mobilization, thereby preventing vasoconstriction of human brain capillaries or microvessels (Chen et al., 2000). Endothalin-1 synthesized by vascular endothelial cells plays a critical role in the regulation of blood circulation in ocular tissues by inducing vasoconstriction, and this effect is suggested to be involved in RGC death in glaucoma (Gray, Battistini, & Webb, 2000; Haefliger, Meyer, Flammer, & Luscher, 1994). Considering that the cannabinoids act primarily through the CB receptors, and that these receptors are present in various ocular tissues, including the retina (Straiker et al., 1999), the cannabinoids may be very useful in the management of glaucoma and glaucoma-mediated neuropathy.

Ocular Tumors

The use of cannabinoids as a neoplastic agent was reported in 1975 (Munson, Harris, Friedman, Dewey, & Carchman, 1975), wherein oral administration of THC, Δ^8-THC, and cannabinol inhibited the growth of Lewis lung adenocarcinoma cells in vitro and in vivo. Since then, antiproliferative, antimetastatic, antiangiogenic, and proapoptotic effects of the cannabinoinds have been reported in various cancer types (Galve-Roperh et al., 2000).

A nonselective CB receptor agonist, WIN 55,212-2, exhibited antineoplastic activity in several cell lines (Blazquez et al., 2006; Ellert-Miklaszewska, Kaminska, & Konarska, 2005; McAllister et al., 2005; Sarfaraz, Afaq, Adhami, & Mukhtar, 2005). Studies with WIN-55,212-2, HU-210, JWH-133, and THC have also been reported to induce vascular endothelial cell apoptosis, inhibit their migration, and inhibit the expression of the proangiogenic factors VEGF and Ang2 (Casanova et al., 2003). CB1/CB2 receptor activation is also reported to block matrix methalloproteinase-2 (Blazquez et al., 2003). JWH-133 administration to C6 glioma-bearing mice altered the expression of 10 genes, all of which were directly or indirectly related to VEGF pathway (Blazquez et al., 2004). One of the mechanisms by which cannabinoids act as an antiangiogenic agent is through the inhibition of the p21ras pathway (Portella et al., 2003).

Although not yet indicated for the treatment of cancer, pharmacological targeting of the endocannabinoid system is emerging as a promising new approach for slowing the progression of cancer (Pacher & Kunos, 2013). In particular, combination therapy utilizing both traditional chemotherapeutics and molecules targeting the endocannabinoid system may be an excellent next generation treatment for cancer. The potential anticancer activity of the cannabinoids can find wide application in the treatment of retinoblastoma, and other ocular tumors.

Ocular Pain and Inflammation

Pain management by cannabinoids is through (1) direct action of cannabinoids on cannabinoid receptors, (2) inhibition of enzymes, such as fatty acid amide hydrolase and monoacyl glycerol lipases that degrade endocannabinoids (Di Marzo, 2008), and (3) antinociceptive properties of cannabinoids (Pertwee et al., 2000). The cannabinoid combination containing THC and cannabidiol (CBD) is reported to reduce pain characterized by allodynia, in patients suffering from pain of neurological origin often refractory to other treatments (Nurmikko et al., 2007). Studies with WIN-55,212-2, CP-55,940, and HU-210 have been shown to suppress neuropathic pain in the chronic constriction injury model (partial sciatic ligation model), primarily via CB1 receptor-mediated mechanisms, and produced a reversal of mechanical hyperalgesia upon subcutaneous administration (Fox et al., 2001).

Thus, the cannabinoids present a wealth of untapped potential in the management of ocular pain, such as in glaucoma, corneal and uveal tract infections, inflammation, and the localized management of injury-related pain.

OCULAR DELIVERY OF TETRAHYDROCANNABINOL

In the past, various routes of cannabinoid administration have been explored to study the effect of these agents on IOP. These routes include inhalation (Merritt, Crawford, Alexander, Anduze, & Gelbart, 1980), oral (Merritt, McKinnon, Armstrong, Hatem, & Reid, 1980), and intravenous administration (Green, Symonds, Oliver, & Elijah, 1982). Although some of these studies ratified the IOP lowering properties of the cannabinoids, the systemic side effects observed far outweighed their benefits in the management of ocular conditions. Thus, topical administration of the cannabinoids is the most logical delivery route for ocular diseases.

Challenges in Ocular Delivery of Cannabinoids Through the Topical Route

Topical delivery to the deeper ocular tissues is, however, a very challenging task. The complicated anatomy of the eye poses a major barrier for the topically administered drug/drug candidates, and requires that the therapeutic agent possess favorable physicochemical characteristics for ocular absorption to occur. Some of these challenges are briefly described later in the chapter (Table 78.1).

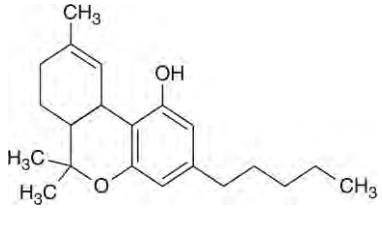
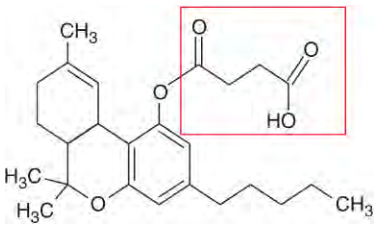
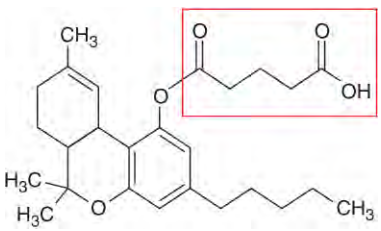
Physiological Factors

The structure of the eye is illustrated in Fig. 78.1. Physiological barriers include both static, such as different layers of cornea, sclera, and retina, as well as dynamic barriers, such as choroidal and conjunctival blood flow, lymphatic clearance, and tear dilution. Drug absorption into the deeper ocular tissues from the ocular surface may involve corneal or noncorneal pathways.

Cornea is the primary absorption restricting membrane in the corneal pathway. It is composed of a lipophilic epithelium, hydrophilic stroma, and lipophilic endothelium and, thus, poses a major barrier for both lipophilic and hydrophilic molecules. Tight junctions (zonula occludens) seal off the gap between the cells, and block the paracellular penetration of the hydrophilic and lipophilic molecules into the deeper ocular tissues (Majumdar & Srirangam, 2010).

The noncorneal route of absorption mainly involves diffusion across the conjunctiva and sclera (Mizuno et al., 2009). The vascular and lymphatic drainage mechanisms in the conjunctiva, sclera, choroid, and retina, however, rapidly clear the topically administered agents

TABLE 78.1 Physicochemical Properties and Chemical Structures of (A) Tetrahydrocannabinol, (B) Tetrahydrocannabinol Hemisuccinate, and (C) Tetrahydrocannabinol Hemiglutarate

Properties	THC	THC-HS	THC-HG
Polar surface area	29.46	72.83	72.3
Hydrogen bond donor	1	1	1
Hydrogen bond acceptor	2	5	5
Rotatable bonds	4	8	10
$logD_{7.4}$	7.07	2.7	4.66
logP	—	3.3	7.77
pKa	9.6	4.25	4.6
Structure			

THC, Tetrahydrocannabinol; THC-HS, tetrahydrocannabinol hemisuccinate; THC-HG, tetrahydrocannabinol hemiglutarate. The physicochemical properties of THC, THC-HS, and THC-HG are shown in the table (calculated using ACD/I-Lab 2.0, Advanced Chemistry Development, Inc., Toronto, ON, Canada, 2014). Compared to THC, both THC-HS and THC-HG are more hydrophilic (increased polar surface area, and lower $logD_{7.4}$ compared to THC).

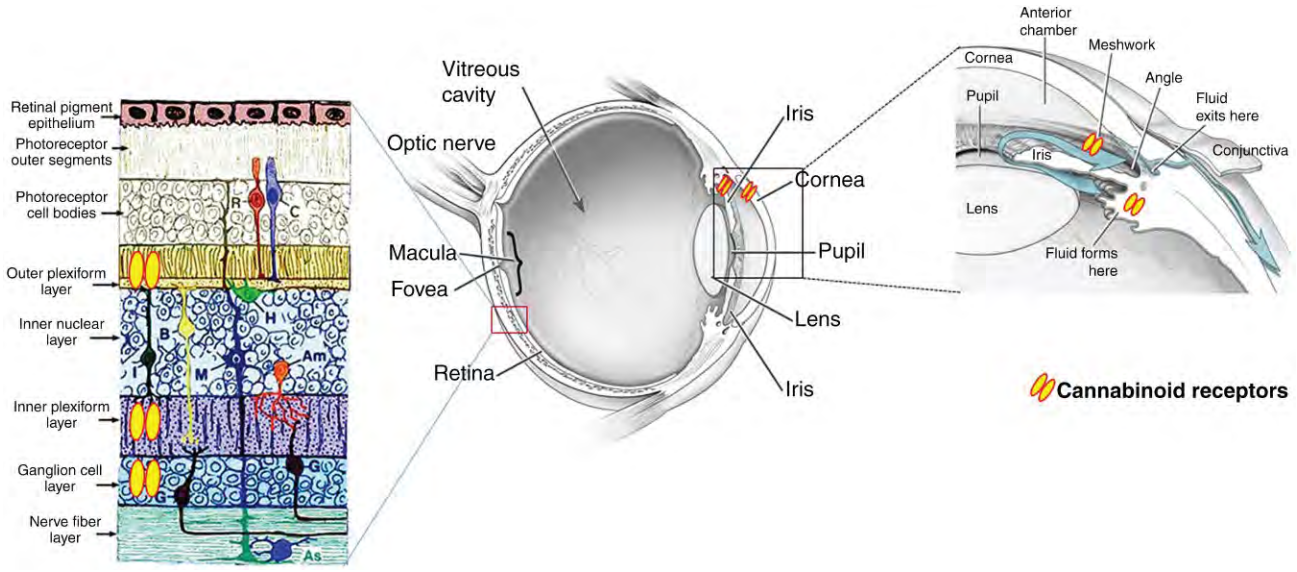


FIGURE 78.1 Structure of the human eye depicting the localization of cannabinoid receptors in various ocular tissues. Anatomical features of the eye, depicting localization of cannabinoid receptor in the cornea, ciliary body, and layers of retina. McDonnell, J. M. (1989). Basic Science and Inherited Retinal Disease. T. E. Ogden. 1, 8. The key target site for tetrahydrocannabinol (THC) action are cannabinoid receptors, localized predominately in the cornea (corneal epithelium and endothelium), ciliary body (ciliary muscle, nonpigmented ciliary epithelium, trabecular meshwork, and ciliary body), and cornea (outer plexiform layer, inner plexiform layer, and ganglionic cell layer). *Source: Straiker et al. (1999). The figure is adapted from NEI/NIH, and modified to show the localization of cannabinoid receptors.*

into the systemic circulation. Furthermore, the outer blood-retinal barrier (BRB)—the retinal pigmented epithelium (RPE)—presents a formidable diffusion barrier in the transscleral pathway, for lipophilic, as well as hydrophilic moieties.

Additionally, various transport systems expressed on biological membranes may modulate ocular absorption. While influx transporters expressed on the corneal, conjunctival, and retinal epithelial cells may aid in the absorption of topically administered substrates, efflux transporters such as P-glycoprotein and multidrug resistance proteins expressed on the corneal, RPE, and conjunctival membranes can restrict transmembrane diffusion of their substrates (Hippalgaonkar, Srirangam, Avula, Khan, & Majumdar, 2010).

Considering the various challenges to ocular absorption of topically administered agents, it is not surprising that, generally, less than 10% of the topically instilled formulation reaches the deeper ocular tissues (Geroski & Edelhauser, 2000). The multiple barriers in the ocular drug absorption pathway from the ocular surface have been illustrated in Fig. 78.2.

Physicochemical Characteristics

Permeation of molecules across biological membranes can take place either through the transcellular or paracellular pathway. Most lipophilic molecules primarily penetrate biological membranes via the transcellular pathway, while the paracellular route is preferred by most hydrophilic molecules (Borchardt, 1990).

There are several factors that affect the permeability of molecules across biological membranes such as the cornea and the conjunctiva. Partition coefficient or logP, aqueous solubility, molecular size, charge and degree of ionization, polar surface area, hydrogen bond acceptors and donors, and stereochemical configuration are some of the critical factors that affect transmembrane passive diffusion (Over et al., 2014).

Effectiveness of Topical THC in Lowering Intraocular Pressure

Green, Bigger, Kim, and Bowman (1977) dissolved 0.1 %w/v THC in four different vehicles: light mineral oil (LMO), mineral oil, sesame oil, and saline (90%)–Tween 80 (10%) combination). Clinically normotensive albino rabbits received 50 µL twice a day, in a 9-day study, and once a week in a 60-day study. The IOP dropped by 20% in all cases. The THC in LMO formulation produced an aqueous humor concentration of 0.05 µg/mL, 1-h post topical application.

Merritt, Olsen, Armstrong, and McKinnon (1981) reported that topical application of 50 µL of THC (0.1 %w/v, 50 µg dose) in mineral oil showed a 5.4 ± 2.6 mm drop in test, and 5.2 ± 2.8 mm drop in contralateral eye, in adult hypertensive human subjects, accompanied by a 12 mm

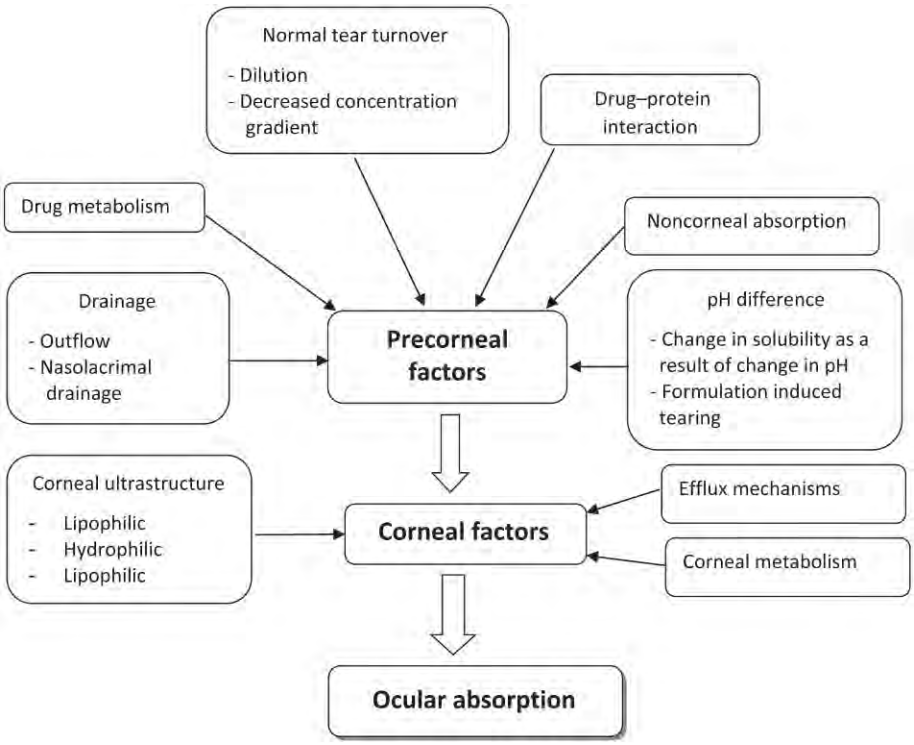


FIGURE 78.2 Illustration depicting the various barriers affecting ocular absorption via the corneal pathway. The illustration depicts the precorneal and corneal barriers that restrict drug availability at the ocular surface, and also limit transcorneal absorption. Considering the multiple barriers involved, less than 10% of the topically administered therapeutic agent generally reaches the deeper ocular tissues (Geroski & Edelhauser, 2000).

drop of systolic blood pressure. With THC in mineral oil (0.05 %w/v), IOP reduced by 4.8 ± 2.8 mm in test eye, and 6.8 ± 2.5 mm in fellow eye from baseline, and did not show any systemic effect. Considering the drop in systolic blood pressure and IOP in the contralateral eye, Merritt et al. (1981) suggested that the IOP lowering effect of THC is through the systemic route, rather than a local mechanism. In yet another study, Green et al. evaluated the IOP lowering efficacy of a single drop of THC in LMO (0.1 %w/v) in normal human volunteers. In this study, a decrease in IOP was not observed (Green & Roth, 1982). In a multidose study, THC in LMO (1 %w/v) was administered four times a day for 1 week, in 28 male human subjects (Jay & Green, 1983). THC did not show any drop in the IOP, compared to the control. A few other reports (Elsohly, Harland, Murphy, Wirth, & Waller, 1981) also reported failure of topical THC to decrease IOP in the rabbit model.

Colasanti, Powell, and Craig (1984b) studied the effect of THC in normotensive cats after a single drop administration, and a chronic study of 9 days. In acute studies, IOP was measured for 6 h after administration of a single drop of THC in light paraffin oil (dose: 1 mg). A minimal drop (approximately 2 mm of Hg) in IOP was observed after a single dose administration (Colasanti et al., 1984b). In the chronic study, a subcutaneous cannula with a mini pump was implanted and delivered 20 µg/h of THC (dissolved in PEG) over a period of 9 days. A drop of 4–7 mm of Hg was observed over a period of 9 days of chronic unilateral delivery of THC.

Some other studies also confirmed the IOP lowering activity of THC. Muchtar, Almog, Torracca, Saettone, and Benita (1992) reported that a Δ^8-THC (0.4 %w/v) in a submicron emulsion formulation produced intense and long lasting reduction in IOP, in an alpha-chymotrypsin induced rabbit model of ocular hypertension. In a more recent randomized longitudinal crossover design study, Fischer, Ward, and Hendrix (2013) reported that 50 µL of THC in olive oil formulation (2 %w/v, dose: 1 mg) showed a drop in ocular pressure in eight clinically normal dogs. Mean IOP drop from baseline in the treated eyes was observed to be 21% in the morning and 15% in the evening sessions.

From the previous discussion, it is apparent that the IOP lowering efficacy of THC from topical ophthalmic formulations is erratic (wide variation in dose-effect) and inconsistent, within and across animal models. In fact, based on the earlier data, a study panel constituted by the National Eye Institute of the National Institutes of Health had concluded that there was insufficient evidence to conclude that THC could be useful in the treatment of glaucoma (NIH, 1981).

Approaches to Improve Ocular Bioavailability of THC Through the Topical Route

Tetrahydrocannabinol has a logP of 6.3, a total number of hydrogen bond donors and acceptors of 3, a polar surface area of 29.46, and a pH independent aqueous solubility of only 1–2 μg/mL. Moreover, stability of THC in aqueous solutions is very low. In view of these unfavorable membrane permeability characteristics, it would be expected that THC would be inefficient in crossing the ocular membranes, and would rather accumulate in the epithelial membranes of the cornea and conjunctiva. In fact, a lipophilic vehicle, as used in most of the earlier pharmacological studies conducted by various researchers, would further hamper partitioning into the ocular tissues. This conclusion is supported by a report from Kearse and Green (2000) wherein in vitro transcorneal permeability of THC from a LMO vehicle was observed to be only 0.18 x 10^{-9} cm/s, which is extremely low.

In vivo ocular tissue distribution of THC using LMO and emulsion-based formulations was investigated in New Zealand white rabbits. At the end of 1-h post topical administration, THC concentrations were found to be below the limit of detection in all ocular tissues tested, except for the cornea and sclera, from both formulations (Hingorani et al., 2013). The data underscores the ocular bioavailability issues encountered with topical THC delivery.

An ophthalmic formulation designed to increase aqueous solubility, retention at the ocular surface, and transcorneal permeability could improve ocular THC concentrations. Based on the in vitro permeability data obtained with various formulations, a THC (0.13 %w/v) formulation containing Cremophor RH 40, ethylenediaminetetraacetic acid (EDTA), benzalkonium chloride, and hydroxypropyl methyl cellulose (HPMC) was tested in vivo (Hingorani et al., 2013). Ocular tissue concentration 1-h post topical administration in rabbits was determined. Except for the cornea and sclera, THC levels were again not observed in any of the ocular tissues (Table 78.2). THC concentrations in the cornea and sclera, however, were much higher in comparison to the LMO and emulsion formulations tested earlier (Hingorani et al., 2013), suggesting that THC partitioned into the ocular tissues from an aqueous formulation better than from an oily vehicle.

Considering that the oil based formulations, used in the earlier studies, were able to deliver very low concentrations of THC to the iris-ciliary bodies and the retina, it is not surprising that in many of these studies topical THC administration did not result in a drop in IOP. Moreover, the high lipophilicity of THC results in its getting trapped in the lipophilic layers of the eye (cornea and sclera). For THC to be successfully employed in the control of IOP, approaches to improve its physicochemical characteristics, so as to improve transmembrane permeability, is needed.

TABLE 78.2 Total Tetrahydrocannabinol Concentrations Observed in Rabbit Ocular Tissues 1-h Post Topical Administration in Light Mineral Oil, Emulsion, or Cremophor Micellar Solutions

Tissue	THC		
	Light mineral oil	Emulsion	0.5% Cremophor RH 40 + 0.1% EDTA + 0.02% BAK + 0.5% HPMC
Drug concentration in terms of THC (% w/v)	0.1	0.37	0.125
Cornea (ng/50 mg tissue)	68.8 ± 14.5	300.6 ± 79.6	553.9 ± 87.4
Aqueous tumor (ng/100 μL)	ND*	ND*	ND*
Iris-ciliary bodies (ng/50 mg tissue)	ND*	ND*	ND*
Vitreous humor (ng/mL)	ND*	ND*	ND*
Retina-choroid (ng/50 mg tissue)	ND*	ND*	ND*
Sclera (ng/250 mg tissue)	104.1 ± 36.1	171.1 ± 66.6	439.3 ± 280.2

ND*, Drug concentration below detection limit. THC levels observed in the cornea and sclera following topical application of all three formulations. However, THC was not detectable in the aqueous humor and the iris-ciliary bodies. The micellar formulations, however, produced significantly higher THC concentrations in the cornea and sclera.
Data are from Hingorani et al. (2013), with permission from the publishers.

Hydrophilic Prodrug Derivatization

Chemical modification of drugs/drug candidates into prodrugs has become an important technique to overcome poor transmembrane permeability of actives. A significant positive aspect of the prodrug approach is that it does not induce any change in the membrane structure, fluidity, or characteristics. The synthesis and development of ganciclovir and acyclovir acyl ester prodrugs to enhance partitioning into the ocular tissues has been previously demonstrated (Hughes & Mitra, 1993; Majumdar, Kansara, & Mitra, 2006). Several other studies have also reported enhanced permeability using a prodrug approach (Majumdar & Mitra, 2006). These reports strongly suggest that a prodrug approach to improve the hydrophilicity of THC can be useful in delivering THC into the deeper ocular tissues.

Two dicarboxylic acid ester prodrugs of THC, THC-hemisuccinate (THC-HS) and THC-hemiglutarate (THC-HG), have been investigated for ocular delivery through the topical route. THC-HS has a logP of 3.3, and pKa of 4.25. However, it demonstrated very poor stability in aqueous solutions. THC-HG ($logD_{7.4}$: 4.66, and polar surface area: 72.3) on the other hand was more stable than THC-HS, and 1.5–2-fold more soluble. To improve the solubility of these prodrugs further, an ion-pair complex formation strategy with L-arginine (ARG) and tromethamine (TRIS), was explored. Solubility of THC-HG increased with increasing concentrations of ARG or TRIS. Surfactants were also tested, and Cremophor RH 40 was observed to be the best solubilizer (Hingorani et al., 2013).

In vitro transcorneal permeability studies revealed that both THC-HS and THC-HG demonstrated pH dependent permeability. At lower pH (5.0), both prodrugs were sixfold more permeable than THC, while at physiological pH (7.4), the permeability of the prodrugs decreased threefold by shielding the negative charge at physiological pH. The counter ions ARG and TRIS significantly improved the transcorneal permeability of the prodrugs (Hingorani, Gul, Elsohly, Repka, & Majumdar, 2011).

Ocular tissue distribution of topical THC-HG was evaluated in vivo in anesthetized rabbits. Surfactant and ion-pair formulations were both tested. THC-HG produced markedly higher total THC concentrations in the ocular tissues compared to THC, 1-h post topical application of the formulations. Among the two formulations tested, the ion-pair formulation appeared to be slightly better than the surfactant formulation (Table 78.3), and maintained significant THC concentrations in the ocular tissues, even after 3-h post topical application. In all cases, THC levels were below detection limits in the vitreous humor and retina-choroid (Hingorani et al., 2011).

Future Directions for Topical THC Delivery for Ocular Diseases

A combination of prodrug design and formulation strategies has significantly improved THC delivery to the anterior segment tissues. This will positively impact therapeutic outcomes in many of the indications associated with the front-of-the eye. THC, however, could still not penetrate into the posterior segment ocular tissues, such as the retina and posterior uvea. Prodrugs with improved solubility, permeability, and chemical and enzymatic stability characteristics could improve THC penetration into the back of the eye. Mono- and di-amino acid conjugated prodrugs may yield compounds with significantly better physicochemical, solubility, and stability characteristics (Elsohly, Gul, Repka, & Majumdar, 2010). Moreover, use of formulation strategies, such as nanoparticulate systems, may help deliver the prodrugs into the deeper ocular tissues. Overall, THC presents itself as a very promising drug candidate for ocular diseases, and needs carefully designed dosage forms to utilize its therapeutic potential.

TABLE 78.3 Total Tetrahydrocannabinol Concentrations Observed in Rabbit Ocular Tissues Post Topical Administration of 50 µL of Tetrahydrocannabinol Hemiglutarate Formulated in Cremophor Micellar Solution (1 h) and Ion Pair Formulations (1- and 3-h Time Points)

	THC-HG		
	0.5% Cremophor RH 40 + 0.1% EDTA + 0.02% BAK + 0.5% HPMC	Ion pair formulation (8 mM tromethamine + 0.5% HPMC)	
Tissue	**1 h**	**1 h**	**3 h**
Drug concentration in terms of THC (% w/v)	0.18	0.16	0.16
Cornea (ng/50 mg tissue)	2451.6 ± 645.6	2245.7 ± 240.2	1382.7 ± 109.8
Aqueous humor (ng/100 µL)	32.1 ± 12.6	52.2 ± 18.7	27.3 ± 11.3
Iris-ciliary bodies (ng/50 mg tissue)	35.6 ± 12.5	93.1 ± 41.4	67.1 ± 55.6
Vitreous humor (ng/mL)	ND*	ND*	ND*
Retina-choroid (ng/50 mg tissue)	ND*	ND*	ND*
Sclera (ng/250 mg tissue)	354.7 ± 86.4	2258.1 ± 1331.1	166.1 ± 49.4

ND*, Drug concentration below detection. The data given in the table shows that hydrophilic derivatization of THC and incorporation into an appropriate formulation markedly improves delivery of tetrahydrocannabinol (THC) to the aqueous humor and the iris-ciliary bodies. The tetrahydrocannabinol hemiglutarate (THC-HG)/tromethamine ion pair formulation delivered significantly higher THC concentrations to the iris-ciliary bodies, compared to the micellar formulations, and significant THC concentrations were still detectable in the aqueous humor and iris-ciliary bodies at the 3-h time point post topical application.
Data are from Hingorani et al. (2013), with permission from the publishers.

MINI-DICTIONARY

Anterior segment of the eye Anterior segment of the eye refers to the anatomical structures between cornea and iris-ciliary bodies, including aqueous humor, pupil, and trabecular meshwork.

Drug delivery Delivery of a drug or drug candidates at therapeutic levels through various approaches, formulations, and technologies, across physiological membranes and to the site of action.

Endocannabinoid system A combination of endocannabinoids (endogenous substances that activate cannabinoid receptors), enzymes involved in their degradation (fatty acid amide hydrolase) and biosynthesis, and cannabinoid receptors (CB1 or CB2).

Neuroprotection A mechanism to protect retinal ganglionic cells from neuronal injury or degeneration due to various ocular disorders (glaucoma, age-related macular degeneration, vitreoretinopathy, and diabetic retinopathy).

Posterior segment of the eye It is also referred to as back-of-the eye segment, and includes the ocular structures such as anterior hyaloid membrane, vitreous humor, retinal pigmented epithelium, choroid, and optic nerve.

Retinal ganglionic cells Retinal ganglionic cells are a type of neurons which help in transmission of the image forming and nonimage forming visual information received by the photoreceptors to the brain.

WIN 55,212-2 WIN 55,212-2 is a synthetic cannabinoid compound which serves as a full agonist at the cannabinoid receptor to produce similar activities as tetrahydrocannabinol.

References

Blazquez, C., Carracedo, A., Barrado, L., Real, P. J., Fernandez-Luna, J. L., & Velasco, G. (2006). Cannabinoid receptors as novel targets for the treatment of melanoma. *FASEB Journal*, *20*(14), 2633–2635.

Blazquez, C., Casanova, M. L., Planas, A., Gomez Del Pulgar, T., Villanueva, C., & Fernandez-Acenero, M. J. (2003). Inhibition of tumor angiogenesis by cannabinoids. *FASEB Journal*, *17*(3), 529–531.

Blazquez, C., Gonzalez-Feria, L., Alvarez, L., Haro, A., Casanova, M. L., & Guzman, M. (2004). Cannabinoids inhibit the vascular endothelial growth factor pathway in gliomas. *Cancer Research*, *64*(16), 5617–5623.

Borchardt, R. T. (1990). Assessment of transport barriers using cell and tissue culture systems. *Drug Development and Industrial Pharmacy*, *16*, 2595–2612.

Casanova, M. L., Blazquez, C., Martinez-Palacio, J., Villanueva, C., Fernandez-Acenero, M. J., & Huffman, J. W. (2003). Inhibition of skin tumor growth and angiogenesis in vivo by activation of cannabinoid receptors. *Journal of Clinical Investigation*, *111*(1), 43–50.

Chen, Y., McCarron, R. M., Ohara, Y., Bembry, J., Azzam, N., & Lenz, F. A. (2000). Human brain capillary endothelium: 2-arachidonoglycerol (endocannabinoid) interacts with endothelin-1. *Circulation Research*, *87*(4), 323–327.

Colasanti, B. K., Craig, C. R., & Allara, R. D. (1984a). Intraocular pressure, ocular toxicity and neurotoxicity after administration of cannabinol or cannabigerol. *Experimental Eye Research*, *39*(3), 251–259.

Colasanti, B. K., Powell, S. R., & Craig, C. R. (1984b). Intraocular pressure, ocular toxicity and neurotoxicity after administration of Δ9-tetrahydrocannabinol or cannabichromene. *Experimental Eye Research*, *38*(1), 63–71.

Cook, C., & Foster, P. (2012). Epidemiology of glaucoma: what's new? *Canadian Journal of Ophthalmology*, *47*(3), 223–226.

Di Marzo, V. (2008). Endocannabinoids: synthesis and degradation. *Reviews of Physiology, Biochemistry and Pharmacology*, *160*, 1–24.

Ellert-Miklaszewska, A., Kaminska, B., & Konarska, L. (2005). Cannabinoids down-regulate PI3K/Akt and Erk signalling pathways and activate proapoptotic function of Bad protein. *Cell Signalling*, *17*(1), 25–37.

Elsohly, M.A., Gul, W., Repka, M.A., & Majumdar, S. (2010). Compositions containing delta-9-thc-amino acid esters and process of preparation: PCT/US2009/062998.

Elsohly, M. A., Harland, E., Murphy, J. C., Wirth, P., & Waller, C. W. (1981). Cannabinoids in glaucoma: a primary screening procedure. *Journal of Clinical Pharmacology*, *21*(8–9 Suppl.), 472S–478S.

Fischer, K. M., Ward, D. A., & Hendrix, D. V. (2013). Effects of a topically applied 2% delta-9-tetrahydrocannabinol ophthalmic solution on intraocular pressure and aqueous humor flow rate in clinically normal dogs. *American Journal of Veterinary Research*, *74*(2), 275–280.

Fox, A., Kesingland, A., Gentry, C., McNair, K., Patel, S., & Urban, L. (2001). The role of central and peripheral cannabinoid1 receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. *Pain*, *92*(1–2), 91–100.

Galve-Roperh, I., Sanchez, C., Cortes, M. L., Gomez del Pulgar, T., Izquierdo, M., & Guzman, M. (2000). Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. *Nature Medicine*, *6*(3), 313–319.

Geroski, D. H., & Edelhauser, H. F. (2000). Drug delivery for posterior segment eye disease. *Investigative Ophthalmology and Visual Science*, *41*(5), 961–964.

Gray, G. A., Battistini, B., & Webb, D. J. (2000). Endothelins are potent vasoconstrictors, and much more besides. *Trends in Pharmacological Sciences*, *21*(2), 38–40.

Green, K. (1979). The ocular effects of cannabinoids. *Current Topics in Eye Research*, *1*, 175–215.

Green, K., Bigger, J. F., Kim, K., & Bowman, K. (1977). Cannabinoid penetration and chronic effects in the eye. *Experimental Eye Research*, *24*(2), 197–205.

Green, K., Kearse, E. C., & McIntyre, O. L. (2001). Interaction between delta-9-tetrahydrocannabinol and indomethacin. *Ophthalmic Research*, *33*(4), 217–220.

Green, K., & Pederson, J. E. (1973). Effect of 1 -tetrahydrocannabinol on aqueous dynamics and ciliary body permeability in the rabbit. *Experimental Eye Research*, *15*(4), 499–507.

Green, K., & Roth, M. (1982). Ocular effects of topical administration of delta 9-tetrahydrocannabinol in man. *Archives of Ophthalmology*, *100*(2), 265–267.

Green, K., Symonds, C. M., Oliver, N. W., & Elijah, R. D. (1982). Intraocular pressure following systemic administration of cannabinoids. *Current Eye Research*, *2*(4), 247–253.

Haefliger, I. O., Meyer, P., Flammer, J., & Luscher, T. F. (1994). The vascular endothelium as a regulator of the ocular circulation: a new concept in ophthalmology? *Survey of Ophthalmology*, *39*(2), 123–132.

He, F., & Song, Z. H. (2007). Molecular and cellular changes induced by the activation of CB2 cannabinoid receptors in trabecular meshwork cells. *Molecular Vision*, *13*, 1348–1356.

Hepler, R. S., & Frank, I. R. (1971). Marihuana smoking and intraocular pressure. *Journal of the American Medical Association*, *217*(10), 1392.

Hingorani, T., Adelli, G. R., Punyamurthula, N., Gul, W., Elsohly, M. A., Repka, M. A., & Majumdar, S. (2013). Ocular disposition of the hemiglutarate ester prodrug of (9)-tetrahydrocannabinol from various ophthalmic formulations. *Pharmaceutical Research*, *30*(8), 2146–2156.

Hingorani, T., Gul, W., Elsohly, M., Repka, M. A., & Majumdar, S. (2011). Effect of ion pairing on in vitro transcorneal permeability of a Delta(9)-tetrahydrocannabinol prodrug: potential in glaucoma therapy. *Journal of Pharmaceutical Sciences*, *101*(2), 616–626.

Hippalgaonkar, K., Srirangam, R., Avula, B., Khan, I. A., & Majumdar, S. (2010). Interaction between topically and systemically coadministered P-glycoprotein substrates/inhibitors: effect on vitreal kinetics. *Drug Metabolism and Disposition*, *38*(10), 1790–1797.

Howlett, A. C., Barth, F., Bonner, T. I., Cabral, G., Casellas, P., & Devane, W. A. (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacological Reviews*, *54*(2), 161–202.

Hughes, P. M., & Mitra, A. K. (1993). Effect of acylation on the ocular disposition of acyclovir. II: Corneal permeability and anti-HSV 1

activity of 2′-esters in rabbit epithelial keratitis. *Journal of Ocular Pharmacology*, *9*(4), 299–309.
Jay, W. M., & Green, K. (1983). Multiple-drop study of topically applied 1% delta 9-tetrahydrocannabinol in human eyes. *Archives of Ophthalmology*, *101*(4), 591–593.
Jin, K. L., Mao, X. O., Goldsmith, P. C., & Greenberg, D. A. (2000). CB1 cannabinoid receptor induction in experimental stroke. *Annals of Neurology*, *48*(2), 257–261.
Kearse, E. C., & Green, K. (2000). Effect of vehicle upon in vitro transcorneal permeability and intracorneal content of Delta9-tetrahydrocannabinol. *Current Eye Research*, *20*(6), 496–501.
Lu, Q., Straiker, A., & Maguire, G. (2000). Expression of CB2 cannabinoid receptor mRNA in adult rat retina. *Visual Neuroscience*, *17*(1), 91–95.
Majumdar, S., Kansara, V., & Mitra, A. K. (2006). Vitreal pharmacokinetics of dipeptide monoester prodrugs of ganciclovir. *Journal of Ocular Pharmacology and Therapeutics*, *22*(4), 231–241.
Majumdar, S., & Mitra, A. K. (2006). Chemical modification and formulation approaches to elevated drug transport across cell membranes. *Expert Opinion on Drug Delivery*, *3*(4), 511–527.
Majumdar, S., & Srirangam, R. (2010). Potential of the bioflavonoids in the prevention/treatment of ocular disorders. *Journal of Pharmacy and Pharmacology*, *62*(8), 951–965.
Marsicano, G., Moosmann, B., Hermann, H., Lutz, B., & Behl, C. (2002). Neuroprotective properties of cannabinoids against oxidative stress: role of the cannabinoid receptor CB1. *Journal of Neurochemistry*, *80*(3), 448–456.
McAllister, S. D., Chan, C., Taft, R. J., Luu, T., Abood, M. E., & Moore, D. H. (2005). Cannabinoids selectively inhibit proliferation and induce death of cultured human glioblastoma multiforme cells. *Journal of Neurooncology*, *74*(1), 31–40.
Merritt, J. C., Crawford, W. J., Alexander, P. C., Anduze, A. L., & Gelbart, S. S. (1980a). Effect of marihuana on intraocular and blood pressure in glaucoma. *Ophthalmology*, *87*(3), 222–228.
Merritt, J. C., McKinnon, S., Armstrong, J., Hatem, G., & Reid, L. (1980b). Oral delta-9-tetrahydrocannabinol in heterogeneous glaucomas. *Annals of Ophthalmology*, *12*(8), 947–950.
Merritt, J. C., Olsen, J. L., Armstrong, J. R., & McKinnon, S. M. (1981). Topical delta 9-tetrahydrocannabinol in hypertensive glaucomas. *Journal of Pharmacy and Pharmacology*, *33*(1), 40–41.
Mizuno, K., Koide, T., Shimada, S., Mori, J., Sawanobori, K., & Araie, M. (2009). Route of penetration of topically instilled nipradilol into the ipsilateral posterior retina. *Investigative Ophthalmology and Visual Science*, *50*(6), 2839–2847.
Muchtar, S., Almog, S., Torracca, M. T., Saettone, M. F., & Benita, S. (1992). A submicron emulsion as ocular vehicle for delta-8-tetrahydrocannabinol: effect on intraocular pressure in rabbits. *Ophthalmic Research*, *24*(3), 142–149.
Munson, A. E., Harris, L. S., Friedman, M. A., Dewey, W. L., & Carchman, R. A. (1975). Antineoplastic activity of cannabinoids. *Journal of the National Cancer Institute*, *55*(3), 597–602.
NIH. (1981). Reports of marijuana for glaucoma treatment are misleading; researchers will study. National Institutes of Health. *Clinical Toxicology*, *18*(2), 245–246.
Nurmikko, T. J., Serpell, M. G., Hoggart, B., Toomey, P. J., Morlion, B. J., & Haines, D. (2007). Sativex successfully treats neuropathic pain characterised by allodynia: a randomised, double-blind, placebo-controlled clinical trial. *Pain*, *133*(1–3), 210–220.
Over, B., McCarren, P., Artursson, P., Foley, M., Giordanetto, F., & Gronberg, G. (2014). Impact of stereospecific intramolecular hydrogen bonding on cell permeability and physicochemical properties. *Journal of Medicinal Chemistry*, *57*(6), 2746–2754.
Pacher, P., & Kunos, G. (2013). Modulating the endocannabinoid system in human health and disease—successes and failures. *FEBS Journal*, *280*(9), 1918–1943.
Pertwee, R. G. (1999). Pharmacology of cannabinoid receptor ligands. *Current Medicinal Chemistry*, *6*(8), 635–664.
Pertwee, R. G., Gibson, T. M., Stevenson, L. A., Ross, R. A., Banner, W. K., & Saha, B. (2000). O-1057, a potent water-soluble cannabinoid receptor agonist with antinociceptive properties. *British Journal of Pharmacology*, *129*(8), 1577–1584.
Porcella, A., Casellas, P., Gessa, G. L., & Pani, L. (1998). Cannabinoid receptor CB1 mRNA is highly expressed in the rat ciliary body: implications for the antiglaucoma properties of marihuana. *Brain Research. Molecular Brain Research*, *58*(1–2), 240–245.
Porcella, A., Maxia, C., Gessa, G. L., & Pani, L. (2000). The human eye expresses high levels of CB1 cannabinoid receptor mRNA and protein. *European Journal of Neuroscience*, *12*(3), 1123–1127.
Portella, G., Laezza, C., Laccetti, P., De Petrocellis, L., Di Marzo, V., & Bifulco, M. (2003). Inhibitory effects of cannabinoid CB1 receptor stimulation on tumor growth and metastatic spreading: actions on signals involved in angiogenesis and metastasis. *FASEB Journal*, *17*(12), 1771–1773.
Sarfaraz, S., Afaq, F., Adhami, V. M., & Mukhtar, H. (2005). Cannabinoid receptor as a novel target for the treatment of prostate cancer. *Cancer Research*, *65*(5), 1635–1641.
Schlicker, E., Timm, J., & Gothert, M. (1996). Cannabinoid receptor-mediated inhibition of dopamine release in the retina. *Naunyn Schmiedebergs Archives of Pharmacology*, *354*(6), 791–795.
Straiker, A. J., Maguire, G., Mackie, K., & Lindsey, J. (1999). Localization of cannabinoid CB1 receptors in the human anterior eye and retina. *Investigative Ophthalmology and Visual Science*, *40*(10), 2442–2448.
Sugrue, M. F. (1997). New approaches to antiglaucoma therapy. *Journal of Medicinal Chemistry*, *40*(18), 2793–2809.
Weinreb, R. N., & Levin, L. A. (1999). Is neuroprotection a viable therapy for glaucoma? *Archives of Ophthalmology*, *117*(11), 1540–1544.
Yoles, E., Belkin, M., & Schwartz, M. (1996). HU-211, a nonpsychotropic cannabinoid, produces short- and long-term neuroprotection after optic nerve axotomy. *Journal of Neurotrauma*, *13*(1), 49–57.

CHAPTER

79

The Role of γ-Aminobutyric Acid in the Interoceptive Effects of Oral Δ^9-Tetrahydrocannabinol in Humans

J.A. Lile, J.S. Fogel**, T.H. Kelly**

***Departments of Behavioral Science and Psychiatry, University of Kentucky College of Medicine, and Department of Psychology, University of Kentucky College of Arts and Sciences, Lexington, KY, United States**

****Department of Psychology, University of Kentucky College of Arts and Sciences, Lexington, KY, United States**

SUMMARY POINTS

- This chapter focuses on the involvement of γ-aminobutyric acid (ie, GABA) in the interoceptive effects of Δ^9-tetrahydrocannabinol (ie, Δ^9-THC) in humans.
- The interoceptive effects of drugs can be assessed using drug discrimination procedures: a selective means to examine neuropharmacological interactions between neurotransmitter systems.
- Δ^9-THC, the primary active constituent of cannabis, can be trained as a discriminative stimulus in humans.
- The GABA reuptake inhibitor tiagabine substituted for Δ^9-THC, and enhanced the discriminative stimulus effects of Δ^9-THC.
- The $GABA_B$ agonist baclofen, but not the $GABA_A$ positive allosteric modulator diazepam, also substituted for Δ^9-THC, and enhanced the discriminative stimulus effects of Δ^9-THC.
- The results from the clinical drug-discrimination studies differ from preclinical data, suggesting that the neuropharmacological interactions between GABA and CB systems differ between human and nonhuman species.
- The studies reviewed here indicate that GABA, particularly the $GABA_B$ receptor subtype, plays a significant part in the interoceptive effects produced by Δ^9-THC, and by extension cannabis, in humans.

KEY FACTS OF MEDICATIONS DEVELOPMENT FOR CANNABIS-USE DISORDERS

- Cannabis accounts for over half of recently used illicit drugs in the United States.
- The number of Americans who meet diagnostic criteria for cannabis-use disorders is similar to that of other illicit drugs.
- For personal health, social, or legal reasons, a subset of users decide, or are compelled, to quit their use.
- Behavioral interventions are moderately successful, but experts believe that a medication could enhance the efficacy of behavioral interventions, and reduce their cost.
- No medications are approved to treat cannabis-use disorders
- Understanding the neuropharmacological mechanisms of the abuse-related effects of cannabis is necessary

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00090-9
Copyright © 2017 Elsevier Inc. All rights reserved.

for the development of medications for cannabis-use disorders.

- Some theorists propose that medications that block of the interoceptive effects of cannabis would result in the extinction of drug-seeking and -taking behaviors.
- Other theorists argue that drugs that produce effects that overlap with cannabis would address some of the signs and symptoms of cannabis withdrawal that are reported to make abstinence difficult.

LIST OF ABBREVIATIONS

CB	Cannabinoid
CNS	Central nervous system
GABA	γ-aminobutyric acid
mg	Milligrams
Δ^9-THC	Δ^9-Tetrahydrocannabinol
VDCC	Voltage-dependent calcium channels

CANNABINOID AND GABA NEUROPHARMACOLOGY AND THE GAP IN OUR UNDERSTANDING OF THE INTERACTIONS OF THESE NEUROTRANSMITTER SYSTEMS RELATED TO THE INTEROCEPTIVE EFFECTS OF CANNABIS

The central effects of cannabis (*Cannabis sativa*, *Cannabis indica*) are mediated primarily through endogenous cannabinoid neurotransmitter system receptors, especially the CB_1 receptor subtype (Compton et al., 1993). However, a principal function of CB receptors is the modulation of non-CB neurotransmitter release via retrograde signaling (Szabo & Schlicker, 2005), so it stands to reason that other neurotransmitter systems play a role in the effects of cannabinoids. Neuroanatomical and neurochemical studies support a functional link between CB and GABA systems. CB_1 receptors are co-localized with GABA receptors in regions throughout the brain (Freund, Katona, & Piomelli, 2003; Mailleaux & Vanderhaeghen, 1992), and modulate synaptic GABA levels (Antonelli et al., 2009; Engler, Freiman, Urbanski, & Szabo, 2006). For example, GABAergic transmission is enhanced by reducing GABA reuptake via stimulation of presynaptic cannabinoid receptors in certain GABA neurons (Romero, de Miguel, Ramos, & Fernández-Ruiz, 1998), and also possibly by direct interactions of cannabinoids with the GABA transporter (Coull, Johnston, Pertwee, & Davies, 1997).

Research to determine the in vivo behavioral and physiological consequences of neuroanatomical and neurochemical interactions between the CB and GABA systems is, however, relatively scarce. Information about the overlap between these systems is generally limited to what can be ascertained from the comparisons of the results of individual studies that tested CB and GABA drugs separately (reviewed later in the chapter). Further, the experimental outcomes from those cross-study comparisons generally lack the pharmacological selectivity required for evidence-based inferences about the interaction of these neurotransmitter systems, and have also not focused on the role that GABA might play in the interoceptive effects of cannabinoids. Considering the growing interest in the use of cannabinoid medications for various therapeutic indications, a better understanding of the function of other neurotransmitters in the interoceptive effects produced by centrally acting cannabinoids would be beneficial, as they represent potential side effects (eg, abuse-related effects) that are undesirable to healthcare professionals, regulatory bodies, and some patients. Furthermore, such information would be informative in the development of medications for cannabis dependence (see Key Facts of Medications for Cannabis-Use Disorders).

DRUG DISCRIMINATION AS AN IN VIVO METHOD FOR DETECTING CNS INTERACTIONS BETWEEN NEUROTRANSMITTER SYSTEMS

A valuable method of studying CNS interactions between neurotransmitter systems, especially as it pertains to the interoceptive effects of psychoactive substances, is drug discrimination. Drug discrimination is trained across multiple sessions, using differential conditioning procedures, in which reinforcement is contingent upon correct identification of the specific drug condition (eg, presence vs absence of the training drug) administered under blind conditions. In this way, the interoceptive effects of the training drug come to control behavior, following its administration. Decades of research have demonstrated that these procedures yield data that are concordant with the neuropharmacological actions of a drug at the receptor level (Holtzman & Locke, 1988). An example germane to the present topic is that, in rodents and monkeys trained to discriminate Δ^9-THC, synthetic cannabinoid agonists such as WIN 55212-2 and CP 55-940 generalized to Δ^9-THC (ie, engendered Δ^9-THC-like responding), whereas drugs from a range of other pharmacological classes having functionally distinct receptor-level effects generally do not (Barrett, Wiley, Balster, & Martin, 1995; Browne & Weissman, 1981; Wiley, Barrett, Lowe, Balster, & Martin, 1995; Wiley, Huffman, Balster, & Martin, 1995). Similarly, in studies in which human subjects were trained to discriminate Δ^9-THC, the cannabinoid agonist nabilone, but not methylphenidate,

triazolam, or hydromorphone, occasioned Δ9-THC-like responding, despite the fact that each of the drugs engendered interoceptive stimulus effects, as evidenced by increased ratings on self-reported drug-effect questionnaire items (Lile, Kelly, Pinsky, & Hays, 2009; Lile, Kelly, & Hays, 2010).

In humans, drug discrimination training and testing generally follows a three-phase design. In an initial sampling phase, subjects receive the training dose, identified as the Drug condition, but are not informed of the capsules' contents. A subsequent control phase is then conducted to determine whether subjects can discriminate the training drug from placebo (ie, the Not Drug condition). During the control phase, subjects receive Drug and Not Drug under double-blind and randomized conditions, and accurate drug identification responding following drug administration is reinforced with money. An acquisition criterion (eg, ≥80% correct responding for multiple consecutive sessions that include both Drug and Not Drug conditions) is imposed, and subjects are typically dismissed from the study if they do not meet the criterion within a predetermined number of sessions. A final test phase is then conducted to test additional doses of the training drug, as well as novel drugs alone and/or in combination with the training drug. During test sessions when drugs and/or doses other than the control conditions are administered, responding is reinforced with money, regardless of the option chosen during task performance.

The interoceptive effects of drugs can also be assessed in humans, using subjective effects questionnaires, and is the more typical method. Advantages include being able to examine a wide range of drug effects with minimal eligibility and training requirements. However, self-report and drug discrimination outcomes are not isomorphic and, importantly, drug discrimination procedures have been found to have greater pharmacological selectivity, compared to subjective effects questionnaires (reviewed in Kelly, Stoops, Perry, Prendergast, & Rush, 2003). A potential explanation for the reduced fidelity of self-report measures for examining neuropharmacological mechanisms of drug action is that an appropriate verbal conditioning history is likely not uniform across subjects. For example, the adjective "stimulated" could be interpreted differently, as it could be associated with a variety of different internal states, such as motivation or interest, or physiological cues related to cardiovascular or motor states. In contrast, the relationship between neuropharmacologically-based interoceptive drug cues and drug-identification responding are formally established during drug discrimination training. Thus, drug discrimination serves as an in vivo method to characterize neuropharmacological interactions, and also informs our understanding of how various neurotransmitter systems contribute to the interoceptive effects of drugs.

THE INFLUENCE OF GLOBAL CNS ELEVATION OF GABA ON Δ9-THC DISCRIMINATION

In order to address the gap in our understanding of the function of GABA in the interoceptive effects of cannabinoids, a series of studies using drug-discrimination and drug combination procedures was undertaken. In the first of these studies, the separate and combined effects of the GABA reuptake inhibitor tiagabine (ie, NO-328; Gabitril) and Δ9-THC was determined (Lile, Kelly, & Hays, 2012a). A GABA reuptake inhibitor was chosen because global elevation of GABA levels would permit an initial, albeit nonselective, probe into whether there was an interaction between GABA and cannabinoid systems. Tiagabine was chosen because it is a commercially available GABA reuptake inhibitor that is approved for human use, and elevates extracellular brain concentrations of GABA (Fig. 79.1).

Previous studies have evaluated potential CB–GABA interactions by comparing or combining CB agonists and drugs that elevate central GABA via blockade of the reuptake transporter, and have demonstrated an overlap in some of their central effects. For instance, GABA reuptake inhibition by tiagabine augmented catalepsy produced by Δ9-THC in rodents (Pertwee, Greentree, & Swift, 1988; Pertwee, Browne, Ross, & Stretton, 1991). Likewise, individual studies have shown that, under certain conditions, both CB agonists and GABA transporter blockers are anxiolytic (Moreira & Wotjak, 2010; Schmitt & Hiemke, 1999), induce hypothermia (Frosini,

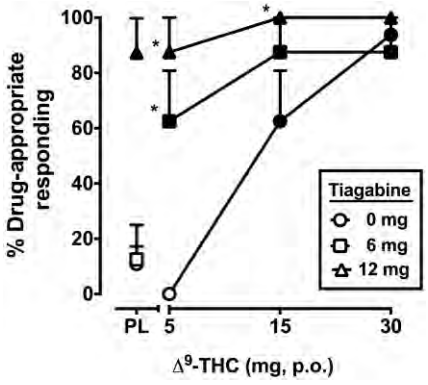


FIGURE 79.1 Global CNS elevation of GABA enhances the discriminative stimulus effects of Δ9-THC. Separate and combined effects of Δ9-THC and tiagabine on Δ9-THC-appropriate responding on the drug-discrimination task. Filled symbols indicate values that are significantly different from placebo. *Asterisks* indicate combinations of Δ9-THC and tiagabine that are significantly different from that dose of Δ9-THC alone. Statistical analyses were conducted using within-subjects ANOVA with $p \leq 0.05$. The *x*-axis represents the Δ9-THC dose in mg; PL denotes placebo. Data points show means of 8 subjects. Unidirectional brackets indicate 1 SEM. *Source: With permission from Lile et al. (2012a).*

Valoti, & Sgaragli, 2004; Wenger & Moldrich, 2002), impair memory processes and motor activity (Castellano, Rossie-Arnaud, Cestari, & Costanzi, 2003; Sañudo-Peña, Romero, Seale, Fernandez-Ruiz, & Walker, 2000; Schmitt & Hiemke, 2002; Suzdak et al., 1992), and produce antinociception (Hohmann, 2002; Laughlin, Tram, Wilcox, & Birnbaum, 2002). In separate clinical studies, CB agonists and tiagabine have each been shown to alleviate pain and anxiety, and promote sleep (Bestard & Toth, 2011; Nakano, Gillespie, & Hollister, 1978; Schwartz & Nihalani, 2006; Steiger, 2007; Todorov, Kolchev, & Todorov, 2005; Ware, Fitzcharles, Joseph, & Shir, 2010).

To further explore these possible CB–GABA interactions within the CNS, tiagabine and Δ^9-THC were administered orally alone and in combination to adult men and women who had been trained to discriminate 30 mg of Δ^9-THC. In addition to the drug discrimination measure, a self-report questionnaire, performance tasks, and physiological assessments were also included to provide a more comprehensive assessment of interactions between tiagabine and Δ^9-THC. The self-report questionnaire consisted of items rated on a Visual Analog Scale (VAS) that assessed positive (eg, Like Drug) and negative (eg, Bad Effects) drug effects, as well as items specific to cannabinoids (eg, Stoned). Performance tasks included a Digit-Symbol-Substitution task and a Repeated Acquisition task to assess impairment in psychomotor function and learning/memory processes. Data were collected immediately prior to drug administration, and at regular intervals after drug administration.

During the test phase of the study, after demonstrating acquisition of the 30 mg Δ^9-THC discrimination, subjects received placebo, 5, 15, and 30 mg Δ^9-THC, and 6 and 12 mg tiagabine, alone and in combination, during separate sessions. For reference, the acute recommended Δ^9-THC dosing range in adults, for appetite stimulation and the prevention of nausea and vomiting is 2.5–20 mg (Marinol Product Information, 2006). The starting dose of tiagabine for epilepsy is 4 mg (Gabitril Product Information, 2009), although acute doses of 12–24 mg tiagabine have been administered without reports of serious adverse events (Zwanzger et al., 2005).

Δ^9-THC dose-dependently functioned as a discriminative stimulus, increased subject ratings on multiple VAS items (eg, High, Stoned, Like Drug), elevated heart rate, and impaired rate and accuracy on psychomotor performance tasks. The higher tiagabine dose substituted for the Δ^9-THC discriminative stimulus, and engendered subjective and performance-impairing effects that overlapped with those of Δ^9-THC, when administered alone. In combination, tiagabine shifted the discriminative stimulus effects of Δ^9-THC leftward/upward, and enhanced Δ^9-THC effects on self-report and performance measures. These results indicated that GABA is involved in the interoceptive and other behavioral effects of Δ^9-THC (ie, a positive nonselective signal was obtained).

The substitution of the higher tiagabine dose, and potentiation of the discriminative stimulus effects of Δ^9-THC by concurrent administration with tiagabine was remarkably similar to what was observed in a previous study in which the CB agonist nabilone was administered alone and in combination with Δ^9-THC (Lile, Kelly, & Hays, 2011). In that study, Δ^9-THC and nabilone shared behavioral and physiological effects, when administered separately. In combination, nabilone significantly shifted the discriminative stimulus effects of Δ^9-THC leftward/upward, and enhanced Δ^9-THC effects on cannabinoid-sensitive measures. It is worth noting that in a previous human study in which 4 and 8 mg of tiagabine were tested in subjects discriminating oral cocaine (Lile et al., 2004), tiagabine did not change the cocaine dose-effect curve. The impact of tiagabine on the discriminative stimulus effects of Δ^9-THC, but not cocaine, suggest a role for GABA in the neuropharmacological effects of cannabinoids, rather than a general impact on all drug effects.

THE INFLUENCE OF $GABA_B$ ACTIVATION ON Δ^9-THC DISCRIMINATION

As described, tiagabine modulates GABA activity by blocking reuptake transporters, thereby producing global elevations in GABA levels, so the contribution of specific GABA receptor subtypes (ie, $GABA_A$ or $GABA_B$) could not be determined in the previous study. With respect to $GABA_B$, some preclinical research has supported its involvement in the effects of cannabinoids. For example, a study found that Δ^9-THC and the $GABA_B$ agonist baclofen (Lioresal) both decreased open field locomotor activity in rodents, and the $GABA_B$ antagonist CGP 35348 reversed the locomotor-decreasing effects of both drugs (Romero, García-Palomero, Fernández-Ruiz & Ramos, 1996). In addition, baclofen augmented the catalepsy produced by Δ^9-THC in rodents (Pertwee et al., 1988). Preclinical studies that have tested cannabinoid and $GABA_B$ agonists separately have demonstrated overlap in their pharmacological profiles; specifically, these drugs are anxiolytic (Moreira & Wotjak, 2010; Pilc & Nowak, 2005), induce hypothermia (Frosini et al., 2004; Wenger & Moldrich, 2002), impair memory processes (Castellano et al., 2003; DeSousa, Beninger, Jhamandas, & Boegman, 1994), and produce peripheral antinociception (Dario, Pisani, Sangiorgi, Pessina, & Tomei, 2007; Hohmann, 2002).

$GABA_B$ participation in the effects of cannabinoids in humans has received less attention. There appears to be only a single clinical research study that has combined

a $GABA_B$ agonist (baclofen) with a cannabinoid, under controlled laboratory conditions (Haney et al., 2010). In that study, daily cannabis users were maintained on 60 and 90 mg/day of baclofen, divided into three 20 or 30 mg doses, and then received active (3.3% Δ^9-THC) and placebo cannabis. Baclofen had minimal impact on the direct effects of active cannabis, except that the low, but not the high, dose of baclofen significantly decreased self-reported ratings of High. In addition, baclofen did not reduce cannabis self-administration, using a laboratory model of relapse in that study. It is worth mentioning, however, that two case report publications described the ability of baclofen treatment to reduce the signs and symptoms of cannabis withdrawal, and facilitate abstinence (Imbert, Labrune, Lancon, & Simon, 2014; Nanjayya, Shivappa, Chand, Murthy, & Benegal, 2010).

A follow-up study was therefore conducted to determine the potential involvement of the $GABA_B$ receptor subtype in the clinical effects of Δ^9-THC by assessing the effects of Δ^9-THC and the $GABA_B$ agonist baclofen, using drug-discrimination procedures (Lile, Kelly, & Hays, 2012b). Baclofen was chosen because it is a $GABA_B$ agonist that is commercially available for use in humans, and has a favorable safety profile. Using procedures similar to those described above, baclofen (25 and 50 mg), Δ^9-THC (5, 15, and 30 mg), and placebo were administered alone and in combination to cannabis users. For reference, the recommended starting dose of baclofen for the treatment of muscle spasticity is 5 mg, taken three times a day, subsequently increased at 3-day intervals by 5 mg, up to an optimum dose range of 30–75 mg daily (Lioresal Product Information, 2008). Single doses of 40 mg and 50 mg have been administered previously in the initial pharmacokinetic and efficacy studies.

As demonstrated previously, Δ^9-THC functioned as a discriminative stimulus, produced subjective effects typically associated with cannabinoids, elevated heart rate, and impaired rate and accuracy on psychomotor performance. The higher dose of baclofen alone substituted for the Δ^9-THC discriminative stimulus, and both baclofen doses shifted the discriminative stimulus effects of Δ^9-THC leftward/upward. Similar results were observed on other cannabinoid-sensitive outcomes, although baclofen generally did not engender Δ^9-THC-like subjective responses, when administered alone. These results suggest that the $GABA_B$ receptor subtype is involved in the interoceptive effects of Δ^9-THC (Fig. 79.2).

The results obtained with baclofen were comparable to tiagabine, suggesting that $GABA_B$ receptors were responsible, at least in part, for the effects of tiagabine-induced elevated GABA on cannabinoid-related behaviors in the previous study. A few differences are worth pointing out, however. With respect to the drug-discrimination data, tiagabine fully (≥80% drug-appropriate responding) substituted for the Δ^9-THC discriminative stimulus, whereas baclofen significantly, but only partially, substituted for Δ^9-THC. Likewise, both doses of tiagabine (6 and 12 mg) significantly increased the discriminative stimulus effects of 5 mg Δ^9-THC, while only the larger dose of baclofen (50 mg) enhanced the low-dose Δ^9-THC discriminative stimulus effects. In addition, 12 mg of tiagabine alone engendered a response on many of the self-reported drug-effect items, but baclofen did not. One possible explanation for these differences is that relative doses of tiagabine were larger than baclofen. However, baclofen, but not tiagabine, augmented Δ^9-THC effects on heart rate, which might not be expected if the differences were simply dose dependent. Another explanation is that activation of the $GABA_A$ receptor is also involved in the effects of cannabinoids in humans, and contributed to the responses observed when GABA was elevated by tiagabine (Fig. 79.3).

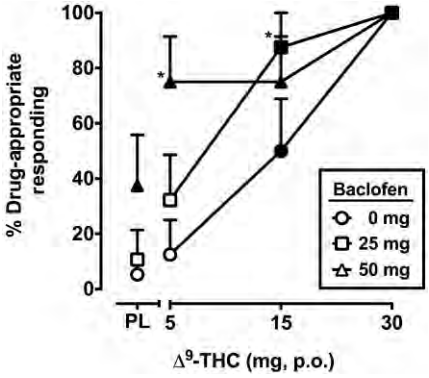


FIGURE 79.2 $GABA_B$ agonism enhances the discriminative stimulus effects of Δ^9-THC. Separate and combined effects of Δ^9-THC and baclofen on Δ^9-THC-appropriate responding on the drug-discrimination task. Other details are as in Fig. 79.1. *Source: With permission from Lile et al. (2012b).*

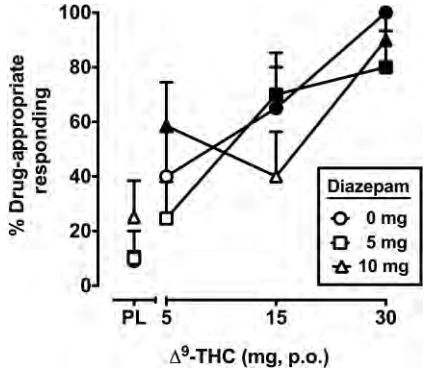


FIGURE 79.3 $GABA_A$ positive allosteric modulation does not influence the discriminative stimulus effects of Δ^9-THC. Separate and combined effects of Δ^9-THC and diazepam on Δ^9-THC-appropriate responding on the drug-discrimination task. Data points show means of 10 subjects. Other details are as in Fig. 79.1. *Source: With permission from Lile et al. (2014).*

THE INFLUENCE OF POSITIVE ALLOSTERIC MODULATION OF $GABA_A$ RECEPTORS ON Δ^9-THC DISCRIMINATION

To address the possibility that the $GABA_A$ receptor subtype is also involved in the effects of cannabinoids in humans, and contributed to the responses observed when GABA was elevated by tiagabine, a third study was conducted to test the interoceptive effects of Δ^9-THC in combination with diazepam (Lile, Kelly, & Hays, 2014). Diazepam, first marketed as Valium, is a benzodiazepine sedative-hypnotic drug that functions as a positive allosteric modulator of $GABA_A$ receptors. A direct $GABA_A$ agonist was not used due to side effects associated with that class of drugs (eg, dissociative hallucinations with muscimol), as well as their limited availability for use in humans. Diazepam was selected over other $GABA_A$ positive allosteric modulators because earlier preclinical work demonstrated that diazepam engendered partial drug-appropriate responding in animals trained to discriminate Δ^9-THC (Barrett et al., 1995; Browne & Weissman, 1981; Mokler, Nelson, Harris, & Rosecrans, 1986; Wiley & Martin, 1999). These Δ^9-THC-like discriminative stimulus effects of diazepam appeared to be mediated by its interaction with $GABA_A$, rather than cannabinoid receptors, because the partial substitution in animals trained to discriminate Δ^9-THC was blocked by the benzodiazepine antagonist flumazenil (Mokler et al., 1986) but not the cannabinoid inverse agonist/antagonist rimonabant (Wiley & Martin, 1999).

Similarities in some of the other behavioral effects of benzodiazepines such as diazepam and cannabinoid agonists have also been documented. For example, diazepam produced a positive signal on three of the four tests included in the "tetrad" traditionally used to assess cannabinoid activity in rodents, specifically catalepsy, locomotor activity, and antinociception (Wiley & Martin, 2003). In addition, impairment of psychomotor performance, as well as learning and memory processes, are well-characterized effects of $GABA_A$ positive allosteric modulators and cannabinoids (Evans, Griffiths, & de Wit, 1996; Kelly, Foltin, & Fischman, 1993). Lastly, cannabinoids promote sleep, and function as anxiolytics under certain conditions and doses, which are therapeutic indication of $GABA_A$ positive allosteric modulators (Nakano et al., 1978; Ware et al., 2010).

In the third study, diazepam (5 and 10 mg), Δ^9-THC (5, 15, and 30 mg), and placebo were administered alone and in combination to cannabis users, using the procedures and outcomes described earlier. For reference, the recommended acute oral dosage of diazepam for the treatment of anxiety is 2–10 mg (Valium Product Information, 2013). Δ^9-THC produced the anticipated effects on the drug-discrimination task, subjective questionnaire, performance measures, and heart rate. Diazepam alone impaired performance on psychomotor performance tasks, and increased ratings on a limited number of self-report questionnaire items (eg, Any Effect, Sedated), but did not substitute for the Δ^9-THC discriminative stimulus or alter the Δ^9-THC discrimination dose-response function, and had little impact on the other behavioral effects of Δ^9-THC. These results suggest that the $GABA_A$ receptor subtype has minimal involvement in the interoceptive effects of Δ^9-THC or cannabis in humans.

This study found no evidence of overlap in the discriminative stimulus effects of diazepam and Δ^9-THC. These results contrast with previous research that demonstrated partial substitution of diazepam for the Δ^9-THC discriminative stimulus in rats and monkeys (Barrett et al., 1995; Browne & Weissman, 1981; Mokler et al., 1986; Wiley & Martin, 1999). However, Δ^9-THC did not occasion drug-appropriate responding in animals trained to discriminate diazepam, demonstrating that the partial cross-generalization was asymmetrical (Wiley & Martin, 1999). In addition, the benzodiazepines chlordiazepoxide and midazolam failed to produce Δ^9-THC-appropriate responding under conditions in which diazepam partially substituted (Barrett et al., 1995). Muscimol, a direct $GABA_A$ agonist, also did not engender drug-appropriate responding in rats trained to discriminate Δ^9-THC (Browne & Weissman, 1981). In humans, triazolam did not occasion Δ^9-THC-like discriminative stimulus effects, up to doses that impaired psychomotor performance (Lile et al., 2009). Together, these results indicate that there is minimal involvement of $GABA_A$ in the interoceptive cue produced by Δ^9-THC in humans, and that shared discriminative stimulus effects between Δ^9-THC and benzodiazepines are limited to diazepam, only under certain training conditions, and in nonhuman species. Also worth noting is that baclofen did not share discriminative stimulus effects with Δ^9-THC in rodents (Browne & Weissman, 1981). Taken in the context of the clinical drug discrimination study with baclofen described above, and the cross-species differences with diazepam, it appears that the neuropharmacological interactions between GABA and CB systems differ between human and nonhuman species.

SUMMARY AND FUTURE CONSIDERATIONS

The studies reviewed here indicate that GABA, particularly the $GABA_B$ receptor subtype, plays a significant part in the interoceptive effects produced by Δ^9-THC in humans, and, by extension, cannabis. Drugs that produce effects that overlap with cannabis have been shown to address some of the signs and symptoms of cannabis withdrawal that are reported to make abstinence

difficult and, as such, could serve as effective pharmacotherapies for cannabis dependence. These results suggest that GABA could be targeted in the development of medications for cannabis dependence. In fact, gabapentin, a GABA analog that is approved for treating neuropathic pain and seizures, has recently emerged as a promising candidate for cannabis-use disorder management (Mason et al., 2012) and, to date, is the only medication that has demonstrated initial pharmacotherapeutic efficacy in clinical trials in adults. In the pilot study by Mason et al. (2012), 1200 mg/day of gabapentin decreased Δ^9-THC metabolite levels in the urine, self-reported cannabis use, craving, and depression, and improved performance on tests of executive function in treatment-seeking cannabis users. Although the therapeutic effects of gabapentin have been linked primarily to VDCCs, there is also evidence that it elevates brain GABA levels (Cai et al., 2012). In addition, the therapeutic effects of gabapentin overlap with those of tiagabine. Specifically, gabapentin and its derivative pregabalin alleviate pain and anxiety, and promote sleep, effects that are also produced by tiagabine (Bestard & Toth, 2011). In a study using drug discrimination procedures similar to those described here, gabapentin substituted for the interoceptive cue produced by Δ^9-THC, and enhanced the discriminative stimulus effects of Δ^9-THC (Lile, Kelly, & Hays, 2016) in a manner similar to what was observed in the studies with tiagabine and baclofen. However, a role for VDCCs in gabapentin's efficacy in cannabis dependence cannot be ruled out. For example, one of the main consequences of CB receptor mediated G-protein activation is the inhibition of VDCCs (Howlett, Blume, & Dalton, 2010). In addition, there is evidence for a CB-receptor-independent modulation of VDCCs by CB ligands, either through interaction with the plasma membrane lipid bilayer, or by direct interaction with a binding site on the ion channel (Lozovaya, Min, Tsintsadze, & Burnashev, 2009). Future research is needed to disentangle the mechanism by which gabapentin reduces cannabis use, and also to determine whether a GABA reuptake inhibitor or $GABA_B$ agonist would be useful for managing cannabis dependence.

MINI-DICTIONARY

Agonist A chemical that activates a receptor.
Allosteric modulator A chemical that binds to a receptor at a site distinct from the primary binding site for the endogenous ligand, and either increases or decreases receptor activity.
Antagonist A chemical that binds to a receptor but does not produce a response, and prevents other chemicals from binding to that receptor.
GABA The major inhibitory neurotransmitter in the CNS.
GABA transporter A protein present in the membrane of GABA neurons that transports recently released GABA back into the neuron.
$GABA_A$ receptor One of the two well-characterized GABA receptors; $GABA_A$ are ionotropic, meaning that binding of an agonist opens a pore formed by the receptor that spans the cell membrane, through which ions may pass; $GABA_A$ receptors permit the influx of negatively charged chloride ions through the channel which hyperpolarizes the neuron, reducing the likelihood of an action potential, and thus having an overall inhibitory effect on neurotransmission.
$GABA_B$ receptor The other of the two well-characterized GABA receptors; $GABA_B$ are metabotropic, meaning they affect cellular communication via intracellular signaling pathways originating with G-proteins. $GABA_B$ activation of G-proteins stimulates the opening of channels that permit the passage of positively charged potassium ions out of the neuron, which hyperpolarizes the cell.
Interoceptive effects Internal stimulus that cannot be directly observed, in this case referring specifically to the perception of drug effects based on specific neuropharmacological mechanisms.
Neurotransmitter An endogenous chemical that transmits signals to and from neurons (nerve cells); typically, these are released by one neuron to travel across the synaptic cleft (space between two neurons) to transmit a chemical signal to another neuron.
Receptor A protein embedded within a cell membrane that binds endogenous (native to the body) or exogenous (foreign to the body) ligand (chemical) to affect cellular communication.
Retrograde signaling In this case, the process by which a neuron sends a chemical message back to a neuron that controls it, in order to provide regulatory feedback of neurotransmission.

References

Antonelli, T., Tomasini, M. C., Mazza, R., Fuxe, K., Gaetani, S., Cuomo, V., Tanganelli, S., & Ferraro, L. (2009). Cannabinoid CB1 and cholecystokinin CCK2 receptors modulate, in an opposing way, electrically evoked [3H]GABA efflux from rat cerebral cortex cell cultures: possible relevance for cortical GABA transmission and anxiety. *Journal of Pharmacology and Experimental Therapeutics*, *329*, 708–717.

Barrett, R. L., Wiley, J. L., Balster, R. L., & Martin, B. R. (1995). Pharmacological specificity of delta 9-tetrahydrocannabinol discrimination in rats. *Psychopharmacology*, *118*, 419–424.

Bestard, J. A., & Toth, C. C. (2011). An open-label comparison of nabilone and gabapentin as adjuvant therapy or monotherapy in the management of neuropathic pain in patients with peripheral neuropathy. *Pain Practice*, *11*, 353–368.

Browne, R. G., & Weissman, A. (1981). Discriminative stimulus properties of delta 9-tetrahydrocannabinol: mechanistic studies. *Journal of Clinical Pharmacology*, *21*, S227–S234.

Cai, K., Nanga, R. P., Lamprou, L., Schinstine, C., Elliott, M., Hariharan, H., Reddy, R., & Epperson, C. N. (2012). The impact of gabapentin administration on brain GABA and glutamate concentrations: a 7T ^{1}H-MRS study. *Neuropsychopharmacology*, *37*, 2764–2771.

Castellano, C., Rossi-Arnaud, C., Cestari, V., & Costanzi, M. (2003). Cannabinoids and memory: animal studies. *Current Drug Targets: CNS Neurological Disorders*, *2*, 389–402.

Compton, D. R., Rice, K. C., DeCosta, B. R., Razdan, R. K., Melvin, L. S., Johnson, M. R., & Martin, B. R. (1993). Cannabinoid structure-activity relationships: correlation of receptor binding and in vivo activities. *Journal of Pharmacology and Experimental Therapeutics*, *265*, 218–226.

Coull, M. A., Johnston, A. T., Pertwee, R. G., & Davies, S. N. (1997). Action of Δ^9-tetrahydrocannabinol on $GABA_A$ receptor-mediated responses in a grease-gap recording preparation of the rat hippocampal slice. *Neuropharmacology*, *36*, 1387–1392.

Dario, A., Pisani, R., Sangiorgi, S., Pessina, F., & Tomei, G. (2007). Relationship between intrathecal baclofen and the central nervous system. *Acta Neurochirurgica Supplement*, *97*(Pt 1), 461–464.

DeSousa, N. J., Beninger, R. J., Jhamandas, K., & Boegman, R. J. (1994). Stimulation of $GABA_B$ receptors in the basal forebrain selectively impairs working memory of rats in the double Y-maze. *Brain Research*, *641*, 29–38.

Engler, B., Freiman, I., Urbanski, M., & Szabo, B. (2006). Effects of exogenous and endogenous cannabinoids on GABAergic neurotransmission between the caudate-putament and the globus pallidus in the mouse. *Journal of Pharmacology and Experimental Therapeutics*, *316*, 608–617.

Evans, S. M., Griffiths, R. R., & de Wit, H. (1996). Preference for diazepam, but not buspirone, in moderate drinkers. *Psychopharmacology*, *123*, 154–163.

Freund, T. F., Katona, I., & Piomelli, D. (2003). Role of endogenous cannabinoids in synaptic signaling. *Physiological Reviews*, *83*, 1017–1066.

Frosini, M., Valoti, M., & Sgaragli, G. (2004). Changes in rectal temperature and ECoG spectral power of sensorimotor cortex elicited in conscious rabbits by i.c.v. injection of GABA, GABA(A) and GABA(B) agonists and antagonists. *British Journal of Pharmacology*, *141*, 152–162.

Gabitril Product Information (2009). Cephalon Inc.

Haney, M., Hart, C. L., Vosburg, S. K., Comer, S. D., Cooper, Z. D., & Foltin, R. W. (2010). Effects of baclofen and mirtazapine on a laboratory model of marijuana withdrawl and relapse. *Psychopharmacology*, *211*, 233–244.

Hohmann, A. G. (2002). Spinal and peripheral mechanisms of cannabinoid antinociception: behavioral, neurophysiological and neuroanatomical perspectives. *Chemistry and Physics of Lipids*, *121*, 173–190.

Holtzman, S. G., & Locke, K. W. (1988). Neural mechanisms of drug stimuli: experimental approaches. *Psychopharmacology*, *4*, 138–153.

Howlett, A. C., Blume, L. C., & Dalton, G. D. (2010). CB(1) cannabinoid receptors and their associated proteins. *Current Medicinal Chemistry*, *17*, 1382–1393.

Imbert, B., Labrune, N., Lancon, C., & Simon, N. (2014). Baclofen in the management of cannabis dependence syndrome. *Therapeutic Advances in Psychopharmacology*, *4*, 50–52.

Kelly, T. H., Foltin, R. W., & Fischman, M. W. (1993). Effects of smoked marijuana on heart rate, drug ratings and task performance by humans. *Behavioural Pharmacology*, *2*, 167–178.

Kelly, T. H., Stoops, W. W., Perry, A. S., Prendergast, M. A., & Rush, C. R. (2003). Clinical neuropharmacology of drugs of abuse: a comparison of drug-discrimination and subject-report measures. *Behavioral and Cognitive Neuroscience Reviews*, *2*, 227–260.

Laughlin, T. M., Tram, K. V., Wilcox, G. L., & Birnbaum, A. K. (2002). Comparison of antiepileptic drugs tiagabine, lamotrigine, and gabapentin in mouse models of acute, prolonged, and chronic nociception. *Journal of Pharmacology and Experimental Therapeutics*, *302*, 1168–1175.

Lile, J. A., Kelly, T. H., & Hays, L. R. (2010). Substitution profile of the cannabinoid agonist nabilone in humans discriminating Δ^9-THC. *Clinical Neuropharmacology*, *33*, 235–242.

Lile, J. A., Kelly, T. H., & Hays, L. R. (2011). Separate and combined effects of the cannabinoid agonists nabilone and Δ^9-THC in humans discriminating Δ(9)-THC. *Drug and Alcohol Dependence*, *116*, 86–92.

Lile, J. A., Kelly, T. H., & Hays, L. R. (2012a). Separate and combined effects of the GABA reuptake inhibitor tiagabine and Δ^9-THC in humans discriminating Δ(9)-THC. *Drug and Alcohol Dependence*, *122*, 61–69.

Lile, J. A., Kelly, T. H., & Hays, L. R. (2012b). Separate and combined effects of the GABA(B) agonist baclofen and Δ9-THC in humans discriminating Δ9-THC. *Drug and Alcohol Dependence*, *126*, 216–223.

Lile, J. A., Kelly, T. H., & Hays, L. R. (2014). Separate and combined effects of the GABA(A) positive allosteric modulator diazepam and Δ9-THC in humans discriminating Δ9-THC. *Drug and Alcohol Dependence*, 143, 141–148.

Lile, J. A., Kelly, T. H., Pinsky, D. J., & Hays, L. R. (2009). Substitution profile of Δ^9-tetrahydrocannabinol, triazolam, hydromorphone and methylphenidate in humans discriminating Δ^9-tetrahydrocannabinol. *Psychopharmacology*, *203*, 241–250.

Lile, J. A., Stoops, W. W., Allen, T. S., Glaser, P. E., Hays, L. R., & Rush, C. R. (2004). Baclofen does not alter the reinforcing, subject-rated or cardiovascular effects of intranasal cocaine in humans. *Psychopharmacology*, *171*, 441–449.

Lile, J. A., Wesley, M. J., Kelly, T. H., & Hays, L. R. (2016). Separate and combined effects of gabapentin and Δ9-THC in humans discriminating Δ9-THC. *Behavioural Pharmacology*, *27*, 215–224.

Lioresal Product Information (2008). Novartis Pharmaceuticals Limited.

Lozovaya, N., Min, R., Tsintsadze, V., & Burnashev, N. (2009). Dual modulation of CNS voltage-gated calcium channels by cannabinoids: focus on CB1 receptor-independent effects. *Cell Calcium*, *46*, 154–162.

Mailleaux, P., & Vanderhaeghen, J. J. (1992). Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and in situ hybridization histochemistry. *Neuroscience*, *48*, 655–658.

Marinol Product Information (2006). Solvay Pharmaceuticals Inc.

Mason, B. J., Crean, R., Goodell, V., Light, J. M., Quello, S., Shadan, F., Buffkins, K., Kyle, M., Adusumalli, M., Begovic, A., & Rao, S. (2012). A proof-of-concept randomized controlled study of gabapentin: effects on cannabis use, withdrawal and executive function deficits in cannabis-dependent adults. *Neuropsychopharmacology*, *37*, 1689–1698.

Mokler, D. J., Nelson, B. D., Harris, L. S., & Rosecrans, J. A. (1986). The role of benzodiazepine receptors in the discriminative stimulus properties of delta 9-tetrahydrocannabinol. *Life Science*, *38*, 1581–1589.

Moreira, F. A., & Wotjak, C. T. (2010). Cannabinoids and anxiety. *Current Topics in Behavioral Neurosciences*, *2*, 429–450.

Nakano, S., Gillespie, H. K., & Hollister, L. E. (1978). A model for evaluation of antianxiety drugs with the use of experimentally induced stress: comparison of nabilone and diazepam. *Clinical Pharmacology and Therapeutics*, *23*, 54–62.

Nanjayya, S. B., Shivappa, M., Chand, P. K., Murthy, P., & Benegal, V. (2010). Baclofen in cannabis dependence syndrome. *Biological Psychiatry*, *68*, 9–10.

Pertwee, R. G., Browne, S. E., Ross, T. M., & Stretton, C. D. (1991). An investigation of the involvement of GABA in certain pharmacological effects of delta-9-tetrahydrocannabinol. *Pharmacology, Biochemistry and Behavior*, *40*, 581–585.

Pertwee, R. G., Greentree, S. G., & Swift, P. A. (1988). Drugs which stimulate or facilitate central GABAergic transmission interact synergistically with delta-9-tetrahydrocannabinol to produce marked catalepsy in mice. *Neuropharmacology*, *27*, 1265–1270.

Pilc, A., & Nowak, G. (2005). GABAergic hypotheses of anxiety and depression: focus on GABA-B receptors. *Drugs Today*, *41*, 755–766.

Romero, J., de Miguel, R., Ramos, J. A., & Fernández-Ruiz, J. J. (1998). The activation of cannabinoid receptors in striatonigral GABAergic neurons inhibited GABA uptake. *Life Science*, *62*, 351–363.

Romero, J., García-Palomero, E., Fernández-Ruiz, J. J., & Ramos, J. A. (1996). Involvement of GABA(B) receptors in the motor inhibition produced by agonists of brain cannabinoid receptors. *Behavioural Pharmacology*, *7*, 299–302.

Sañudo-Peña, M. C., Romero, J., Seale, G. E., Fernandez-Ruiz, J. J., & Walker, J. M. (2000). Activational role of cannabinoids on movement. *European Journal of Pharmacology*, *391*, 269–274.

Schmitt, U., & Hiemke, C. (1999). Effects of GABA-transporter (GAT) inhibitors on rat behaviour in open-field and elevated plus-maze. *Behavioral Pharmacology*, *10*, 131–137.

Schmitt, U., & Hiemke, C. (2002). Tiagabine, a gamma-amino-butyric acid transporter inhibitor impairs spatial learning of rats in the Morris water-maze. *Behavioural Brain Research*, *133*, 391–394.

Schwartz, T. L., & Nihalani, N. (2006). Tiagabine in anxiety disorders. *Expert Opinion on Pharmacotherapy*, *7*, 1977–1987.

Steiger, A. (2007). Neurochemical regulation of sleep. *Journal of Psychiatric Research*, *41*, 537–552.

Suzdak, P. D., Frederiksen, K., Andersen, K. E., Sørensen, P. O., Knutsen, L. J., & Nielsen, E. B. (1992). NNC-711, a novel potent and selective gamma-aminobutyric acid uptake inhibitor: pharmacological characterization. *European Journal of Pharmacology*, *224*, 189–198.

Szabo, B., & Schlicker, E. (2005). Effects of cannabinoids on neurotransmission. *Handbook of Experimental Pharmacology*, *168*, 327–365.

Todorov, A. A., Kolchev, C. B., & Todorov, A. B. (2005). Tiagabine and gabapentin for the management of chronic pain. *Clinical Journal of Pain*, *21*, 358–361.

Valium Product Information (2013). Genentech, Inc.

Ware, M. A., Fitzcharles, M. A., Joseph, L., & Shir, Y. (2010). The effects of nabilone on sleep in fibromyalgia: results of a randomized controlled trial. *Anesthesia and Analgesia*, *110*, 604–610.

Wenger, T., & Moldrich, G. (2002). The role of endocannabinoids in the hypothalamic regulation of visceral function. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *66*, 301–307.

Wiley, J. L., Barrett, R. L., Lowe, J., Balster, R. L., & Martin, B. R. (1995a). Discriminative stimulus effects of CP 55, 940 and structurally dissimilar cannabinoids in rats. *Neuropharmacology*, *34*, 669–676.

Wiley, J. L., Huffman, J. W., Balster, R. L., & Martin, B. R. (1995b). Pharmacological specificity of the discriminative stimulus effects of delta 9-tetrahydrocannabinol in rhesus monkeys. *Drug and Alcohol Dependence*, *40*, 81–86.

Wiley, J. L., & Martin, B. R. (1999). Effects of SR 141716A on diazepam substitution for delta 9-tetrahydrocannabinol in rat drug discrimination. *Pharmacology, Biochemistry and Behavior*, *64*, 519–522.

Wiley, J. L., & Martin, B. R. (2003). Cannabinoid pharmacological properties common to other centrally acting drugs. *European Journal of Pharmacology*, *471*, 185–193.

Zwanzger, P., Schule, C., Eser, D., Baghai, T. C., Padberg, F., Ella, R., Moller, H. J., Rupprecht, R., & Kathmann, N. (2005). Saccadic eye velocity after selective GABAergic treatment with tiagabine in healthy volunteers. *Neuropsychobiology*, *52*, 147–150.

CHAPTER

80

The Role of Δ^9-Tetrahydrocannabinol in Diabetes Mellitus

Z.M. Coskun, S. Bolkent***

***Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Istanbul Bilim University, Istanbul, Turkey**

****Department of Medical Biology, Cerrahpasa Faculty of Medicine, Istanbul University, Istanbul, Turkey**

SUMMARY POINTS

- This chapter focuses on diabetes treated with Δ^9-tetrahydrocannabinol (Δ^9-THC), the main constituent of *Cannabis sativa*.
- Diabetes mellitus is one of the metabolic diseases that are characterized by hyperglycemia due to deficiency in production of insulin by the pancreas.
- In clinical and experimental studies, dyslipidemia, oxidative stress, and inflammation have critically key roles in diabetes and its complications.
- Δ^9-THC demonstrates therapeutic effects by means of cannabinoid receptors.
- Cannabinoid receptors are present in the brain, pancreas, lung, heart, thymus and testicles.
- Hence, Δ^9-THC may be effective against oxidative stress, inflammation, food intake, energy balance, and secretion of endocrine hormones.

KEY FACTS OF DIABETES

- The pancreas consists of the exocrine and endocrine portions.
- The endocrine portion, consists of the pancreatic islets that secrete glucagon and insulin hormones.
- Beta (β) cells produce the insulin hormone in pancreas.
- The degeneration of β cells causes the deficiency of insulin.
- Therefore, the blood glucose levels are elevated in diabetic patients.
- In addition, lipid profile is changed in diabetes.
- Diabetes is a critical medical problem that is dramatically on-the-rise.

KEY FACTS OF Δ^9-THC

- Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the principal cannabinoid of the *Cannabis sativa*.
- Δ^9-THC has pharmaceutical properties.
- The therapeutic features of Δ^9-THC result from the agonist activity of the cannabinoid receptors.
- There are two cannabinoid (CB1 and CB2) receptors in the endocannabinoid system.
- Δ^9-THC accumulates in adipose tissue.
- The excretion of Δ^9-THC is provided via urine.

LIST OF ABBREVIATIONS

α	Alpha
β	Beta
CAT	Catalase
CART	Cocaine- and amphetamine-regulated transcript
CB1 receptor	Cannabinoid 1 receptor

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00091-0
Copyright © 2017 Elsevier Inc. All rights reserved.

CB2 receptor	Cannabinoid 2 receptor
CGRP	Calcitonin gene-related peptide
DM	Diabetes mellitus
δ	Delta
GHRL	Ghrelin
GPx	Glutathione peroxidase
GSH	Glutathione
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
MDA	Malondialdehyde
PP	Pancreatic polypeptide
PPAR-γ	Peroxisome proliferator-activated receptor gamma
PPARs	Peroxisome proliferator-activated receptors
PYY	Peptide YY
SOD	Superoxide dismutase
STZ	Streptozotocin
Δ^9-THC	Δ^9-Tetrahydrocannabinol
VLDL	Very low density lipoprotein

INTRODUCTION

Diabetes mellitus (DM) is a multifactorial disease characterized by high levels of glucose in the blood. Hyperglycemia is caused by reduced insulin secretion and enhanced insulin resistance. It is also due to the disturbances of carbohydrate, protein, and fat metabolisms. A high level of blood glucose is frequently accompanied by hyperlipidemia (Derek, Taylor, & Olefsky, 2000; Kahn et al., 2008; Kim & Caprio, 2011).

Pancreas is a glandular organ responsible for the digestion and homeostasis of glucose. Pancreas is the well-known main source of insulin. Pancreas consists of the exocrine and endocrine portions. The endocrine functions of pancreas are led by the cells in islets of Langerhans. There are classically four types of cells in islets of Langerhans. It has been indicated that in addition to the classical islet hormones, such as insulin (secretion of β cells), glucagon (secretion of α cells), somatostatin (secretion of δ cells), and pancreatic polypeptide (PP) (secretion of PP cells), the islets also express nonclassical islet hormone-like peptides such as islet calcitonin gene-related peptide (CGRP), peptide YY (PYY), ghrelin (GHRL), and cocaine- and amphetamine-regulated transcript (CART) (Yildirim, Sundler, & Bolkent, 2007; Yildirim, Bolkent, & Sundler, 2008). In an adult human, the total islet cells contain ~70–80% β cells, ~15–20% α cells, ~5% δ cells and ~ 1% PP cells, and other cells in the remaining percentage. β cell impairment is due to development and progression of diabetes (Derek et al., 2000; Kahn et al., 2008).

It is known that the islets of Langerhans are regulators of glucose homeostasis. The main hormones in the regulation of glycemia are insulin and glucagon. In diabetes, the number of β cells is decreased in the islets of Langerhans (Fig. 80.1). On the contrary, the number of glucagon-secreting cells is increased in the islets of Langerhans (Fig. 80.2) (Coskun & Bolkent, 2014). The level of plasma glucose is increased, while the level of plasma insulin is decreased in streptozotocin (STZ)-induced diabetes (Bolkent, Bolkent, Yanardag, & Tunali, 2005; Havel et al., 2000). In a study by Sefi et al. (2012), the serum insulin level decreased, and the blood glucose level was elevated in alloxan-induced diabetic rats, as compared to healthy rats. The body weights of rats were decreased in both diabetic animal models (Duarte et al., 2007; Sefi et al., 2012).

Increasing evidence suggests that the development and progression of DM and its complications are derived from increased oxidative stress. Oxidative stress is a lack of balance between the prooxidative and antioxidative stress in an organism (Derek et al., 2000; Kopáni, Celec, Danisovic, Michalka, & Biró, 2006). The clinical and experimental studies have demonstrated that oxidative

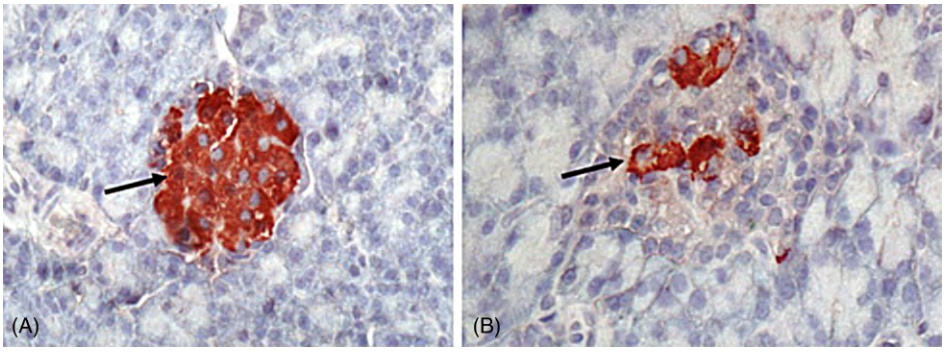


FIGURE 80.1 The expression of insulin reduced due to beta cells dysfunction. The immunopositive cells of insulin *(arrow)* were observed in islets of Langerhans. (A) Healthy pancreas, (B) diabetic pancreas of rat by using the Streptavidin–biotin–peroxidase technique, hematoxylin counterstain; magnification ×400 (Coskun & Bolkent, 2014).

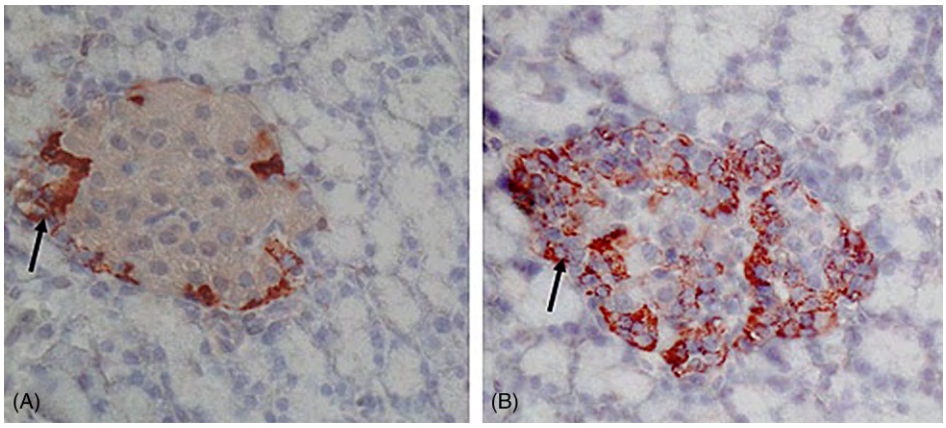


FIGURE 80.2 **The expression of glucagon increased in the Langerhans islet of diabetic pancreas.** The immunopositive cells of glucagon *(arrow)* were shown in islets of Langerhans. (A) Healthy pancreas, (B) diabetic pancreas of rat by using the Streptavidin–biotin–peroxidase technique, hematoxylin counterstain; magnification ×400 (Coskun & Bolkent, 2014).

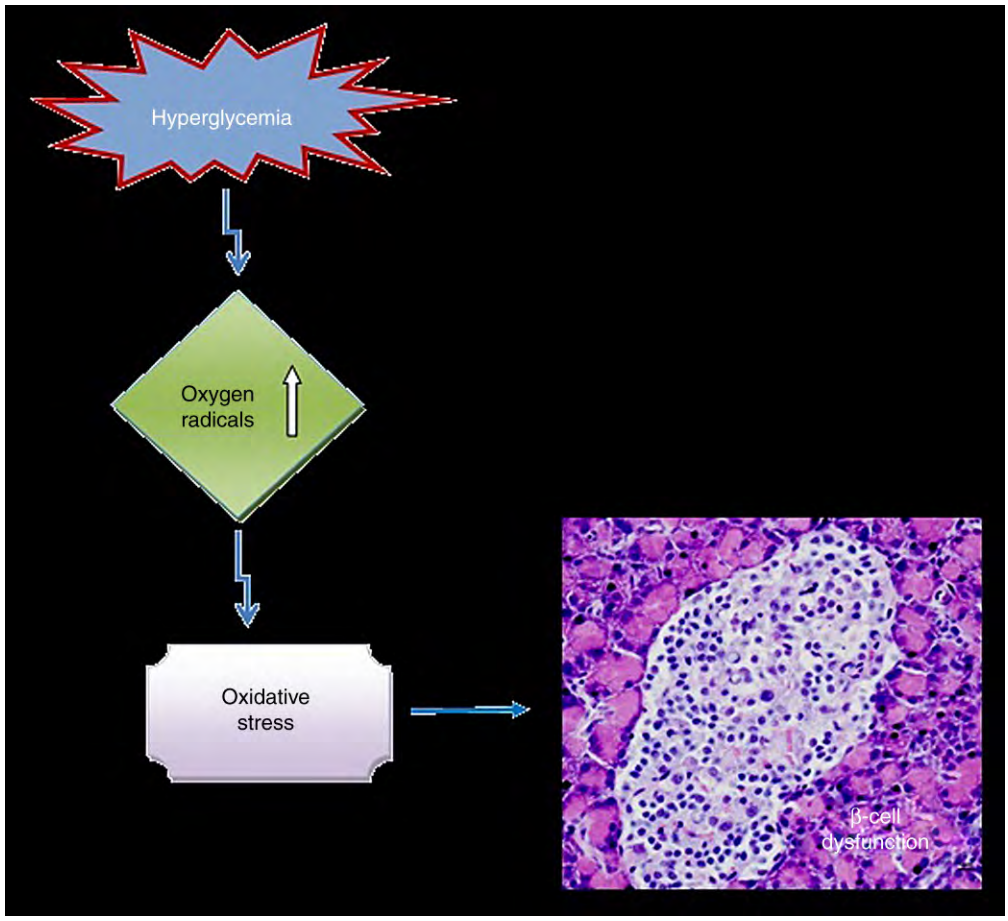


FIGURE 80.3 **Beta cells dysfunction is a consequence of the oxidative stress increased due to hyperglycemia.** The increased oxygen radicals induce oxidative stress and damage in islets of Langerhans (Coskun & Bolkent, 2014).

stress has a central role in the progression of diabetes (Fig. 80.3). The many pathways of diabetic complications include oxidative stress (Ávila de Lima et al., 2013; Baburao Jain & Anand Jain, 2012; Coskun et al., 2013; Giacco & Brownlee, 2010; Kassab & Piwowar, 2012; Li et al., 2011c; Stadler, 2012). Vascular problems such as cardiomyopathy, nephropathy, neuropathy, and retinopathy are among major complications of diabetes (Frostegård, 2013; Tiwari & Ndisang, 2014; Turenkov, Voronkov, & Slietsans, 2013). Diabetic dyslipidemia is one of the major risk factors for vascular problems. Although the precise pathogenesis of diabetic dyslipidemia is not

known, the lipid changes were shown to be associated with DM (Bolkent et al., 2005; Coskun & Bolkent, 2014).

The main constituent of *Cannabis sativa* is known to be Δ^9-tetrahydrocannabinol (Δ^9-THC), a natural phytocannabinoid. It has been known that Δ^9-THC is an agonist of cannabinoid receptors (Waldman et al., 2013). The cannabinoids are mediated by the activation of cannabinoid receptors. The endocannabioid system consists of cannabinoid 1 (CB1) and cannabinoid 2 (CB2) receptors (Li, Jones, & Persaud, 2011b). The CB1 and CB2 receptors are expressed in brain and peripheral organs, including pancreas, lung, heart, thymus, and testicles (Herkenham et al., 1991; Howlett et al., 2002; Li et al., 2011b; Munro, Thomas, & Abu-Shaar, 1993; Pertwee, 1997). Most research studies on the identification of over diabetes have been focused on glucose homeostasis in which cannabinoid receptors agonists or antagonists are useful on glucose homeostasis (Coskun & Bolkent, 2014; Li et al., 2011a; Tharp, Lee, Maple, & Pratley, 2008; Kim et al., 2011; Vilches-Flores, Delgado-Buenrostro, Navarrete-Vázquez, & Villalobos-Molina, 2010).

In this chapter, we will consider the roles of Δ^9-THC in diabetes, and its therapeutic potential of regulation regarding insulin secretion, lipid alterations, and energy homeostasis, as well as its properties as an antioxidant, and immunosuppressive agent.

Δ^9-THC IN OXIDATIVE STRESS IN DIABETES

The antioxidative defense system involves the activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) enzymes. Lenzen, Drinkgern, and Tiedge (1996) have shown that pancreatic islets have a weak antioxidative defense mechanism. Hence, it can easily be affected by overproduction of reactive species. The levels of glutathione (GSH) and the activities of CAT, SOD, and GPx enzymes in the pancreas were decreased in diabetic rats, when compared to healthy rats (Coskun et al., 2013; Perez Gutierrez et al., 2014). The marker of lipid peroxidation index, such as malondialdehyde (MDA) formation, can play a role in tissue damage related with diabetes. The MDA level in pancreas increases during diabetes (Turk, Dagistanli, Sacan, Yanardag, & Bolkent, 2012). In many diseases, Δ^9-THC can provide protection as an antioxidant (Carroll, Zeissler, Hanemann, & Zajicek, 2012; Chen et al., 2005; Hampson, Grilmaldi, Axelrod, & Wink, 1998; Waldman et al., 2013). Δ^9-THC was protective against diabetes-induced by oxidative stress. The decreased GSH level in pancreas showed an increase, when treated with Δ^9-THC in diabetes, which also applied to the activities of CAT and SOD. However, the level of MDA was not changed in diabetic pancreas with Δ^9-THC (unpublished data). In diabetic pancreas treated with Δ^9-THC, the increasing activities of SOD and CAT enzymes, and increased GSH level, demonstrated that Δ^9-THC may protect the cells against reactive species. Δ^9-THC may reduce oxidative damage up to a point, during diabetes.

Δ^9-THC IN DYSLIPIDEMIA IN DIABETES

Diabetic dyslipidemia is characterized by the high plasma triglyceride concentration, reduced high density lipoprotein (HDL) cholesterol concentration, and increased concentration of low density lipoprotein (LDL) cholesterol level, as well as overproduction of very low density lipoprotein (VLDL) (Mooradian, 2009). An increase in total cholesterol, triglyceride, LDL cholesterol, and VLDL cholesterol levels of the blood was observed in STZ-induced diabetic rats (Krishnamurthy, Lakshman, Pruthvi, & Chandrika, 2011). Increased VLDL, LDL, total cholesterol, and triglyceride levels of serum were reduced in diabetes treated with Δ^9-THC (Coskun & Bolkent, 2014).

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptors family, and they play essential roles in regulating gene expression, differentiation, and development of cells, as well as metabolism of lipid, carbohydrate, and protein. There are three subtypes (α, β, and γ) of PPARs (Shearer & Hoekstra, 2003). PPAR-γ is associated with insulin resistance and adipocytes, and it is expressed in the β cells of the islets (Celi & Shuldiner, 2002; Yildirim et al., 2008). Recent evidence indicates that cannabinoids act on PPARs (Burstein, 2005; O'Sullivan, 2007). Thus, an exchange in the lipid profile can be provided by Δ^9-THC-induced activation of PPARs in diabetes.

Δ^9-THC IN THE SECRETION OF INSULIN IN DIABETES

Cannabinoid receptors may play a role in the endocrine secretion by pancreas (Bermúdez-Silva et al., 2008). An increase in insulin secretion was observed in rat insulinoma β-pancreatic cells, islet cells of mice and rats, which was induced by the cannabinoid receptor agonist (Matias et al., 2006; Vilches-Flores et al., 2010; Li, Bowe, Jones, & Persaud, 2010). It may be suggested that Δ^9-THC demonstrates therapeutic effects, which result from cannabinoid receptors. Both receptors are present in the pancreas of rodent species. However, their localization is different in pancreas. While the CB1 receptors are mostly expressed in the islets of Langerhans, the CB2 receptors are expressed in pancreatic ducts (unpublished data) (Figs. 80.4 and 80.5). In diabetes treated with Δ^9-THC, the expressions of insulin and

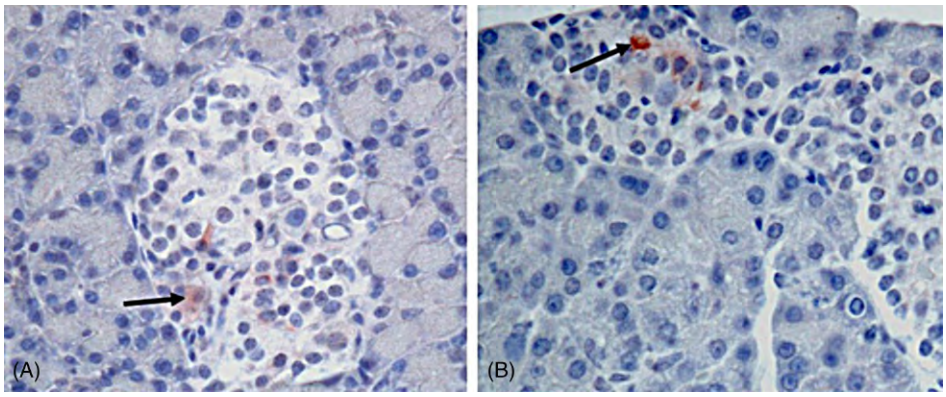


FIGURE 80.4 The localization of CB1 receptor in rat pancreas by Δ^9-THC. CB1 receptor signals *(arrow)* are seen in the Langerhans islets of pancreas immunohistochemically. (A) Nontreated diabetes, (B) diabetes treated with Δ^9-THC. Streptavidin–biotin–peroxidase technique, hematoxylin counterstain; magnification ×400 (Coskun & Bolkent, 2014).

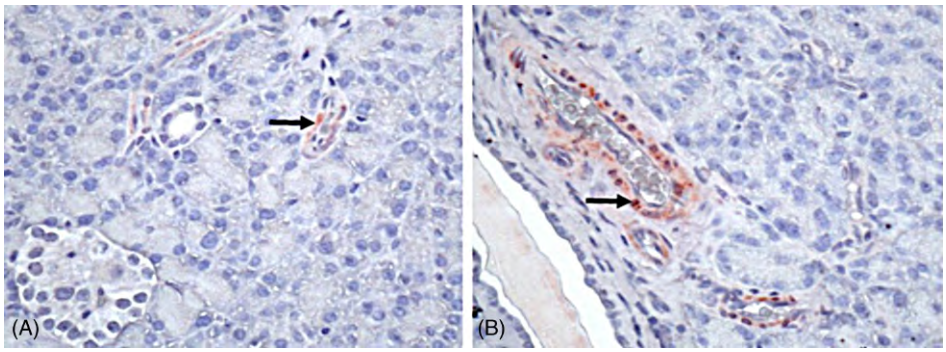


FIGURE 80.5 The localization of CB2 receptor in rat pancreas by Δ^9-THC. CB2 receptor signals *(arrow)* are observed in pancreatic ducts. (A) Nontreated diabetes, (B) diabetes treated with Δ^9-THC. Streptavidin–biotin–peroxidase technique, hematoxylin counterstain; magnification ×400 (Coskun & Bolkent, 2014).

glucagon hormones was changed in pancreas (Coskun & Bolkent, 2014). These observations demonstrate that Δ^9-THC has a potential role in regulating the secretion of pancreatic hormones. Specifically, Δ^9-THC can influence the pancreatic hormone mediated by CB1 receptors in the islets of Langerhans.

Δ^9-THC IN FOOD INTAKE AND ENERGY HOMEOSTASIS IN DIABETES

The CB1 and CB2 receptors may provide control of food intake and regulation of energy homeostasis. Hence, appetite regulation and body weight gain are affected by the endocannabinoid system (Doyle, 2011; Jesudason & Wittert, 2008; Li et al., 2010; Romero-Zerbo et al., 2011). It is suggested that Δ^9-THC reduced weight loss in a rat model of activity-based anorexia (Verty et al., 2011). On the other hand, studies on obesity showed that cannabinoid receptor antagonists could be used to lose weight (Alonso et al., 2012; Verty, Lockie, Stefanidis, & Oldfield, 2013). Body weight was increased in Δ^9-THC-treated diabetic rats (unpublished data). Therefore, Δ^9-THC may aid in controlling weight loss in diabetes.

IMMUNOSUPPRESSIVE EFFECTS OF Δ^9-THC

Studies have demonstrated that Δ^9-THC may be used as a therapeutic agent for the treatment of immune-mediated diseases. Δ^9-THC is capable of reducing inflammation (Lyman, Sonett, Brosnan, Elkin, & Bornstein, 1989; Hegde, Nagarkatti, & Nagarkatti, 2010). β cell damage is caused by STZ-induced diabetes. Moreover, the immune system enhances the destruction of β cells. According to Li, Kaminski, and Fischer (2001), the immunosuppressive influence of Δ^9-THC can attenuate the autoimmune response in the mouse model of autoimmune diabetes. Most probably, the immune suppression effect of Δ^9-THC is capable of ensuring the activation of cannabinoid receptors.

EXCRETION OF Δ9THC METABOLITES IN THE URINE OF DIABETICS

Δ^9-THC is highly lipophilic, and accumulates in adipose tissue from where it is dispersed into the blood stream for long durations (Kreuz & Axelrod, 1973). The metabolic degradation of Δ^9-THC is in the liver (Bland, Haining, Tracy, & Callery, 2005). The Δ^9-THC metabolites can be detected in serum and urine of cannabis consumers (Jung, Kempf, Mahler, & Weinmann, 2007).

Furthermore, in our experimental study, the level of Δ^9-THC metabolites in the urine was elevated in diabetic rats treated with Δ^9-THC, as compared to healthy rats that were given Δ^9-THC (unpublished data). The reduced level of Δ^9-THC metabolites in urine may be induced by weight loss in diabetes (unpublished data). Hence, the accumulation of Δ^9-THC metabolites in adipose tissue can be reduced in diabetic status.

In summary, studies have shown that Δ^9-THC may be effective against diabetes and its complications. Δ^9-THC, a natural cannabinoid receptor agonist, may decrease blood glucose levels, and increase body weight in diabetes. The administration of Δ^9-THC can attenuate the oxidative damage induced by diabetes. The protection provided by Δ^9-THC in diabetes is mediated by cannabinoid receptors and antioxidant properties. Thus, it is suggested that Δ^9-THC can be considered for its pharmaceutical effects against symptoms of diabetes such as regulating food intake, glucose homeostasis, oxidative damage, and attenuation of the severity of the autoimmune response. In the future, Δ^9-THC may be preferred for its protective benefits in diabetes.

MINI-DICTIONARY

Alloxan Alloxan is a toxic chemical. It has been used to induce diabetes in experimental animals by destroying the insulin-secreting cells of Langerhans islet in pancreas.
Antioxidant It provides protection against some of the damage induced by free radicals. It may neutralize free radicals.
Dyslipidemia A disorder of lipoprotein metabolism in the blood that involves lipoprotein overproduction or deficiency.
Enzyme A biomolecule that triggers chemical reactions very quickly. They are made of amino acids.
Hormone A regulator of the functions of tissues and organs that is derived from glands in organisms.
Metabolite A metabolite is the byproduct of a breakdown of any substance in tissues and organs.
Oxidative stress A disturbance in the balance between antioxidant defense system and the production of free radicals and peroxides.
Streptozotocin It is a toxic agent that causes degeneration in β cells of Langerhans islet, and induces the experimental diabetes model.
Δ9-Tetrahydrocannabinol (Δ9-THC) It is the main psychoactive ingredient in the plant *Cannabis*.
Therapeutic effect The beneficial effect that is the consequence of a medical treatment.

References

Alonso, M., Serrano, A., Vida, M., Crespillo, A., Hernandez-Folgado, L., Jagerovic, N., Goya, P., Reyes-Cabello, C., Perez-Valero, V., Decara, J., Macías-González, M., Bermúdez-Silva, F. J., Suárez, J., Rodríguez de Fonseca, F., & Pavón, F. J. (2012). Anti-obesity efficacy of LH-21, a cannabinoid CB(1) receptor antagonist with poor brain penetration, in diet-induced obese rats. *British Journal of Pharmacology*, *165*, 2274–2291.

Ávila de Lima, D. ., Araújo, G. R., Silva, M., Miranda, P. H., Diniz, M. F., Pedrosa, M. L., Silva, M. E., de Lima, W. G., & Costa, D. C. (2013). Vildagliptin ameliorates oxidative stress and pancreatic beta cell destruction in type 1 diabetic rats. *Archives of Medical Research*, *44*, 194–202.

Baburao Jain, A., & Anand Jain, V. (2012). Vitamin E, its beneficial role in diabetes mellitus (DM) and its complications. *Journal of Clinical and Diagnostic Research*, *6*, 1624–1628.

Bermúdez-Silva, F. J., Suárez, J., Baixeras, E., Cobo, N., Bautista, D., Cuesta-Muñoz, A. L., Fuentes, E., Juan-Pico, P., Castro, M. J., Milman, G., Mechoulam, R., Nadal, A., & Rodríguez de Fonseca, F. (2008). Presence of functional cannabinoid receptors inhuman endocrine pancreas. *Diabetologia*, *51*, 476–487.

Bland, T. M., Haining, R. L., Tracy, T. S., & Callery, P. S. (2005). CYP2C-catalyzed delta9-tetrahydrocannabinol metabolism: kinetics, pharmacogenetics and interaction with phenytoin. *Biochemical Pharmacology*, *70*, 1096–1103.

Bolkent, S., Bolkent, S., Yanardag, R., & Tunali, S. (2005). Protective effect of vanadyl sulfate on the pancreas of streptozotocin-induced diabetic rats. *Diabetes Research and Clinical Practice*, *70*, 103–109.

Burstein, S. (2005). PPAR-gamma: a nuclear receptor with affinity for cannabinoids. *Life Sciences*, *77*, 1674–1684.

Carroll, C. B., Zeissler, M. L., Hanemann, C. O., & Zajicek, J. P. (2012). Δ^9-Tetrahydrocannabinol (Δ^9-THC) exerts a direct neuroprotective effect in a human cell culture model of Parkinson's disease. *Neuropathology and Applied Neurobiology*, *38*, 535–547.

Celi, F. S., & Shuldiner, A. R. (2002). The role of peroxisome proliferator-activated receptor gamma in diabetes and obesity. *Current Diabetes Reports*, *2*, 179–185.

Chen, J., Lee, C. T., Errico, S., Deng, X., Cadet, J. L., & Freed, W. J. (2005). Protective effects of Delta(9)-tetrahydrocannabinol against *N*-methyl-d-aspartate-induced AF5 cell death. *Brain Research. Molecular Brain Research*, *134*, 215–225.

Coskun, Z. M., & Bolkent, S. (2014). Biochemical and immunohistochemical changes in delta-9-tetrahydrocannabinol-treated type 2 diabetic rats. *Acta Histochemica*, *116*, 112–116.

Coskun, Z. M., Sacan, O., Karatug, A., Turk, N., Yanardag, R., Bolkent, S., & Bolkent, S. (2013). Regulation of oxidative stress and somatostatin, cholecystokinin, apelin gene expressions by ghrelin in stomach of newborn diabetic rats. *Acta Histochemica*, *115*, 740–747.

Derek, L., Taylor, S. I., & Olefsky, J. M. (2000). *Diabetes mellitus: A fundamental and clinical text* (2nd ed.). Philadelphia, PA: Lippincott Williams & Wilkins.

Doyle, M. E. (2011). The role of the endocannabinoid system in islet biology. *Current Opinion in Endocrinology Diabetes and Obesity*, *18*, 153–158.

Duarte, J. M., Nogueira, C., Mackie, K., Oliveira, C. R., Cunha, R. A., & Köfalvi, A. (2007). Increase of cannabinoid CB1 receptor density in the hippocampus of streptozotocin-induced diabetic rats. *Experimental Neurology*, *204*, 479–484.

Frostegård, J. (2013). Immune mechanisms in atherosclerosis, especially in diabetes type 2. *Frontiers in Endocrinology (Lausanne)*, *4*, 162.

Giacco, F., & Brownlee, M. (2010). Oxidative stress and diabetic complications. *Circulation Research*, *107*, 1058–1070.

Hampson, A. J., Grilmaldi, M., Axelrod, J., & Wink, D. (1998). Cannabidiol and (−)Δ^9-tetrahydrocannabinol are neuroprotective antioxidants. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 8268–8273.

Havel, P. J., Hahn, T. M., Sindelar, D. K., Baskin, D. G., Dallman, M. F., Weigle, D. S., & Schwartz, M. W. (2000). Effects of streptozotocin-induced diabetes and insulin treatment on the hypothalamic melanocortin system and muscle uncoupling protein 3 expression in rats. *Diabetes*, *49*, 244–252.

Hegde, V. L., Nagarkatti, M., & Nagarkatti, P. S. (2010). Cannabinoid receptor activation leads to massive mobilization of myeloid-derived suppressor cells with potent immunosuppressive properties. *European Journal of Immunology*, *40*, 3358–3371.

Herkenham, M., Lynn, A. B., Johnson, M. R., Melvin, L. S., de Costa, B. R., & Rice, K. C. (1991). Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *The Journal of Neuroscience*, *11*, 563–583.

Howlett, A. C., Barth, F., Bonner, T. I., Cabral, G., Casellas, P., Devane, W. A., Felder, C. C., Herkenham, M., Mackie, K., Martin, B. R., Mechoulam, R., & Pertwee, R. G. (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacological Reviews*, *54*, 161–202.

Jesudason, D., & Wittert, G. (2008). Endocannabinoid system in food intake and metabolic regulation. *Current Opinion in Lipidology*, *19*, 344–348.

Jung, J., Kempf, J., Mahler, H., & Weinmann, W. (2007). Detection of Delta9-tetrahydrocannabinolic acid A in human urine and blood serum by LC-MS/MS. *Journal of Mass Spectrometry*, *42*, 354–360.

Kahn, C. R., Weir, G. C., King, G. L., Jacobson, A. M., Moses, A. C., & Smith, R. J. (2008). *Joslin's diabetes mellitus* (14th ed.). Philadelphia, PA: Lippincott Williams & Wilkins.

Kassab, A., & Piwowar, A. (2012). Cell oxidant stress delivery and cell dysfunction onset in type 2 diabetes. *Biochimie*, *94*, 1837–1848.

Kim, G., & Caprio, S. (2011). Diabetes and insulin resistance in pediatric obesity. *Pediatric Clinics of North America*, *58*, 1355–1361.

Kim, W., Doyle, M. E., Liu, Z., Lao, Q., Shin, Y. K., Carlson, O. D., Kim, H. S., Thomas, S., Napora, J. K., Lee, E. K., Moaddel, R., Wang, Y., Maudsley, S., Martin, B., Kulkarni, R. N., & Egan, J. M. (2011). Cannabinoids inhibit insulin receptor signaling in pancreatic β-cells. *Diabetes*, *60*, 1198–1209.

Kopáni, M., Celec, P., Danisovic, L., Michalka, P., & Biró, C. (2006). Oxidative stress and electron spin resonance. *Clinica Chimica Acta*, *364*, 61–66.

Kreuz, D. S., & Axelrod, J. (1973). Delta-9-tetrahydrocannabinol: localization in body fat. *Science*, *179*, 391–393.

Krishnamurthy, G., Lakshman, K., Pruthvi, N., & Chandrika, P. U. (2011). Antihyperglycemic and hypolipidemic activity of methanolic extract of *Amaranthus viridis* leaves in experimental diabetes. *Indian Journal of Pharmacology*, *43*, 450–454.

Lenzen, S., Drinkgern, J., & Tiedge, M. (1996). Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. *Free Radical Biology and Medicine*, *20*, 463–466.

Li, C., Bowe, J. E., Huang, G. C., Amiel, S. A., Jones, P. M., & Persaud, S. J. (2011a). Cannabinoid receptor agonists and antagonists stimulate insulin secretion from isolated human islets of Langerhans. *Diabetes, Obesity and Metabolism*, *13*, 903–910.

Li, C., Bowe, J. E., Jones, P. M., & Persaud, S. J. (2010). Expression and function of cannabinoid receptors in mouse islets. *Islets*, *2*, 293–302.

Li, C., Jones, P. M., & Persaud, S. J. (2011b). Role of the endocannabinoid system in food intake, energy homeostasis and regulation of the endocrine pancreas. *Pharmacology and Therapeutics*, *129*, 307–320.

Li, F., Tang, H., Xiao, F., Gong, J., Peng, Y., & Meng, X. (2011c). Protective effect of salidroside from Rhodiolae Radix on diabetes-induced oxidative stress in mice. *Molecules*, *16*, 9912–9924.

Li, X., Kaminski, N. E., & Fischer, L. J. (2001). Examination of the immunosuppressive effect of delta9-tetrahydrocannabinol in streptozotocin-induced autoimmune diabetes. *International Immunopharmacology*, *1*, 699–712.

Lyman, W. D., Sonett, J. R., Brosnan, C. F., Elkin, R., & Bornstein, M. B. (1989). Delta 9-tetrahydrocannabinol: a novel treatment for experimental autoimmune encephalomyelitis. *Journal of Neuroimmunology*, *23*, 73–81.

Matias, I., Gonthier, M. P., Orlando, P., Martiadis, V., De Petrocellis, L., Cervino, C., Petrosino, S., Hoareau, L., Festy, F., Pasquali, R., Roche, R., Maj, M., Pagotto, U., Monteleone, P., & Di Marzo, V. (2006). Regulation, function, and dysregulation of endocannabinoids in models of adipose and beta-pancreatic cells and in obesity and hyperglycemia. *The Journal of Clinical Endocrinology and Metabolism*, *91*, 3171–3180.

Mooradian, A. D. (2009). Dyslipidemia in type 2 diabetes mellitus. *Nature Clinical Practice. Endocrinology and Metabolism*, *5*, 150–159.

Munro, S., Thomas, K. L., & Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, *365*, 61–65.

O'Sullivan, S. E. (2007). Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. *British Journal of Pharmacology*, *152*, 576–582.

Perez Gutierrez, R. M., Madrigales Ahuatzi, D., Horcacitas, M. del C., Garcia Baez, E., Cruz Victoria, T., & Mota-Flores, J. M. (2014). Ameliorative effect of hexane extract of *Phalaris canariensis* on high fat diet-induced obese and streptozotocin-induced diabetic mice. *Evidence-Based Complementary and Alternative Medicine*, *2014*, 145901.

Pertwee, R. G. (1997). Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacology and Therapeutics*, *74*, 129–180.

Romero-Zerbo, S. Y., Rafacho, A., Díaz-Arteaga, A., Suárez, J., QuesadaI, Imbernon, M., Ross, R. A., Dieguez, C., Rodríguez de Fonseca, F., Nogueiras, R., Nadal, A., & Bermúdez-Silva, F. J. (2011). A role for the putative cannabinoidreceptor GPR55 in the islets of Langerhans. *The Journal of Endocrinology*, *211*, 177–185.

Sefi, M., Fetoui, H., Soudani, N., Chtourou, Y., Makni, M., & Zeghal, N. (2012). *Artemisia campestris* leaf extract alleviates early diabetic nephropathy in rats by inhibiting protein oxidation and nitric oxide end products. *Pathology Research and Practice*, *208*, 157–162.

Shearer, B. G., & Hoekstra, W. J. (2003). Recent advances in peroxisome proliferator-activated receptor science. *Current Medicinal Chemistry*, *10*, 267–280.

Stadler, K. (2012). Oxidative stress in diabetes. *Advances in Experimental Medicine and Biology*, *771*, 272–287.

Tharp, W. G., Lee, Y. H., Maple, R. L., & Pratley, R. E. (2008). The cannabinoid CB1 receptor is expressed in pancreatic delta-cells. *Biochemical and Biophysical Research Communications*, *372*, 595–600.

Tiwari, S., & Ndisang, J. F. (2014). The heme oxygenase system and type-1 diabetes. *Current Pharmaceutical Design*, *20*, 1328–1337.

Turenkov, I. N., Voronkov, A. V., & Slietsans, A. A. (2013). Role of endothelial dysfunction in the development of vascular complications of diabetes. *Patologicheskaia Fiziologiia i Eksperimental'naia Terapiia*, *2*, 80–84.

Turk, N., Dagistanli, F. K., Sacan, O., Yanardag, R., & Bolkent, S. (2012). Obestatin and insulin in pancreas of newborn diabetic rats treated with exogenous ghrelin. *Acta Histochemica*, *114*, 349–357.

Verty, A. N., Evetts, M. J., Crouch, G. J., McGregor, I. S., Stefanidis, A., & Oldfield, B. J. (2011). The cannabinoid receptor agonist THC attenuates weight loss in a rodent model of activity-based anorexia. *Neuropsychopharmacology*, *36*, 1349–1358.

Verty, A. N., Lockie, S. H., Stefanidis, A., & Oldfield, B. J. (2013). Anti-obesity effects of the combined administration of CB1 receptor antagonist rimonabant and melanin-concentrating hormone antagonist SNAP-94847 in diet-induced obese mice. *International Journal of Obesity*, *37*, 279–287.

Vilches-Flores, A., Delgado-Buenrostro, N. L., Navarrete-Vázquez, G., & Villalobos-Molina, R. (2010). CB1 cannabinoid receptor expression isregulated by glucose and feeding in rat pancreatic islets. *Regulatory Peptides*, *163*, 81–87.

Waldman, M., Hochhauser, E., Fishbein, M., Aravot, D., Shainberg, A., & Sarne, Y. (2013). An ultra-low dose of tetrahydrocannabinol provides cardioprotection. *Biochemical Pharmacology*, *85*, 1626–1633.

Yildirim, S., Bolkent, S., & Sundler, F. (2008). The role of rosiglitazone treatment in the modulation of islet hormones and hormone-like peptides: a combined in situ hybridization and immunohistochemical study. *Journal of Molecular Histology*, *39*, 635–642.

Yildirim, S., Sundler, F., & Bolkent, S. (2007). Ghrelin and insulin gene expression changes in streptozotocin-induced diabetic rats after rosiglitazone pretreatment. *European Journal of Histochemistry*, *51*, 11–18.

CHAPTER

81

Cannabidiol: An Overview of its Antipsychotic Properties

F.F. Peres, V. Almeida, V.C. Abilio

Department of Pharmacology, Federal University of Sao Paulo, Sao Paulo, Brazil; Integrated Laboratory of Clinical Neurosciences (LiNC), Federal University of Sao Paulo, Sao Paulo, Brazil

SUMMARY POINTS

- Schizophrenia presents three categories of symptoms: positive, negative, and cognitive symptoms.
- The available antipsychotic drugs treat mainly the positive symptoms, presenting low efficacy against the negative and cognitive symptoms, and induce significant side effects.
- Alterations in the endocannabinoid system seem to be implicated in the pathophysiology of schizophrenia, suggesting that this system might be a target for therapeutic strategies.
- CBD (cannabidiol), a nonpsychotomimetic compound of cannabis, seems to be able to treat the positive symptoms of schizophrenia, and represents an advance with regard to the existing antipsychotics, since it might be effective also against the negative and cognitive symptoms of schizophrenia.
- CBD treatment does not induce significant side effects.

KEY FACTS OF CANNABIDIOL AS AN ANTIPSYCHOTIC DRUG

- CBD (cannabidiol), when administered prior or simultaneously to Δ^9-tetrahydrocannabinol (THC), prevents THC psychotomimetic effects.
- Cannabis with high CBD content induces less psychotic symptoms and memory impairments than those with low CBD levels.
- In animal models of schizophrenia, CBD attenuates behavioral abnormalities that model the positive, negative, and cognitive symptoms.
- In a case-report, CBD significantly reduced the symptoms of a schizophrenia patient in the brief psychiatric rating scale (BPRS).
- CBD treatment decreases psychotic symptoms of patients with Parkinson disease (PD).
- In a double-blind controlled clinical trial with schizophrenia patients, it was seen that CBD reduces the patients' symptoms in a way similar to the atypical antipsychotic amisulpride.
- Unlike typical and atypical antipsychotics, CBD does not induce significant side effects.

LIST OF ABBREVIATIONS

5-HT$_{1A}$	5-Hydroxytryptamine receptor 1A
AEA	Anandamide
BAD	Bipolar affective disorder
BPRS	Brief psychiatric rating scales
CB$_1$	Cannabinoid-1 receptor
CB$_2$	Cannabinoid-2 receptor
CBD	Cannabidiol
CFS	Cerebrospinal fluid
D$_2$	Dopamine 2 receptor
GPR55	G-protein coupled receptor 55
MK-801	Dizocilpine

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00092-2
Copyright © 2017 Elsevier Inc. All rights reserved.

NMDA *N*-Methyl-D-aspartate
PANSS Positive and negative syndrome scale
pCO_2 Carbon dioxide partial pressure
PD Parkinson disease
pH Potential of hydrogen
pO_2 Oxygen partial pressure
PPI Prepulse inhibition of startle
SHR Spontaneously hypertensive rats
THC Δ^9-Tetrahydrocannabinol
TRPV1 Potential vanilloid receptor type 1
TRPV2 Potential vanilloid receptor type 2
YMRS Young mania rating scale

INTRODUCTION

Schizophrenia is a complex and severe psychiatric disorder that affects around 0.7% of the world's population. Its onset occurs between late adolescence and early adulthood. Schizophrenia patients may experience three categories of symptoms: positive, negative, and cognitive symptoms. The positive symptoms, also known as psychotic symptoms, comprise hallucinations, delusions, and thought disorders. It is worth mentioning that psychosis is seen not only in schizophrenia, but also in other psychiatric conditions, such as bipolar affective disorder (BAD) and major depressive disorder, and it may be induced by dopaminergic agonists and glutamatergic antagonists. The negative symptoms of schizophrenia include social withdrawal, anhedonia, and apathy. With respect to the cognitive symptoms, patients present mainly deficits in working and attentional memories, as well as impairments in the sensorimotor gating functioning.

Schizophrenia and psychotic states can be devastating to the patients and their caregivers. In this context, the advent of antipsychotic drugs has brought enormous benefits, and is therefore considered one of the major advances in psychopharmacology. However, the therapeutic effectiveness of the antipsychotics seems to be restricted to the positive symptoms of schizophrenia. Moreover, the drugs available are not able to benefit all patients, and induce significant side effects that impair the quality of life, and are even life-shortening. Consequently, the search for new therapeutic strategies for schizophrenia is required.

In this scenario, cannabidiol (CBD)—a nonpsychotomimetic compound of cannabis—emerges as a potential new therapeutic strategy to schizophrenia and other psychotic states. This chapter will discuss the latest evidence on CBD's antipsychotic profile, focusing on its discovery, and the subsequent studies with animal models, healthy individuals, and psychiatric patients. In addition, we review here the data concerning CBD's possible side effects. Fig. 81.1 summarizes the aim of this chapter.

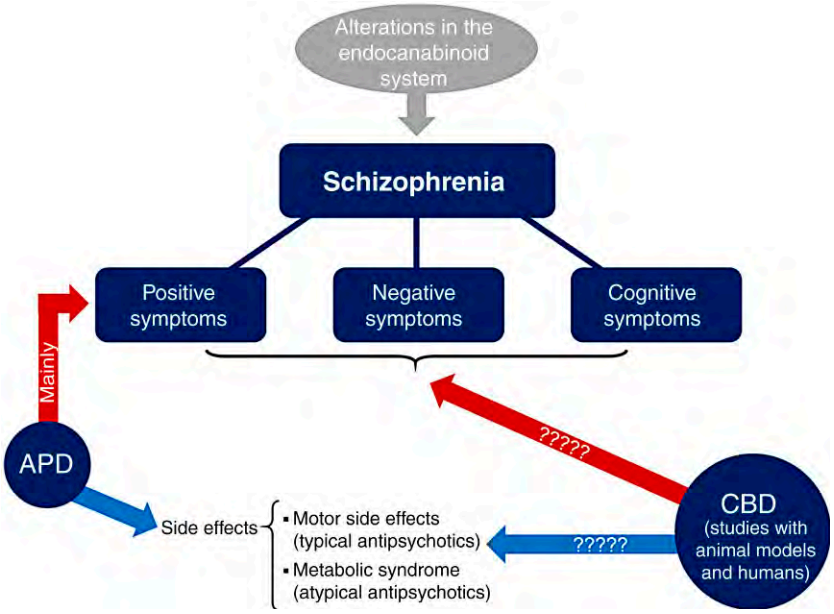


FIGURE 81.1 **Summary of the aims of this chapter.** Schizophrenia presents three categories of symptoms: positive, negative, and cognitive symptoms. The available antipsychotic drugs (APD) treat mainly the positive symptoms, having a low therapeutic efficacy against the negative and cognitive symptoms. In addition, APD induce important motor and metabolic side effects. The motor side effects are promoted primarily by the typical antipsychotics, while the metabolic syndrome is provoked mostly by the atypical antipsychotics. Therefore, the search for new therapeutic strategies is needed. In parallel, alterations in the endocannabinoid system seem to be implicated in the pathophysiology of schizophrenia. In this scenario, cannabidiol (CBD)—a nonpsychotomimetic compound of cannabis—emerges as a potential new therapeutic strategy for schizophrenia. Preclinical and clinical studies are essential to elucidate its therapeutic profile, as well as possible side effects.

ENDOCANNABINOID SYSTEM AND SCHIZOPHRENIA

The endocannabinoid system has recently emerged as an important neurotransmitter system in schizophrenia: both its involvement in the pathophysiology of this disorder, as well as its potential as a therapeutic target have been extensively studied. This interest is based on some compelling facts: first, the exposure to Δ^9-tetrahydrocannabinol (THC), the main psychotropic component of cannabis, may induce transient psychotic states in healthy individuals. Second, cannabis consumption provokes more and earlier psychotic relapses in schizophrenia patients, even under antipsychotic treatment, and cannabis abuse has been shown to be a risk factor for psychotic outcomes. Conversely, the rates of cannabis abuse are higher in schizophrenia patients than in the general population, and its use has been suggested as a self-medication strategy.

In parallel, the endocannabinoid system modulates neurotransmissions related to the pathophysiology of schizophrenia, such as dopaminergic and glutamatergic systems. Neurochemical evaluations point to alterations in the endocannabinoid system in this disorder: (1) the levels of anandamide (AEA), one of the main endocannabinoids, are increased in cerebrospinal fluid (CSF) and peripheral blood of schizophrenia patients. The increase of AEA levels in CSF is also seen in individuals at high risk of developing schizophrenia; (2) in nonmedicated schizophrenia patients, the psychotic symptoms are negatively correlated to CSF AEA levels, and it has been demonstrated that some successful treatments with antipsychotics are accompanied by a decrease of these levels; (3) in vivo studies using positron emission tomography (PET) demonstrate increased density of cannabinoid-1 receptors (CB_1) in both medicated and nonmedicated schizophrenia patients. In addition, the CB_1 density in the nonmedicated group is negatively correlated to negative symptoms; (4) several postmortem studies show increased density of CB_1 in the dorsolateral prefrontal, anterior cingulated, and posterior cingulated cortices of schizophrenia patients; (5) there is an association between schizophrenia phenotype and polymorphisms of the genes that codify the cannabinoid receptors CB_1 and CB_2 [for a broad review of the role of the endocannabinoid system in schizophrenia, see: Fernandez-Espejo, Viveros, Nunez, Ellenbroek, & Rodriguez de Fonseca (2009); Zamberletti, Rubino, and Parolaro (2012)].

CBD AS AN ANTIPSYCHOTIC DRUG

CBD is one of over 60 cannabinoids identified in *Cannabis sativa*, being the most abundant after THC. CBD constitutes up to 40% of the plant's extract, but its proportion in cannabis is highly variable, and depends on the phenotype and the growing conditions of the plant. CBD was first isolated in 1940 by Adams, Hunt, and Clark (1940), and its structure (depicted on Fig. 81.2) was only elucidated 23 years later by Mechoulam and Shvo (1963). Perez-Reyes, Timmons, Davis, and Wall (1973) described that, unlike THC, CBD is not a psychotomimetic compound. Since then, several pharmacological effects in the central nervous system and in the periphery have been described for CBD, including anxiolytic, antiinflammatory, neuroprotective, and antipsychotic actions.

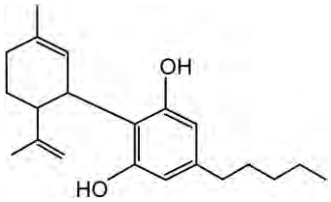


FIGURE 81.2 Chemical structure of cannabidiol. The chemical structure of cannabidiol, first described by Mechoulam and Shvo (1963).

The mechanisms underlying CBD effects are still not precisely known, but it is clear that it presents many molecular targets. CBD can act as an antagonist of CB_1 and CB_2 receptors, and as an inverse agonist of CB_2. CBD also inhibits the reuptake and degradation of AEA, therefore increasing its levels. Furthermore, CBD may interfere on other neurotransmitter systems, besides the endocannabinoid: it has been described, among other actions, that it works as antagonist of the orphan receptor GPR55, as well as agonist of the serotonin receptor 5-HT_{1A}, and of the vanilloid receptors TRPV1 and TRPV2. Only few studies addressed the molecular targets involved in the antipsychotic effects of CBD, but undoubtedly elucidating it is paramount. The main CBD mechanisms of action are summarized in Fig. 81.3 [for a review, see Izzo, Borrelli, Capasso, Di Marzo, and Mechoulam (2009)].

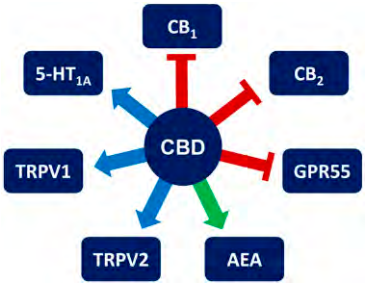


FIGURE 81.3 Proposed mechanisms of action of cannabidiol. Cannabidiol (CBD) activates the 5-hydroxytryptamine receptor 1A (5-HT_{1A}), the potential vanilloid receptor type 1 (TRPV1), and the potential vanilloid receptor type 2 (TRPV2). On the other hand, CBD blocks the cannabinoid-1 receptor (CB_1), the cannabinoid-2 receptor (CB_2) and the G protein-coupled receptor 55 (GPR55). In addition, CBD inhibits the reuptake and degradation of anandamide (AEA), therefore increasing its levels. The *blue arrows* represent agonism, the *red bars* indicate antagonism and the *green arrow* represents inhibition of reuptake and degradation.

Evidence From Healthy Subjects

The first evidence of CBD's antipsychotic profile comes from studies with healthy subjects. Perez-Reyes et al. (1973) demonstrated that, unlike THC and cannabinol, the intravenous administration of CBD does not induce psychological changes in healthy individuals, indicating, therefore that CBD is not a psychotomimetic compound. One year later, Karniol, Shirakawa, Kasinski, Pfeferman, and Carlini (1974) showed that CBD, when administered simultaneously to THC, blocks the THC effects on psychological reactions, pulse rate, and time production tasks. Moreover, the administration of CBD prior or concomitant to THC reduces the psychotomimetic symptoms induced by THC (Bhattacharyya et al., 2010; Zuardi, Shirakawa, Finkelfarb, & Karniol, 1982). These results indicated that not only is CBD devoid of psychotomimetic effects, but it is also able to inhibit the psychotic symptoms induced by other cannabinoids, leading to the suggestion that CBD might act as an antipsychotic. CBD also attenuates the binocular depth inversion—a model of impaired perception during psychotic states—provoked by nabilone, a synthetic analog of THC (Leweke, Schneider, Radwan, Schmidt, & Emrich, 2000), and shows a trend to reduce the depersonalization symptoms induced by ketamine (Hallak et al., 2011). Ketamine is an antagonist of NMDA glutamatergic receptors, and presents a psychotomimetic profile.

In addition, Rottanburg, Robins, Ben-Arie, Teggin, and Elk (1982) described a higher frequency of cannabis-related psychosis in a cohort of patients who used a variety of cannabis virtually devoid of CBD, pointing to the protective effect of CBD against THC's psychotomimetic effects. Reinforcing this suggestion, it was seen that individuals who have only THC in hair present higher levels of schizophrenia-like symptoms than those with CBD and THC, and that the CBD + THC group displays better recognition memory (Morgan & Curran, 2008; Morgan et al., 2012). Likewise, CBD's proportion in cannabis influences its effects: cannabis with high CBD content induces less psychotic symptoms and memory impairments than cannabis with low CBD levels (Morgan, Schafer, Freeman, & Curran, 2010; Schubart et al., 2011).

The evidence of antipsychotic effects of CBD in healthy subjects is summarized in Table 81.1.

TABLE 81.1 Antipsychotic Effects of CBD in Humans

References	Results
Studies with healthy subjects	
Perez-Reyes et al. (1973)	Unlike THC or cannabinol, CBD does not induce psychological changes
Karniol et al. (1974)	Coadministration of CBD and THC reduces the THC effects on pulse rate, time production tasks and psychological reactions
Rottanburg et al. (1982)	Higher frequency of cannabis-related psychosis among patients who used a variety of cannabis virtually devoid of CBD
Zuardi et al. (1982)	Coadministration of CBD and THC reduces the psychotomimetic effects of THC
Leweke et al. (2000)	CBD attenuates impaired perception induced by nabilone, a synthetic analog of THC
Morgan and Curran (2008)	Subjects with only THC in hair present higher levels of schizophrenia-like symptoms than those with THC and CBD, or with no cannabinoid
Bhattacharyya et al. (2010)	The administration of CBD prior to THC reduces the psychotomimetic effects of THC
Morgan et al. (2010)	Cannabis with high CBD content induces less memory impairments than those with low CBD levels
Hallak et al. (2011)	CBD reduces the depersonalization symptoms induced by ketamine (nonsignificant trend)
Schubart et al. (2011)	Cannabis with high CBD content induces less psychotic symptoms than that with low CBD levels
Morgan et al. (2012)	Subjects with only THC in hair present higher levels of schizophrenia-like symptoms and memory recognition impairment than those with THC and CBD
Studies with psychiatric patients	
Zuardi, Morais, Guimaraes, and Mechoulam (1995)	Chronic CBD treatment (4 weeks) reduced psychotic symptoms in a female schizophrenia patient
Zuardi et al. (2009)	Chronic CBD treatment (4 weeks) reduced psychotic symptoms in six patients with Parkinson disease, without worsening their motor function
Leweke et al. (2012)	Chronic CBD treatment (4 weeks) reduced psychotic symptoms in 21 schizophrenia patients, in the same way as amisulpride. CBD induced fewer side effects

Summary of the studies indicating the antipsychotic effects of CBD in healthy subjects and in psychiatric patients. CBD, Cannabidiol; THC, Δ^9-tetrahydrocannabinol.

Evidence From Animal Studies

Considering the compelling evidence of CBD antipsychotic profile derived from observations with healthy subjects, the next step was to evaluate effects of CBD on animal models of schizophrenia. These models display some behavioral abnormalities that resemble the symptoms observed in patients, and are reversed by the administration of antipsychotic drugs. It is the case of diminished social interaction—that mimics social withdrawal—and deficits in prepulse inhibition of startle (PPI), a paradigm that evaluates the sensorimotor gating functioning, and that is also impaired in schizophrenia patients. Alongside these, hyperlocomotion and stereotyped behavior are used in animals to model the positive symptoms of the disorder, since they are all related to increased dopaminergic transmission. In addition, animal models of schizophrenia, similar to patients, might present cognitive deficits. The behavioral abnormalities seen in animal models of schizophrenia, and the corresponding symptoms observed in patients, are depicted on Fig. 81.4 [for a review, see Lipska and Weinberger (2000)].

The first study evaluating the effects of CBD administration on an animal model of schizophrenia was performed in 1991. The authors demonstrated that CBD, as the antipsychotic drug haloperidol, is able to reduce the stereotyped behavior induced by apomorphine in rats (Zuardi, Rodrigues, & Cunha, 1991). Later, it was seen that CBD is also capable of inhibiting, in rodents, the hyperlocomotion induced by dopamine agonists or NMDA antagonists (Gururajan, Taylor, & Malone, 2012; Long et al., 2010; Moreira & Guimaraes, 2005). This inhibition is comparable to the one induced by the antipsychotic clozapine. It is noteworthy, however, that CBD was not able to reverse the hyperlocomotion induced by MK-801 (an NMDA antagonist) in another study (Gururajan, Taylor, & Malone, 2011), nor the spontaneous hyperlocomotion presented by the SHR strain (Almeida et al., 2013), suggested as an animal model to study several aspects of schizophrenia (Calzavara et al., 2009, 2011; Levin et al., 2011). Moreover, despite a trend, CBD inhibition of the hyperlocomotion induced by ketamine failed to reach significance (Moreira & Guimaraes, 2005).

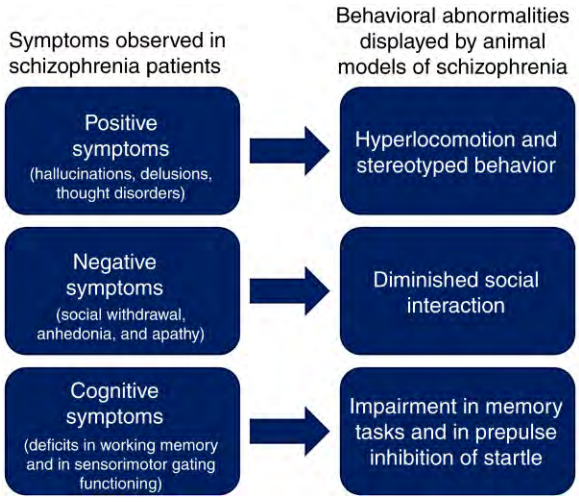


FIGURE 81.4 The relationship between schizophrenia symptomatology and the behavioral abnormalities presented by animal models of schizophrenia. Animal models of schizophrenia display behavioral abnormalities (depicted in the right column) that model its symptoms (the corresponding symptom is listed in the left column).

CBD also reverses the deficits in social interaction induced by THC or MK-801 (Gururajan et al., 2012; Malone, Jongejan, & Taylor, 2009). On the other hand, it failed to restore the spontaneous deficit on social interaction presented by the SHR strain (Almeida et al., 2013). Deficits in PPI, induced by MK-801 or spontaneously presented by the SHR strain, are also attenuated by CBD (Levin et al., 2014; Long, Malone, & Taylor, 2006). Conversely, one study did not see this beneficial effect of CBD on the PPI deficit promoted by MK-801 (Gururajan et al., 2011). Finally, CBD restores the spontaneous impairment in emotional context processing presented by the SHR strain (Levin et al., 2012).

The discrepancies found in the previous studies are probably due to differences in the rodent species, and in the drug regimen. However, even with conflicting results, preclinical data reinforce the CBD antipsychotic profile, suggesting it is effective against several schizophrenia-like behavioral abnormalities. The data concerning the antipsychotic profile of CBD from animal models of schizophrenia are summarized in Table 81.2.

Evidence From Clinical Studies

Taken as a whole, the striking evidence of the antipsychotic profile of CBD described earlier led to studies with patients. The first one was a case-study, and it was only performed in 1995. A 19-year-old female schizophrenia patient who was experiencing serious side effects with the previous antipsychotic treatment was treated with increasing doses of CBD, for 4 weeks. CBD significantly reduced the patient's symptoms in the brief psychiatric rating scale (BPRS), in a way superior to the antipsychotic drug haloperidol (Zuardi et al., 1995).

In 2006, a second study was conducted with three male patients with treatment-resistant schizophrenia. They received CBD for 4 weeks, with the doses increasing throughout the treatment. Only one patient had a mild improvement under CBD treatment, while the others showed no improvement. The authors argue that the lack of CBD effect is probably due to the therapeutic approach, different from the one used in the previous case-study. Another possible explanation is that the patients were resistant to conventional antipsychotic treatments, and CBD might be ineffective on these individuals (Zuardi et al., 2006).

TABLE 81.2 Antipsychotic Effects of CBD in Animal Models

References	Animal	Results
Zuardi et al. (1991)	Rats	CBD attenuates stereotyped behavior induced by apomorphine, as well as the antipsychotic drug haloperidol
Moreira and Guimaraes (2005)	Mice	CBD attenuates hyperlocomotion induced by amphetamine, as well as the antipsychotic drugs haloperidol and clozapine
Long et al. (2006)	Mice	CBD attenuates the PPI deficit induced by MK-801
Malone et al. (2009)	Rats	CBD attenuates the deficit in social interaction induced by THC
Long et al. (2010)	Mice	Chronic treatment with CBD attenuates the hyperlocomotion induced by dexamphetamine
Gururajan et al. (2012)	Rats	CBD attenuates hyperlocomotion and social withdrawal induced by MK-801
Levin et al. (2012)	Rats	CBD attenuates the spontaneous impairment in emotional context processing presented by SHR strain
Levin et al. (2014)	Rats	CBD attenuates the spontaneous PPI deficit presented by SHR strain

Summary of the studies indicating the antipsychotic effects of CBD in animal models of schizophrenia. CBD, Cannabidiol; MK-801, dizocilpine; PPI, prepulse inhibition of startle; SHR, spontaneously hypertensive rats; THC, Δ^9-tetrahydrocannabinol.

CBD antipsychotic effects were also evaluated in patients with Parkinson disease (PD). An open-label study with six PD patients showed that CBD administration for 4 weeks, in addition to their usual treatment, decreases psychotic symptoms—evaluated by BPRS and by Parkinson Psychosis Questionnaire. It was also seen that CBD treatment does not worsen the motor function of the patients (Zuardi et al., 2009).

Another study performed in 2010 investigated the effects of acute CBD administration on selective attention, in 28 schizophrenia patients. No beneficial effects of CBD were seen, although it cannot be disregarded that chronic CBD administration might have had a different outcome (Hallak et al., 2010). The lack of CBD effectiveness was also observed in a case report with two patients with BAD. The 4-week treatment with CBD showed no improvement on patient's symptomatology—accessed by BPRS and Young mania rating scale (YMRS)—indicating that CBD is probably ineffective against the psychosis associated to BAD (Zuardi et al., 2010).

The first double-blind controlled clinical trial with CBD in schizophrenia patients was performed in 2012. Forty-two patients were randomly assigned to receive CBD or amisulpride (a potent atypical antipsychotic) for 4 weeks. Patients' symptoms, evaluated by BPRS and positive and negative syndrome scale (PANSS), significantly decreased under both treatments, and no difference was seen between them. Nonetheless, CBD induced fewer side effects (weight gain, changes in prolactin levels, and extrapyramidal symptoms) than amisulpride. Furthermore, the treatment with CBD, but not with amisulpride, increased the levels of AEA in serum, and this increase was associated to the decrease of psychotic symptoms (Leweke et al., 2012).

Taken together, these data strengthen the suggestion of CBD as an antipsychotic drug, and point to its use on the treatment of schizophrenia, as well as other psychiatric conditions that present psychotic symptoms. Table 81.1 summarizes the positive results from clinical studies.

SIDE EFFECTS OF CBD

As commented earlier, the available antipsychotic drugs induce significant side effects that impair the patients' quality of life. It is the case of motor disturbances—also known as extrapyramidal symptoms—induced mainly by typical or first generation antipsychotics, and of metabolic syndrome, promoted primarily by atypical antipsychotics. It is important, therefore not only to evaluate the therapeutic profile of potential new antipsychotic drugs, but also to assess their side effects.

Several studies conducted with CBD address its toxicity, and potential side effects. With regard to the motor side effects, studies with rodents show that CBD does not modify motor activity or induce catalepsy (which parallels the acute motor side effects induced by typical antipsychotics), even at high doses. In accordance, CBD treatment in humans does not promote extrapyramidal symptoms. CBD also does not alter some metabolic parameters such as body weight gain and blood levels of glucose, gonadal hormones, or prolactin. Several other blood analyses are also not affected by CBD: in rodents, pH, pO_2, pCO_2, hematocrit, K^+, and Na^+ levels were evaluated after CBD administration, and were not different from control group. The assessment of hematocrit, hemoglobin, leukocyte, and erythrocyte counts, bilirubin and creatinine blood levels in humans treated with CBD also did not yield any significant effect of the cannabinoid. CBD also does not modify urine osmolarity, pH, albumin, leukocyte, and erythrocyte counts, as well as cylinders and crystals levels in humans.

Moreover, some studies investigated the effects of CBD on physiological parameters. CBD does not alter blood pressure or heart rate in rodents; human studies corroborate these findings, and show that CBD does not induce changes in electrocardiogram or electroencephalogram, either. In addition, studies with animals demonstrate that CBD does not affect gastrointestinal motility or rectal temperature. In humans, it was also seen that CBD does not impair psychomotor performance. Finally, some animal studies demonstrate that CBD does not affect the vitality of nontumor cell lines, nor does it alter embryonic development [For a broad review of the side effects of CBD, see: Bergamaschi, Queiroz, Zuardi, and Crippa (2011)].

CONCLUSIONS

Several evidence reinforce the therapeutic potential of CBD in the treatment of schizophrenia's symptomatology. The available data demonstrate that CBD, as the current antipsychotic drugs, is able to treat the positive symptoms of schizophrenia. It is noteworthy that CBD represents an advance with regard to the existing antipsychotics, since it might also be effective against the negative and cognitive symptoms of schizophrenia. Furthermore, significant side effects are not seen with CBD, a common outcome of treatment with antipsychotic drugs.

It is worth mentioning that, although much evidence point to CBD as a safe compound with extensive antipsychotic properties, not all reports are concurring. Therefore, further studies are still necessary to strengthen CBD's antipsychotic profile. Accordingly, it is important to emphasize the relevance of using animal models and translational approaches in the investigation of new therapeutic strategies, such as CBD.

MINI-DICTIONARY

Anandamide (AEA) An endogenous cannabinoid neurotransmitter (endocannabinoid) derived from arachidonic acid.
Antipsychotic drugs Class of drugs used mainly to treat psychotic states, in particular schizophrenia and bipolar disorder. There are two classes of antipsychotic drugs: typical and atypical. The typical antipsychotics are linked to extrapyramidal side effects, while the main side effects of the atypical ones are metabolic disturbances.
Cannabidiol (CBD) One of over 60 cannabinoids identified in cannabis. It is devoid of psychotomimetic actions, but presents several pharmacological effects in the central nervous system and periphery.
Extrapyramidal symptoms Motor dysfunctions that are a common side effect of typical antipsychotic drugs.
Hyperlocomotion Increase in locomotion, evaluated in behavioral tasks, used to model the positive symptoms of schizophrenia in rodents.
Prepulse Inhibition of Startle (PPI) Behavioral paradigm that assess the sensorimotor gating functioning in humans and animals. It is impaired in some psychiatric conditions, including schizophrenia, and is a marker of schizophrenia animal models.
Psychotomimetic drugs Class of drugs that induce psychotic symptoms, such as hallucinations and delusions. It is the case of dopaminergic agonists and glutamatergic antagonists (eg, amphetamine, apomorphine, MK-801, ketamine).
Social interaction A series of behaviors evaluated in rodents, indicating their social performance. Its impairment models the negative symptoms of schizophrenia.
Stereotyped behavior Mechanical repetition of movements or speech, seen in schizophrenia patients. The stereotyped behavior is also observed in animal models of schizophrenia.
Δ^9-Tetrahydrocannabinol (THC) The main psychoactive compound of cannabis. It may be obtained from the plant, or made synthetically, and presents psychotomimetic effects.

References

Adams, R., Hunt, M., & Clark, J. (1940). Structure of cannabidiol, a product isolated from the marihuana extract of Minnesota wild hemp. I. *Journal of the American Chemical Society*, *62*(1), 196–200.

Almeida, V., Levin, R., Peres, F. F., Niigaki, S. T., Calzavara, M. B., Zuardi, A. W., Hallak, J. E., Crippa, J. A., & Abilio, V. C. (2013). Cannabidiol exhibits anxiolytic but not antipsychotic property evaluated in the social interaction test. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *41*, 30–35.

Bergamaschi, M. M., Queiroz, R. H., Zuardi, A. W., & Crippa, J. A. (2011). Safety and side effects of cannabidiol, a *Cannabis sativa* constituent. *Current Drug Safety*, *6*(4), 237–249.

Bhattacharyya, S., Morrison, P. D., Fusar-Poli, P., Martin-Santos, R., Borgwardt, S., Winton-Brown, T., Nosarti, C., Cm, O. C., Seal, M., Allen, P., Mehta, M. A., Stone, J. M., Tunstall, N., Giampietro, V., Kapur, S., Murray, R. M., Zuardi, A. W., Crippa, J. A., Atakan, Z., & McGuire, P. K. (2010). Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology*, *35*(3), 764–774.

Calzavara, M. B., Levin, R., Medrano, W. A., Almeida, V., Sampaio, A. P., Barone, L. C., Frussa-Filho, R., & Abilio, V. C. (2011). Effects of antipsychotics and amphetamine on social behaviors in spontaneously hypertensive rats. *Behavioral Brain Research*, *225*(1), 15–22.

Calzavara, M. B., Medrano, W. A., Levin, R., Kameda, S. R., Andersen, M. L., Tufik, S., Silva, R. H., Frussa-Filho, R., & Abilio, V. C. (2009). Neuroleptic drugs revert the contextual fear conditioning deficit presented by spontaneously hypertensive rats: a potential animal model of emotional context processing in schizophrenia? *Schizophrenia Bulletin*, *35*(4), 748–759.

Fernandez-Espejo, E., Viveros, M. P., Nunez, L., Ellenbroek, B. A., & Rodriguez de Fonseca, F. (2009). Role of cannabis and endocannabinoids in the genesis of schizophrenia. *Psychopharmacology (Berlin)*, *206*(4), 531–549.

Gururajan, A., Taylor, D. A., & Malone, D. T. (2011). Effect of cannabidiol in a MK-801-rodent model of aspects of schizophrenia. *Behavioural Brain Research*, *222*(2), 299–308.

Gururajan, A., Taylor, D. A., & Malone, D. T. (2012). Cannabidiol and clozapine reverse MK-801-induced deficits in social interaction and hyperactivity in Sprague-Dawley rats. *Journal of Psychopharmacology*, *26*(10), 1317–1332.

Hallak, J. E., Dursun, S. M., Bosi, D. C., de Macedo, L. R., Machado-de-Sousa, J. P., Abrao, J., Crippa, J. A., McGuire, P., Krystal, J. H., Baker, G. B., & Zuardi, A. W. (2011). The interplay of cannabinoid and NMDA glutamate receptor systems in humans: preliminary evidence of interactive effects of cannabidiol and ketamine in healthy

human subjects. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *35*(1), 198–202.
Hallak, J. E., Machado-de-Sousa, J. P., Crippa, J. A., Sanches, R. F., Trzesniak, C., Chaves, C., Bernardo, S. A., Regalo, S. C., & Zuardi, A. W. (2010). Performance of schizophrenic patients in the Stroop Color Word Test and electrodermal responsiveness after acute administration of cannabidiol (CBD). *Revista Brasileira de Psiquiatria*, *32*(1), 56–61.
Izzo, A. A., Borrelli, F., Capasso, R., Di Marzo, V., & Mechoulam, R. (2009). Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends in Pharmacological Science*, *30*(10), 515–527.
Karniol, I. G., Shirakawa, I., Kasinski, N., Pfeferman, A., & Carlini, E. A. (1974). Cannabidiol interferes with the effects of delta 9-tetrahydrocannabinol in man. *European Journal Pharmacology*, *28*(1), 172–177.
Levin, R., Almeida, V., Peres, F. F., Calzavara, M. B., da Silva, N. D., Suiama, M. A., Niigaki, S. T., Zuardi, A. W., Hallak, J. E., Crippa, J. A., & Abilio, V. C. (2012). Antipsychotic profile of cannabidiol and rimonabant in an animal model of emotional context processing in schizophrenia. *Current Pharmaceutical Design*, *18*(32), 4960–4965.
Levin, R., Calzavara, M. B., Santos, C. M., Medrano, W. A., Niigaki, S. T., & Abilio, V. C. (2011). Spontaneously Hypertensive Rats (SHR) present deficits in prepulse inhibition of startle specifically reverted by clozapine. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *35*(7), 1748–1752.
Levin, R., Peres, F. F., Almeida, V., Calzavara, M. B., Zuardi, A. W., Hallak, J. E., Crippa, J. A., & Abilio, V. C. (2014). Effects of cannabinoid drugs on the deficit of prepulse inhibition of startle in an animal model of schizophrenia: the SHR strain. *Frontiers in Pharmacology*, *5*, 10.
Leweke, F. M., Piomelli, D., Pahlisch, F., Muhl, D., Gerth, C. W., Hoyer, C., Klosterkotter, J., Hellmich, M., & Koethe, D. (2012). Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. *Translational Psychiatry*, *2*, e94.
Leweke, F. M., Schneider, U., Radwan, M., Schmidt, E., & Emrich, H. M. (2000). Different effects of nabilone and cannabidiol on binocular depth inversion in man. *Pharmacology Biochemistry and Behaviour*, *66*(1), 175–181.
Lipska, B. K., & Weinberger, D. R. (2000). To model a psychiatric disorder in animals: schizophrenia as a reality test. *Neuropsychopharmacology*, *23*(3), 223–239.
Long, L. E., Chesworth, R., Huang, X. F., McGregor, I. S., Arnold, J. C., & Karl, T. (2010). A behavioural comparison of acute and chronic delta9-tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice. *International Journal of Neuropsychopharmacology*, *13*(7), 861–876.
Long, L. E., Malone, D. T., & Taylor, D. A. (2006). Cannabidiol reverses MK-801-induced disruption of prepulse inhibition in mice. *Neuropsychopharmacology*, *31*(4), 795–803.
Malone, D. T., Jongejan, D., & Taylor, D. A. (2009). Cannabidiol reverses the reduction in social interaction produced by low dose Delta(9)-tetrahydrocannabinol in rats. *Pharmacology Biochemistry and Behavior*, *93*(2), 91–96.
Mechoulam, R., & Shvo, Y. (1963). Hashish. I. The structure of cannabidiol. *Tetrahedron*, *19*(12), 2073–2078.
Moreira, F. A., & Guimaraes, F. S. (2005). Cannabidiol inhibits the hyperlocomotion induced by psychotomimetic drugs in mice. *European Journal of Pharmacology*, *512*(2–3), 199–205.
Morgan, C. J., & Curran, H. V. (2008). Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. *British Journal of Psychiatry*, *192*(4), 306–307.
Morgan, C. J., Gardener, C., Schafer, G., Swan, S., Demarchi, C., Freeman, T. P., Warrington, P., Rupasinghe, I., Ramoutar, A., Tan, N., Wingham, G., Lewis, S., & Curran, H. V. (2012). Sub-chronic impact of cannabinoids in street cannabis on cognition, psychotic-like symptoms and psychological well-being. *Psychological Medicine*, *42*(2), 391–400.
Morgan, C. J., Schafer, G., Freeman, T. P., & Curran, H. V. (2010). Impact of cannabidiol on the acute memory and psychotomimetic effects of smoked cannabis: naturalistic study: naturalistic study [corrected]. *British Journal of Psychiatry*, *197*(4), 285–290.
Perez-Reyes, M., Timmons, M. C., Davis, K. H., & Wall, E. M. (1973). A comparison of the pharmacological activity in man of intravenously administered delta9-tetrahydrocannabinol, cannabinol, and cannabidiol. *Experientia*, *29*(11), 1368–1369.
Rottanburg, D., Robins, A. H., Ben-Arie, O., Teggin, A., & Elk, R. (1982). Cannabis-associated psychosis with hypomanic features. *Lancet*, *2*(8312), 1364–1366.
Schubart, C. D., Sommer, I. E., van Gastel, W. A., Goetgebuer, R. L., Kahn, R. S., & Boks, M. P. (2011). Cannabis with high cannabidiol content is associated with fewer psychotic experiences. *Schizophrenia Research*, *130*(1–3), 216–221.
Zamberletti, E., Rubino, T., & Parolaro, D. (2012). The endocannabinoid system and schizophrenia: integration of evidence. *Current Pharmaceutical Design*, *18*(32), 4980–4990.
Zuardi, A. W., Crippa, J., Dursun, S., Morais, S., Vilela, J., Sanches, R., & Hallak, J. (2010). Cannabidiol was ineffective for manic episode of bipolar affective disorder. *Journal of Psychopharmacology*, *24*(1), 135–137.
Zuardi, A. W., Crippa, J. A., Hallak, J. E., Pinto, J. P., Chagas, M. H., Rodrigues, G. G., Dursun, S. M., & Tumas, V. (2009). Cannabidiol for the treatment of psychosis in Parkinson's disease. *Journal Psychopharmacology*, *23*(8), 979–983.
Zuardi, A. W., Hallak, J. E., Dursun, S. M., Morais, S. L., Sanches, R. F., Musty, R. E., & Crippa, J. A. (2006). Cannabidiol monotherapy for treatment-resistant schizophrenia. *Journal of Psychopharmacology*, *20*(5), 683–686.
Zuardi, A. W., Morais, S. L., Guimaraes, F. S., & Mechoulam, R. (1995). Antipsychotic effect of cannabidiol. *Journal of Clinical Psychiatry*, *56*(10), 485–486.
Zuardi, A. W., Rodrigues, J. A., & Cunha, J. M. (1991). Effects of cannabidiol in animal models predictive of antipsychotic activity. *Psychopharmacology (Berlin)*, *104*(2), 260–264.
Zuardi, A. W., Shirakawa, I., Finkelfarb, E., & Karniol, I. G. (1982). Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects. *Psychopharmacology (Berlin)*, *76*(3), 245–250.

CHAPTER

82

Cannabidiol for the Treatment of Epilepsy: An Overview of Possible Mechanisms of Action and Preclinical and Human Studies

R. Guimarães dos Santos, J.E.C. Hallak*, A.W. Zuardi*, A.C. de Souza Crippa**, J.A. de Souza Crippa**

***Department of Neuroscience and Behavior, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Sao Paulo, Brazil**

****Health Sciences Sector, Federal University of Paraná, Curitiba, Parana, Brazil**

SUMMARY POINTS

- This chapter focuses on cannabidiol (CBD), a naturally occurring plant-derived compound.
- CBD is produced by the cannabis plant, which produces several other chemically related compounds, collectively called phytocannabinoids or simply cannabinoids.
- CBD does not produce the psychoactive effects typically produced by Δ^9-tetrhydrocannabinol (THC), the main active compound in cannabis.
- Preclinical and human studies suggest that CBD has unique anticonvulsant effects, and presents good safety and tolerability, producing few side effects.
- Despite its promising anticonvulsant effects, only a small number of placebo-controlled clinical trials testing the antiepileptic potentials of CBD were published.
- Given the good safety profile of CBD and its promising anticonvulsant potentials, there is a clear need to perform new randomized, controlled clinical trials with this phytocannabinoid.

KEY FACTS OF CANNABIDIOL

- Cannabis is a plant original from Central Asia that has been used for ritual and medicinal purposes for thousands of years.
- The cannabis plant is rich in unique chemical compounds called cannabinoids.
- The main cannabinoids are Δ^9-tetradydrocannabinol (THC), responsible for producing most of the subjective effects of cannabis, and cannabidiol (CBD), which lacks the psychoactivity of THC.
- The chemical structure of CBD was elucidated in 1963, while THC, the main active component in cannabis, was isolated in a pure form, and its structure determined, in 1964.
- The antiepileptic/anticonvulsant actions of CBD were first described in the 1970s, and the first clinical trial was published in the 1980s.
- The isolation and structure of anandamide, the brain's first identified endogenous constituent that binds to the cannabinoid receptors, occurred in 1992.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00094-6
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

4-AP	4-Aminopyridine
5-HT$_{1A}$	Serotonergic receptor 1A
CB$_1$	Cannabinoid receptor 1
CB$_2$	Cannabinoid receptor 2
CBD	Cannabidiol
CNS	Central nervous system
COX	Cyclooxygenase
DG	Dentate gyrus
EEG	Electroencephalography
IFN-γ	Interferon γ
LFP	Local field potential
NO	Nitric oxide
PGE2	Prostaglandin E2
THC	Δ^9-Terahydrocannabinol
TNF	Tumor necrosis factor
TRPV$_1$	Transient receptor potential vanilloid 1 (vanilloid receptor 1)
TRPV$_2$	Transient receptor potential vanilloid 2 (vanilloid receptor 2)

INTRODUCTION

Cannabis (*Cannabis sativa*), a plant of the Cannabaceae botanical family, has been used for thousands of years for medicinal, religious, and recreational uses by cultures around the world (Mechoulam & Carlini, 1978; Schultes & Hofmann, 1992; Furst, 1994; Robinson, 1995; Iversen, 2000; Spinella, 2001; Russo et al., 2008; Crippa, Zuardi, & Hallak, 2010; Godlaski, 2012). Nowadays, cannabis is the most consumed illegal recreational drug worldwide (Degenhardt et al., 2013; Greydanus, Hawver, Greydanus, & Merrick, 2013; Hall & Degenhardt, 2014).

Besides terpenoids, flavonoids, steroids, and enzymes, cannabis contains over 100 compounds called cannabinoids, which are unique to the plant (Mechoulam & Carlini, 1978; Mechoulam & Hanus, 2012). Several preclinical and human studies suggest that the main cannabinoid responsible for producing the subjective effects associated with cannabis consumption is Δ^9-tetrahydrocannabinol (THC), while the other main cannabinoid present in cannabis is cannabidiol (CBD), which lacks the psychoactivity of THC (Mechoulam & Carlini, 1978; Iversen, 2000; Spinella, 2001; Zuardi, 2008; Zuurman et al., 2008, 2010; Crippa et al., 2010; Morgan, Freeman, Schafer, & Curran, 2010; Carbuto et al., 2012; Mechoulam & Hanus, 2012; Zhornitsky & Potvin, 2012; Robson, 2014).

In the last decades, a growing number of studies have reported that CBD has a number of therapeutic potentials, including: antiepileptic, sedative, anxiolytic, antipsychotic, antiinflammatory, antioxidative, neuroprotective, antiemetic, anticancer, antidepressant, and mood stabilizer, and with therapeutic action on movement disorders, ischemia, diabetes, and on cannabis withdrawal syndrome (Mechoulam & Carlini, 1978; Ben Amar, 2006; Zuardi, 2008; Crippa et al., 2010; Hazekamp & Grotenhermen, 2010; Mechoulam & Hanus, 2012; Zhornitsky & Potvin, 2012; Robson, 2014). A recent review reported that there are 18 clinical trials involving the administration of CBD to clinical populations, including patients with multiple sclerosis (six studies), schizophrenia and bipolar mania (four studies), social anxiety disorder (two studies), neuropathic and cancer pain (two studies), cancer anorexia (one study), Huntington disease (one study), insomnia (one study), and epilepsy (one study) (Zhornitsky & Potvin, 2012).

Epilepsy is a common neurological disorder characterized by recurrent, unprovoked seizures. Seizures are caused by excessive brain electrical activity, and not all seizures are characterized by convulsions, an abnormal and involuntary contraction of the muscles. There are several types of epilepsy, and available treatments include antiepileptic drugs, steroids, or surgery. Antiepileptic drugs currently available do not benefit all patients, and many produce several side effects, limiting their clinical use. Thus, the present text will introduce an overview of the antiepileptic effects of CBD, both in preclinical and human studies. The text will focus specifically on the antiepileptic effects of CBD because, although this was one of the first pharmacological actions described for this compound in the 1970s, there is only a small number of case reports and placebo-controlled clinical trials testing the antiepileptic potentials of CBD. Therefore, there is a clear necessity to keep investigating the antiepileptic actions of CBD.

CBD'S MECHANISMS OF ACTION

CBD has multiple mechanisms of action, and its pharmacology is not completely understood. In the endocannabinoid system, THC acts as a partial agonist at cannabinoid CB$_1$ receptors, found primarily in the central nervous system (CNS), and CB$_2$ receptors, found primarily on cells of the immune system (Iversen, 2000; Zuurman et al., 2008, 2010; Carbuto et al., 2012). On the other hand, CBD has little affinity to cannabinoids receptors CB$_{1/2}$, although it is capable of antagonizing CB$_{1/2}$ receptor agonists. Moreover, CBD blocks the uptake of the endocannabinoid anandamide, and the inhibition of its enzymatic hydrolysis, and antagonizes the putative novel cannabinoid receptor GPR55 (Zuardi, 2008; Zhornitsky & Potvin, 2012).

Regarding other pharmacological mechanisms that are not directly mediated by the endocannabinoid system, CBD activates 5-HT$_{1A}$ serotonergic and TRPV$_{1\text{-}2}$ vanilloid receptors, antagonizes α_1 adrenergic

and μ-opioid receptors, inhibits the uptake of adenosine, noradrenaline, dopamine, serotonin, and γ-aminobutyric acid, and stimulates the activity of the inhibitory glycine-receptor. Moreover, CBD reduces hydroperoxide-induced oxidative damage, T cell responses, the release of bioactive tumor necrosis factor (TNF), the production of prostaglandin E2 (PGE2), and cytokines interferon γ (IFN-γ) and tumor necrosis factor (TNF), reduces tissue cyclooxygenase (COX) activity and the production of nitric oxide (NO) (Zuardi, 2008; Zhornitsky & Potvin, 2012).

The wide range of pharmacological actions produced by CBD clearly indicates the need for further research to clarify the precise mechanisms that underlie its therapeutic effects, including its antiepileptic potentials.

PRECLINICAL STUDIES SUPPORTING CBD'S ANTICONVULSANT EFFECTS

The antiepileptic effects of CBD were one of the first pharmacological actions described for this compound, still in the 1970s (Carlini, Leite, Tannhauser, & Barardi, 1973; Izquierdo, Orsingher, & Berardi, 1973; Turkanis, Cely, Olsen, & Karler, 1974; Consroe & Wolkin, 1977; Mechoulam & Carlini, 1978; Consroe, Benedito, Leite, Carlini, & Mechoulam, 1982). According to previous studies in rodents, CBD is an effective and relatively potent anticonvulsant (Carlini et al., 1973; Izquierdo et al., 1973; Turkanis et al., 1974; Consroe & Wolkin, 1977; Consroe et al., 1982).

CBD decreased the susceptibility of rat dorsal hippocampus to seizures discharges caused by afferent stimulation (Izquierdo et al., 1973), and significantly protected mice from the convulsant and lethal effects of leptazol (Carlini et al., 1973).

According to Consroe and Wolkin (1977), CBD might be an effective anticonvulsant with specificity more comparable to drugs clinically effective in major (eg, tonic-clonic seizures) than minor seizures. Using the maximal electroshock (a model of partial seizure with secondary generalization) and audiogenic seizures tests, CBD enhanced the anticonvulsant effects of drugs clinically effective in major seizures, and reduced the effects of drugs effective in minor seizures. The anticonvulsant potency of phenytoin was significantly increased when combined with phenobarbital, CBD, and phenobarbital plus CBD, but CBD reduced the anticonvulsant potencies of chlordiazepoxide, clonazepam, trimethadione, and ethosuximide (Consroe & Wolkin, 1977). It is uncertain whether the interactions of CBD with other antiepileptic drugs results from pharmacokinetic or pharmacodynamic mechanisms, but since CBD is a potent inhibitor of multiple cytochrome P450 enzymes including CYP1A2, CYP2B6, CYP2C9, CYP2D6, and CYP3A4 (Zhornitsky & Potvin, 2012), it is possible that pharmacokinetic mechanisms are involved.

Another study in rodents, using transcorneal electroshock current or proconvulsant drugs to induce seizures, reported that CBD selectively prevented tonic, but not clonic, convulsions (Consroe et al., 1982).

More recently, a study reported that CBD caused concentration-related and region-dependent attenuation of chemically induced epileptiform activity in hippocampal brain slices, using in vitro electrophysiological recordings (Jones et al., 2010). This study used extracellular multielectrode array recordings to show that CBD decreased epileptiform activity in the Mg^{2+}-free and 4-aminopyridine (4-AP) in vitro models of epilepsy in the mammalian hippocampus, which is a key epileptogenic brain region. Specifically, in the Mg^{2+}-free model, CBD decreased epileptiform local field potential (LFP) in CA1 and dentate gyrus (DG) regions, while in the 4-AP model CBD decreased LFP in CA1, CA3, and DG regions. Overall, CBD induced more prominent effects in the Mg^{2+}-free than in 4-AP model, which could reflect inherent differences between the two models, which affect NMDA glutamate receptors and K^+ channels, respectively (Jones et al., 2010). Furthermore, this study also examined the effects of CBD in vivo, using the pentylenetetrazole (PTZ) model of generalized seizures, and reported that CBD reduced the incidence of severe seizures and mortality in rodents. Moreover, the antiepileptic effects associated with CBD were suggested to be produced by a potentially CB_1-independent mechanism, since CBD acted with only low affinity at CB_1 receptors (Jones et al., 2010).

In another study (Jones et al., 2012), the same research group examined the anticonvulsant potential of CBD in rodents, using the acute pilocarpine model of temporal lobe seizure, and the penicillin model of partial seizure. In the pilocarpine model, CBD showed modest anticonvulsant effects, reducing the incidence of the most severe seizures, but failing to reduce mortality. On the other hand, CDB produced strong anticonvulsant effects in the penicillin model, with significant reductions in the lethality of seizures, and in the proportions of animals developing the most severe (tonic-clonic) seizure types. Furthermore, CBD had very little effect on motor function tests, suggesting that CBD may present a better safety profile, when compared to currently available antiepileptic drugs, which cause significant and strong motor side effects. Taken together, the studies by Jones et al. (2010, 2012) suggest that CBD appears to hold the greatest potential for the treatment of partial and generalized seizures, rather than temporal lobe seizures.

A recent rodent study using the maximal electroshock and the PTZ seizure models reported anticonvulsant effects of CBD, in both seizure models (Shirazi-zand,

Ahmad-Molaei, Motamedi, & Naderi, 2013). Moreover, this study also evaluated the possible interactions between CBD and the potassium BK channel blocker paxilline. Paxilline did not produce significant change in the PTZ model, but co-administration of CBD and paxilline attenuated the antiseizure effect of CBD in PTZ-induced tonic seizures. In the MES model, both CBD and paxilline produced anticonvulsant effect, but there was no interaction between both substances. According to the authors, these results suggest a BK channel-mediated anticonvulsant action of CBD in the PTZ model of seizure, where CBD could act by decreasing intracellular calcium levels.

Table 82.1 shows that preclinical evidence clearly attests the protective effect of CBD in respect to seizures induced by a number of agents in laboratory animals.

HUMAN STUDIES SUPPORTING CBD'S ANTICONVULSANT EFFECTS

Only a small number of placebo-controlled clinical trials testing the antiepileptic potentials of CBD were published (Mechoulam & Carlini, 1978; Cunha et al., 1980; Ben Amar, 2006; Zuardi, 2008; Hazekamp & Grotenhermen, 2010; Gloss & Vickrey, 2014; Zhornitsky & Potvin, 2012; Robson, 2014). All these trials used CBD as an add-on medication at doses ranging from 200 to 300 mg/day, over treatment periods ranging from 4 weeks to 12 months. Most trials reported reduction in seizures and few side effects after CBD administration (Mechoulam & Carlini, 1978; Cunha et al., 1980; Ben Amar, 2006; Zuardi, 2008; Hazekamp & Grotenhermen, 2010; Gloss & Vickrey, 2014; Zhornitsky & Potvin, 2012; Robson, 2014).

TABLE 82.1 Antiepileptic Effects of CBD in Animal Models

Model	Animals	Results (symbol)[a]		Dose with significant results	References
Convulsant hippocampal discharges	Rats	Reduced susceptibility	(+)	5 mg/kg	Izquierdo et al. (1973)
Leptazol-induced seizures	Mice	Seizure and mortality reduction	(+)	200 mg/kg	Carlini et al. (1973)
Electroshock-induced seizure	Mice	Seizure reduction	(+)	ED_{50}[b] = 120 mg/kg	Turkanis et al. (1974)
Electroshock-induced seizure	Rats	Seizure reduction	(+)	Oral (?)	Consroe and Wolkin (1977)
Electroshock-induced seizure transcorneal	Mice	Reduction of tonic seizures	(+)	ED_{50} = 267 mg/kg	Consroe et al. (1982)
Seizures induced by strychnine sulfate	Mice	No protection against seizures or death	(−)	—	Consroe et al. (1982)
Picrotoxin-induced seizures	Mice	Reduction of tonic seizures	(+)	ED_{50} = 194.4 mg/kg	Consroe et al. (1982)
Seizures induced by 3-mercaptopropionic acid	Mice	Reduction of tonic seizures	(+)	ED_{50} = 122.5 mg/kg	Consroe et al. (1982)
Pentylenetetrazol-induced seizures	Mice	Reduction of tonic seizures	(+)	ED_{50} = 304.8 mg/kg	Consroe et al. (1982)
Seizures induced by isonicotinic acid hydrazide	Mice	Reduction of tonic seizures	(+)	ED_{50} = 266.1 mg/kg	Consroe et al. (1982)
Bicuculline-induced seizures	Mice	Reduction of tonic seizures	(+)	ED_{50} = 379.9 mg/kg	Consroe et al. (1982)
Epileptiform activity in hippocampal tissue	In vitro (rats)	Protection	(+)	1–100 μM	Jones et al. (2010)
Pentylenetetrazol-induced seizures	Rats	Seizure and mortality reduction	(+)	100 mg/kg	Jones et al. (2010)
Pilocarpine-induced seizures	Rats	Seizure reduction	(+)	1–100 mg/kg	Jones et al. (2012)
Partial seizures induced by intraventricular penicillin	Rats	Seizure reduction	(+)	10–100 mg/kg	Jones et al. (2012)
Electroshock-induced seizure	Mice	Increased threshold	(+)	20–200 ng (intraventricular)	Shirazi-zand et al. (2013)
Pentylenetetrazol-induced seizures	Mice	Increased threshold	(+)	200 ng (intraventricular)	Shirazi-zand et al. (2013)

The antiepileptic effects of cannabidiol (CBD) were demonstrated in several animal models.
[a] *(+), Anticonvulsant action; (−), no anticonvulsant action.*
[b] *Median effective doses.*

According to several reviews (Ben Amar, 2006; Hazekamp & Grotenhermen, 2010; Gloss & Vickrey, 2014; Zhornitsky & Potvin, 2012; Robson, 2014), the study by Cunha et al. (1980) seems to be the only double-blind, placebo-controlled clinical trial on the antiepileptic effects of CBD that was fully published in a peer-reviewed journal. There are other studies cited by Zuardi (2008), Robson (2014), and by Gloss and Vickrey (2014). However, one of these studies was published as a one-page letter to the editor, while another study was published as a one-page abstract from a conference. In both reports, few methodological details are given, and the overall quality of the reports is low (for details, see Zuardi, 2008; Robson, 2014; Gloss & Vickrey, 2014). Another study seems to be a preliminary report from nine volunteers from the study by Cunha et al. (1980) (See paragraph below). Thus, only the study by Cunha et al. (1980) will be further discussed.

Cunha et al. (1980) evaluated 15 patients (11 women/4 men; aged 14–49 years; average 24 years) suffering from secondary generalized epilepsy with temporal lobe focus that was unresponsive to prescribed antiepileptic drugs. Patients continued to take their regular antiepileptic drugs throughout the study period. Patients participated in a double-blind, placebo-controlled study, where eight patients received 200–300 mg/day oral CBD for 8–18 weeks, and the other seven individuals received placebo. In this study, four out of eight CBD-treated patients evidenced significant improvement in their condition, remaining virtually convulsion-free for the duration of the study. The other three CBD-treated subjects exhibited partial improvement in their clinical condition. Therefore, only one of the eight patients getting CBD showed no improvement. Moreover, three CDB-treated patients showed improvements in electroencephalographic (EEG) measures. Among the patients who received placebo, only one improved, while seven remained unchanged. CBD was well tolerated by all participants. No further clinical trials investigating the antiepileptic effects of CBD have been published since then.

More recently, a study explored the use of CBD-enriched cannabis in children with treatment-resistant epilepsy (Porter & Jacobson, 2013). The researchers presented a survey to parents belonging to a Facebook group dedicated to sharing information about the use of CBD-enriched cannabis to treat their child's seizures. Nineteen responses met the following inclusion criteria for the study: a diagnosis of epilepsy, and current use of CBD-enriched cannabis. Thirteen children had Dravet syndrome, four had Doose syndrome, one had Lennox–Gastaut syndrome, and one had idiopathic epilepsy. Before using CBD-enriched cannabis, the average number of antiepileptic drugs tried was 12 (range 4–17), and seizure frequency ranged from 2 per week to 250 per day. The children experienced a variety of seizure types, including focal, tonic-clonic, myoclonic, atonic, and infantile spasms. In most cases, the children experienced treatment-resistant epilepsy for more than 3 years before trying CBD-enriched cannabis. The treatment period with CBD-enriched cannabis ranged from 2 weeks to over 1 year. Sixteen (84%) of the 19 parents reported a reduction in their child's seizure frequency. Of these, two (11%) reported complete seizure freedom, eight (42%) reported reduction greater than 80% in seizure frequency, and six (32%) reported a 25–60% seizure reduction. Other beneficial effects included increased alertness, better mood, and improved sleep. Side effects were mild, and included drowsiness and fatigue.

Clinical studies with CBD focusing on children with intractable epileptic syndromes, such as Dravet and Lennox–Gastaut syndromes, are currently underway (ClinicalTrials.gov Identifier: NCT02091206, NCT02091375, NCT02224560, NCT02224573, NCT02224690, and NCT02224703).

The clinical trials available on the effectiveness of CBD in epilepsy are shown in Table 82.2.

CONCLUSIONS

CBD definitely has anticonvulsant effects, as reported by several preclinical studies. In humans, case reports, and at least one randomized controlled study, suggest that CBD is well tolerated, and has substantial therapeutic potentials to be further explored. Moreover, CBD has a good safety and tolerability profile, as reported by several preclinical and human studies. The small number of clinical trials suggests that no reliable conclusions can be drawn at present regarding the efficacy of CBD as a treatment for epilepsy.

CBD has a polypharmacological profile, resulting in multiple mechanisms of action possibly responsible for its anticonvulsant effects. CBD's anticonvulsant effects may result from numerous cannabinoid receptor-independent mechanisms. CBD may potentially modulate neuronal hyperexcitability via a number of different mechanisms, which include regulation of Ca^{2+} homeostasis, agonistic properties at 5-HT_{1A} receptors, and reduction in adenosine reuptake (Zuardi, 2008; Jones et al., 2010, 2012; Zhornitsky & Potvin, 2012; Shirazi-zand et al., 2013). Investigating CBD's mechanisms of action may improve its efficacy and safety profile, and may stimulate research with new antiepileptic agents with fewer side effects than current available antiepileptic drugs.

Given the good safety profile of CBD, and its promising anticonvulsant potentials, there is a clear need to perform new randomized, controlled clinical trials with this phytocannabinoid. Research should focus on determining safety, optimal dosing, tolerability, and possible interactions with other antiepileptic drugs. Moreover, clinical trials

TABLE 82.2 Clinical Trials with CBD in Epilepsy

Study design	Sample	Results (symbol)[a]		Dose with significant results	References
Double blind, placebo-controlled trial	15 patients with treatment-resistant epilepsy	7 of 8 patients improved with CBD; 1 of 7 improved with placebo	(+)	200–300 mg/day (add-on) for 8–18 weeks	Cunha et al. (1980)
Placebo-controlled trial (*letter* with few details)	12 patients with mental retardation and seizures	No differences between groups (6 and 6?)	(−)	300 mg/day for 4 weeks	Ames and Cridland (1986), cited in Gloss and Vickrey (2014)
Double blind trial (unpublished congress abstract)	12 patients with epilepsy	No statistical analysis (does preliminary analysis suggests any reduction?)	(+/−)	300 mg/day for 12 months (6 on drug and 6 on placebo)	Trembly and Sherman (1990), cited in Gloss and Vickrey (2014)
Retrospective assessment with questionnaires completed by parents of children treated with CBD	19 children with treatment-resistant seizures[b]	84% reported seizure reduction	(+)	*Cannabis* extract rich in CBD	Porter and Jacobson (2013)
Open label trial	27 treatment-resistant children or young adults (up to 18 years), predominantly with Dravet syndrome ($n = 9$)	Compared with baseline: 15%—no seizures; 22%—90% reduction, 41%—70% reduction; 48%—50% reduction	(+)	5–20 mg/kg per day for 12 weeks	GW Pharmaceuticals (2014)[c]

Few studies assessed the antiepileptic effects of cannabidiol (CBD) in patients, and many of these studies have important methodological limitations, such as small sample size. Thus, it is difficult to have a definitive opinion regarding the clinical efficacy of CBD in epileptic patients.

[a] *(+), Anticonvulsant action; (−), no anticonvulsant action.*

[b] *12 children with Dravet syndrome, 4 with Doose syndrome, 1 with Lennox–Gastaut syndrome, 1 with mental retardation, and 1 with early-onset idiopathic epilepsy.*

[c] *Personal communication.*

should be conducted among different populations of patients, such as children with treatment-resistant epilepsy.

MINI-DICTIONARY

Anandamide *N*-arachidonoylethanolamine. From the Sanskrit word "ananda": bliss, delight. Endogenous cannabinoid neurotransmitter.

Antiepileptic/anticonvulsant drugs Drugs used for the treatment of epilepsy. These drugs control the occurrence of seizures in epileptic patients.

Cannabidiol (CBD) One of the main phytocannabinoids. It lacks the psychoactive characteristics of Δ^9-tetradydrocannbinol (THC) and has several therapeutic potentials.

Cannabinoid receptors G-protein-coupled receptors present in the endocannabinoid system.

Cannabinoids Chemical compounds present exclusively in the cannabis plant. More than 100 cannabinoids have been identified. Cannabinoids are also called phytocannabinoids, in opposition to endocannabinoids.

Cannabis A plant of the Cannabaceae botanical family, with three different species: *Cannabis sativa*, *C. indica*, and *C. ruderalis*.

CB_1 cannabinoid receptor Cannabinoid receptors mainly distributed in the central nervous system. CB_1 receptors are densely distributed in the frontal cerebral cortex, basal ganglia, cerebellum, hippocampus, hypothalamus, and anterior cingulate cortex, but rarely in the brainstem nuclei.

CB_2 cannabinoid receptor Cannabinoid receptors mainly distributed in immune cells. CB_2 receptors are also expressed in microglia, spinal cord, and sparsely in several brain regions, such as the cerebellum, cortex, and brainstem.

Convulsion An abnormal and involuntary contraction of the muscles. The term is often used interchangeably with "seizure," which is an abnormal electrical activity in the brain, but not all seizures are characterized by convulsions.

Endocannabinoids Endogenous cannabinoid neurotransmitters. Endocannabinoids are chemically and pharmacologically related to phytocannabinoids, both groups of substances acting at the endocannabinoid system, which is basically composed by endocannabinoids, cannabinoid receptors, and the enzymes responsible for endocannabinoid synthesis and degradation.

Epilepsy A group of neurological diseases characterized by the presentation of several types of seizures.

Δ^9-Tetrahydrocannabinol (THC) The main chemical in the cannabis plant responsible for producing the typical subjective effects reported for cannabis consumption.

References

Ames, F. R., & Cridland, S. (1986). Anticonvulsant effect of cannabidiol. *South African Medical Journal*, *69*, 14.

Ben Amar, M. (2006). Cannabinoids in medicine: a review of their therapeutic potential. *Journal of Ethnopharmacology*, *105*, 1–25.

Carbuto, M., Sewell, R. A., Williams, A., Forselius-Bielen, K., Braley, G., Elander, J., Pittman, B., Schnakenberg, A., Bhakta, S., Perry, E., Ranganathan, M., & D'Souza, D. C. Yale THC Study Group. (2012). The safety of studies with intravenous Δ^9-tetrahydrocannabinol in humans, with case histories. *Psychopharmacology*, *219*, 885–896.

Carlini, E. A., Leite, J. R., Tannhauser, M., & Berardi, A. C. (1973). Cannabidiol and *Cannabis sativa* extract protect mice and rats against convulsive agents. *Journal of Pharmacy and Pharmacology*, *25*, 664–665.

Consroe, P., Benedito, M. A., Leite, J. R., Carlini, E. A., & Mechoulam, R. (1982). Effects of cannabidiol on behavioral seizures caused by convulsant drugs or current in mice. *European Journal of Pharmacology*, *83*, 293–298.

Consroe, P., & Wolkin, A. (1977). Cannabidiol—antiepileptic drug comparisons and interactions in experimentally induced seizures in rats. *Journal of Pharmacology and Experimental Therapeutics*, *201*, 26–32.

Crippa, J. A., Zuardi, A. W., & Hallak, J. E. (2010). Therapeutical use of the cannabinoids in psychiatry. *Revista Brasileira de Psiquiatria*, *32*, S56–S66.

Cunha, J. M., Carlini, E. A., Pereira, A. E., Ramos, O. L., Pimentel, C., Gagliardi, R., Sanvito, W. L., Lander, N., & Mechoulam, R. (1980). Chronic administration of cannabidiol to healthy volunteers and epileptic patients. *Pharmacology*, *21*, 175–185.

Degenhardt, L., Ferrari, A. J., Calabria, B., Hall, W. D., Norman, R. E., McGrath, J., Flaxman, A. D., Engell, R. E., Freedman, G. D., Whiteford, H. A., & Vos, T. (2013). The global epidemiology and contribution of cannabis use and dependence to the global burden of disease: results from the GBD 2010 study. *PLoS One*, *8*, e76635.

Furst, P. T. (1994). *Alucinógenos y cultura*. México, Mexico: Fondo de Cultura Económica.

Gloss, D., & Vickrey, B. (2014). Cannabinoids for epilepsy. *Cochrane Database of Systematic Reviews*, *3*, CD009270.

Godlaski, T. M. (2012). Shiva, Lord of Bhang. *Substance Use and Misuse*, *47*, 1067–1072.

Greydanus, D. E., Hawver, E. K., Greydanus, M. M., & Merrick, J. (2013). Marijuana: current concepts. *Front Public Health*, *1*, 42.

Hall, W., & Degenhardt, L. (2014). The adverse health effects of chronic cannabis use. *Drug Testing and Analysis*, *6*, 39–45.

Hazekamp, A., & Grotenhermen, F. (2010). Review on clinical studies with cannabis and cannabinoids 2005–2009. *Cannabinoids*, *5*, 1–21.

Iversen, L. (Ed.). (2000). *The science of marijuana*. Oxford, UK: Oxford University Press.

Izquierdo, I., Orsingher, O. A., & Berardi, A. C. (1973). Effect of cannabidiol and of other *Cannabis sativa* compounds on hippocampal seizure discharges. *Psychopharmacologia*, *28*, 95–102.

Jones, N. A., Glyn, S. E., Akiyama, S., Hill, T. D., Hill, A. J., Weston, S. E., Burnett, M. D., Yamasaki, Y., Stephens, G. J., Whalley, B. J., & Williams, C. M. (2012). Cannabidiol exerts anti-convulsant effects in animal models of temporal lobe and partial seizures. *Seizure*, *21*, 344–352.

Jones, N. A., Hill, A. J., Smith, I., Bevan, S. A., Williams, C. M., Whalley, B. J., & Stephens, G. J. (2010). Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo. *Journal of Pharmacology and Experimental Therapeutics*, *332*, 569–577.

Mechoulam, R., & Carlini, E. A. (1978). Toward drugs derived from cannabis. *Naturwissenschaften*, *65*, 174–179.

Mechoulam, R., & Hanus, L. (2012). Other cannabinoids. In D. Castle, R. M. Murray, & D. C. D'Souza (Eds.), *Marijuana and madness* (2nd ed., pp. 17–22). Cambridge, UK: Cambridge University Press.

Morgan, C. J., Freeman, T. P., Schafer, G. L., & Curran, H. V. (2010). Cannabidiol attenuates the appetitive effects of delta-9-tetrahydrocannabinol in humans smoking their chosen cannabis. *Neuropsychopharmacology*, *35*, 1879–1885.

Porter, B. E., & Jacobson, C. (2013). Report of a parent survey of cannabidiol-enriched cannabis use in pediatric treatment-resistant epilepsy. *Epilepsy and Behavior*, *29*, 574–577.

Robson, P. J. (2014). Therapeutic potential of cannabinoid medicines. *Drug Testing and Analysis*, *6*, 24–30.

Robinson, R. (1995). *The Great Book of Hemp: the complete guide to the environmental, commercial, and medicinal uses of the world's most extraordinary plant*. Rochester: Park Street Press.

Russo, E. B., Jiang, H. E., Li, X., Sutton, A., Carboni, A., del Bianco, F., Mandolino, G., Potter, D. J., Zhao, Y. X., Bera, S., Zhang, Y. B., Lü, E. G., Ferguson, D. K., Hueber, F., Zhao, L. C., Liu, C. J., Wang, Y. F., & Li, C. S. (2008). Phytochemical and genetic analyses of ancient cannabis from Central Asia. *Journal of Experimental Botany*, *59*, 4171–4182.

Schultes, R. E., & Hofmann, A. (1992). *Plants of the gods: their sacred, healing, and hallucinogenic powers*. Rochester, VT: Healing Arts Press.

Shirazi-zand, Z., Ahmad-Molaei, L., Motamedi, F., & Naderi, N. (2013). The role of potassium BK channels in anticonvulsant effect of cannabidiol in pentylenetetrazole and maximal electroshock models of seizure in mice. *Epilepsy and Behavior*, *28*, 1–7.

Spinella, M. (2001). *The psychopharmacology of herbal medicine: plant drugs that alter mind, brain and behavior*. Cambridge, MA: The MIT Press.

Trembly, B., & Sherman, M. (1990). *Double-blind clinical study of cannabidiol as a secondary anticonvulsant*. Marijuana '90 International Conference on Cannabis and Cannabinoids (p. 5). Kolympari, Crete: International Association for Cannabinoid Medicines.

Turkanis, S. A., Cely, W., Olsen, D. M., & Karler, R. (1974). Anticonvulsant properties of cannabidiol. *Research Communication in Chemical Pathology and Pharmacology*, *8*, 231–246.

Zhornitsky, S., & Potvin, S. (2012). Cannabidiol in humans—the quest for therapeutic targets. *Pharmaceuticals*, *5*, 529–552.

Zuardi, A. W. (2008). Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. *Revista Brasileira de Psiquiatria*, *30*, 271–280.

Zuurman, L., Roy, C., Schoemaker, R. C., Amatsaleh, A., Guimaeres, L., Pinquier, J. L., Cohen, A. F., & van Gerven, J. M. (2010). Inhibition of THC-induced effects on the central nervous system and heart rate by a novel CB1 receptor antagonist AVE1625. *Journal of Psychopharmacology*, *24*, 363–371.

Zuurman, L., Roy, C., Schoemaker, R. C., Hazekamp, A., den Hartigh, J., Bender, J. C., Verpoorte, R., Pinquier, J. L., Cohen, A. F., & van Gerven, J. M. (2008). Effect of intrapulmonary tetrahydrocannabinol administration in humans. *Journal of Psychopharmacology*, *22*, 707–716.

CHAPTER

83

Cannabidiol and Neuroprotection: Evidence from Preclinical Studies

*N. Schröder**, *V.K. da Silva**, *J.E.C. Hallak***, *A.W. Zuardi***, *J.A. de Souza Crippa****

Neurobiology and Developmental Biology Laboratory, Faculty of Biosciences, Pontifical Catholic University, Porto Alegre, Rio Grande do Sul, Brazil

****Department of Neuroscience and Behavior, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Sao Paulo, Brazil**

SUMMARY POINTS

- This chapter focuses on the potential use of cannabidiol (CBD), one of the chemical compounds found in *Cannabis sativa*, as a neuroprotective agent, for the treatment of neurodegenerative disorders.
- Evidence from preclinical studies indicates that CBD displays antioxidant, antiinflammatory, and antiapoptotic properties.
- CBD was also shown to hinder excitotoxicity, and protect mitochondria against mitochondrial toxins.
- Evidence supports the view that CBD may increase progenitor cell proliferation and neurogenesis.
- CBD was shown to improve and ameliorate damage observed in animal models of neurodegeneration associated to hypoxic-ischemic brain injury, multiple sclerosis, brain iron overload, Alzheimer's, Parkinson's, and Huntington's disease.
- Since neurodegeneration is considered a multifactorial process, and CBD acts on multiple pharmacological targets implicated on neurodegeneration, CBD may constitute a promising therapeutic agent for the prevention/treatment of neurodegenerative disorders.

KEY FACTS OF HYPOXIA-ISCHEMIA

- Hypoxia-ischemia (HI) encephalopathy results from stroke, which is a focal disruption of blood supply to a part of the brain, or from global ischemia, which can affect the entire brain.
- HI encephalopathy may also result from perinatal asphyxia in neonates, and may cause long-term neurologic sequelae or death.
- Physiopathology of HI includes energy failure due to reductions in cerebral blood flow and oxygen.
- Energy failure may lead to excitotoxic damage.
- Oxidative stress, mitochondrial failure, and apoptotic cell death are also associated to HI.

KEY FACTS OF ALZHEIMER'S DISEASE

- Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder, affecting 7–10% of individuals over 65 years of age, and 50–60% of people over 85 years of age.
- Features include memory loss, followed by a progressive deterioration of other domains of cognitive functioning.
- Degeneration affects primarily the hippocampus and the neocortex.
- Neuropathological hallmarks of AD are neuritic plaques and neurofibrilary tangles.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00095-8
Copyright © 2017 Elsevier Inc. All rights reserved.

- Neurofibrillary tangles are filamentous inclusions, formed of hyperphosphorylated tau.
- Neuritic plaques are extracellular deposits of aggregated amyloid-β peptide, which results from aberrant cleavage of amyloid precursor protein.

KEY FACTS OF IRON INVOLVEMENT IN NEURODEGENERATIVE DISORDERS

- Iron is the most abundant metal in the human brain.
- It progressively accumulates in selective brain regions affected by neurodegenerative disorders (NDD) such as PD, AD, among others.
- Due to its redox states, iron can catalyze free radical formation, leading to oxidative stress.
- Iron accumulation in the hippocampus and the basal ganglia has been related to impairments in spatial, aversive, and recognition memory in rodents.
- In humans, performance in cognitive tests negatively correlates with iron deposition, assessed by resonance magnetic imaging, in brain regions such as the hippocampus, cortical areas, and basal ganglia.

LIST OF ABBREVIATIONS

AD	Alzheimer's disease
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
APP	Amyloid precursor protein
BV2	Immortalized murine microglial cell line
CA1	Cornu ammonis area 1
CB1	Cannabinoid receptor type 1
CB2	Cannabinoid receptor type 2
CBD	Cannabidiol
COX2	Cyclooxygenase-2
Cu,Zn SOD	Copper and zinc superoxide dismutase
CX3CR1	Chemokine (C-X3-C motif) receptor 1
DNA	Deoxyribonucleic acid
DNM1L	Dynamin-1-like protein
FCCP	Carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone
GABA	γ-Aminobutyric acid
GSK3β	Glycogen synthase kinase 3 beta
HD	Huntington's disease
HI	Hypoxic-ischemic
5HT1A	5-Hydroxytryptamine (serotonin) receptor 1A
iNOS	Inducible oxide nitric synthase
mRNA	Messenger ribonucleic acid
MS	Multiple sclerosis
NDD	Neurodegenerative disorders
NMDA	*N*-Methyl-D-aspartate
3NP	3-Nitropropionic acid
NO	Nitric oxide
6-OHDA	6-Hydroxydopamine
PC12	Pheocromocytoma cells
PD	Parkinson's disease
PPARγ	Peroxisome proliferator-activated receptor-γ
PS1	Presenilin-1
ROS	Reactive oxygen species
S100B	S100 calcium binding protein B
SHSY5Y	Human neuroblastoma cells type
SOD	Superoxide dismutase
THC	Δ^9-Tetrahydrocannabinol
TNFα	Tumor necrosis factor alpha

INTRODUCTION

There are two main neuroactive components in *Cannabis sativa*: the psychoactive Δ^9-Tetrahydrocannabinol (THC), and the nonpsychoactive Cannabidiol (CBD). CBD was firstly isolated during late 1930s, but its structure was only elucidated by Mechoulam and Shvo (1963). Nowadays, many researchers are studying this compound, and some of its pharmacological effects are already reported, including antiinflammatory, antioxidative, anxiolytic, antipsychotic, and neuroprotective effects, although its mechanisms of action are not completely elucidated (Zuardi, 2008). In view of these effects, CBD has been considered as an interesting compound with potential therapeutic application in a number of neurological and neuropsychiatric disorders. Studies have been conducted to verify the effects of CBD in nervous system pathologies. In recent years, preclinical in vitro and in vivo studies have investigated the potential of CBD in experimental models, focusing mainly on neurodegeneration associated to hypoxic-ischemic (HI) injury and Alzheimer's disease (AD). The purpose of this chapter is to provide a comprehensive overview on the main studies reporting the effects of CBD in the context of HI injury and AD, as well as other relevant experimental models of neurodegeneration, and to discuss its putative mechanisms. Studies in which CBD was used in combination with other cannabinoid compounds were not included in this chapter.

EXCITOTOXICITY AND HYPOXIC-ISCHEMIC BRAIN INJURY

Hampson, Grimaldi, Axelrod, and Wink (1998) analyzed the effects of CBD in rat cortical neuron cultures exposed to glutamatergic excitotoxicity. CBD was able to block *N*-methyl-D-aspartate receptor (NMDAR) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor-mediated neurotoxicity, and to protect neurons against reactive oxygen species (ROS)-

induced cell death. The results also indicated that the effects of CBD were independent of cannabinoid receptors. From these results, the authors proposed that CBD could be a useful therapeutic agent for the treatment of neurological disorders associated to excitotoxicity and oxidative stress, such as cerebral ischemia. Therapeutic options to treating HI brain injury are very limited, and in vitro and in vivo preclinical studies indicate that CBD would be a candidate to be tested to treat this condition. Accordingly, Castillo, Tolón, Fernández-Ruiz, Romero, and Martinez-Orgado (2010) investigated the effects of CBD in HI immature brain of newborn mice, as a model of neonatal HI. Forebrain slices from newborn mice underwent glucose and oxygen deprivation, and the effects of CBD on acute and apoptotic cell death, excitotoxicity, inflammatory markers, and inducible oxide nitric synthase (iNOS) expression were evaluated. By using selective antagonists, they also analyzed cannabinoid receptors CB1 and CB2, and adenosine A_{1A} and A_{2A} receptors in mediating these effects. They observed that CBD was able to reduce acute and apoptotic damage in immature brain slices by reducing glutamate and interleukin-6 concentration, and tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (COX-2), and iNOS expression. These effects were reversed by CB2 and A_{2A} antagonists, suggesting that CBD-induced neuroprotection in HI cell death was mediated by CB2 and adenosine receptors.

Hayakawa et al. (2007) examined the neuroprotective effects of CBD, using a 4 h middle cerebral artery occlusion model of ischemia in mice. Results indicated that both pre- and postischemic treatment with CBD decreased infarct volume, reversed the reduction in cerebral blood flow, and increases in myeloperoxidase activity and number of myeloperoxidase positive cells, which are markers of neutrophil response, induced by ischemia. Thus, this study provided evidence that CBD conferred long-lasting neuroprotection, through an antiinflammatory mechanism against ischemic brain damage. The effect of CBD on behavioral end-point of neuroprotection was assessed in the rota-rod test for motor coordination, showing that CBD was able to significantly improve motor coordination in mice submitted to ischemia.

The effects of CBD were also verified in newborn piglets submitted to acute HI (Lafuente et al., 2011). In this study, the authors found that CBD recovered brain activity, assessed by amplitude-integrated electroencephalography, reduced neuronal damage and astrocytosis in the cortex, decreased the percentage of TNF-α positive cells, and normalized general neurobehavioral changes induced by ischemia. CBD administration displayed a neuroprotective effect, which included both neurons and astrocytes, possibly mediated by an antiinflammatory effect in the newborn brain. Pazos et al. (2013) have also analyzed the effects of CBD on HI brain injury using a HI model in newborn pigs. CBD was able to prevent all the alterations induced by HI, increasing the number of viable neurons, recovering brain activity, reducing excitotoxicity assessed by glutamate/*N*-acetylaspartate ratio, oxidative stress assessed by glutathione/creatine ratio and protein carbonylation, and inflammation assessed by brain levels of interleukin-10. The effects of CBD were reversed by CB2 and $5HT_{1A}$ antagonists, suggesting that these receptors are implicated in CBD's neuroprotective profile.

The same group has also analyzed the effects of CBD on neurobehavioral parameters in rats, 30 days after HI injury. Once again, neuroprotective effects of CBD were associated to reductions in excitotoxicity, oxidative stress, and inflammation. Neuroprotection was further confirmed by magnetic resonance imaging and histological evaluation to measure the extension of brain damage. At the functional level, results showed that CBD improved motor and coordination deficits tested in the rota-rod, and short-term object recognition memory (Pazos et al., 2012).

Neuroprotection against excitotoxic insult was also investigated using a diverse model of glutamatergic excitotoxicity in retinal neurons (El-Remessy et al., 2003). Intravitreal injection of NMDA in rats induced a dose- and time-dependent accumulation of nitrite/nitrate, lipid peroxidation, and nitrotyrosine (mark of peroxynitrite), and a dose-dependent apoptosis and loss of inner retinal neurons. CBD injected immediately before the intravitreal injection of NMDA significantly attenuated NMDA-induced tyrosine nitration and apoptosis. Given the involvement of excitotoxicity in retinal cell death observed in glaucoma, CBD has been proposed as a candidate therapy for its treatment.

NEURODEGENERATIVE DISORDERS

Neurodegenerative disorders (NDD) are characterized by progressive loss of neurons in selected areas of the nervous system, which determine their clinical presentation. Although the etiology of NDD is not completely elucidated, there is a general consensus that they are multifactorial diseases that involve oxidative stress, iron accumulation, mitochondrial dysfunction leading to energetic and subsequent synaptic failure, protein misfolding, and formation of protein aggregates, neuroinflammation, excitotoxicity, and apoptosis (Duncan, 2011; Li, O, Li, Jiang, & Ghanbari, 2013).

In spite of tremendous effort put into the investigation of therapeutic agents to treat NDD, no effective disease-modifying drugs that can interrupt the progressive course of the disease are currently available. Thus, due to the already characterized pharmacological properties of CBD, this compound may be promising for the treatment of NDD.

Alzheimer's Disease

In order to investigate cellular and molecular alterations related to AD, many in vitro studies, in which cells are exposed to amyloid-β peptide, have been performed. Iuvone et al. (2004) investigated the effects of CBD in reversing amyloid-β peptide-induced cell death in cultured rat pheocromocytoma PC12 cells. Pretreatment with CBD significantly increased cell survival, and decreased ROS production, lipid peroxidation, as well as the active form of caspase 3 (a key enzyme in the effector phase of apoptosis) appearance, DNA fragmentation, and reversed increases in intracellular calcium induced by amyloid-β exposure. Those results suggested that antioxidant and antiapoptotic properties may underlie CBD-mediated neuroprotection against amyloid-β toxicity. Another study using PC12 neuronal cells reported that CBD inhibits hyperphosphorylation of tau in amyloid-β-stimulated cells. CBD dose-dependently reversed amyloid-β-induced glicogen synthase kinase 3β (GSK-3β) phosphorylation, and reduction in the expression of β-catenin, suggesting that the effects of CBD in rescuing amyloid-β-challenged PC12 cells are mediated through the Wnt/β-catenin pathway (Esposito, De Filippis, Carnuccio, Izzo, & Iuvone, 2006). In the same line, recently, Janefjord, Måag, Harvey, and Smid (2014) have shown that CBD was able to protect SH-SY5Y cells against amyloid-β_{1-42} neurotoxicity.

Esposito et al. (2011) investigated the effects of CBD on the release of inflammatory mediators induced by amyloid-β challenge in rat primary astroglial cultures. CBD antagonized the enhanced release of the proinflammatory molecules, nitric oxide (NO), interleukin-1β, TNFα, and S100B (S100 calcium binding protein B), and the blockade of peroxisome proliferator-activated receptor-γ (PPARγ) reversed this effect. In the same study, the authors stereotaxically inoculated human amyloid-β into the hippocampus of adult rats that were subsequently treated with chronic CBD. CBD rescued CA1 pyramidal neurons integrity, and downregulated reactive gliosis, as shown by a significant decrease of glial fibrillary acidic protein immunostaining. PPARγ antagonism completely abolished CBD neuroprotective effects. Remarkably, CBD restored neurogenesis in dentate gyrus of amyloid-β injected rat hippocampi through PPARγ activation. These findings provide evidence that the neuroprotective effects of CBD against AD neuroinflammation may be related to PPARγ activation. Along this line, the effects of CBD in a cellular model of AD were investigated using $SHSY5Y^{APP+}$ cells (Scuderi, Steardo, & Esposito, 2013). CBD induced the ubiquitination of amyloid precursor protein (APP), with subsequent reduction of amyloid-β production. In addition, CBD was able to reduce apoptosis, increasing survival of $SHSY5Y^{APP+}$ neurons. Results also indicated that these effects were related to selective activation of PPARγ. PPARs have been recently related to AD (Kitamura et al., 1999), and studies have proposed that CBD translocates into the nucleus, thereby interacting with PPARs (O'Sullivan, Sun, Bennett, Randall, & Kendall, 2009).

Martín-Moreno et al. (2011) investigated the effects of CBD in primary rat microglial cultures. CBD promoted microglial cell migration, and decreased NO generation in the culture media of lipopolysaccharides-stimulated microglia. Migration of microglial cells that may subserve a beneficial function as a requisite for phagocytosing aggregated amyloid-β. They also demonstrated that CBD could rescue spatial memory deficits, tested in the Morris water maze, in amyloid-β-injected mice, and to significantly reduce interleukin-6 mRNA expression, which was markedly increased by amyloid-β injection.

Cheng, Low, Logge, Garner, and Karl (2014) analyzed the effects of chronic CBD in a transgenic mouse model of AD. They used APP/PS1 mutant mice and these animals underwent a behavioral test battery. CBD reversed deficits in social recognition and object recognition displayed by APP/PS1 mice, without affecting fear-associated memory and anxiety-related behavior.

While in vitro and ex vivo studies are useful in the search of cellular and molecular mechanisms associated to CBD's neuroprotective actions, in vivo behavioral studies are essential to identify the potential of this drug in reversing neurofunctional deficits associated with the disease. Table 83.1 summarizes findings from the main behavioral studies investigating the effects of CBD in the context of preclinical models of NDD.

Parkinson's Disease

Parkinson's disease (PD) is the second most common neurodegenerative disease, characterized by a selective degeneration of dopaminergic neurons of the substantia nigra pars compacta, that leads to motor deficits, including tremor, rigidity, bradykinesia, and postural instability (Kim, Ko, Dawson, & Dawson, 2005). The effects of CBD were investigated in the 6-hydroxy-dopamine (6-OHDA) rat model of PD (García-Arencibia et al., 2007). Results showed that CBD administration immediately after the lesion, but not 1 week later, was able to recover 6-OHDA-induced dopamine depletion, and upregulate mRNA levels for Cu,Zn-superoxide dismutase (SOD), a key enzyme in endogenous defenses against oxidative stress, and a representative parameter of the degree of neuronal injury caused by oxidative stress in PD.

Although only a few studies investigated the effects of CBD in preclinical models, CBD was already tested in patients suffering from PD. Zuardi et al. (2009) investigated safety, tolerability, and efficacy of CBD on PD patients. In this study they observed that CBD was able to decrease psychotic symptoms scores evaluated

TABLE 83.1 Summary of Studies Testing the Effects of Cannabidiol (CBD) on Behavioral Parameters

Experimental Model	Regimen of Treatment	Effects	Receptor(s) Involved	References
Ischemic mouse	Pre/postischemia, 1 or 3 mg/kg, intraperitoneally	Improved motor coordination	Not investigated	Hayakawa et al. (2007)
Neonatal hypoxic-ischemic rat	Acute (10 min after ischemia), 1 mg/kg, subcutaneous	Improved motor coordination deficits and short-term recognition memory	Not investigated	Pazos et al. (2012)
Mouse model of Alzheimer's disease (hippocampal amyloid-β injection)	Chronic, 24 h after procedure (first week: daily injection, second and third weeks: 3 days/week), 20 mg/kg, intraperitoneally	Rescued spatial memory deficits	Not investigated	Martín-Moreno et al. (2011)
Mouse model of Alzheimer's disease (APP/PS1 mutant mice)	Chronic (daily/3 weeks prior behavioral tests and 8 weeks in total duration), 20 mg/kg, intraperitoneally	Reversed social recognition and object recognition deficits	Not investigated	Cheng et al. (2014)
Mouse model of multiple sclerosis	Chronic, after infection (daily/10 days), 5 mg/kg, intraperitoneally	Improved motor deficits	Not investigated	Mecha et al. (2013)
Rat model of cognitive impairment induced by iron overload	Acute and chronic, in adult age (daily/2 weeks), 5 or 10 mg/kg, intraperitoneally	Rescued recognition memory deficits	Not investigated	Fagherazzi et al. (2012)

CBD improved motor or cognitive parameters when administered acute or chronically to rodent models of neurodegenerative disorders or hypoxic-ischemia. APP/PS1, amyloid precursor protein/presenilin-1.

by questionnaires in PD patients, but this compound did not affect any cognitive or motor function. Given these positive results, further studies investigating the neuroprotective effects of CBD in PD are warranted.

Huntington's Disease

Huntington's disease (HD) is an inherited NDD characterized by loss of striatal projection neurons, characterized by chorea, cognitive, and psychiatric disturbances involving the basal ganglia and cerebral cortex. HD has been modeled in rodents by lesioning the striatum with the inhibitor of the mitochondrial complex II, 3-nitropropionic acid (3NP) (Kumar, Kalonia, & Kumar, 2010). Administration of CBD completely reversed 3NP-induced reductions in γ-aminobutyric acid (GABA) contents and mRNA levels for substance P, markers of striatal GABAergic projection neurons, neuronal-specific enolase, and SOD-2. This study demonstrated that CBD provided neuroprotection, mediated by cannabinoid receptor-independent antioxidant properties, against 3NP-induced striatal damage, which may be relevant for HD (Sagredo, Ramos, Decio, Mechoulam, & Fernández-Ruiz, 2007).

Multiple Sclerosis

Multiple sclerosis (MS) is an inflammatory and degenerative disease affecting the central nervous system, characterized by demyelination and axonal degeneration (Messina & Patti, 2014). Mecha et al. (2013) investigated the effects of CBD using a viral model of MS. It was observed that CBD reduced the expression of inflammatory molecules, such as vascular cell adhesion molecule-1, chemokines, and the proinflammatory cytokine interleukin-1β, and attenuated microglia activation. CBD has also improved motor deficits. Some of the anti-inflammatory effects were blocked by an A_{2A} adenosine receptor antagonist. This study highlights the potential of CBD for the treatment of neuropathologies with an inflammatory component, such as MS.

OTHER MODELS OF NEURODEGENERATION

Cognitive Impairment Associated to Brain Iron Overload

Rats and mice treated with iron show severe memory impairment, and our research group has been using this animal model to investigate the involvement of iron in NDD-related cognitive impairment, as well as a tool for the search of compounds with therapeutic potential (for a review, see Schröder, Figueiredo, & de Lima, 2013). We have tested the effects of CBD in reversing cognitive dysfunction associated with brain iron overload (Fagherazzi et al., 2012). Male adult rats, treated with iron, received acute or chronic injections of CBD in adulthood, and were tested in the object recognition task. We showed

that CBD rescued recognition memory deficits in iron-overloaded animals (Fig. 83.1).

Subsequently, we decided to investigate the cellular and molecular mechanisms associated with CBD's ability in restoring the damage caused by iron loading in rats, using the same dose previously shown to ameliorate memory deficits associated with iron loading (da Silva et al., 2014). We analyzed proteins involved in mitochondrial fusion and fission mechanisms, which are crucial processes for mitochondrial recycling and survival of healthy mitochondria, synaptophysin, a transmembrane protein of synaptic vesicles, often regarded as a synaptic marker, and caspase 3, an apoptosis-related protein. Iron overload resulted in mitochondrial dynamics imbalance,

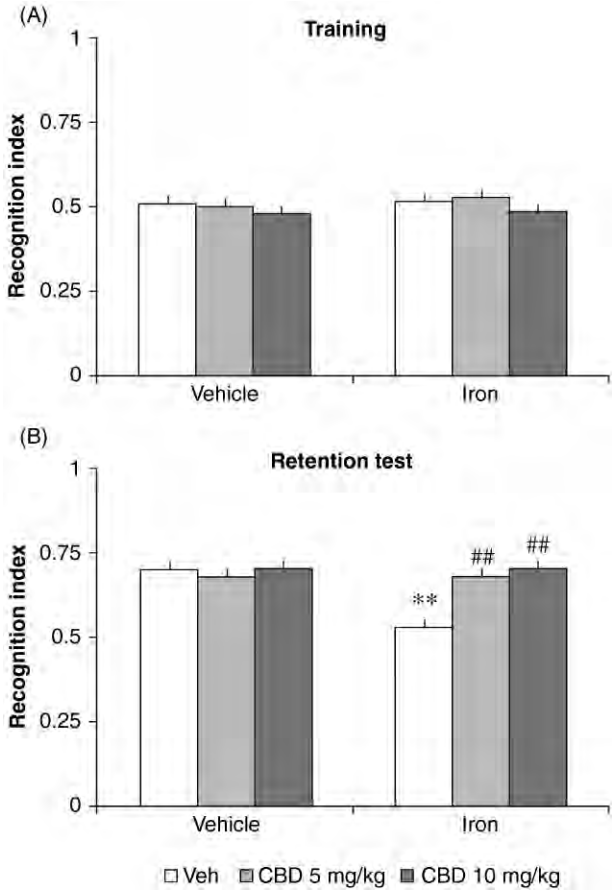


FIGURE 83.1 Effects of chronic CBD on iron-induced recognition memory deficits. Effects of chronic cannabidiol *(CBD)* on iron-induced recognition memory deficits. Recognition indexes in (A) training and (B) retention test session are expressed as mean ± SEM. A daily single injection of vehicle or CBD (5.0 or 10.0 mg/kg) was administered for 14 days. Animals were trained in the object recognition task 24 h after the last injection. N = 12–15 per group. Differences between vehicle–vehicle versus other groups are indicated as $^{**}p < 0.001$; difference between iron–vehicle versus iron–CBD is indicated as $\#\#p < 0.001$. *Adapted from* Springer and Psychopharmacology *(Berlin)*, 219*(4)*, *2012, 1133–1140, "Memory-rescuing effects of cannabidiol in an animal model of cognitive impairment relevant to neurodegenerative disorders." (Fagherazzi et al., 2012, Fig. 3). With kind permission from Springer Science and Business Media.*

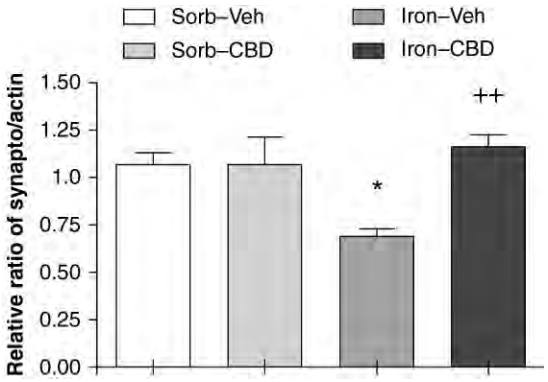


FIGURE 83.2 Effects of chronic CBD on Synaptophysin expression in hippocampus of iron-treated rats. Western blot of synaptophysin in the hippocampus of 3-month-old rats treated with sorbitol *(Sorb)* or iron neonatally, and treated with vehicle *(Veh)* or cannabidiol *(CBD)* chronically; 25 μg of protein, normalized to β-actin, were separated on SDS–PAGE and probed with antisynaptophysin. Statistical analysis was performed using one-way ANOVA followed by Tukey's HSD posthoc. Data expressed as mean ± SEM. N = 4–6/group. $^{*}p < 0.05$, differences between Sorb–Veh versus iron–Veh; $^{++}p < 0.01$, difference between iron–Veh versus iron–CBD. *Adapted from* Springer and Molecular Neurobiology, 49*(1)*, *2014, 222–233, Cannabidiol normalizes caspase 3, synaptophysin, and mitochondrial fission protein DNM1L expression levels in rats with brain iron overload: implications for neuroprotection (da Silva et al., 2014, Fig. 5a). With kind permission from Springer Science and Business Media.*

possibly triggering synaptic loss, expressed as a reduction of synaptophysin levels, and apoptotic cell death, related to increased caspase 3 levels. We found that CBD rescued iron-induced effects, bringing hippocampal synaptophysin levels (Fig. 83.2) and caspase 3 (Fig. 83.3) back to values comparable to the control group. We also showed that CBD was able to restore hippocampal levels of mitochondrial fusion protein, dynamin 1-like (DNM1L) in iron-treated rats. Altogether, our results suggest that CBD should be considered as a potential molecule with memory-rescuing and neuroprotective properties to be used in the treatment of cognitive deficits observed in NDD.

The neuroprotective potential of CBD was evaluated in an animal model of sciatic nerve transection in neonatal rats (Perez et al., 2013). In neonatal rats, transection of a peripheral nerve leads to an intense retrograde degeneration of both motor and sensory neurons, which is prominently related to neuronal loss resulting of apoptotic processes. Neuronal counting revealed both motor and sensory neuron rescue following treatment with CBD. Administration of CBD decreased the astroglial and microglial reaction, and number of apoptotic cells, mostly located in the spinal cord intermediate zone. CBD also induced synaptic preservation within the spinal cord, revealed by synaptophysin staining.

Recently, the effects of transdermal delivery CBD against alcohol-induced neurodegeneration were studied in a rodent model of alcohol use disorder

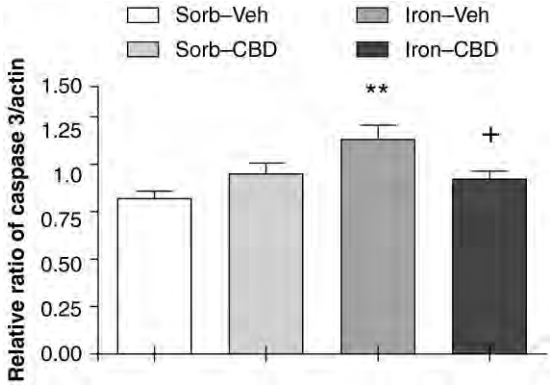


FIGURE 83.3 Effects of chronic CBD on caspase 3 expression in hippocampus of iron-treated rats. Western blot of caspase 3 in the hippocampus of 3-month-old rats treated with sorbitol *(Sorb)* or iron neonatally, and treated with vehicle *(Veh)* or cannabidiol *(CBD)* chronically; 25 μg of protein, normalized to β-actin, were separated on SDS–PAGE and probed with anticaspase 3. Statistical analysis was performed using one-way ANOVA followed by Tukey's HSD posthoc. Data expressed as mean ± SEM. $N = 4–6$/group. $^{**}p < 0.01$, differences between Sorb–Veh versus other groups; $+p < 0.05$, difference between Iron–Veh versus Iron–CBD. *Adapted from* Springer and Molecular Neurobiology, *49(1), 2014, 222–233, Cannabidiol normalizes caspase 3, synaptophysin, and mitochondrial fission protein DNM1L expression levels in rats with brain iron overload: implications for neuroprotection (da Silva et al., 2014; Fig. 7a). With kind permission from Springer Science and Business Media.*

(Liput, Hammell, Stinchcomb, & Nixon, 2013). Ethanol exposure resulted in neurodegeneration as indicated by the presence of Fluoro-Jade B positive cells along the entorhinal cortex. Treatment with CBD by either intraperitoneal injection or a second generation transdermal CBD gel resulted in a reduction in Fluoro-Jade B positive cells in the entorhinal cortex following binge ethanol treatment. Although the mechanisms involved in protection against alcohol-induced neurodegeneration are not clear, previous studies had indicated that impairment in mitochondrial function, and subsequent oxidative stress, are likely causal factors contributing to alcohol-induced neurodegeneration. It is possible that CBD attenuates oxidative stress caused by impairments in the mitochondrial electron transport chain.

SUMMARY OF THE MECHANISMS OF ACTION OF CBD

Using in vitro and ex vivo models, it is possible to investigate the molecular and cellular pathways targeted by CBD that mediate its neuroprotective effects. Some studies aimed to elucidate whether CBD's effects were related to its interaction with membrane receptors. Thus, the possibility of blocking CBD's effects by using cannabinoid receptor, adenosine receptor, and serotonin receptor antagonists was investigated. Remarkably, many studies have reported effects of CBD in specific intracellular pathways, which are probably receptor-independent effects. Table 83.2 presents a summary of the in vivo studies investigating the neuroprotective effects of CBD, indicating doses and regimen of treatment, and dependence on membrane receptors, when applicable.

Many studies report the antioxidant properties of CBD in brain tissue or neural cell cultures, using different molecular markers of oxidative stress, such as glutathione/creatine ratio, reduction of oxidative damage to proteins and lipids, assessed by levels of protein carbonylation and lipid peroxidation, respectively, ROS production, and upregulation of enzymatic antioxidant defense, such as SOD. The chemical structure of CBD, including a resorcinol, confers potent antioxidant effects (Mechoulan, Peters, Murillo-Rodriguez, & Hanus, 2007). CBD is also well known for its antiinflammatory actions. In vitro and in vivo studies have shown that CBD reduces numerous inflammatory markers, including cytokines, such as interleukin-6, TNF-α, and interleukin-1β, chemokines, and vascular cell adhesion molecule-1. Reductions in enzymes related to the inflammatory pathway, such as COX-2, and NOS, as well as attenuation of microglia activation were also demonstrated. Excitotoxicity and apoptotic cell death were also demonstrated to be hindered by CBD.

To characterize the transcriptional effects of CBD, Juknat et al. (2012) treated BV-2 microglial cells with CBD, and performed microarray analysis. They identified 1204 differentially expressed genes in response to CBD treatment, which were associated with many different functions, including stress, inflammatory response, membrane transport, adhesion and migration, cell cycle, and proliferation. CBD repressed the expression of an important subset of proinflammatory genes, especially the chemokine ligands, and the chemokine receptor CX-3CR1.

Intracellularly CBD was shown to interact with the GSK-3β pathway, thereby inhibiting hyperphosphorylation of tau, and also to activate nuclear receptors of the PPAR family. Increasing evidence also supports the view that CBD may have protective effects against mitochondrial damage. CBD restored hippocampal levels of a mitochondrial fusion protein, DNM1L, in iron-loaded rats, and increased the activity of mitochondrial complexes (I, II, II–III, and IV) in the rat brain, raising the possibility that CBD modulates brain energy production (Valvassori et al., 2013). CBD was shown to protect SH-SY5Y or cultured hippocampal neurons against the mitochondrial toxin FCCP (carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone) (Ryan, Drysdale, Lafourcade, Pertwee, & Platt, 2009).

Interestingly, Campos et al. (2013) have shown that CBD was able to significantly increase hippocampal progenitor proliferation and neurogenesis, in mice submitted to chronic unpredictable stress. Experiments

TABLE 83.2 Summary of In Vivo/Ex Vivo Studies Testing Cellular and Molecular Effects of CBD.

Experimental Model	Regimen of Treatment (Effective Dose)	Effects	Receptor(s) Involved	References
Ischemic mouse	Doses injection pre/postischemia, 1 or 3 mg/kg, intraperitoneally	⇩ Infarct volume, ⇧ myeloperoxidase activity	Cannabinoid receptor type 1 and 2-independent	Hayakawa et al. (2007)
Neonatal hypoxic-ischemic pig	Two doses (15/240 min after ischemia), 0.1 mg/kg, intravenous	⇧ Brain activity, ⇩ neuronal damage/astrocytosis in the cortex, ⇩ tumor necrosis factor α positive cells	Not investigated	Lafuente et al. (2011)
Neonatal hypoxic-ischemic pig	Acute (30 min after ischemia), 1 mg/kg, intravenous	⇧ Number of viable neurons, ⇧ brain activity, ⇩ excitotoxicity, ⇩ Oxidative stress, ⇩ Inflammation	Effects dependent on cannabinoid receptor type 2 and serotonin receptor 1A	Pazos et al. (2013)
Neonatal hypoxic-ischemic rat	Acute (10 min after ischemia), 1 mg/kg, subcutaneous	⇩ Excitotoxicity, ⇩ oxidative stress, ⇩ inflammation, ⇩ brain damage	Not investigated	Pazos et al. (2012)
Retinal neurotoxicity in rat	Acute before induced excitotoxicity, 2 mg/kg, intravenous	⇩ Nitrotyrosine formation, ⇩ apoptosis	Not investigated	El-Remessy et al. (2003)
Rat model of Alzheimer's disease (hippocampal amyloid-β injection)	Chronic, after surgery (daily/15 days), 10 mg/kg, intraperitoneally	⇧ Neurons integrity, ⇩ reactive gliosis, ⇧ neurogenesis	Not investigated	Esposito et al. (2011)
Mouse model of Alzheimer's disease (hippocampal amyloid-β injection)	Chronic, 24 h after procedure (first week: daily injection, second and third weeks: 3 days/week), 20 mg/kg, intraperitoneally	⇩ Interleukin 6 expression	Not investigated	Martín-Moreno et al. (2011)
Rat model of Parkinson's disease (6-OHDA)	Chronic, 16 h after procedure (daily/2 weeks) 3 mg/kg, intraperitoneally	⇧ Dopamine, ⇧ levels of Cu,Zn-superoxide dismutase	Cannabinoid receptor-independent	García-Arencibia et al. (2007)
Rat model of Huntington's disease	Chronic, 4 h after first procedure (daily/5 days), 5 mg/kg, intraperitoneally	⇩ Striatal degeneration	Effects dependent on cannabinoid receptor type 1/transient receptor potential vanilloid 1/ adenosine A_{2A}-receptor-independent	Sagredo et al. (2007)
Mouse model of multiple sclerosis	Chronic, after infection (daily/10 days), 5 mg/kg, intraperitonealy	⇩ Expression of inflammatory molecules, ⇩ microglia activation	Effects dependent on A_{2A} adenosine receptor	Mecha et al. (2013)
Rat model of cognitive impairment induced by iron overload	Chronic in adult age (daily/2 weeks), 10 mg/kg, intraperitoneally	⇩ Hippocampal caspase 3, ⇧ hippocampal synaptophysin, ⇧ hippocampal dynamin-1-like protein	Not investigated	da Silva et al. (2014)
Rat model of sciatic nerve transection	Chronic, after transection (daily/5 days), 15 or 30 mg/kg, intraperitoneally	⇧ Motor and sensory neuron intregality, ⇩ astrogliosis and microgliois, ⇩ apoptotic cells, ⇧ synaptic preservation	Not investigated	Perez et al. (2013)
Rat model of alcoholism	Chronic, after 3rd ethanol dose (transdermal: daily/3 days; intraperitoneally: twice a day/3 days), 2.5 or 5%, transdermal gel; 20 mg/kg, intraperitoneally	⇩ Fluoro-Jade B positive cells in the entorhinal cortex	Not investigated	Liput et al. (2013)
Energetic metabolism in rat	Acute and chronic (daily/2 weeks), 15, 30, or 60 mg/kg, intraperitoneally	⇧ Activity of mitochondrial complexes (I, II, II–III and IV), ⇧ creatine kinase	Not investigated	Valvassori et al. (2013)
Mouse model of chronic unpredictable stress	Chronic, 2 h after daily stress (daily/2 weeks), 30 mg/kg, intraperitoneally	⇧ Neurogenesis	Not investigated	Campos et al. (2013)

Summary of studies designed to investigate cellular and molecular aspects of neuroprotective effects of CBD. CBD displays antiapoptotic, antiinflammatory, and antioxidant properties. CBD also reduces excitotoxicity, reactive astrocytosis, and improves brain activity and neuronal survival, synaptic and mitochondrial parameters, and neurogenesis. (6-OHDA: 6-hydroxydopamine; CBD, cannabidiol.)

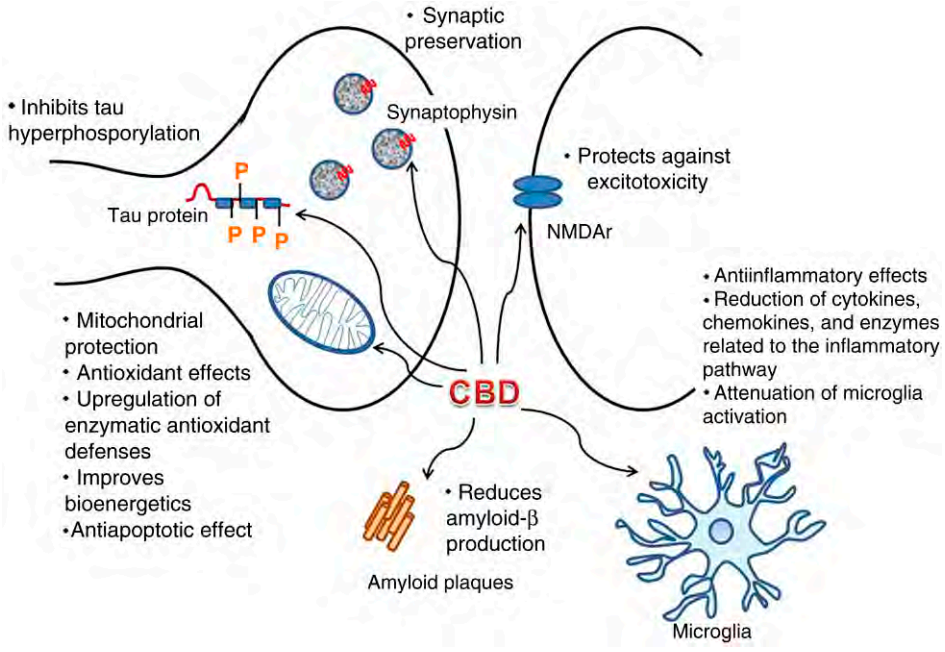


FIGURE 83.4 Summary of the main mechanisms of action of CBD. Using in vitro and ex vivo approaches, studies have shown that cannabidiol *(CBD)* exhibits antioxidant, antiinflammatory, and antiapoptotic effects. CBD was also demonstrated to exert a protective action on mitochondria, thus preserving energy production. CBD prevents synaptic loss, demonstrated by the maintenance of the synaptic marker, synaptophysin. CBD also prevents hyperphosphorylation of tau and amyloid-β production, and protects against excitotoxic cell death. *NMDAr*, NMDA receptor.

conducted with hippocampal progenitor cells in culture showed that CBD promoted progenitor proliferation and cell cycle progression, and mimicked the proliferative effect of CB1 and CB2 cannabinoid receptor activation, supporting the view that CBD may promote neurogenesis in the hippocampus.

Fig. 83.4 summarizes the putative mechanisms of action of CBD.

CONCLUSIONS

Despite the fact that the mechanisms of action of CBD are not fully understood, a great deal of evidence suggests that, by protecting against oxidative damage and neuroinflammation, and possibly by protecting the mitochondria, thereby maintaining energy supply, CBD may provide resistance against synaptic loss, and subsequent cell death. Neurodegeneration is considered a multifactorial process, involving oxidative stress, energetic failure, and synaptic dysfunction that may ultimately culminate in cell death. CBD combines multitarget pharmacological properties that might make it a promising candidate compound for the prevention/treatment of NDD.

Since CBD has been already tested in humans (Consroe et al., 1991; Zuardi et al., 2009) showing safety, with no toxic side effects, and while preclinical tests continue, in order to provide a better understanding of mechanisms, clinical tests with CBD should proceed, perhaps as a multitarget drug for the treatment of NDD.

MINI-DICTIONARY

Excitotoxic damage Damage resulting from excessive glutamate release and membrane depolarization associated to stimulation of NMDA receptors, which mediate an increase in intracellular calcium levels. Calcium activates enzymes involved in the catabolism of proteins, phospholipids, and nucleic acids.

Oxidative stress Imbalance between ROS production and a biological system's ability to remove or repair damage caused by ROS.

Apoptotic cell death A type of programmed cell death, characterized by ordered cellular destruction without inflammatory response, having an important role in development and tissue homeostasis; however, deregulation in this process results in pathological conditions.

Rota-rod Equipment used to evaluate motor behavior, including balance, coordination, and motor learning in rodents, particularly to test experimental drugs' effects, analyze brain damage, and diseases. In this performance test, rodents are placed on a rotating rod with a controlled speed.

Astrocytosis An abnormal increase in the number of astrocytes, in response to all forms of central nervous system injury and disease.

Object recognition Behavioral test used to evaluate the recognition memory in rodents, based on the proportion of time that the animal explores a novel object, when presented together with a familiar object.

PPARs Ligand-activated transcription factors belonging to the nuclear receptor superfamily. They play an essential role in energy homeostasis and other important biological processes, such as inflammation, cell proliferation, and differentiation.

APP/PS1 mutant mice Mice carrying mutations in amyloid precursor protein (APP) and presenilin-1 (PS1) genes, known as a double transgenic AD model.
6-OHDA model of PD 6-hydroxydopamine is a neurotoxic synthetic compound involved in massive destruction of nigrostriatal dopaminergic neurons, widely used to induce parkinsonism in laboratory animals.
Fluoro-Jade B positive cells Fluoro-Jade staining, an anion derived from fluorescein and highly acidic, is a specific marker for neurons that are in the process of degeneration.

References

Campos, A. C., Ortega, Z., Palazuelos, J., Fogaça, M. V., Aguiar, D. C., Díaz-Alonso, J., Ortega-Gutiérrez, S., Vázquez-Villa, H., Moreira, F. A., Guzmán, M., Galve-Roperh, I., & Guimarães, F. S. (2013). The anxiolytic effect of cannabidiol on chronically stressed mice depends on hippocampal neurogenesis: involvement of the endocannabinoid system. *International Journal of Neuropsychopharmacology*, *16*, 1407–1419.

Castillo, A., Tolón, M. R., Fernández-Ruiz, J., Romero, J., & Martinez-Orgado, J. (2010). The neuroprotective effect of cannabidiol in an in vitro model of newborn hypoxic-ischemic brain damage in mice is mediated by CB(2) and adenosine receptors. *Neurobiology of Disease*, *37*, 434–440.

Cheng, D., Low, J. K., Logge, W., Garner, B., & Karl, T. (2014). Chronic cannabidiol treatment improves social and object recognition in double transgenic APPswe/PS1ΔE9 mice. *Psychopharmacology*, *231*, 3009–3017.

Consroe, P., Laguna, J., Allender, J., Snider, S., Stern, L., Sandyk, R., Kennedy, K., & Schram, K. (1991). Controlled clinical trial of cannabidiol in Huntington's disease. *Pharmacology Biochemistry and Behavior*, *40*, 701–708.

da Silva, V. K., de Freitas, B. S., da Silva Dornelles, A., Nery, L. R., Falavigna, L., Ferreira, R. D., Bogo, M. R., Hallak, J. E., Zuardi, A. W., Crippa, J. A., & Schröder, N. (2014). Cannabidiol normalizes caspase 3, synaptophysin, and mitochondrial fission protein DNM1L expression levels in rats with brain iron overload: implications for neuroprotection. *Molecular Neurobiology*, *49*, 222–233.

Duncan, G. W. (2011). The aging brain and neurodegenerative diseases. *Clinics in Geriatric Medicine*, *27*, 629–644.

El-Remessy, A. B., Khalil, I. E., Matragoon, S., Abou-Mohamed, G., Tsai, N. J., Roon, P., Caldwell, R. B., Caldwell, R. W., Green, K., & Liou, G. I. (2003). Neuroprotective effect of (−)Delta9-tetrahydrocannabinol and cannabidiol in *N*-methyl-D-aspartate-induced retinal neurotoxicity: involvement of peroxynitrite. *American Journal of Pathology*, *163*, 1997–2008.

Esposito, G., De Filippis, D., Carnuccio, R., Izzo, A. A., & Iuvone, T. (2006). The marijuana component cannabidiol inhibits beta-amyloid-induced tau protein hyperphosphorylation through Wnt/beta-catenin pathway rescue in PC12 cells. *Journal of Molecular Medicine*, *84*, 253–258.

Esposito, G., Scuderi, C., Valenza, M., Togna, G. I., Latina, V., De Filippis, D., Cipriano, M., Carratù, M. R., Iuvone, T., & Steardo, L. (2011). Cannabidiol reduces Aβ-induced neuroinflammation and promotes hippocampal neurogenesis through PPARγ involvement. *PLoS One*, *6*(12), e28668.

Fagherazzi, E. V., Garcia, V. A., Maurmann, N., Bervanger, T., Halmenschlager, L. H., Busato, S. B., Hallak, J. E., Zuardi, A. W., Crippa, J. A., & Schröder, N. (2012). Memory-rescuing effects of cannabidiol in an animal model of cognitive impairment relevant to neurodegenerative disorders. *Psychopharmacology*, *219*, 1133–1140.

García-Arencibia, M., González, S., de Lago, E., Ramos, J. A., Mechoulam, R., & Fernández-Ruiz, J. (2007). Evaluation of the neuroprotective effect of cannabinoids in a rat model of Parkinson's disease: importance of antioxidant and cannabinoid receptor-independent properties. *Brain Research*, *1134*, 162–170.

Hampson, A. J., Grimaldi, M., Axelrod, J., & Wink, D. (1998). Cannabidiol and (−)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 8268–8273.

Hayakawa, K., Mishima, K., Nozako, M., Hazekawa, M., Irie, K., Fujioka, M., Orito, K., Abe, K., Hasebe, N., Egashira, N., Iwasaki, K., & Fujiwara, M. (2007). Delayed treatment with cannabidiol has a cerebroprotective action via a cannabinoid receptor-independent myeloperoxidase-inhibiting mechanism. *Journal of Neurochemistry*, *102*, 1488–1496.

Iuvone, T., Esposito, G., Esposito, R., Santamaria, R., Di Rosa, M., & Izzo, A. A. (2004). Neuroprotective effect of cannabidiol, a non-psychoactive component from Cannabis sativa, on beta-amyloid-induced toxicity in PC12 cells. *Journal of Neurochemistry*, *89*, 134–141.

Janefjord, E., Mååg, J. L., Harvey, B. S., & Smid, S. D. (2014). Cannabinoid effects on β amyloid fibril and aggregate formation, neuronal and microglial-activated neurotoxicity in vitro. *Cellular and Molecular Neurobiology*, *34*, 31–42.

Juknat, A., Pietr, M., Kozela, E., Rimmerman, N., Levy, R., Coppola, G., Geschwind, D., & Vogel, Z. (2012). Differential transcriptional profiles mediated by exposure to the cannabinoids cannabidiol and Δ9-tetrahydrocannabinol in BV-2 microglial cells. *British Journal of Pharmacology*, *165*, 2512–2528.

Kim, S. W., Ko, H. S., Dawson, V. L., & Dawson, T. M. (2005). Recent advances in our understanding of Parkinson's disease. *Drug Discovery Today*, *2*, 427–433.

Kitamura, Y., Shimohama, S., Koike, H., Kakimura, J., Matsuoka, Y., Nomura, Y., Gebicke-Haerter, P. J., & Taniguchi, T. (1999). Increased expression of cyclooxygenases and peroxisome proliferator-activated receptor-gamma in Alzheimer's disease brains. *Biochemical and Biophysical Research Communications*, *254*, 582–586.

Kumar, P., Kalonia, H., & Kumar, A. (2010). Huntington's disease: pathogenesis to animal models. *Pharmacological Reports*, *62*, 1–14.

Lafuente, H., Alvarez, F. J., Pazos, M. R., Alvarez, A., Rey-Santano, M. C., Mielgo, V., Murgia-Esteve, X., Hilario, E., & Martinez-Orgado, J. (2011). Cannabidiol reduces brain damage and improves functional recovery after acute hypoxia-ischemia in newborn pigs. *Pediatric Research*, *70*, 272–277.

Li, J., O, W., Li, W., Jiang, Z. G., & Ghanbari, H. A. (2013). Oxidative stress and neurodegenerative disorders. *International Journal of Molecular Sciences*, *14*, 24438–24475.

Liput, D. J., Hammell, D. C., Stinchcomb, A. L., & Nixon, K. (2013). Transdermal delivery of cannabidiol attenuates binge alcohol-induced neurodegeneration in a rodent model of an alcohol use disorder. *Pharmacology Biochemistry and Behavior*, *111*, 120–127.

Martín-Moreno, A. M., Reigada, D., Ramírez, B. G., Mechoulam, R., Innamorato, N., Cuadrado, A., & de Ceballos, M. L. (2011). Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: relevance to Alzheimer's disease. *Molecular Pharmacology*, *79*, 964–973.

Mecha, M., Feliú, A., Iñigo, P. M., Mestre, L., Carrillo-Salinas, F. J., & Guaza, C. (2013). Cannabidiol provides long-lasting protection against the deleterious effects of inflammation in a viral model of multiple sclerosis: a role for A2A receptors. *Neurobiology of Disease*, *59*, 141–150.

Mechoulam, R., Peters, M., Murillo-Rodriguez, E., & Hanus, L. O. (2007). Cannabidiol-recent advances. *Chemistry and Biodiversity*, *4*(8), 1678–1692.

Mechoulam, R., & Shvo, Y. (1963). Hashish. I. The structure of cannabidiol. *Tetrahedron*, *19*(12), 2073–2078.

Messina, S., & Patti, F. (2014). Gray matters in multiple sclerosis: cognitive impairment and structural MRI. *Multiple Sclerosis International*, *2014*, 609694.

O'Sullivan, S. E., Sun, Y., Bennett, A. J., Randall, M. D., & Kendall, D. A. (2009). Time-dependent vascular actions of cannabidiol in the rat aorta. *European Journal of Pharmacology*, *612*, 61–68.

Pazos, M. R., Cinquina, V., Gómez, A., Layunta, R., Santos, M., Fernández-Ruiz, J., & Martínez-Orgado, J. (2012). Cannabidiol administration after hypoxia-ischemia to newborn rats reduces long-term brain injury and restores neurobehavioral function. *Neuropharmacology*, *63*, 776–783.

Pazos, M. R., Mohammed, N., Lafuente, H., Santos, M., Martínez-Pinilla, E., Moreno, E., Valdizan, E., Romero, J., Pazos, A., Franco, R., Hillard, C. J., Alvarez, F. J., & Martínez-Orgado, J. (2013). Mechanisms of cannabidiol neuroprotection in hypoxic-ischemic newborn pigs: role of 5HT(1A) and CB2 receptors. *Neuropharmacology*, *71*, 282–291.

Perez, M., Benitez, S. U., Cartarozzi, L. P., Del Bel, E., Guimarães, F. S., & Oliveira, A. L. (2013). Neuroprotection and reduction of glial reaction by cannabidiol treatment after sciatic nerve transection in neonatal rats. *European Journal of Neuroscience*, *38*, 3424–3434.

Ryan, D., Drysdale, A. J., Lafourcade, C., Pertwee, R. G., & Platt, B. (2009). Cannabidiol targets mitochondria to regulate intracellular Ca^{2+} levels. *Journal of Neuroscience*, *29*, 2053–2063.

Sagredo, O., Ramos, J. A., Decio, A., Mechoulam, R., & Fernández-Ruiz, J. (2007). Cannabidiol reduced the striatal atrophy caused 3-nitropropionic acid in vivo by mechanisms independent of the activation of cannabinoid, vanilloid TRPV1 and adenosine A2A receptors. *European Journal of Neuroscience*, *26*, 843–851.

Schröder, N., Figueiredo, L. S., & de Lima, M. N. M. (2013). Role of brain iron accumulation in cognitive dysfunction: evidence from animal models and human studies. *Journal of Alzheimer's Disease*, *34*, 797–812.

Scuderi, C., Steardo, L., & Esposito, G. (2013). Cannabidiol promotes amyloid precursor protein ubiquitination and reduction of beta amyloid expression in SHSY5YAPP+ cells through PPARγ involvement. *Phytotherapy Research*, *28*, 1007–1013.

Valvassori, S. S., Bavaresco, D. V., Scaini, G., Varela, R. B., Streck, E. L., Chagas, M. H., Hallak, J. E., Zuardi, A. W., Crippa, J. A., & Quevedo, J. (2013). Acute and chronic administration of cannabidiol increases mitochondrial complex and creatine kinase activity in the rat brain. *Revista Brasileira de Psiquiatria*, *35*, 380–386.

Zuardi, A. W. (2008). Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. *Revista Brasileira de Psiquiatria*, *30*, 271–280.

Zuardi, A. W., Crippa, J. A., Hallak, J. E., Pinto, J. P., Chagas, M. H., Rodrigues, G. G., Dursun, S. M., & Tumas, V. (2009). Cannabidiol for the treatment of psychosis in Parkinson's disease. *Journal of Psychopharmacology*, *23*, 979–983.

CHAPTER

84

Cannabinoids as Potent Inhibitors of Human CYP1 Enzymes

K. Watanabe[*,††], *S. Yamaori*[**], *K. Masuda*[†], *T. Katsu*[‡], *S. Narimatsu*[§], *I. Yamamoto*[¶]

[*]Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa, Japan

[**]Department of Pharmacy, Shinshu University Hospital, Matsumoto, Japan

[†]Department of Physical Chemistry, Graduate School of Clinical Pharmacy, Shujitsu University, Okayama, Japan

[‡]Department of Pharmacy, Faculty of Pharmacy, Yasuda Women's University, Hiroshima, Japan

[§]Department of Health Chemistry, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan

[¶]Hokuriku University, Kanazawa, Japan

[††]Pharmaceutical Education Support Center, Daiichi University of Pharmacy, Fukuoka, Japan

SUMMARY POINTS

- Three major phytocannabinoids, Δ^9-Tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), and cannabinol (CBN), inhibit the catalytic activities of CYP1 enzymes.
- CBD is a potent CYP1A1 inhibitor.
- CBN is a potent inhibitor of CYP1A2 and CYP1B1.
- CBD is a potent mechanism-based inhibitor of CYP1A1.
- The torsionable structure and two phenolic hydroxyl groups have important roles in the interaction between CBD and CYP1A1.
- Two phenyl rings are important for the interactions between CBN and CYP1A2 and CYP1B1.
- CBD may be a promising lead candidate for anticancer drugs, due to its inhibition of CYP1 enzymes.

KEY FACTS FOR THE INHIBITION OF CYP1 ENZYMES BY PHYTOCANNABINOIDS

- Three major phytocannabinoids (Δ^9-THC, CBD, and CBN) inhibit the catalytic activities of CYP1 enzymes.
- CBD is a potent inhibitor of CYP1A1, and its inhibitory potency is similar to those of 2,4,3′,5′-tetramethylstilbene and rhapontigenin.
- CBN is a potent inhibitor of CYP1A2 and CYP1B1. The inhibitory potency of CBN for CYP1A2 is similar to that of fluvoxamine, while that for CYP1B1 is markedly higher than those of *trans*-resveratrol and flutamide.
- CBD is a potent mechanism-based inhibitor of CYP1 enzymes, and its K_{inact}/K_I value for CYP1A1 is similar to that of rhapontigenin.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00096-X
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

CBD Cannabidiol
CBN Cannabinol
CYP Cytochrome P450
EROD 7-Ethoxyresorufin O-deethylase
THC Tetrahydrocannabinol

INTRODUCTION

The three major phytocannabinoids, Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), and cannabinol (CBN), are highly lipophilic compounds that are distributed in the membrane fractions of animal tissues in vivo. Thus, these cannabinoids are efficiently metabolized by drug-metabolizing enzymes involving cytochrome P450 (CYP) (Agurell et al., 1986; Harvey, 1984; Huestis, 2005; Yamamoto, Watanabe, Narimatsu, & Yoshimura, 1995). Hundreds of metabolites have been identified for these cannabinoids, some of which are known to be active metabolites that contribute to their biological effects, particularly in the case of Δ^9-THC. CYP2C9, CYP2C19, and CYP3A4 were found to be mainly involved in the metabolism of the three major phytocannabinoids in the human liver (Jiang, Yamaori, Okamoto, Yamamoto, & Watanabe, 2013; Watanabe, Matsunaga, Yamamoto, & Yoshimura, 2005; Watanabe, Yamaori, Funahashi, Kimura, & Yamamoto, 2006; Watanabe, Yamaori, Funahashi, Kimura, & Yamamoto, 2007). A high-affinity substrate of CYPs could be a strong inhibitor of the corresponding CYP enzymes (Hollenberg, 2002). These major cannabinoids were previously shown to inhibit CYP-mediated drug metabolism in experimental animals, both in vitro and in vivo (Hamajima et al., 1983; Paton & Pertwee, 1972; Siemens, Kalant, Khanna, Marshman, & Ho, 1974; Watanabe, Hamajima, Narimatsu, Yamamoto, & Yoshimura, 1986; Watanabe, Arai, Narimatsu, Yamamoto, & Yoshimura, 1987; Watanabe, Narimatsu, Gohda, Yamamoto, & Yoshimura, 1988). These findings suggested that these cannabinoids in marijuana may cause pharmacokinetic alterations to other coadministered drugs, and also that more attention should be paid to marijuana (or cannabinoid)-drug interactions, in some cases. The drug interactions that can potentially occur between cannabinoids (or cannabis) and other drugs have been attributed to changes in pharmacokinetics (Benowitz, Nguyen, Jones, Herning, & Bachman, 1980; Chetty, Miller, & Moodley, 1994; Jusko, Schentag, Clark, Gardner, & Yurchak, 1978; Roth et al., 2001). Our research group more recently systematically evaluated the inhibitory potencies of the major phytocannabinoids on human CYP enzymes (Yamaori, Kushihara, Yamamoto, & Watanabe, 2010; Yamaori, Maeda, Yamamoto, & Watanabe, 2011; Yamaori, Ebisawa, Okushima, Yamamoto, & Watanabe, 2011a; Yamaori, Okamoto, Yamamoto, & Watanabe, 2011c; Yamaori et al., 2012, 2013; Yamaori, Okushima, Yamamoto, & Watanabe, 2014; Jiang et al., 2013).

The human CYP 1 family consists of three members: CYP1A1, CYP1A2, and CYP1B1. CYP1A2 is almost exclusively expressed in the liver, while CYP1A1 and CYP1B1 are expressed in extrahepatic tissues (Shimada et al., 1996). CYP1A2 has been shown to play an important role in the metabolism of caffeine and melatonin, as well as many marketed drugs (Agundez, 2004; Ding & Kaminsky, 2003). CYP1A1 and CYP1B1 are responsible for the metabolic activation of polycyclic aromatic hydrocarbons, heterocyclic amines, and estradiol to carcinogenic intermediates (Shimada & Fujii-Kuriyama, 2004; Sissung, Price, Sparreboom, & Figg, 2006). The 4-hydroxylation of estradiol by CYP1 enzymes is known to be critical for estrogen-related carcinogenesis, such as breast cancer (Badawi, Cavalieri, & Rogan, 2001; Chun & Kim, 2003). A growing body of research has indicated that the overexpression of CYP1 enzymes, in particular CYP1B1, in several tumor cells may be used as a novel target for anticancer chemotherapy (Purnapatre, Khattar, & Saini, 2008; Androutsopoulos et al., 2013).

Human CYP1 enzymes are able to metabolize cannabinoids, while these were not the main enzymes responsible for cannabinoid metabolism in the human liver (Watanabe et al., 2007; Jiang, Yamaori, Takeda, Yamamoto, & Watanabe, 2011). In this review, we summarized the inhibitory potencies of Δ^9-THC, CBD, and CBN on human CYP1 enzymes, with a focus on drug interactions caused by marijuana, as well as the implications of the potent inhibition of CYP1 enzymes by the major phytocannabinoids.

INHIBITION OF CYP1 ENZYMES BY MAJOR PHYTOCANNABINOIDS

CYP1 enzymes are not the main enzymes responsible for metabolizing cannabinoids in mammalian species. Therefore, a limited amount of research has been undertaken to evaluate the ability of cannabinoids to induce or inhibit CYP1 enzymes. In an earlier study, Witschi and Saint-Francois (1972) reported that the oral administration of Δ^9-THC to rats caused a threefold increase in the activity of arylhydrocarbon hydroxylase, which is a marker of CYP1 activity, in lung homogenates. Roth et al. (2001) demonstrated that marijuana tar and Δ^9-THC induced CYP1A1 mRNA in murine Hepa-1 cells, while the 7-ethoxyresorfin *O*-deethylase (EROD) assay, a typical marker of CYP1 activity, revealed that Δ^9-THC competitively inhibited CYP activity. However, the interactions between cannabinoids and human CYP1 enzymes have not yet been examined in detail. We recently demonstrated that

TABLE 84.1 Kinetic Parameters for the Inhibition of EROD Activity by the Major Phytocannabinoids

Cannabinoids	Enzymes	K_i (μM)
Δ^9-THC	CYP1A1	4.78
	CYP1A2	7.54
	CYP1B1	2.47
	Human liver microsomes	4.72
CBD	CYP1A1	0.155
	CYP1A2	2.69
	CYP1B1	3.63
	Human liver microsomes	1.75
CBN	CYP1A1	0.541
	CYP1A2	0.0790
	CYP1B1	0.148
	Human liver microsomes	0.0808

TABLE 84.2 Comparison of K_i Values for Inhibition of the EROD Activity of Human CYP1A1 by the Major Phytocannabinoids and Other CYP1A1 Inhibitors

Inhibitors	K_i (nM)	References
α-Naphthoflavone	2	Pastrakuljic et al. (1997)
Isorhamnetin	25	Chang et al. (2006)
Kampferol	52	Chang et al. (2006)
Quercetin	77	Chang et al. (2006)
2,3,3′,5′-Tetramethoxystilbene	130	Chun et al. (2001a)
CBD	155	
Rhapontigenin	210	Chun et al. (2001b)
Apigenin	320	Pastrakuljic et al. (1997)
CBN	541	
trans-Resveratrol	1,200	Chang et al. (2001)
Δ^9-THC	4,780	
Isosafrole	10,000	Pastrakuljic et al. (1997)
Flutamide	10,300	Rochat et al. (2001)
Fluvoxamine	33,000	Pastrakuljic et al. (1997)

TABLE 84.3 Comparison of K_i Values for Inhibition of the EROD Activity of Human CYP1A2 by the Major Phytocannabinoids and Other CYP1A2 Inhibitors

Inhibitors	K_i (nM)	References
α-Naphthoflavone	2	Pastrakuljic et al. (1997)
Fluvoxamine	40	Pastrakuljic et al. (1997)
CBN	79	
Kampferol	109	Chang et al. (2006)
Isorhamnetin	137	Chang et al. (2006)
Apigenin	360	Pastrakuljic et al. (1997)
Quercetin	418	Chang et al. (2006)
Isosafrole	700	Pastrakuljic et al. (1997)
Flutamide	1,400	Rochat et al. (2001)
Oltipraz	1,500	Langouet et al. (2000)
CBD	2,690	
Tannic acid	4,800	Yao et al. (2008)
Δ^9-THC	7,540	
trans-Resveratrol	15,500	Chang et al. (2001)

the three major phytocannabinoids, Δ^9-THC, CBD, and CBN, could inhibit recombinant human CYP enzymes (Yamaori et al., 2010, 2011a, 2011b, 2011c, 2012, 2013, 2014; Jiang et al., 2013), in particular CYP1 enzymes (Table 84.1). Among the three cannabinoids, CBD showed the highest inhibitory effect on CYP1A1 (K_i = 0.155 μM), whereas CBN showed the highest inhibitory effect on CYP1A2 (K_i = 0.0790 μM), and CYP1B1 (K_i = 0.148 μM). Table 84.2 summarizes the K_i values of known CYP1A1 inhibitors (Chang, Chen, & Lee, 2001; Chang, Chen, & Yeung, 2006; Chun, Ryu, Jeong, & Kim, 2001b; Pastrakuljic, Tang, Roberts, & Kalow, 1997; Rochat, Morsman, Murray, Figg, & McLeod, 2001) with those of the three phytocannabinoids. When compared to known inhibitors of CYP1 enzymes, the inhibitory potency of CBD toward human CYP1A1 was similar to those of 2,4,3′,5′-tetramethylstilbene (Chun, Kim, Kim, Lee, & Guengerich, 2001a) and rhapontigenin (Pastrakuljic et al., 1997), which are known to be potent CYP1A1 inhibitors with K_i values of 0.130 and 0.210 μM, respectively. The inhibitory potency of CBN was markedly higher than that of *trans*-resveratrol. The K_i value of Δ^9-THC was markedly lower than those of isosafrole and fluvoxamine, which are typical CYP1A1 inhibitors. Table 84.3 summarizes the K_i values of known CYP1A2 inhibitors (Chang et al., 2001, 2006; Langouet et al., 2000; Pastrakuljic et al., 1997; Rochat et al., 2001; Yao, Chang, Lan, & Yeh, 2008) with those of the three phytocannabinoids. The inhibitory potency of CBN toward human CYP1A2 was similar to that of fluvoxamine (Pastrakuljic et al., 1997), which is a potent CYP1A2 inhibitor, and more effective than kaempferol (K_i = 0.109 μM) and isorhamnetin (K_i = 0.137 μM) (Chang et al., 2006). The inhibitory potencies of Δ^9-THC and CBD were lower than those of isosafrole, quercetin, and flutamide. Table 84.4 summarizes the K_i values of known CYP1B1 inhibitors (Chang et al., 2001, 2006; Roberts et al., 2004; Rochat et al., 2001; Sparfel, Van Grevenynghe, Le Vee, Aninat, & Fardel, 2006) with those of the three phytocannabinoids. The K_i value of CBN for the inhibition of CYP1B1

TABLE 84.4 Comparison of K_i Values for Inhibition of the EROD Activity of Human CYP1B1 by the Major Phytocannabinoids and Other CYP1B1 Inhibitors

Inhibitors	K_i (nM)	References
Isorhamnetin	3	Chang et al. (2006)
2,4,3′,5′-Tetramethoxystilbene	3	Chun et al. (2001a)
Pifithrinα	4	Sparfel et al. (2006)
Kampferol	14	Chang et al. (2006)
Quercetin	23	Chang et al. (2006)
CBN	148	
trans-Resveratrol	800	Chang et al. (2001)
Flutamide	1,000	Rochat et al. (2001)
Genistein	1,900	Roberts et al. (2004)
Daunomycin	2,100	Rochat et al. (2001)
Δ^9-THC	2,470	
Doxorubicin	2,600	Rochat et al. (2001)
CBD	3,630	
Daidzein	3,700	Roberts et al. (2004)
Tamoxifen	5,000	Rochat et al. (2001)
Paclitaxel	31,600	Rochat et al. (2001)

was markedly lower than those of *trans*-resveratrol (K_i = 0.8 μM) (Chang et al., 2001) and flutamide (K_i = 1 μM) (Rochat et al., 2001), but higher than those of isorhamnetin, 2,4,3′,5′-tetramethoxystilbene, kampferol, and quercetin. The K_i values of Δ^9-THC and CBD were similar to those of doxorubicin and daidzein, respectively, but lower than those of tamoxifen and paclitaxel.

However, the inhibitory potencies of these cannabinoids toward human CYP1 enzymes were markedly less potent than that of α-naphthoflavone (K_i = 2 nM), the most potent inhibitor of CYP1 enzymes (Shimada et al., 1998; Pastrakuljic et al., 1997; Liu, Sridhar, & Foroozesh, 2013) (Tables 84.2 and 84.3).

MECHANISM-BASED INHIBITION OF CYP1 ENZYMES BY MAJOR PHYTOCANNABINOIDS

Earlier studies suggested that the inhibitory effects of CBD on liver microsomal drug-metabolizing enzymes may be attributed to the formation of inhibitory metabolites (Bornheim & Grillo, 1998; Karler, Sangdee, Turkanis, & Borys, 1979; Narimatsu et al., 1990; Watanabe et al., 1987, 1988). CBD is known to decrease mouse liver CYPs in a metabolism-dependent manner, while active species of CBD metabolites have not yet been specified (Watanabe et al., 1987, 1988). Δ^9-THC, CBD, and CBN are

TABLE 84.5 Kinetic Parameters for the Mechanism-Based Inhibition of Human CYP1 Enzymes by the Major Phytocannabinoids

Cannabinoids	Enzymes	K_{inact} (min^{-1})	K_i (μM)	K_{inact}/K_i (L/mmol/min)
Δ^9-THC	CYP1A1	0.0239	1.22	19.6
CBD	CYP1A1	0.316	0.585	540
	CYP1A2	0.109	3.09	35.3
	CYP1B1	0.0267	2.12	12.6
CBN	CYP1A1	0.0191	0.0856	223

able to inhibit recombinant human CYP1 enzymes in a metabolism-dependent manner (Table 84.5) (Yamaori et al., 2010). CBD inhibited EROD activities by the three CYP1 enzymes, in preincubation time- and concentration-dependent manners, whereas Δ^9-THC and CBN only inhibited EROD activity by CYP1A1, in a metabolism-dependent manner. The K_i values of CBD for the inactivation of CYP1A1, CYP1A2, and CYP1B1 were 0.585, 3.09, and 2.12 μM, respectively. CBD showed the highest K_{inact} value for the inactivation of CYP1A1 (0.316 min^{-1}), among the CYP enzymes and phytocannabinoids investigated. The K_{inact} values of Δ^9-THC and CBN for CYP1A1 were 13- and 17-fold lower, respectively, than that of CBD. CBD for the inactivation of CYP1A1 showed the highest ratio of K_{inact} to K_i (540 L/mmol/min), followed by CBN (223 L/mmol/min). The alkyl resorcinol structure of CBD was previously shown to be important for the inactivation of CYP1A1 by the cannabinoid (Yamaori et al., 2014). Thus, CBD is a potent mechanism-based inhibitor of CYP1 enzymes, and its K_{inact}/K_i value for CYP1A1 was shown to be similar to that of rhapontigenin (K_{inact}/K_i value = 667 L/mmol/min) (Shimada et al., 2007).

INTERACTIONS BETWEEN MAJOR PHYTOCANNABINOIDS AND CYP1 ENZYMES

The structures of CYP1A2 and CYP1B1 could be constructed using the crystallographic data of the complex between α-naphthoflavone and CYP1 enzymes (Sansen et al., 2007; Wang, Savas, Stout, & Johnson, 2011). The conformations of the three cannabinoids have been constructed using the Insight II/Search-Compare module on a SGI Indigo 2 workstation. The amino acid residues at the catalytic sites of the CYP1 enzymes were drawn using RasMol version 2.6-ucb-1.0, as reported previously (Masuda et al., 2005). Possible interactions between the three major phytocannabinoids and CYP1 enzymes could be searched for using this model (Fig. 84.1). In the case of CYP1A1, the phenyl ring of the three cannabinoids could form a stacking interaction with

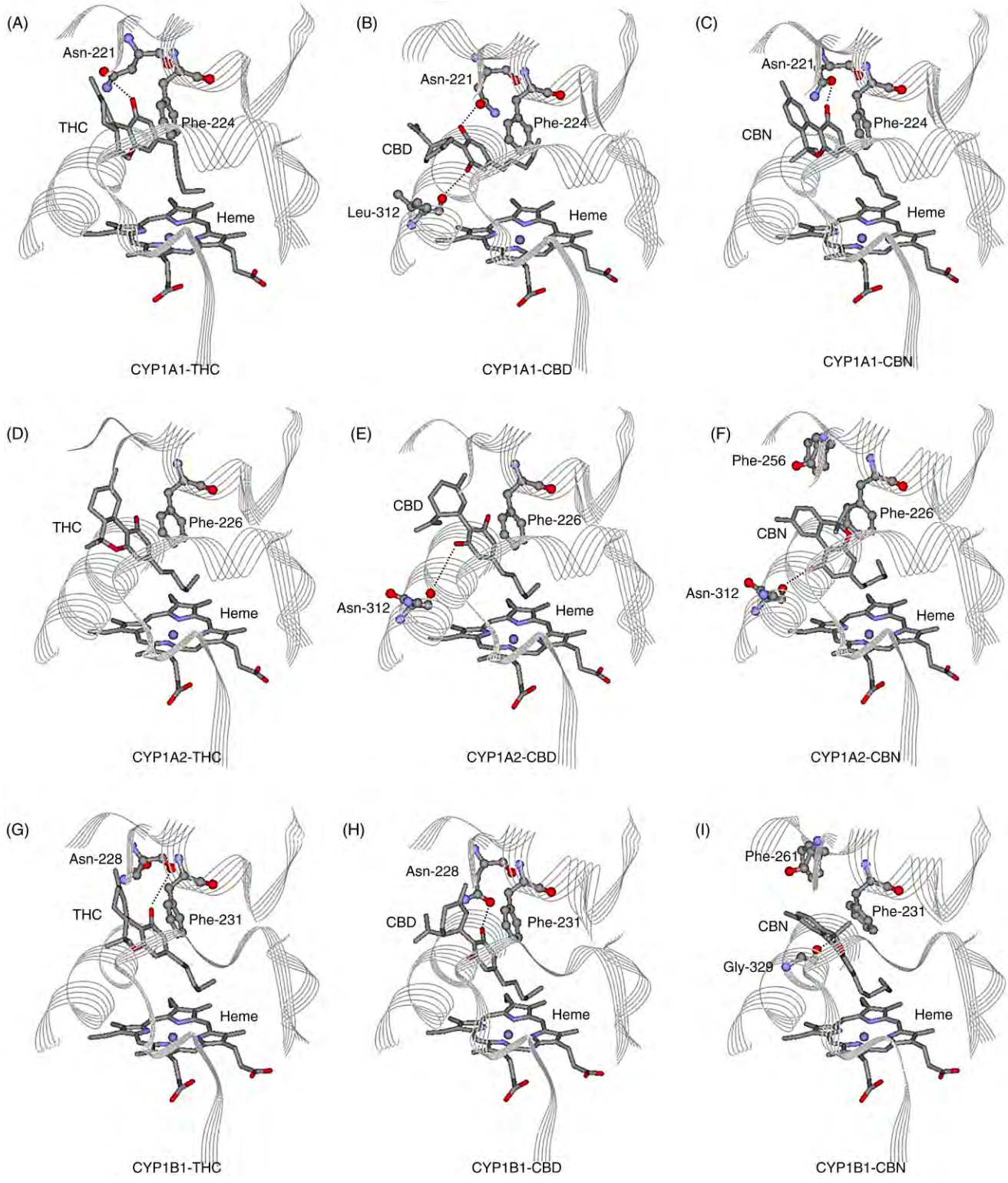


FIGURE 84.1 Proposed models for the interactions between the major phytocannabinoids and amino acid residues in the active-site cavity of humanCYP1 enzymes. (A) CYP1A1-Δ^9-THC, (B) CYP1A1-CBD, (C) CYP1A1-CBN, (D) CYP1A2-Δ^9-THC, (E) CYP1A2-CBD, (F) CYP1A2-CBN, (G) CYP1B1-Δ^9-THC, (H) CYP1B1-CBD, (I) CYP1B1-CBN.

Phe-224, and a hydrogen bond with Asn-221 (the calculated distances for hydrogen bonding were 2.53–3.57 Å) (Fig. 84.1A–C). CBD had two phenolic hydroxyl groups, one of which was able to form an additional hydrogen bond with the carbonyl group of Leu-312 (the calculated distance for hydrogen bonding was 3.72 Å) (Fig. 84.1B). In the case of CYP1A2, the phenyl ring of the three cannabinoids may form a stacking interaction with Phe-226 (Fig. 84.1D–F). In addition, another phenyl ring of CBN could make a stacking interaction with Phe-256, in the catalytic site of CYP1A2 (Fig. 84.1F). In the same manner as CYP1A2, the phenyl ring of the three cannabinoids formed a stacking interaction with Phe-231, and their phenolic hydroxyl groups formed a hydrogen bond with Asn-228 for Δ^9-THC and CBD (Fig. 84.1G and H), or Gly-329 for CBN (Fig. 84.1I) (the calculated distances for hydrogen bonding were 2.70–3.72 Å) in the active site of CYP1B1 (Fig. 84.1G–I). In addition, another phenyl ring of CBN could provide additional stabilization by a stacking interaction with Phe-261, in the active site of CYP1B1 (Fig. 84.1I). These multiple stabilizations of CBD (CYP1A1) and CBN (CYP1A2 and CYP1B1) within the catalytic site of CYP enzymes suggest that these cannabinoids act as strong inhibitors of particular CYP1 enzymes.

SIGNIFICANCE OF CYP1 INHIBITION BY MAJOR PHYTOCANNABINOIDS

The potent inhibition of CYP1 enzymes by these cannabinoids indicated that cannabinoid-mediated drug interactions may occur. The peak plasma levels of the three major cannabinoids were previously reported to be from 84.3 ng/mL (268 nM) to 126 ng/mL (406 nM) after smoke inhalation from a marijuana cigarette containing these cannabinoids (15.8–20 mg) (Huestis, Henningfield, & Cone, 1992; Johansson et al., 1987; Ohlsson et al., 1984). In clinical settings using treatments with Marinol or Sativex (http://www.marinol.com/; http://www.bayer.ca/), the C_{max} values of Δ^9-THC and CBD were shown to be from 3.02 ng/mL (9.62 nM) to 7.88 ng/mL (25.1 nM) in healthy humans. Δ^9-THC may not cause a significant drug interaction through CYP1 enzymes because the K_i values of Δ^9-THC were found to be markedly higher than the blood concentrations of the cannabinoid, as reported in previous studies. The K_i values of CBD for CYP1A1, and those of CBN for CYP1A2 and CYP1B1, were markedly lower than the blood levels of these cannabinoids, after smoking marijuana, but were slightly higher than the blood concentrations of CBD in the clinical settings described previously. These cannabinoids have high lipid solubility, and are known to be selectively distributed in various tissues, in particular the membrane fractions. Thus, the local tissue concentrations of these cannabinoids may be markedly higher than the blood concentrations in particular cases. These findings suggested that the inhibition of CYP1 enzymes by CBD and CBN may exert a cannabinoid-drug interaction through CYP1 enzymes.

CYP1 enzymes are able to activate procarcinogens, such as polycyclic aromatic hydrocarbons, heterocyclic amines, aromatic amines, and nitropolycyclic hydrocarbons, in metabolic reactions, resulting in the formation of highly mutagenic compounds. CYP1A1 and CYP1B1 have also been shown to be expressed in certain cancer cells, and are known to be involved in the regulation of tumor development (Androutsopoulos et al., 2013). CYP1B1 also plays an important role in the metabolism of estradiol to 4-hydroxyestradiol, which is a reactive metabolite that plays a critical role in estrogen-mediated carcinogenesis (Liehr & Ricci, 1996; Guengerich, Chun, Kim, Gillam, & Shimada, 2003). In addition, CYP1B1 was suggested to be involved in angiotensin-induced hypertension, and the resulting renal failure (Jennings et al., 2012), as well as in the pathogenesis of glaucoma, and other anterior segment dysgenesis disorders (Vasiliou & Gonzalez, 2007). These findings suggested that the inactivation of CYP1A1 and CYP1B1 may be a potential target of chemopreventive agents (Badal & Delgoda, 2013; Purnapatre et al., 2008; Sissung et al., 2006). Previous studies reported that resveratrol exhibited anticancer properties (Chang et al., 2001; Jang et al., 1997), whereas its inhibitory potency for CYP1A1 and CYP1B1 was less effective than those of CBD and/or CBN, respectively. Therefore, potent and selective inhibitors of CYP1 enzymes, such as CBD and CBN, may represent a new class of anticancer agents. CBD, in particular, which is a potent mechanism-based inhibitor of CYP1A1, may be a promising candidate as a lead compound of chemopreventive drugs.

MINI-DICTIONARY

CYP1A1 A member of the CYP1 family, which is expressed in extrahepatic tissues, and catalyzes the oxidation of granisetron and dacarbazine as well as the bioactivation of carcinogenic polycyclic aromatic hydrocarbons and heterocyclic amines.
CYP1A2 A member of the CYP1 family that is principally expressed in the human liver, and contributes to the bioactivation of carcinogenic aromatic and heterocyclic amines, as well as the metabolism of caffeine, flutamide, lidocaine, melatonin, phenacetin, and theophylline.
CYP1B1 A member of the CYP1 family that is expressed in extrahepatic tissues, and overexpressed in many cancer cells. It is involved in the metabolic activation of carcinogens, such as polycyclic aromatic hydrocarbons, and heterocyclic amines.
Insight II A graphical modeling program developed by Accelrys, Inc. In conjunction with the use of Discover, a dynamic manipulation of a molecular system can be achieved.
K_{inact} The maximal rate of enzyme inactivation at the saturating concentration of an inhibitor.
K_i value The concentration of an inhibitor that achieved half the

maximal rate of inhibition.

Mechanism-based inhibition Irreversible or quasi-irreversible loss of enzyme function when a substrate binds to an enzyme by irreversible or stable complex formation during the catalytic reaction of the enzyme.

α-Naphthoflavone A typical and strong inhibitor of CYP1 enzymes and CYP19, also known as 7,8-benzoflavone.

Phytocannabinoids A series of lipophilic compounds composed solely of carbon, hydrogen, and oxygen, which are the constituents of *cannabis*, and biosynthesized by corresponding synthases in the plant.

References

Agundez, J. A. (2004). Cytochrome P450 gene polymorphism and cancer. *Current Drug Metabolism*, *5*, 211–224.

Agurell, S., Halldin, M., Lindgren, J. -E., Ohlsson, A., Widman, M., Gillespie, H., & Hollister, H. (1986). Pharmacokinetics and metabolism of Δ^1-tetrahydrocannabinol and other cannabinoids with emphasis on man. *Pharmacological Reviews*, *38*, 21–43.

Androutsopoulos, V. P., Spyou, I., Ploumidis, A., Papalampros, A. E., Kyriakakis, M., Delakas, D., Spandidos, D. A., & Tsatsakis, A. M. (2013). Expression profile of CYP1A1 and CYP1B1 enzymes in colon and bladder tumors. *PLoS One*, *8*, e82487.

Badal, S., & Delgoda, R. (2013). CYP1B1: friend or foe? A critical review. *OA Biochemistry*, *1*(1), 8.

Badawi, A. F., Cavalieri, E. L., & Rogan, E. G. (2001). Role of human cytochrome P450 1A1, 1A2, 1B1, and 3A4 in the 2-, 4-, and 16α-hydroxylation of 17ß-estradiol. *Metabolism*, *50*, 1001–1003.

Benowitz, N. L., Nguyen, T. L., Jones, R. T., Herning, R. I., & Bachman, J. (1980). Metabolic and psychophysiologic studies of cannabidiol–hexobarbital interaction. *Clinical Pharmacology and Therapeutics*, *28*, 115–120.

Bornheim, L. M., & Grillo, M. P. (1998). Characterization of cytochrome P450 3A inactivation by cannabidiol: possible involvement of cannabidiol-hydroxyquinone as a P450 inactivator. *Chemical Research in Toxicology*, *11*, 1209–1216.

Chang, T. K., Chen, J., & Lee, W. B. (2001). Differential inhibition and inactivation of human CYP1 enzymes by *trans*-resveratrol; evidence for mechanism-based inactivation of CYP1A2. *Journal of Pharmacology and Experimental Therapeutics*, *299*, 874–882.

Chang, T. K., Chen, J., & Yeung, E. Y. (2006). Effect of *Ginkgo biloba* extract on procarcinogen-bioactivating human CYP1 enzymes: identification of isorhamnetin, kaempferol, and quercetin as potent inhibitors of CYP1B1. *Toxicology and Applied Pharmacology*, *213*, 18–26.

Chetty, M., Miller, R., & Moodley, S. V. (1994). Smoking and body weight influence the clearance of chlorpromazine. *European Journal of Clinical Pharmacology*, *46*, 523–526.

Chun, Y. J., & Kim, S. (2003). Discovery of cytochrome P450 1B1 inhibitors as new promising anti-cancer agents. *Medical Research Reviews*, *23*, 657–668.

Chun, Y. J., Kim, S., Kim, D., Lee, S. K., & Guengerich, F. P. (2001a). A new selective and potent inhibitor of human cytochrome P450 1B1 and its application to antimutagenesis. *Cancer Research*, *61*, 8164–8170.

Chun, Y. J., Ryu, S. Y., Jeong, T. C., & Kim, M. Y. (2001b). Mechanism-based inhibition of human cytochrome P450 1A1 by rhapontigenin. *Drug Metabolism and Disposition*, *29*, 389–393.

Ding, X., & Kaminsky, L. S. (2003). Human extrahepatic cytochromes P450: function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. *Annual Review of Pharmacology and Toxicology*, *43*, 140–173.

Guengerich, F. P., Chun, Y. J., Kim, D., Gillam, E. M., & Shimada, T. (2003). Cytochrome P450 1B1: a target for inhibition in anticarcinogenesis strategies. *Mutation Research*, *523–524*, 173–182.

Hamajima, K., Watanabe, K., Narimatsu, S., Tateoka, Y., Yamamoto, I., & Yoshimura, H. (1983). Sex difference in the effect of Δ^9-tetrahydrocannabinol and cannabidiol on pentobarbital-induced sleeping time and hepatic microsomal drug metabolizing enzyme systems in mice. *Yakugaku Zasshi*, *103*, 1289–1297.

Harvey, D. J. (1984). Chemistry, metabolism, and pharmacokinetics of the cannabinoids. In G. G. Nahas (Ed.), *Marijuana in science and medicine* (pp. 37–107). New York, NY: Raven Press.

Hollenberg, P. F. (2002). Characteristics and common properties of inhibitors, inducers, and activators of CYP enzymes. *Drug Metabolism Reviews*, *34*, 17–37.

Huestis, M. A. (2005). Pharmacokinetics and metabolism of the plant cannabinoids. Δ^9-tetrahydrocannabinol, cannabidiol and cannabinol. *Handbook of Experimental Pharmacology*, *168*, 657–690.

Huestis, M. A., Henningfield, J. E., & Cone, E. J. (1992). Blood cannabinoids. 1. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. *Journal of Analytical Toxicology*, *16*, 276–282.

Jang, M., Cai, L., Udeani, G. O., Slowing, K. V., Thomas, C. F., Beecher, C. W., Fong, H. H., Farnsworth, N. R., Kinghorn, A. D., Mehta, R. G., Moon, R. C., & Pezzuto, J. M. (1997). Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*, *275*, 218–220.

Jennings, B. L., Anderson, L. J., Estes, A. M., Yaghini, F. A., Fang, X. R., Porter, J., Gonzalez, F. J., Campbell, W. B., & Malik, K. U. (2012). Cytochrome P450 1B1 contributes to renal dysfunction and damage caused by angiotensin II in mice. *Hypertension*, *59*, 348–354.

Jiang, R. R., Yamaori, S., Okamoto, Y., Yamamoto, I., & Watanabe, K. (2013). Cannabidiol is a potent inhibitor of the catalytic activity of cytochrome P450 2C19. *Drug Metabolism and Pharmacokinetics*, *28*, 332–338.

Jiang, R. R., Yamaori, S., Takeda, S., Yamamoto, I., & Watanabe, K. (2011). Identification of cytochrome P450 enzymes responsible for metabolism of cannabidiol by human liver microsomes. *Life Sciences*, *89*, 165–170.

Johansson, E., Ohlsson, A., Lindgren, J. -E., Agurell, S., Gillepsie, H., & Hollister, L. E. (1987). Single-dose kinetics of deuterium-labelled cannabinol in man after intravenous administration and smoking. *Biomedical and Environmental Mass Spectrometry*, *14*, 495–499.

Jusko, W. J., Schentag, J. J., Clark, J. H., Gardner, M., & Yurchak, A. M. (1978). Enhanced biotransformation of theophylline in marihuana and tobacco smokers. *Clinical Pharmacology and Therapeutics*, *24*, 405–410.

Karler, R., Sangdee, P., Turkanis, S. A., & Borys, H. K. (1979). The pharmacokinetics fate of cannabidiol and its relationship to barbiturate sleeping time. *Biochemical Pharmacology*, *28*, 777–784.

Langouet, S., Furge, L. L., Kerriguy, N., Nakamura, K., Guillouzo, A., & Guengerich, F. P. (2000). Inhibition of human cytochrome P450 enzymes by 1,2-dithiole-3-thione, oltipraz and its derivatives, and sulforaphane. *Chemical Research in Toxicology*, *13*, 245–252.

Liehr, J. G., & Ricci, M. J. (1996). 4-Hydroxylation of estrogens as marker of human mammary tumors. *Proceeding of the National Academy of Sciences of the United States of America*, *93*, 3294–3296.

Liu, J., Sridhar, J., & Foroozesh, M. (2013). Cytochrome P450 family 1 inhibitors and structure-activity relationships. *Molecules*, *18*, 14470–14495.

Masuda, K., Tamagake, K., Okuda, Y., Torigoe, F., Tsuzuki, D., Isobe, T., Hichiya, H., Hanioka, N., Yamamoto, S., & Narimatsu, S. (2005). Change in enantioselectivity in bufuralol 1″-hydroxylation by the substitution of phenylalanine-120 by alanine in cytochrome P450 2D6. *Chirality*, *17*, 37–43.

Narimatsu, S., Watanabe, K., Matsunaga, T., Yamamoto, I., Imaoka, S., Funae, Y., & Yoshimura, H. (1990). Inhibition of hepatic microsomal cytochrome P450 by cannabidiol in adult male rats. *Chemical and Pharmaceutical Bulletin*, *38*, 1365–1368.

Ohlsson, A., Lindgren, J. -E., Andersson, S., Agurell, S., Gillepsie, H., & Hollister, L. E. (1984). Single dose kinetics of cannabinoid in man. In S. Agurell, W. L. Dewey, & R. Willette (Eds.), *The cannabinoids: Chemical, pharmacologic, and therapeutic aspects* (pp. 219–225). New York, NY: Academic Press.

Pastrakuljic, A., Tang, B. K., Roberts, E. A., & Kalow, W. (1997). Distinction of CYP1A1 and CYP1A2 activity by selective inhibition using fluvoxamine and isosafrole. *Biochemical Pharmacology*, *53*, 531–538.

Paton, W. D. M., & Pertwee, R. G. (1972). Effect of cannabis and certain of its constituents on pentobarbitone sleeping time and phenazone metabolism. *British Journal of Pharmacology*, *44*, 250–261.

Purnapatre, K., Khattar, S. K., & Saini, K. S. (2008). Cytochrome P450s in the development of target-based anticancer drugs. *Cancer Letters*, *259*, 1–15.

Roberts, D. W., Doerge, D. R., Churchwell, M. I., Gamboa da Costa, G., Marques, M. M., & Tolleson, W. H. (2004). Inhibition of extrahepatic human cytochrome P450 1A1 and 1B1 by metabolism of isoflavones found in *Trifolium pratense* (red clover). *Journal of Agricultural and Food Chemistry*, *52*, 6623–6632.

Rochat, B., Morsman, J. M., Murray, G. I., Figg, W. D., & McLeod, H. L. (2001). Human CYP1B1 and anticancer agent metabolism: mechanism for tumor-specific drug inactivation? *Journal of Pharmacology and Experimental Therapeutics*, *296*, 537–541.

Roth, M. D., Marques-Magallanes, J. A., Yuan, M., Sun, W., Tashkin, D. P., & Hankinson, O. (2001). Induction and regulation of the carcinogen-metabolizing enzyme CYP1A1 by marijuana smoke and Δ^9-tetrahydrocannabinol. *American Journal of Respiratory Cell and Molecular Biology*, *24*, 330–344.

Sansen, S., Yano, J. K., Reynald, R. L., Schoch, G. A., Griffin, K. J., Stout, C. D., & Jonson, E. F. (2007). Adaptations for the oxidation of polycyclic aromatic hydrocarbons exhibited by the structure of human P450 1A2. *Journal of Biological Chemistry*, *282*, 14348–14355.

Shimada, T., & Fujii-Kuriyama, Y. (2004). Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochrome P450 1A1 and 1B1. *Cancer Science*, *95*, 1–6.

Shimada, T., Hayes, C. L., Yamazaki, H., Amin, S., Hecht, S. S., Guengerich, F. P., & Sutter, T. R. (1996). Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. *Cancer Research*, *56*, 2979–2984.

Shimada, T., Murayama, N., Okada, K., Funae, Y., Yamazaki, H., & Guengerich, F. P. (2007). Different mechanisms for inhibition of human cytochrome P450 1A1, 1A2, and 1B1 by polycyclic aromatic inhibitors. *Chemical Research in Toxicology*, *20*, 489–496.

Shimada, T., Yamazaki, H., Foroozesh, M., Hopkins, N. E., Alworth, W. L., & Guengerich, F. P. (1998). Selectivity of polycyclic inhibitors for human cytochrome P450s 1A1, 1A2 and 1B1. *Chemical Research in Toxicology*, *11*, 1048–1056.

Siemens, A. J., Kalant, H., Khanna, J. M., Marshman, J., & Ho, G. (1974). Effect of cannabis on pentobarbital-induced sleeping time and pentobarbital metabolism in the rat. *Biochemical Pharmacology*, *23*, 477–488.

Sissung, T. M., Price, D. K., Sparreboom, A., & Figg, W. D. (2006). Pharmacogenetics and regulation of human cytochrome P450 1B1: implications in hormone-mediated tumor metabolism and a novel target for therapeutic intervention. *Molecular Cancer Research*, *4*, 135–150.

Sparfel, L., Van Grevenynghe, J., Le Vee, M., Aninat, C., & Fardel, O. (2006). Potent inhibition of carcinogen-bioactivating cytochrome P450 1B1 by the *p53* inhibitor pifithrin α. *Carcinogenesis*, *27*, 656–663.

Vasiliou, V., & Gonzalez, F. J. (2007). Role of CYP1B1 in glaucoma. *Annual Review of Pharmacology and Toxicology*, *48*, 333–358.

Wang, A., Savas, U., Stout, C. D., & Johnson, E. L. (2011). Structural characterization of the complex between α-naphthoflavone and human cytochrome P450 1B1. *Journal of Biological Chemistry*, *286*, 5736–5743.

Watanabe, K., Arai, M., Narimatsu, S., Yamamoto, I., & Yoshimura, H. (1987). Self-catalyzed inactivation of cytochrome P-450 during microsomal metabolism of cannabidiol. *Biochemical Pharmacology*, *36*, 3371–3377.

Watanabe, K., Hamajima, K., Narimatsu, S., Yamamoto, I., & Yoshimura, H. (1986). Effect of two cannabinoids on hepatic microsomal cytochrome P-450. *Journal of Pharmacobio Dynamics*, *9*, 39–45.

Watanabe, K., Matsunaga, T., Yamamoto, I., & Yoshimura, H. (2005). Involvement of CYP2C in the metabolism of cannabinoids by human hepatic microsomes from an old woman. *Biological and Pharmaceutical Bulletin*, *18*, 1138–1141.

Watanabe, K., Narimatsu, S., Gohda, H., Yamamoto, I., & Yoshimura, H. (1988). Formation of similar species to carbon monoxide during hepatic microsomal metabolism of cannabidiol on the basis of spectral interaction with cytochrome P-450. *Biochemical Pharmacology*, *37*, 4719–4726.

Watanabe, K., Yamaori, S., Funahashi, T., Kimura, T., & Yamamoto, I. (2006). 8-Hydroxycannabinol; a new metabolite of cannabinol formed by human hepatic microsomes. *Forensic Toxicology*, *24*, 80–82.

Watanabe, K., Yamaori, S., Funahashi, T., Kimura, T., & Yamamoto, I. (2007). Cytochrome P450 enzymes involved in the metabolism of tetrahydrocannabinols and cannabinol by human hepatic microsomes. *Life Sciences*, *80*, 1415–1419.

Witschi, H. P., & Saint-Francois, B. (1972). Enhanced activity of benzpyrene hydroxylase in rat liver and lung after acute cannabis administration. *Toxicology and Applied Pharmacology*, *23*, 165–168.

Yamamoto, I., Watanabe, K., Narimatsu, S., & Yoshimura, H. (1995). Recent advances in the metabolism of cannabinoids. *International Journal of Biochemistry and Cell Biology*, *27*, 741–746.

Yamaori, S., Ebisawa, Y., Okushima, Y., Yamamoto, I., & Watanabe, K. (2011a). Potent inhibition of human cytochrome P450 3A isoforms by cannabidiol: role of phenolic hydroxyl groups in the resorcinol moiety. *Life Sciences*, *88*, 730–736.

Yamaori, S., Koeda, K., Kushihara, M., Hada, Y., Yamamoto, I., & Watanabe, K. (2012). Comparison in the in vitro inhibitory effects of major phytocannabinoids and polycyclic aromatic hydrocarbons contained in marijuana smoke on cytochrome P450 2C9 activity. *Drug Metabolism and Pharmacokinetics*, *27*, 294–300.

Yamaori, S., Kushihara, M., Yamamoto, I., & Watanabe, K. (2010). Characterization of major phytocannabinoids, cannabidiol and cannabinol, as isoform-selective and potent inhibitors of human CYP1 enzymes. *Biochemical Pharmacology*, *79*, 1691–1698.

Yamaori, S., Maeda, C., Yamamoto, I., & Watanabe, K. (2011b). Differential inhibition of human cytochrome P450 2A6 and 2B6 by major phytocannabinoids. *Forensic Toxicology*, *29*, 117–124.

Yamaori, S., Okamoto, Y., Yamamoto, I., & Watanabe, K. (2011c). Cannabidiol, a major phytocannabinoid, as a potent atypical inhibitor for CYP2D6. *Drug Metabolism and Disposition*, *39*, 2049–2056.

Yamaori, S., Okushima, Y., Masuda, K., Kushihara, M., Katsu, T., Narimatsu, S., Yamamoto, I., & Watanabe, K. (2013). Structural requirements for potent direct inhibition of human cytochrome P450 1A1 by cannabidiol: role of pentylresorcinol moiety. *Biological and Pharmaceutical Bulletin*, *36*, 1197–1203.

Yamaori, S., Okushima, Y., Yamamoto, I., & Watanabe, K. (2014). Characterization of the structural determinants required for potent mechanism-based inhibition of human cytochrome P450 1A1. *Chemico Biological Interactions*, *215*, 62–68.

Yao, H. T., Chang, Y. W., Lan, S. J., & Yeh, T. K. (2008). The inhibitory effect of tannic acid on cytochrome P450 enzymes and NADPH-CYP reductase in rat and human liver microsomes. *Food and Chemical Toxicology*, *46*, 645–653.

CHAPTER

85

The Synthetic Analog of Δ^9-Tetrahydrocannabinol (THC): Nabilone. Pharmacology and Clinical Application

*R.E Balter**, *M. Haney***

***Division on Substance Abuse, New York State Psychiatric Institute and Department of Psychiatry, Columbia University Medical Center, New York, NY, United States**

****Division on Substance Abuse, New York State Psychiatric Institute and Department of Psychiatry, College of Physicians and Surgeons of Columbia University, New York, NY, United States**

SUMMARY POINTS

- Nabilone (Cesamet), a synthetic analog of Δ^9-tetrahydrocannabinol (Δ^9-THC) developed in the early 1970s, is approved for the treatment of nausea and vomiting suffered by cancer patients undergoing chemotherapy.
- Nabilone is a potent cannabinoid receptor agonist with high oral bioavailability, and a long duration of action suitable for once or twice daily dosing (1–2 mg).
- At higher doses (3–8 mg), nabilone can produce moderate cognitive impairment, and feelings of "high"and "drug effect" similar to that of THC.
- In individuals with cannabis use disorder, laboratory studies suggest nabilone may have utility as a treatment for cannabis withdrawal and relapse.
- Nabilone is effective in treating pain and insomnia in some patients with chronic pain conditions though it has not been shown effective as an analgesic for acute pain.
- Nabilone has also shown promise for the treatment of dyskinesia resulting from Huntington disease, spinal cord injury, and levodopa treatment in patients with Parkinson disease.
- Finally, nabilone may have utility for long-term management of anxiety, but not for the treatment of acute anxiety.

KEY FACTS

- Nabilone is an orally available, synthetic analog of THC with better potency, bioavailability, and a longer duration of action than dronabinol (perhaps due to an active metabolite).
- Although nabilone produces some subjective intoxication and increased heart rate, its slow rate of onset relative to smoked cannabis results in low abuse liability.
- Placebo-controlled studies demonstrate nabilone can be used in the treatment of chronic and neuropathic pain.
- Placebo-controlled research are needed to demonstrate nabilone's potential efficacy for the treatment of cannabis use disorder, posttraumatic stress disorder, insomnia, and dyskinesias.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00099-5
Copyright © 2017 Elsevier Inc. All rights reserved.

ABBREVIATIONS

cAMP	Cyclic adenosine monophosphate
CB1 and CB2	Cannabinoid receptor 1 and 2
GABA	Gamma-aminobutyric acid
GTPγS	Guanosine 5′-O-[gamma-thio] triphosphate
POMS	Profile of mood states
PTSD	Posttraumatic stress disorder
Δ9-THC	Δ9-Tetrahydrocannabinol

INTRODUCTION

Medical uses of cannabis and cannabinoids, the primary psychoactive chemicals in the *Cannabis sativa* plant, are a topic of great interest and continued debate. Although the arc of science points toward the potential benefits of this class of compounds, controlled clinical studies are clearly needed. Currently, there are two oral cannabinoids available for medical use and research: dronabinol, a synthetic Δ9-tetrahydrocannabinol (Δ9-THC), and nabilone, a synthetic analog of THC. In 1985, the United States Food and Drug Administration (FDA) approved nabilone, marketed as Cesamet, for the treatment of nausea and vomiting suffered by cancer patients undergoing chemotherapy. The Medicines and Healthcare Products Regulatory Agency (MHRA) in the United Kingdom licensed nabilone in 1995. Compared to dronabinol, nabilone has a higher bioavailability and a longer duration of action (Ben Amar, 2006; Glass, Uhlenhuth, & Hartel, 1979; Lemberger et al., 1982; Matsuda, Lolait, Brownstein, Young, & Bonner, 1990; McGilveray, 2005). Nabilone's most common adverse effects, such as drowsiness, dry mouth, intoxication, vertigo, and tachycardia, are generally mild enough, so as to not prevent continued use. Beyond extensive research of nabilone's use as an antiemetic, it has been evaluated for use in a number of other medical and psychiatric conditions. The objective of this chapter is to describe the potential utility of nabilone for a range of disorders: cannabis use disorder, analgesia, motor disorders, and anxiety.

PHARMACOLOGY

Nabilone was developed in the early 1970s by Lilly Research Laboratories, and its synthesis is described by Archer et al. (1977). Nabilone's structure is quite similar to that of THC (Fig. 85.1). Both compounds are dibenzopyrans, with a dimethyl at the 6 position, and a hydroxyl at the 1 position. Unlike THC, nabilone has a dimethylheptyl side chain at the 3 position, a saturated ring with no double bond in the 9–10 position, and a ketone group rather than a methyl group in the 9 position. Because of these structural differences, nabilone is not converted into THC or vice versa (Stark, 1982). Further, nabilone administration does not produce a positive urine toxicology result for THC (Fraser & Meatherall, 1989).

Nabilone is a potent cannabinoid agonist and binds with high affinity to both the CB1 and CB2 cannabinoid receptors, with dissociation constants of 2.2 and 1.8 nM, respectively (Gareau et al., 1996). Across most measures, 1 mg of nabilone is equivalent to approximately 7 mg of dronabinol (Johnson & Jasinski, 1983). In addition to high binding affinity, nabilone is more efficacious than THC, producing greater cAMP inhibition and [^{35}S]GTPγS binding than THC, in mouse brain tissue (Matsuda et al., 1990; Baillie, Ross, & Pertwee, 2010).

Lemberger and Rowe (1975) provide the first description of nabilone administration to healthy human subjects, showing that the onset of behavioral effects begin at about 1 h after dosing, and last for 8–9 h. Five milligram of nabilone produced dry mouth, euphoria, tachycardia, and postural hypotension. These effects were minimal at 2.5 mg, and absent at 1 mg. All doses produced relaxation and sedative effects. Tolerance to side effects developed following 3 days of dosing. The findings from this early study are fairly consistent with the results from later work, although side effects have been reported at 1 and 2 mg doses, in some studies (Ware, Daeninck, & Maida, 2008). However, side effects generally do not seem to deter patients from continuing to take nabilone.

Following oral administration, nabilone is readily absorbed, and has a short plasma half-life of approximately 2 h (Rubin et al., 1977). However, the metabolites of

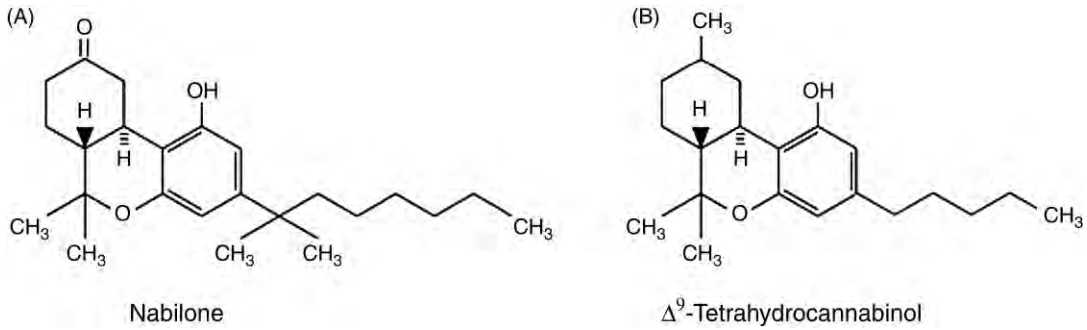


FIGURE 85.1 Nabilone and Δ9-tetrahydrocannabinol (THC). Chemical structures for nabilone (left) and THC (right).

nabilone, including isomeric carbinols, persist in plasma with a half-life of over 20 h. The long duration of action of nabilone, despite rapid metabolism, suggests that at least one or more of these metabolites are pharmacologically active (Rubin et al., 1977).

COGNITIVE AND SUBJECTIVE EFFECTS

A series of studies has also sought to better understand the cognitive and subjective effects of nabilone. Wesnes et al. (2010) found that high (3 mg) but not lower (1 or 2 mg) nabilone doses produced significant changes in measures of attention, working memory, and episodic memory, at 4 h after drug administration in healthy, drug-naïve participants, as compared to placebo capsules. Participants also reported feelings of "high" and "drug effect" following all doses, but did not report a desire to consume more of the drug.

These results are consistent with the findings from a study by Bedi, Cooper, and Haney (2013) comparing the acute effects of nabilone (2, 4, 6, 8 mg) to dronabinol (10, 20 mg) in current cannabis smokers. Note: because of cross tolerance, a higher nabilone dose range was tested relative to those used for drug-naïve participants in earlier studies. The highest doses of nabilone (6 and 8 mg) produced modest decreases in psychomotor speed, and 4, 6, and 8 mg doses increased ratings of "good drug effect," and "high." Nabilone also increased heart rate dose dependently. Interestingly, in comparison to dronabinol, the subjective effects of nabilone had a slower onset to peak effect (180–240 vs 90 min), a longer duration of action (6 vs 4 h), and were more dose-related, supportive of nabilone's better bioavailability.

In a drug discrimination study, the interoceptive effects of 3 and 5 mg of nabilone substituted for 25 mg of dronabinol in regular cannabis users, again demonstrating nabilone's higher potency relative to dronabinol (Lile, Kelly, & Hays, 2010). A follow-up study replicated these results, and also showed that low dose nabilone in combination with low dose THC could substitute for higher training doses of THC (Lile, Kelly, & Hays, 2011). The additive nature of the interoceptive effects suggests a shared mechanism of action, that is, the CB1 receptor.

CANNABIS USE DISORDER

Given the shared pharmacology and subjective effects of nabilone and THC, nabilone has been tested as an agonist substitution treatment for cannabis use disorder. In one promising study, Haney et al. (2013) found that nabilone (6 or 8 mg/day) significantly reversed cannabis withdrawal-induced irritability, sleep disruption, and anorexia in nontreatment seeking cannabis smokers. Nabilone also attenuated a laboratory measure of cannabis relapse, defined as cannabis self-administration after several days of abstinence. This is particularly significant because few medications, including dronabinol, have been shown to disrupt cannabis self-administration in a comparable manner.

Adding to the appeal of nabilone as a treatment option is the fact that, unlike dronabinol, nabilone produces urinary metabolites that are distinct from those of cannabis (Fraser & Meatherall, 1989), which would allow clinicians to distinguish medication compliance from ongoing cannabis use in a clinical setting. There is always a risk that a CB1 agonist would have abuse liability that, if substantial, would lessen enthusiasm for its use to treat cannabis use disorder. However, in a comprehensive review of scientific literature as well as popular press and interviews with medical professionals and law enforcement officials suggest this is unlikely: Ware and St Arnaud-Trempe (2010) found that there is little evidence for nabilone abuse—it has no known street value and there are almost no instances of diversion, most likely due to its slow rate of onset relative to smoked cannabis.

ANALGESIA

Beyond the treatment of nausea, analgesia is one of the most commonly proposed indications for cannabinoids (Russo, 2008). Yet, in a placebo-controlled clinical trial of nabilone (1–2 mg every 8 h) for acute postoperative pain, patients reported higher pain scores, compared to placebo-treated patients, suggesting an enhancement rather than attenuation of pain (Beaulieu, 2006). This is consistent with laboratory studies showing that nabilone failed to attenuate thermal or capsaicin-induced acute pain (Redmond, Goffaux, Potvin, & Marchand, 2008; Kalliomaki et al., 2012). These results are somewhat surprising, as both smoked cannabis and oral THC (dronabinol) have shown moderate analgesia following acute experimenter-induced pain (Cooper, Comer, & Haney, 2013).

In contrast to acute pain studies, nabilone has shown more promise for the treatment of chronic pain. Pinsger et al. (2006) published a placebo-controlled crossover design study of nabilone (up to 4 mg/day) as an add-on treatment for patients with chronic musculoskeletal pain ($n = 30$). Over the 4-week treatment period, patients taking nabilone reported significantly less pain as compared to those receiving placebo. A 6-week crossover design study in patients with neuropathic pain ($n = 96$) showed that nabilone (2 mg/day) reduced pain ratings relative to baseline. However, dihydrocodeine (240 mg), a relatively weak opioid medication, was able to produce greater reductions in pain ratings than nabilone (Frank, Serpell,

Hughes, Matthews, & Kapur, 2008). A longer 6-month open label study reported that nabilone was comparable to gabapentin for management of neuropathic pain (Bestard & Toth, 2011). Nabilone (up to 2 mg twice per day) was also well tolerated and effective in an open label study showing that 26 of 37 patients had reduced neuropathic pain associated with diabetes (Toth et al., 2012). During a 5-week, double-blind, placebo-controlled phase, following the initial 4-week trial, patients maintained on nabilone continued to report analgesic effects, while patients switched to placebo reported increases in pain scores. The longest treatment durations are reported in a chart review from Berlach, Shir, and Ware (2006), in which nabilone was used as an off-label analgesic for up to 4 years (average 1.5 years). Fifteen of 20 patients reported subjective overall improvement with nabilone, and 9 reported reduced pain intensity; however, patients were not blinded to the medication.

Nabilone (1 mg) has also been shown to be more effective than ibuprofen in treating medication overuse for rebound headaches, which result from long-term use of therapeutic agents in patients with frequent migraines (Pini et al., 2012), and more effective than placebo as an adjunct therapy for pain management in advanced cancer patients (Maida, Ennis, Irani, Corbo, & Dolzhykov, 2008).

Finally, nabilone (1–2 mg/day) was assessed in a 4-week, placebo-controlled, randomized trial for the treatment of fibromyalgia. Patients treated with nabilone reported significant decreases in pain severity as well as anxiety, while no changes were seen in placebo-treated patients (Skrabek, Galimova, Ethans, & Perry, 2008). Ware, Fitzcharles, Joseph, and Shir (2010) studied nabilone's effects on sleep in fibromyalgia patients. They found that low dose nabilone (0.5–1 mg) prior to bedtime significantly improved sleep quality, compared to baseline, as well as compared to amitriptyline, a common treatment for insomnia. However, no effect on pain was observed in this study. Improved sleep in fibromyalgia patients is consistent with the results of multiple other studies reporting that nabilone improved sleep quality for other chronic pain patients (Bestard & Toth, 2011; Toth et al., 2012).

Limited research has also been done on the effectiveness of nabilone in treating spasticity-associated pain. This line of research began with a case report of a 45-year-old man with relapsing-remitting multiple sclerosis (Martyn, Illis, & Thom, 1995). Both pain from muscle spasms and nocturia decreased during a 4-week treatment with nabilone (1 mg), compared to alternating placebo treatment periods. Nabilone has not been further assessed as a treatment for nocturia in humans, though the result may be consistent with the findings of Pertwee and Fernando (1996) that nabilone dose-dependently attenuated electrically-evoked contractions of isolated mouse urinary bladder tissue. A chart review from Notcutt, Price, Blossfeldt, and Chapman (1999) reported that only 6 out of 16 patients experienced significant analgesic effects from nabilone for multiple sclerosis-associated pain. However, in a placebo-controlled crossover design in patients (n = 13) with spasticity-associated pain, nabilone (1 mg/day) significantly decreased pain following 4 weeks of active treatment compared to placebo (Wissel et al., 2006).

Overall, these limited studies show that nabilone can reduce chronic pain and improve quality of life by its effects on sleep or perhaps nocturia, for example, in a variety of pain conditions.

MOTOR DISORDERS

Although spasticity in patients with multiple sclerosis was not significantly decreased by nabilone (1 mg/day; Wissel et al., 2006), there is evidence that it may show promise as a treatment for other types of motor disorders. In patients with spasticity resulting from spinal cord injury, 4 weeks of treatment with nabilone (0.5–1 mg/day) produced significant improvements in the Ashworth scale for spasticity, as compared to placebo (Pooyania, Ethans, Szturm, Casey, & Perry, 2010). Dyskinesia, involuntary movements that are a common side effect of levodopa, results from dopaminergic hyperactivity and is often treated with medications that upregulate GABA. Sieradzan et al. (2001) found in an in vivo study that nabilone dose-dependently decreased GABA reuptake, possibly through activation of CB1 receptors expressed on GABAergic neurons. They went on to conduct a small placebo-controlled study demonstrating that nabilone (0.03 mg/kg) significantly reduced levodopa-induced dyskinesia in patients with Parkinson disease. Importantly, a study in a nonhuman primate model of Parkinson disease showed that nabilone treatment for dyskinesia did not disrupt the antiparkinsonian effects of levodopa (Fox, Henry, Hill, Crossman, & Brotchie, 2002). In a randomized, placebo-controlled trial of patients with Huntington disease, 5 weeks of treatment with nabilone (1 or 2 mg/day) produced modest but significant decreases in the severity of dyskinesia (Curtis, Mitchell, Patel, Ives, & Rickards, 2009). Thus, results with nabilone on dyskinesias are promising, suggesting that further research is warranted in this area.

ANXIETY

Three small studies failed to show significant effects of nabilone on measures of acute anxiety, following experimentally induced anxiety in healthy (2 mg; Nakano,

Gillespie, & Hollister, 1978), as well as in anxious research volunteers (1–5 mg; Glass, Uhlenhuth, Hartel, Schuster, & Fischman, 1980; Glass, Uhlenhuth, Hartel, Schuster, & Fischman, 1981). At the highest doses (4–5 mg) tested in the studies by Glass and coworkers, significant effects on heart rate and sedation were observed, but no change in anxiety was observed.

However, nabilone may have utility in the treatment of chronic anxiety. One study found that participants with general anxiety disorder ($n = 25$) treated with nabilone (1 mg TID) over 1 month had significant reductions in multiple measures of anxiety, compared to both baseline ratings and a placebo-treated group (Fabre & McLendon, 1981). Side effects, such as dry mouth and drowsiness were reported, but there were no reports of euphoria or feelings of a drug "high." In another study, nabilone-treated patients with advanced cancer reported significant improvements in overall distress and anxiety compared to untreated controls (Maida et al., 2008).

In addition to general anxiety, a chart review report found that nabilone (0.2–4 mg) provided relief from PTSD-associated nightmares in 34 out of 47 (72%) patients, none of whom had responded to conventional therapies (Fraser, 2009). The effect of nabilone lasted for the duration of treatment (4–12 months), but nightmares returned following the cessation of drug. Research to date suggests that nabilone is not appropriate for the treatment of acute anxiety, but may have utility for long-term management of anxiety, although again, large placebo-controlled clinical trials are needed.

CONCLUSIONS

The body of literature from the past 40 years provides clear evidence for nabilone's efficacy as an antiemetic, particularly for patients who do not respond to other treatments. In addition, there is evidence that nabilone has moderate efficacy in the treatment of chronic pain, pain-associated insomnia, PTSD-induced nightmares, dyskinesia, chronic anxiety, and cannabis use disorder. For all of these conditions, more research, especially large, placebo-controlled, clinical trials, is warranted. It is worth noting that medications exist to address many of these conditions; however, nabilone can have utility as either an adjunct treatment or as an alternative for patients who are unresponsive to other therapies. Additionally, in some cases, nabilone can address multiple symptoms thus reducing the number of medications required (Cameron, Watson, & Robinson, 2014).

The potential therapeutic uses for nabilone are comparable to the other FDA-approved oral cannabinoid, dronabinol. However, nabilone seems to be superior to dronabinol as a medication due to its high bioavailability and long duration of action. In addition, although nabilone can produce euphoric effects at therapeutic doses, it seems to have very minimal abuse liability or potential for diversion (Lemberger et al., 1982; Ware & St Arnaud-Trempe, 2010). Looking to the future, new uses for nabilone may emerge as our understanding of the cannabinoid system expands.

MINI-DICTIONARY

Analgesic A drug used for pain relief.
Antiemetic A drug that is effective against nausea and vomiting. Often used to treat chemotherapy-associated nausea and vomiting.
Cannabinoids A class of diverse chemicals that act on the cannabinoid receptors, CB1 and CB2. Phytocannabinoids, such as Δ^9-tetrahydrocannabinol (Δ^9-THC), are produced by the cannabis plant.
Dronabinol A pharmaceutical formulation of Δ^9-tetrahydrocannabinol (Δ^9-THC), the primary psychoactive cannabinoid produced by the cannabis plant. Marketed as Marinol, it is FDA approved to treat chemotherapy-associated nausea and vomiting, as well as loss of appetite in people with AIDS.
Drug discrimination study An experimental paradigm used to identify drugs with similar interoceptive effects. Humans or animals are trained to discriminate between a drug and placebo. Subjects then respond to indicate whether a new test drug "feels" more like the first drug or placebo.
Dyskinesia A category of movement disorders that are characterized by involuntary muscle movements, such as those seen in patients with Parkinson disease, Huntington disease, or following certain spinal cord injuries.
Fibromyalgia A syndrome characterized by a chronic, widespread pain in joints, muscles, tendons, and other soft tissue.
Multiple sclerosis An autoimmune disease that leads to demyelination and death of the neurons in the brain and spinal cord. The disease is associated with a wide range of symptoms, including spasticity.
Nabilone A synthetic analog of Δ^9-tetrahydrocannabinol (Δ^9-THC). Marketed as Cesamet, it is FDA approved to treat chemotherapy-associate nausea and vomiting.
Nocturia Waking often during the night to urinate. This can be a symptom of multiple sclerosis.

References

Archer, R. A., Blanchard, W. B., Day, W. A., Johnson, D. W., Lavagnino, E. R., Ryan, C. W., & Baldwin, J. E. (1977). Cannabinoids. 3. Synthetic approaches to 9-ketocannabinoids. Total synthesis of nabilone. *Journal of Organic Chemistry*, *42*(13), 2277–2284.

Baillie, G. L., Ross, R. A., & Pertwee, R. G. (2010). The ability of nabilone to interact with cannabinoid CB1 and CB2 receptors. In *20th annual symposium of the International Cannabinoid Research Society; Lund, Sweden. 2010*. Research Triangle Park, NC: International Cannabinoid Research Society.

Beaulieu, P. (2006). Effects of nabilone, a synthetic cannabinoid, on postoperative pain. *Canadian Journal of Anaesthesia*, *53*(8), 769–775.

Bedi, G., Cooper, Z. D., & Haney, M. (2013). Subjective, cognitive and cardiovascular dose-effect profile of nabilone and dronabinol in marijuana smokers. *Addiction Biology*, *18*(5), 872–881.

Ben Amar, M. (2006). Cannabinoids in medicine: a review of their therapeutic potential. *Journal of Ethnopharmacology*, *105*(1–2), 1–25.

Berlach, D. M., Shir, Y., & Ware, M. A. (2006). Experience with the synthetic cannabinoid nabilone in chronic noncancer pain. *Pain Medicine*, *7*(1), 25–29.

Bestard, J. A., & Toth, C. C. (2011). An open-label comparison of nabilone and gabapentin as adjuvant therapy or monotherapy in the management of neuropathic pain in patients with peripheral neuropathy. *Pain Practice*, *11*(4), 353–368.

Cameron, C., Watson, D., & Robinson, J. (2014). Use of a synthetic cannabinoid in a correctional population for posttraumatic stress disorder-related insomnia and nightmares, chronic pain, harm reduction, and other indications: a retrospective evaluation. *Journal of Clinical Psychopharmacology*, *34*(5), 559–564.

Cooper, Z. D., Comer, S. D., & Haney, M. (2013). Comparison of the analgesic effects of dronabinol and smoked marijuana in daily marijuana smokers. *Neuropsychopharmacology*, *38*(10), 1984–1992.

Curtis, A., Mitchell, I., Patel, S., Ives, N., & Rickards, H. (2009). A pilot study using nabilone for symptomatic treatment in Huntington's disease. *Movement Disorders*, *24*(15), 2254–2259.

Fabre, L. F., & McLendon, D. (1981). The efficacy and safety of nabilone (a synthetic cannabinoid) in the treatment of anxiety. *Journal of Clinical Pharmacology*, *21*(Suppl. 8–9), 377S–382S.

Fox, S. H., Henry, B., Hill, M., Crossman, A., & Brotchie, J. (2002). Stimulation of cannabinoid receptors reduces levodopa-induced dyskinesia in the MPTP-lesioned nonhuman primate model of Parkinson's disease. *Movement Disorders*, *17*(6), 1180–1187.

Frank, B., Serpell, M. G., Hughes, J., Matthews, J. N., & Kapur, D. (2008). Comparison of analgesic effects and patient tolerability of nabilone and dihydrocodeine for chronic neuropathic pain: randomised, crossover, double blind study. *British Medical Journal*, *336*(7637), 199–201.

Fraser, A. D., & Meatherall, R. (1989). Lack of interference by nabilone in the EMIT d.a.u. cannabinoid assay, Abbott TDx cannabinoid assay, and a sensitive TLC assay for delta 9-THC-carboxylic acid. *Journal of Analytic Toxicology*, *13*(4), 240.

Fraser, G. A. (2009). The use of a synthetic cannabinoid in the management of treatment-resistant nightmares in posttraumatic stress disorder (PTSD). *CNS Neuroscience and Therapeutics*, *15*(1), 84–88.

Gareau, Y., Dufresne, C., Gallant, M., Rochette, C., Sawyer, N., Slipetz, D. M., …, Labelle, M. (1996). Structure activity relationships of tetrahydrocannabinol analogues on human cannabinoid receptors. *Bioorganic and Medicinal Chemistry Letters*, *6*(2), 189–194.

Glass, R. M., Uhlenhuth, E. H., & Hartel, F. W. (1979). The effects of nabilone, a synthetic cannabinoid, on anxious human volunteers [proceedings]. *Psychopharmacology Bulletin*, *15*(2), 88–90.

Glass, R. M., Uhlenhuth, E. H., Hartel, F. W., Schuster, C. R., & Fischman, M. W. (1980). A single dose study of nabilone, a synthetic cannabinoid. *Psychopharmacology (Berlin)*, *71*(2), 137–142.

Glass, R. M., Uhlenhuth, E. H., Hartel, F. W., Schuster, C. R., & Fischman, M. W. (1981). Single-dose study of nabilone in anxious volunteers. *Journal of Clinical Pharmacology*, *21*(Suppl. 8–9), 383S–396S.

Haney, M., Cooper, Z. D., Bedi, G., Vosburg, S. K., Comer, S. D., & Foltin, R. W. (2013). Nabilone decreases marijuana withdrawal and a laboratory measure of marijuana relapse. *Neuropsychopharmacology*, *38*(8), 1557–1565.

Johnson, R., & Jasinski, D. (1983). Abuse potential of nabilone. In L. Lemberger, & M. M. Reidenberg (Eds.), *Proceedings of the 2nd World Conference on Clinical Pharmacology and Therapeutics, Washington, DC* (pp. 90). Bethesda, MD: American Society for Pharmacology and Experimental Therapeutics.

Kalliomaki, J., Philipp, A., Baxendale, J., Annas, P., Karlsten, R., & Segerdahl, M. (2012). Lack of effect of central nervous system-active doses of nabilone on capsaicin-induced pain and hyperalgesia. *Clinical and Experimental Pharmacology and Physiology*, *39*(4), 336–342.

Lemberger, L., & Rowe, H. (1975). Clinical pharmacology of nabilone, a cannabinol derivative. *Clinical Pharmacology and Therapeutics*, *18*(6), 720–726.

Lemberger, L., Rubin, A., Wolen, R., DeSante, K., Rowe, H., Forney, R., & Pence, P. (1982). Pharmacokinetics, metabolism and drug-abuse potential of nabilone. *Cancer Treatment Reviews*, *9*(Suppl. B), 17–23.

Lile, J. A., Kelly, T. H., & Hays, L. R. (2010). Substitution profile of the cannabinoid agonist nabilone in human subjects discriminating delta9-tetrahydrocannabinol. *Clinical Neuropharmacology*, *33*(5), 235–242.

Lile, J. A., Kelly, T. H., & Hays, L. R. (2011). Separate and combined effects of the cannabinoid agonists nabilone and Delta(9)-THC in humans discriminating Delta(9)-THC. *Drug and Alcohol Dependence*, *116*(1–3), 86–92.

Maida, V., Ennis, M., Irani, S., Corbo, M., & Dolzhykov, M. (2008). Adjunctive nabilone in cancer pain and symptom management: a prospective observational study using propensity scoring. *Journal of Supportive Oncology*, *6*(3), 119–124.

Martyn, C. N., Illis, L. S., & Thom, J. (1995). Nabilone in the treatment of multiple sclerosis. *Lancet*, *345*(8949), 579.

Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., & Bonner, T. I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature*, *346*, 561–564.

McGilveray, I. J. (2005). Pharmacokinetics of cannabinoids. *Pain Research and Management*, *10*(Suppl. A), 15A–22A.

Nakano, S., Gillespie, H. K., & Hollister, L. E. (1978). A model for evaluation of antianxiety drugs with the use of experimentally induced stress: Comparison of nabilone and diazepam. *Clinical Pharmacology and Therapeutics*, *23*(1), 54–62.

Notcutt, W., Price, M., Blossfeldt, P., & Chapman, G. (1999). *Clinical experience of the synthetic cannabinoid nabilone for chronic pain*. Totowa, NJ: Humana Press Inc.

Pertwee, R. G., & Fernando, S. R. (1996). Evidence for the presence of cannabinoid CB1 receptors in mouse urinary bladder. *British Journal of Pharmacology*, *118*(8), 2053–2058.

Pini, L. A., Guerzoni, S., Cainazzo, M. M., Ferrari, A., Sarchielli, P., Tiraferri, I., …, Zappaterra, M. (2012). Nabilone for the treatment of medication overuse headache: results of a preliminary double-blind, active-controlled, randomized trial. *Journal of Headache Pain*, *13*(8), 677–684.

Pinsger, M., Schimetta, W., Volc, D., Hiermann, E., Riederer, F., & Polz, W. (2006). Benefits of an add-on treatment with the synthetic cannabinomimetic nabilone on patients with chronic pain--a randomized controlled trial. *Wiener Klinische Wochenschrift*, *118*(11–12), 327–335.

Pooyania, S., Ethans, K., Szturm, T., Casey, A., & Perry, D. (2010). A randomized, double-blinded, crossover pilot study assessing the effect of nabilone on spasticity in persons with spinal cord injury. *Archives of Physical Medicine and Rehabilitation*, *91*(5), 703–707.

Redmond, W. J., Goffaux, P., Potvin, S., & Marchand, S. (2008). Analgesic and antihyperalgesic effects of nabilone on experimental heat pain. *Current Medical Research and Opinion*, *24*(4), 1017–1024.

Rubin, A., Lemberger, L., Warrick, P., Crabtree, R. E., Sullivan, H., Rowe, H., & Obermeyer, B. D. (1977). Physiologic disposition of nabilone, a cannabinol derivative, in man. *Clinical Pharmacology and Therapeutics*, *22*(1), 85–91.

Russo, E. B. (2008). Cannabinoids in the management of difficult to treat pain. *Therapeutics and Clinical Risk Management*, *4*(1), 245–259.

Sieradzan, K. A., Fox, S. H., Hill, M., Dick, J. P., Crossman, A. R., & Brotchie, J. M. (2001). Cannabinoids reduce levodopa-induced dyskinesia in Parkinson's disease: a pilot study. *Neurology*, *57*(11), 2108–2111.

Skrabek, R. Q., Galimova, L., Ethans, K., & Perry, D. (2008). Nabilone for the treatment of pain in fibromyalgia. *Journal of Pain*, *9*(2), 164–173.

Stark, P. (1982). The pharmacologic profile of nabilone: a new antiemetic agent. *Cancer Treatment Review*, *9*(Suppl. B), 11–16.

Toth, C., Mawani, S., Brady, S., Chan, C., Liu, C., Mehina, E., …, Korngut, L. (2012). An enriched-enrolment, randomized withdrawal, flexible-dose, double-blind, placebo-controlled, parallel assignment efficacy study of nabilone as adjuvant in the treatment of diabetic peripheral neuropathic pain. *Pain*, *153*(10), 2073–2082.

Ware, M. A., Daeninck, P., & Maida, V. (2008). A review of nabilone in the treatment of chemotherapy-induced nausea and vomiting. *Therapeutics and Clinical Risk Management*, *4*(1), 99–107.

Ware, M. A., Fitzcharles, M. A., Joseph, L., & Shir, Y. (2010). The effects of nabilone on sleep in fibromyalgia: results of a randomized controlled trial. *Anesthesia and Analgesia*, *110*(2), 604–610.

Ware, M. A., & St Arnaud-Trempe, E. (2010). The abuse potential of the synthetic cannabinoid nabilone. *Addiction*, *105*(3), 494–503.

Wesnes, K. A., Annas, P., Edgar, C. J., Deeprose, C., Karlsten, R., Philipp, A., …, Segerdahl, M. (2010). Nabilone produces marked impairments to cognitive function and changes in subjective state in healthy volunteers. *Journal of Psychopharmacology*, *24*(11), 1659–1669.

Wissel, J., Haydn, T., Muller, J., Brenneis, C., Berger, T., Poewe, W., & Schelosky, L. D. (2006). Low dose treatment with the synthetic cannabinoid Nabilone significantly reduces spasticity-related pain: a double-blind placebo-controlled cross-over trial. *Journal of Neurology*, *253*(10), 1337–1341.

CHAPTER

86

Synthetic Cannabinoids in Dementia

S. Amanullah[*], *K. Shivakumar*[**], *S. Hassan*[†], *A. Canfield*[‡], *J. Cole*[§]

*Woodstock General Hospital, Woodstock, ON, Canada; School of Medicine, University of Western Ontario, London, ON, Canada; and Faculty of Medicine, Dalhousie University, NS, Canada
**Health Sciences North, Department of Psychiatry, and Northern Ontario School of Medicine, Sudbury, ON, Canada
†Dalhousie University, Dartmouth, NS, Canada
‡University of Toronto, Toronto, ON, Canada
§Queen Elizabeth Hospital, Charlottetown, PE, Canada

SUMMARY POINTS

1. The burden of dementia and its complications is largely still unaffected by the current therapeutic modalities.
2. Cannabinoids exert a number of mechanisms of action that could positively affect neuronal resilience.
3. That positive effect may cover the different types of dementing illnesses.
4. The scientific community should endeavor to bridge the gap in the evidence base supporting the use of cannabinoids in dementia.

KEY FACTS

- Dementia is a severe, irreversible, and very expensive disease with rising prevalence rates globally.
- There is no effective strategy to effectively slow down or stop the progress of the illness while maintaining quality of life.
- Cannabinoids have been used for centuries, and can also be synthesized in the laboratory to ensure standardization. Used in various medical conditions, cannabinoids have a positive role in dementia with agitation.
- Current treatment strategies are largely unsatisfactory.
- Cannabinoids seem to be a potential helpful adjunctive treatment.
- Side effects with current interventions include an increased risk of cardiovascular events.
- The reported side effects of synthetic cannabinoids, as noted by the authors, have been restricted to an increased appetite and sedation.

AREAS OF INTEREST

1. Cannabinoids and cognitive functioning.
2. Their effect on various neurotransmitter systems, as well as neuronal homeostatic mechanisms.
3. Effect of these agents on different brain pathologies, including dementias.
4. Potential for affecting phenotypic presentation of dementia, including its complications, using both synthetic and natural cannabinoids.

LIST OF ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
ACh	Acetylcholine
AChE	Acetylhydrolase
AD	Alzheimer dementia
AEA	Anandamide

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00100-9
Copyright © 2017 Elsevier Inc. All rights reserved.

BPSD	Behavioral and psychological symptoms in dementia
CB_1/CB_2	Cannabinoid receptor 1 and 2
DLB	Dementia of Lewy Body
eCBs	Endocannabinoid system
FTD	Frontotemporal dementia
HD	Huntington disease
PD	Parkinson disease
PDD	Parkinson Disease Dementia
THC	Δ^9-Tetrahydocannabinol
THCV	Tetrahydocannabivarin
TNFα	Tumor necrosis factor alpha
TVRP1	Transient receptor potential vanilloid type 1

INTRODUCTION

In the field of medicine, the use of synthetic cannabinoids have been studied (Ben Amar, 2006) by way of its constituent cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC). Cannabinoids have increasingly been reported to be of benefit in a number of conditions such as Parkinson's (FD), frontotemporal dementia (FTD), Huntington disease (HD) dementia (Amanullah, MacDougall, Sweeney, Coffin, & Cole, 2013; Campbell & Gowran, 2007; Robson, 2001). Evidence for routine use is limited, however, and this chapter will hopefully educate the reader on the current understanding and role of synthetic cannabinoids in dementia.

BACKGROUND

Dementia includes all neurocognitive disorders that are characterized by a decline in memory, other cognitive skills, and independent functioning, estimated to affect 14% (35 million) of people aged 71 and older in more developed countries. The number is expected to reach 70 million in 2030 (Plassman et al., 2007).

Various mechanisms and theories of dementia include cholinergic theories, apoptosis, oxidative stress, neuroendocrine alterations, shortening of telomere, damage to mitochondria, and autoimmune changes (Wilson & Nicoll, 2002).

The oldest among such theories is the cholinergic theory. It states that there is a reduction of about 40–70% in cholinergic neurons in the basal forebrain region (Kanof, Greenwald, Mohs, & Davis, 1985). Continued use of medications that inhibit cholinesterases can increase phosphorylation of tau protein (Chalmers et al., 2009), a process that can affect the long term effectiveness of cholinesterase inhibitors. Clinical presentation occurs only after significant neuronal loss, highlighting the importance of early detection.

The process seen in Alzheimer's dementia (AD) is due to the neurodegenerative process that is different from normal age related changes. Different species of Aβ (fibrillar β amyloid) produced by the cleavage of Aβ precursor proteins by β and γ secretases enzymes are a feature of AD (Aso & Ferrer, 2014).

Agonists cause an inhibition of GABA, and glutamate release is modulated (Walther & Halpern, 2010). Studies have shown lower anandamide (AEA) and 2-arachidonoylglycerol (2-AG) levels in Alzheimer's versus normal subjects (Koppel et al., 2009), but the lower levels of AEA in mid-frontal and temporal cortex of patients with AD in post mortem studies suggests a contribution of Aβ 42 dependent AEA impairment by the inverse correlation with Aβ 42 neurotoxic levels (Jung et al., 2012).

MECHANISMS OF ACTION OF CANNABINOIDS IN DEMENTIA

Much of our scientific data is derived from experimental animal models that mimic several traits of the neurodegenerative processes that lead to dementia. These animal models have provided some information about the mechanism of action and the therapeutic properties of cannabis derivatives.

The improvements observed were mainly in cognition and behavior.(Aso et al., 2012; Aso, Juvés, Maldonado, & Ferrer, 2013; Aso & Ferrer, 2014; Fakhfouri et al., 2012; Haghani, Shabani, Javan, Motamedi, & Janahmadi, 2012; Ramírez, Blázquez, Gómez del Pulgar, Guzmán, & de Ceballos, 2005).

Location of Cannabinoid Receptors in the Brain

It can be hypothesized that the potential role of cannabinoids in dementia relies on their capacity to concomitantly mitigate the pathological mechanisms seen in most types of dementia. These include toxicity, oxidative stress, excitotoxicity, mitochondrial dysfunction, abnormal protein folding, and inflammation at the neuronal level. It is reported that this can occur with 20 times the potency of aspirin (Aso & Ferrer, 2014; Fagan & Campbell, 2014; Russo, 2011).

Table 86.1 summarizes the main mechanisms, as currently understood, modulated by cannabinoids that are involved in AD pathology, which can be extrapolated to other pathological conditions (but, as one would expect, clinical trials are needed with larger sample sizes).

Toxicity Secondary to Abnormalities in Protein Folding

Several in vitro and in vivo studies demonstrate that certain cannabinoid compounds, including CB_1 and CB_2 receptor synthetic agonists, endocannabinoids, and natural cannabinoids such as Δ^9-THC and CBD, confer neuroprotection against Aβ. A peptide derived from aberrant

TABLE 86.1 Anatomical Localization and Associated Central Functions of Cannabinoid Receptors in the Mammalian Brain

Receptor class	Intensity	Localization	Functions
CB_1	Dense	Hippocampus, caudate, putamen, nucleus accumbens (ventral striatum), globus pallidus, substantia nigra pars reticulata, horizontal limb of the diagonal band, periaqueductal gray, dorsal horn of spinal cord, some olfactory regions	Cognition, pain modulation, regulation of food intake, motor coordination, mood, autonomic function, memory, sensation, lipid and glucose metabolism
	Moderate	Neocortex, basal amygdala, medial hypothalamus, solitary nucleus	
	Low	Human cerebellum, thalamus, brain stem	
CB_2	Dense	Anterior olfactory nucleus, neurons of layers III and V of the piriform, orbital, visual, motor, and auditory cortex, pyramidal neurons of the hippocampal allocortex, reticular thalamic nucleus, Purkinje cell bodies	Involved in immune function and inflammatory responses, including modulation of cytokine release and control of proliferation, differentiation and survival of neuronal and nonneuronal cells
	Moderate	Periaqueductal gray, substantia nigra pars reticulata, other nuclear structures of brain stem, dendrites of Purkinje cells in the molecular layer	
	Low	Ventromedial and arcuate hypothalamic nuclei	

Cannabinoid receptors CB_1 and CB_2 in the mammalian brain each have unique regional distributions, with receptor densities in brain areas found to be high, moderate, or low. These anatomical localizations play a role in the differing set of functions of each receptor type.
Note. Data compiled from Svíženská, Dubový, and Šulcová (2009).

processing of amyloid precursor protein (APP)—one of the hallmarks of AD (Aso et al., 2012; Harvey, Ohlsson, Mååg, Musgrave, & Smid, 2012; Iuvone et al., 2004).

Protective actions of cannabinoids:

1. Indirectly reducing the Aβ harmful effects on inflammation, oxidative stress, excitotoxicity, and cellular signaling.
2. Directly affecting Aβ processing by stimulating Aβ removal (Bachmeier, Beaulieu-Abdelahad, Mullen, & Paris, 2013; Martín-Moreno et al., 2012; Tolón et al., 2009; Wu et al., 2013).
3. Reducing Aβ levels (Aso et al., 2012; Aso & Ferrer, 2014).

A role for cannabinoids has also been reported in:

1. Tau hyperphosphorylation (Aso et al., 2012, 2013; Casarejos et al., 2013; Esposito et al., 2011).
2. Inhibition of formation of mutant huntingtin-aggregates that accumulate, leading on to neuronal cell death in HD (Blázquez et al., 2011; Scotter, Goodfellow, Graham, Dragunow, & Glass, 2010).

Preclinical studies also suggests the disease-modifying effects of cannabinoid-based medicines in HD (Fernandez-Ruiz et al., 2012).

Modulation of Neurotransmission

Certain cannabinoids may act on the same target receptors as current medications for cognitive impairment; eg, Δ^9-THC competitively inhibits acetylhydrolase (AChE), thus increasing acetylcholine (ACh) levels in the brain (Eubanks et al., 2006). Synthetic cannabinoid HU-211 acts as a stereoselective inhibitor of NMDA receptors and protects cells from NMDA-induced neurotoxicity (Eshhar, Streim, & Biegon, 1993; Feigenbaum et al., 1989; Nadler, Mechoulam, & Sokolovsky, 1993).

Cannabinoids mediate neuroprotection against excitotoxicity by:

1. Inhibiting the presynaptic glutamate release (Marsicano et al., 2003).
2. Blocking the voltage dependent calcium channels (Mackie & Hille, 1992).
3. Inhibiting the calcium release from ryanodine sensitive stores (Zhuang et al., 2005), mechanisms in which CB_1 receptors play a prominent role.
4. Promotion of neurogenesis (Esposito et al., 2011; Tanveer et al., 2012).
5. Facilitation of cerebral blood flow may also contribute to the therapeutic effects of cannabinoids in dementia, although their contribution remains to be fully elucidated. (Ellis, Moore & Willoughby, 1995; Iring et al., 2013).
6. Control of apoptosis pathways through neuroprotection (Noonan et al., 2010).

Mechanism of Action of CB_1 and CB_2 (Table 86.2)

Limitations:

1. Complexity of the human cognitive functions makes it irreproducible in animal models.

TABLE 86.2 Summary of the Basic Mechanism of Actions of CB_1 and CB_2 Receptors

Shared mechanism of action	CB_1 mechanism of action	CB_2 mechanism of action
Coupled to G proteins G_i or G_o Coupled positively to mitogen-activated protein Coupled negatively to adenylyl cyclase Net result: decreased production of cAMP and subsequent inhibition of cAMP-dependent protein kinase (PKA)	Coupled to ion channels via G_i/G_o mechanism • Positively: A type, and inwardly rectifying potassium channels • Negatively: N type, and P/Q type calcium channels, D type potassium channels Net result of PKA inhibition: reduction to ion channel protein phosphorylation • Inhibition of calcium channels on CB_1 containing presynaptic terminals likely reduces neurotransmitter release, including glutamate, acetylcholine, and noradrenaline	PKA inhibition results in decreased phosphorylation of cAMP response element-binding protein cAMP response element binding protein is a transcription factor that regulates a variety of cellular responses including survival, proliferation and differentiation of immune cells Net result: immune inhibition

Cannabinoid receptors CB_1 and CB_2 belong to the same superfamily of receptors that couple to guanine-nucleotide binding proteins (G proteins). Some of the basic mechanism of action for CB_1 receptors is the same as that of CB_2 receptors. Cannabinoids have been suggested to play a role in the regulation of neurotransmitter release, including glutamate, acetylcholine, and noradrenaline. This has been suggested to occur through CB_1 receptors on presynaptic terminals coupled to ion channels.

Note. Data for shared mechanism for action and CB_1 mechanism of action from Sviženská et al. (2009), and data for CB_2 mechanism of action from Malfitano, Basu, Maresz, Bifulco, and Dittel (2014).

2. Mammalian models have limitations due to highly advanced language skills in humans.
3. Moreover, the efficacy of cannabinoids in curbing the cognitive impairment was inversely proportional to the stage of disease at the onset of treatment in the animal models (Aso et al., 2012; Aso & Ferrer, 2014). This will have important ramifications in human studies and interventions.

Early detection and intervention in dementia in patients is critical, in order to take advantage of cannabinoids as potential therapeutic agents.

A series of studies (Solowij, Grenyer, Chesher, & Lewis, 1995), revealed relevant and replicable evidence linking the duration of cannabis use and sensitive measures of brain function; event-related potentials during a complex selective attention task. The results showed impairments in the ability to focus attention, and also abnormalities in filtering out relevant from irrelevant information.

Neuroinflammation

Neuroinflammation is a major contributor to progressive neuronal damage, leading on to dementia. Ongoing research on neuroinflammation focuses on compounds that act on CB_2 receptors, such as THC and THCV (tetrahydrocannabivarin) (Cabral & Griffin-Thomas, 2009), mainly due to expression in the immune system, and activation leading to:

1. Reduced microglial response and proinflammatory molecule production, including cytokines and TNF-α.
2. Attenuation of nitric oxide oversynthesis (Aso et al., 2013; Esposito et al., 2007; Fakhfouri et al., 2012; Martín-Moreno et al., 2012; Ramírez et al., 2005; Russo, 2011; van der Stelt et al., 2006; Wu et al., 2013).

Several CB_1 receptors agonists and natural CBD have shown antiinflammatory properties in certain neurodegenerative models (Aso et al., 2012; Casarejos et al., 2013; Esposito, De Filippis, Carnuccio, Izzo, & Iuvone, 2006a; Martín-Moreno et al., 2011).

Oxidative Stress and Dysfunction of Mitochondria

Mitochondrial dysfunction is one of the earliest events in neurodegenerative processes. Mitochondrial dysfunction leads to energy deficiency and increased production of oxidants that compromise neuronal cell function (Burchell et al., 2010; Ferrer, 2009). The cannabinoid derivatives, mainly CBD, have shown antioxidant properties in cell cultures exposed to toxic proteins like Aβ (Esposito et al., 2006b, 2011; Hampson, Grimaldi, Axelrod, & Wink, 1998; Hampson et al., 2000; Iuvone et al., 2004).

Similar findings were also observed in tauopathy animal models (Casarejos et al., 2013) that were treated with CB_2 agonist or a combination of Δ^9-THC and CBD.

TAUOPATHIES AND CANNABINOIDS

Tauopathy is a term that encompasses all neurodegenerative diseases associated with the pathological modification of tau protein by hyperphosphorylation, leading to tau protein aggregation, and neurotoxicity.

Different types of tauopathies can develop, based on the specific tau modifications, brain areas affected, and the existence of other pathological traits:

1. AD, Down's syndrome (which are also beta-amyloidopathies).
2. Dementia with only tangles—Pick's disease, dementia pugilistica, frontotemporal lobe degeneration associated with MAPT mutations, progressive supranuclear palsy, corticobasal degeneration, argyrophilic grain disease, myotonic dystrophy, and other rarer tauopathies.
3. Studies on animal models have shown potential benefits in taupathies, however no specific information is available about the effects of cannabinoids in patients affected by tauopathies different from AD.

Prolonged exposure to a combination of Δ^9-THC and CBD (natural extracts) have shown a significant reduction in neurofibrillary tangles in murine models that had parkinsonism, complex frontotemporal dementia, and lower motor neuron disease (Casarejos et al., 2013). Reduction in the deposition of tau and amyloid proteins in the hippocampus and the cerebral cortex was also observed in this study. This is of clinical significance, given the paucity of conventional interventions. However, one would also need to look at the prolonged exposure in relation to those who use cannabinoids culturally for protracted periods, to see if there is a reduction in the formation of tangles (Fig. 86.1).

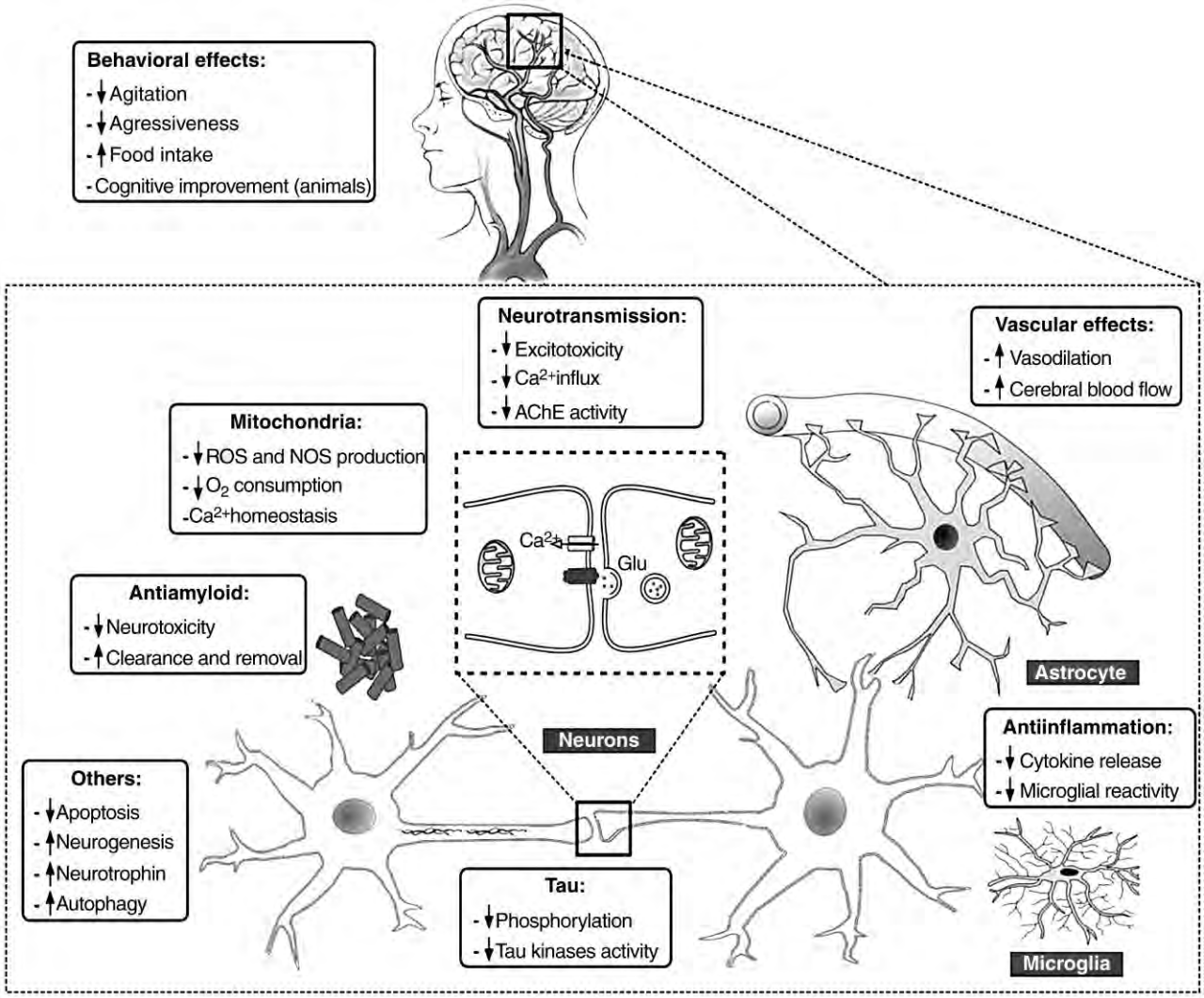


FIGURE 86.1 **Summary of the main findings demonstrating beneficial effects of cannabinoid compounds in AD models.** Cannabinoids may target in parallel several processes that play key roles in AD, including Aβ and tau aberrant processing, chronic inflammatory responses, excitotoxicity, mitochondrial dysfunction, and oxidative stress, among others. Clinical data also reveal an improvement in behavioral in patients with AD, after treatment with cannabinoids. *Source: Reproduced with permission from Aso and Ferrer (2014).*

TABLE 86.3 Cortical and Subcortical Dementia Types

Cortical dementias	Subcortical dementias
Alzheimer disease	***Vascular dementia***
Most common type of dementia Neuropathic process: intracellular neurofibrillary tangles, extracellular β-amyloid plaques Early disease: hippocampus and temporal lobe affected—memory impairment Later disease: tangles and plaques progress to frontal and parietal regions—impairment of: judgment, word finding, temporal orientation, route navigation Eventually, patients may exhibit agitated behavior and/or personality change Typical disease course is 6 to 9 years from symptom onset to death	Second most common type of dementia Cognitive impairment varies depending on location and degree of tissue damage Pathogenesis: ischemia to large or small vessels Large vessel ischemia: abrupt symptom onset; results in stroke Small vessel ischemia: gradual symptom onset as lesions accumulate; often misdiagnosed as cortical dementia Risk factors: smoking, atherosclerosis, hypertension, hypercholesterolemia, and diabetes mellitus
Dementia with lewy bodies	***Parkinson disease dementia***
Neuropathic process: Lewy body protein deposits throughout the cortex, paralimbic areas, substantia nigra Prominent impairment in: attention, visuospatial and executive functioning Other signs and symptoms: sleep disturbance, extrapyramidal symptoms, visual hallucinations, fluctuations in attention and alertness More rapid progression than AD	Slowly progressing dementia that can develop in individuals with Parkinson disease Typically presents 10 years after onset of disease Occurs in approximately 30% of patients with Parkinson's disease; more common in those with greater physical impairment Impairment to: executive functioning, attention, visuospatial functioning, memory
Frontotemporal dementia	
Group of heterogenous disorders Neuropathic process: frontal cortical atrophy, temporal cortical atrophy First symptom: personality change Two subtypes: Behavioral—impaired insight; distinct patterns of behavioral dysfunction Language—distinct patterns of language dysfunction Rapid progression over 5–10 year course	

Cortical dementias result from disease causing diffuse cortical neuronal atrophy, and are typically progressive and degenerative. Subcortical dementias result from diseases involving subcortical structures, including vasculature, the thalamus, and the basal ganglia, and may be progressive, static, or reversible.
Note. Data from Buffington, Lipski, and Westfall (2013).

CANNABINOIDS IN SUBCORTICAL DEMENTIAS AND PARKINSON DISEASE

Although a controversial term, the term subcortical dementia is useful for the clinician in diagnostics and, to some extent, management. The evidence is primarily in AD and HD (Table 86.3).

The role of the cannabinoid system in movement warrants special attention. This encompasses both types of CB receptors, and the related vanilloid TRPV1 receptor. These are all densely located in the output nuclei of the basal ganglia (Mackie, 2005). The various positive effects of cannabinoids on the basal ganglia support their potential therapeutic effects in hyperkinetic movement disorders such as HD, hypokinetic disorders such as PD, and also other disorders of different etiologies, such as primary dystonias, dykinesias, and Tourette syndrome.

Cannabinoids increase GABAergic transmission, inhibit glutamate release, and positively affect dopaminergic uptake.

Tables 86.4 and 86.5 show the available evidence for its use in diseases associated with it.

CONCLUSIONS

The incidence of dementia is expected to quadruple by 2050, with an incidence rate of BPSD of over 90% in sufferers (Steinberg et al., 2008).

Agitation, which presents as a major management challenge, occurs in 60% of patients with dementia, and is associated with the severity of the disease (Senanarong et al., 2004). The lack of satisfactory and enduring results with current mainstream treatments has forced clinicians to look for less studied alternatives, such as

TABLE 86.4 Cannabinoids Suggested for Use in Alzheimer Disease (AD), Vascular Dementia (VD), Huntington Disease (HD) and Parkinson Disease (PD) Using Clinical Trial Findings

Neurodegenerative disease	Cannabinoids suggested for use	Mechanism of action	Suggested benefits	Possible side effects	Number of subjects	References
AD	Dronabinol (THC)	$CB_{1/2}$ agonism	↓ Agitation	Seizure, infection, ↑ body weight, anxiety/nervousness, tiredness, emotional liability, somnolence, euphoria, balance disorder, GI disorder, blurred vision, muscle weakness	15	Volicier, Stelly, Morris, McLaughlin, and Volicier (1997)
	Dronabinol (THC)	$CB_{1/2}$ agonism	↓ Nocturnal motor activity, strengthened circadian rhythms	Not assessed; however, found a slight ↓ in mini mental state exam (MMSE)	2	Walther, Schupbach, Seifritz, Homan, and Strik (2011)
VD	No clinical trials conducted					
HD	Nabilone (mimics THC)	$CB_{1/2}$ agonism	Positive effects on cognition, behavior, and chorea symptoms	Suicidal ideation, drowsiness and forgetfulness, severe sedation	44	Curtis, Mitchell, Patel, Ives, and Rickards (2009)
PD	Nabilone (mimics THC)	$CB_{1/2}$ agonism	Reduced levodopa-induced dyskinesia	Well tolerated	7	Sieradzan et al. (2001)
	Oral cannabis extract (THC: Cannabidiol)	$CB_{1/2}$ agonism and unknown cannabidiol mechanism	Hopeful for reduced levodopa-induced dyskinesia, but no effect found	Drowsiness/lethargy, detachment, dry mouth	19	Carroll et al. (2004)

All studies ($N = 5$) were conducted using randomized double-blind placebo controlled crossover trials; however, study sample sizes were small, and treatment dosages varied. Limited evidence is available on the medicinal use of cannabinoids in dementia and, as a result, there is little guidance available to practitioners regarding its use.
Note. Data from Walther et al. (2011) and van den Elsen et al. (2014).

TABLE 86.5 Cannabinoids Suggested for Potential Use in Neurodegenerative Diseases Using Preclinical Findings

Neurodegenerative disease	Intervention	Possible benefits	Mechanism of action if known
AD	CB_1 agonists	1. Prevention and protection against excitotoxicity 2. Prevention of Aβ-induced neurotoxicity	Inhibition of the presynaptic release of glutamate via G-protein mechanisms 1. Reduction of tau protein hyperphosphorylation via reduction of nitric oxide production 2. Brain-derived neurotrophic factor mediation of CB_1 agonist neuroprotective effects
	N-methyl-D-aspartate	Protection mechanism against neurotoxicity	Increase of endogenous cannabinoid anandamide
	Δ^9-THC or AEA	1. Reduction to neuronal damage 2. Prevention to Aβ-aggregation	CB_1 receptor mediated effects Inhibition of acetylcholine esterase
	CB_2 agonists	1. Suppression to the neuroinflammatory process	1. Reduction of CD40 expression 2. Reduction of nitric oxide and tumor necrosis factor α production in activated microglia
		2. β-amyloid removal	1. Stimulation of human macrophages 2. Suppression of CD40-mediated inhibition of microglial phagocytosis

TABLE 86.5 Cannabinoids Suggested for Potential Use in Neurodegenerative Diseases Using Preclinical Findings (*cont.*)

Neurodegenerative disease	Intervention	Possible benefits	Mechanism of action if known
	CB_1/CB_2 agonists WIN55212-2 and HU-210	1. Prevention of microglial activation 2. Improvement to memory performance	
	Cannabidiol	Antioxidant effects including protection against Aβ-induced neurotoxicity	Mechanism remains unclear
VD	Inconsistent findings	Presently no potential cannabinoid-based intervention for cerebral ischemia	
HD	CB_2 agonists	Possible reduction to neuroinflammation, but still unclear	
PD	CB_1/CB_2 agonist CP55,940	Protection against paraquat toxicity which induces parkinsonism	Inactivation of the oxidative stress response Jun-*N*-terminal kinase signaling
	CB_1 agonist Δ^9-THC, cannabidiol, and endocannabinoid inactivation inhibitor AM404	Amelioration of nigrostriatal lesion effects	Likely the result of antioxidant properties
	CB_2 receptor agonist HU-308	Small recovery to nigrostriatal lesion effects	Contribution to neuroprotection via activation of CB_2 receptors
	CB_1/CB_2 agonist WIN55, 212-2	Amelioration of nigrostriatal lesion effects independent of CB_1 receptor activation	
	CB_1/CB_2 agonist WIN55, 212-2 and CB_2 agonist JWH015	Reduction to lesion-induced microglial activation	
	Coinjection of D_2 receptor agonist quinpirole and CB_1/CB_2 agonist WIN 55, 212-2	Alleviation of akinesia	
	CB_1 antagonists	Alleviation of motor dysfunction	Augmentation of levodopa
	Coadministration of D2 agonist quinpirole and CB_1 receptor antagonist SR141716A	Restoration of locomotion	SR141716A augmentation of quinpirole effects
	Δ^9-THC	Improvement to motor deficits in mild PD	
	Coadministration of CB agonists HU-210 and nabilone with levodopa	Reduction to dyskinesia	CB agonists prevent the reduction of extracellular glutamate caused by levodopa; extracellular glutamate is inversely correlated with dyskinesia
	Anandamide membrane transporter inhibitors, fatty acid amide hydrolase inhibitors, or CB_1 agonists	Decrease to elevated glutamatergic transmission; CB_1 agonists only—decrease to glutamate release from afferent terminals in the striatum	

Endocannabinoids have been suggested as interventions for neurodegenerative diseases including Alzheimer disease (AD), Vascular dementia (VD), Huntington disease (HD), and Parkinson disease (PD), due to their impact on neurotransmission via retrograde signaling. Despite an increase in preclinical data suggesting the potential benefits of cannabinoids in neurodegenerative diseases, treatment plans for those with AD, VD, HD, and PD do not currently incorporate these findings.
Note. Data from Walther and Halpern (2010).

cannabinoids and electroconvulsive therapy (ECT); although a randomized controlled trial in 2011 assessing the latter yielded satisfactory results.

Cannabinoids can potentially alter the progression of the underlying pathology in dementia by different mechanisms. Moreover, they should spur serious randomized controlled trials in assessing their efficacy in clinical populations with dementia.

MINI-DICTIONARY

Alzheimer disease A chronic progressive condition characterized by cognitive decline not in keeping with age, changes in personality, loss of visuospatial skills, language dysfunction, disturbances in planning, judgment, and planning.
Dementia A condition characterized by cognitive dysfunction, changes in personality, loss of visuospatial skills, language dysfunction, disturbances in planning, judgment, and planning. They may occur in isolation, or in varying combinations based on the type of dementia.
Subcortical dementia A controversial term that refers to dementia involving the subcortical regions and presenting with significant slowing of cognition, affective, and motor symptoms.
Synthetic cannabinoids Synthetic cannabinoids are strictly defined as any designer drug that can mimic the effects of cannabis and can be often found as "spice products."
Tauopathies Tauopathy is a general term that encompasses all the neurodegenerative diseases associated with the pathological modification of tau protein by hyperphosphorylation that leads to tau protein aggregation and neurotoxicity.
Vascular dementia This term generally refers to dementia in which vascular causation is seen as the primary etiology

Acknowledgment

The authors would like to acknowledge Ms Ester Aso Perez and Prof I. Ferrer for their input that was instrumental in writing this chapter.

References

Amanullah, S., MacDougall, K., Sweeney, N., Coffin, J., & Cole, J. (2013). Synthetic cannabinoids in dementia with agitation: case studies and literature review. *Clinical Neuropsychiatry*, *10*(3–4), 142–147.

Aso, E., & Ferrer, I. (2014). Cannabinoids for treatment of Alzheimer's disease: moving toward the clinic. *Frontiers in Pharmacology*, *5*, 37.

Aso, E., Palomer, E., Juvés, S., Maldonado, R., Muñoz, F. J., & Ferrer, I. (2012). CB1 agonist ACEA protects neurons and reduces the cognitive impairment of A(PP/PS1 mice. *Journal of Alzheimer's Disease*, *30*(2), 439–459.

Aso, E., Juvés, S., Maldonado, R., & Ferrer, I. (2013). CB2 cannabinoid receptor agonist ameliorates Alzheimer-like phenotype in AβPP/PS1 mice. *Journal of Alzheimer's Disease*, *35*(4), 847–858.

Bachmeier, C., Beaulieu-Abdelahad, D., Mullen, M., & Paris, D. (2013). Role of the cannabinoid system in the transit of beta-amyloid across the blood–brain barrier. *Molecular and Cellular Neurosciences*, *56*, 255–262.

Ben Amar, M. (2006). Cannabinoids in medicine: a review of their therapeutic potential. *Journal of Ethnopharmacology*, *105*(1–2), 1–25.

Blázquez, C., Chiarlone, A., Sagredo, O., Aguado, T., Pazos, M. R., Resel, E., …, Guzmán, M. (2011). Loss of striatal type 1 cannabinoid receptors is a key pathogenic factor in Hunnington's disease. *Brain*, *134*(Pt 1), 119–136.

Buffington, A. L. H., Lipski, D. M., & Westfall, E. (2013). Dementia: an evidence-based review of common presentations and family-based interventions. *The Journal of the American Osteopathic Association*, *113*(10), 768–775.

Burchell, V. S., Gandhi, S., Deas, E., Wood, N. W., Abramov, A. Y., & Plun-Favreau, H. (2010). Targeting mitochondrial dysfunction in neurodegenerative disease: Part I. *Expert Opinion on Therapeutic Targets*, *14*(4), 369–385.

Cabral, G. A., & Griffin-Thomas, L. (2009). Emerging role of the cannabinoid receptor CB2 in immune regulation: therapeutic prospects for neuroinflammation. *Expert Reviews in Molecular Medicine*, *11*, e3.

Campbell, V. A., & Gowran, A. (2007). Alzheimer's disease; taking the edge off with cannabinoids? *British Journal of Pharmacology*, *152*(5), 655–662.

Carroll, C. B., Bain, P. G., Teare, L., Liu, X., Joint, C., Wroath, C., Parkin, S. G., Fox, P., Wright, D., Hobart, J., & Zajicek, J. P. (2004). Cannabis for dyskinesia in Parkinson disease: a randomized double-blind crossover study. *Neurology*, *63*, 1245–1250.

Casarejos, M. J., Perucho, J., Gomez, A., Muñoz, M. P., Fernandez-Estevez, M., Sagredo, O., & Mena, M. A. (2013). Natural cannabinoids improve dopamine neurotransmission and tau and amyloid pathology in a mouse model of tauopathy. *Journal of Alzheimer's Disease*, *35*(3), 525–539.

Chalmers, K. A., Wilcock, G. K., Vinters, H. V., Perry, E. K., Perry, R., Ballard, C. G., & Love, S. (2009). Cholinesterase inhibitors may increase phosphorylated tau in Alzheimer's disease. *Journal of Neurology*, *256*(5), 717–720.

Curtis, A., Mitchell, I., Patel, S., Ives, N., & Rickards, H. (2009). A pilot study using nabilone for symptomatic treatment in Huntington's disease. *Movement Disorders*, *24*, 2254–2259.

Ellis, E. F., Moore, S. F., & Willoughby, K. A. (1995). Anandamide and delta 9-THC dilation of cerebral arterioles is blocked by indomethacin. *American Journal of Physiology*, *269*(6 Pt 2), H1859–H1864.

Eshhar, N., Striem, S., & Biegon, A. (1993). HU-211, a non-psychotropic cannabinoid, rescues cortical neurones from excitatory amino acid toxicity in culture. *Neuroreport*, *5*(3), 237–240.

Esposito, G., De Filippis, D., Carnuccio, R., Izzo, A. A., & Iuvone, T. (2006a). The marijuana component cannabidiol inhibits beta-amyloid-induced tau protein hyperphosphorylation through Wnt/beta-catenin pathway rescue in PC12 cells. *Journal of Molecular Medicine*, *84*(3), 253–258.

Esposito, G., De Filippis, D., Steardo, L., Scuderi, C., Savani, C., Cuomo, V., & Iuvone, T. (2006b). CB1 receptor selective activation inhibits beta-amyloid-induced iNOS protein expression in C6 cells and subsequently blunts tau protein hyperphosphorylation in co-cultured neurons. *Neuroscience Letters*, *404*(3), 342–346.

Esposito, G., Iuvone, T., Savani, C., Scuderi, C., De Filippis, D., Papa, M., …, Steardo, L. (2007). Opposing control of cannabinoid receptor stimulation on amyloid-beta-induced reactive gliosis: in vitro and in vivo evidence. *The Journal of Pharmacology and Experimental Therapeutics*, *322*(3), 1144–1152.

Esposito, G., Scuderi, C., Valenza, M., Togna, G. I., Latina, V., De Filippis, D., …, Steardo, L. (2011). Cannabidiol reduces Aβ-induced neuroinflammation and promotes hippocampal neurogenesis through PPARγ involvement. *PLoS One*, *6*(12), e28668.

Eubanks, L. M., Rogers, C. J., Beuscher, A. E., Koob, G. F., Olson, A. J., Dickerson, T. J., & Janda, K. D. (2006). A molecular link between the active component of marijuana and Alzheimer's disease pathology. *Molecular Pharmaceutics*, *3*(6), 773–777.

Fagan, S. G., & Campbell, V. A. (2014). The influence of cannabinoids on generic traits of neurodegeneration. *British Journal of Pharmacology*, *171*(6), 1347–1360.

Fakhfouri, G., Ahmadiani, A., Rahimian, R., Grolla, A. A., Moradi, F., & Haeri, A. (2012). WIN55212-2 attenuates amyloid-beta-induced neuroinflammation in rats through activation of cannabinoid receptors and PPAR-γ pathway. *Neuropharmacology*, *63*(4), 653–666.

Feigenbaum, J. J., Bergmann, F., Richmond, S. A., Mechoulam, R., Nadler, V., Kloog, Y., & Sokolovsky, M. (1989). Nonpsychotropic cannabinoid acts as a functional *N*-methyl-D-aspartate receptor blocker. *Proceedings of the National Academy of Sciences of the United States of America*, *86*(23), 9584–9587.

Fernandez-Ruiz, J., Sagredo, O., Pazos, M., Garcia, C., Pertwe, R., Mechoulam, R., & Martinez-Orgado, J. (2012). Cannabidiol for neurodegenerative disorders: important new clinical applications for this phytocannabinoid? *British Journal of Clinical Pharmacology*, *75*(2), 323–333.

Ferrer, I. (2009). Altered mitochondria, energy metabolism, voltage-dependent anion channel, and lipid rafts converge to exhaust neurons in Alzheimer's disease. *Journal of Bioenergetics and Biomembranes*, *41*(5), 425–431.

Haghani, M., Shabani, M., Javan, M., Motamedi, F., & Janahmadi, M. (2012). CB1 cannabinoid receptor activation rescues amyloid β-induced alterations in behaviour and intrinsic electrophysiological properties of rat hippocampal CA1 pyramidal neurones. *Cellular Physiology and Biochemistry*, *29*(3–4), 391–406.

Hampson, A. J., Grimaldi, M., Axelrod, J., & Wink, D. (1998). Cannabidiol and (−)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(14), 8268–8273.

Hampson, A. J., Grimaldi, M., Lolic, M., Wink, D., Rosenthal, R., & Axelrod, J. (2000). Neuroprotective antioxidants from marijuana. *Annals of the New York Academy of Sciences*, *899*, 274–282.

Harvey, B. S., Ohlsson, K. S., Mååg, J. L. V., Musgrave, I. F., & Smid, S. D. (2012). Contrasting protective effects of cannabinoids against oxidative stress and amyloid-β evoked neurotoxicity in vitro. *Neurotoxicology*, *33*(1), 138–146.

Iring, A., Ruisanchez, É., Leszl-Ishiguro, M., Horváth, B., Benkő, R., Lacza, Z., …, Benyó, Z. (2013). Role of endocannabinoids and cannabinoid-1 receptors in cerebrocortical blood flow regulation. *PLoS One*, *8*(1), e53390.

Iuvone, T., Esposito, G., Esposito, R., Santamaria, R., Di Rosa, M., & Izzo, A. A. (2004). Neuroprotective effect of cannabidiol, a non-psychoactive component from *Cannabis sativa*, on beta-amyloid-induced toxicity in PC12 cells. *Journal of Neurochemistry*, *89*(1), 134–141.

Jung, K. -M., Astarita, G., Yasar, S., Vasilevko, V., Cribbs, D. H., Head, E., …, Piomelli, D. (2012). An amyloid β42-dependent deficit in anandamide mobilization is associated with cognitive dysfunction in Alzheimer's disease. *Neurobiology of Aging*, *33*(8), 1522–1532.

Kanof, P. D., Greenwald, B. S., Mohs, R. C., & Davis, K. L. (1985). Red blood cell choline. II: kinetics in Alzheimer's disease. *Biological Psychiatry*, *20*(4), 375–383.

Koppel, J., Bradshaw, H., Goldberg, T. E., Khalili, H., Marambaud, P., Walker, M. J., …, Davies, P. (2009). Endocannabinoids in Alzheimer's disease and their impact on normative cognitive performance: a case-control and cohort study. *Lipids in Health and Disease*, *8*, 2.

Mackie, K. (2005). Distribution of cannabinoid receptors in the central and peripheral nervous system. In R. Pertwee (Ed.), *Handbook of experimental pharmacology—168—Cannabinoids* (pp. 299–325). Heidelberg: Springer-Verlag.

Mackie, K., & Hille, B. (1992). Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(9), 3825–3829.

Malfitano, A. M., Basu, S., Maresz, K., Bifulco, M., & Dittel, B. N. (2014). What we know and do not know about the cannabinoid receptor 2 (CB2). *Seminars in Immunology*, *26*(5), 369–379.

Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., …, Lutz, B. (2003). CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science (New York, N.Y.)*, *302*(5642), 84–88.

Martín-Moreno, A. M., Reigada, D., Ramírez, B. G., Mechoulam, R., Innamorato, N., Cuadrado, A., & de Ceballos, M. L. (2011). Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: relevance to Alzheimer's disease. *Molecular Pharmacology*, *79*(6), 964–973.

Martín-Moreno, A. M., Brera, B., Spuch, C., Carro, E., García-García, L., Delgado, M., …, de Ceballos, M. L. (2012). Prolonged oral cannabinoid administration prevents neuroinflammation, lowers (-amyloid levels and improves cognitive performance in Tg APP 2576 mice. *Journal of Neuroinflammation*, *9*, 8.

Nadler, V., Mechoulam, R., & Sokolovsky, M. (1993). The non-psychotropic cannabinoid (+)-(3S,4S)-7-hydroxy-delta 6- tetrahydrocannabinol 1,1-dimethylheptyl (HU-211) attenuates *N*-methyl-D-aspartate receptor-mediated neurotoxicity in primary cultures of rat forebrain. *Neuroscience Letters*, *162*(1–2), 43–45.

Noonan, J., Tanveer, R., Klompas, A., Gowran, A., McKiernan, J., & Campbell, V. A. (2010). Endocannabinoids prevent β-amyloid-mediated lysosomal destabilization in cultured neurons. *Journal of Biological Chemistry*, *285*(49), 38543–38554.

Plassman, B. L., Langa, K. M., Fisher, G. G., Heeringa, S. G., Weir, D. R., Ofstedal, M. B., …, Wallace, R. B. (2007). Prevalence of dementia in the United States: the aging, demographics, and memory study. *Neuroepidemiology*, *29*(1–2), 125–132.

Ramírez, B. G., Blázquez, C., Gómez del Pulgar, T., Guzmán, M., & de Ceballos, M. L. (2005). Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *Journal of Neuroscience*, *25*(8), 1904–1913.

Robson, P. (2001). Therapeutic aspects of cannabis and cannabinoids. *British Journal of Psychiatry*, *178*, 107–115.

Russo, E. B. (2011). Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *British Journal of Pharmacology*, *163*, 1344–1364.

Scotter, E., Goodfellow, C., Graham, E., Dragunow, M., & Glass, M. (2010). Neuroprotective potential of CB_1 receptor agonists in an in vitro model of Huntington's disease. *British Journal of Pharmacology*, *160*(3), 747–761.

Senanarong, V., Cummings, J. L., Fairbanks, L., Mega, M., Masterman, D. M., O'Connor, S. M., & Strickland, T. L. (2004). Agitation in Alzheimer's disease is a manifestation of frontal lobe dysfunction. *Dementia and Geriatric Cognitive Disorders*, *17*(1–2), 14–20.

Sieradzan, K. A., Fox, S. H., Hill, M., Dick, J. P., Crossman, A. R., & Brotchie, J. M. (2001). Cannabinoids reduce levodopa-induced dyskinesia in Parkinson's disease: a pilot study. *Neurology*, *57*, 2108–2111.

Solowij, N., Grenyer, B., Chesher, G., & Lewis, J. (1995). Biopsychosocial changes associated with cessation of cannabis use: a single case study of acute and chronic cognitive effects, withdrawal and treatment. *Life Sciences*, *56*, 2127–2134.

Steinberg, M., Shao, H., Zandi, P., Lyketsos, C. G., Welsh-Bohmer, K. A., Norton, M. C., …, Cache County Investigators. (2008). Point and 5-year period prevalence of neuropsychiatric symptoms in dementia: the Cache County Study. *International Journal of Geriatric Psychiatry*, *23*(2), 170–177.

Sviženská, I., Dubový, P., & Šulcová, A. (2009). Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures—a short review. *Pharmacology Biochemistry, and Behaviour*, *90*, 501–511.

Tanveer, R., Gowran, A., Noonan, J., Keating, S. E., Bowie, A. G., & Campbell, V. A. (2012). The endocannabinoid, anandamide, augments Notch-1 signaling in cultured cortical neurons exposed to amyloid-β and in the cortex of aged rats. *Journal of Biological Chemistry*, *287*(41), 34709–34721.

Tolón, R. M., Núñez, E., Pazos, M. R., Benito, C., Castillo, A. I., Martínez-Orgado, J. A., & Romero, J. (2009). The activation of cannabinoid CB2 receptors stimulates in situ and in vitro beta-amyloid removal by human macrophages. *Brain Research*, *1283*, 148–154.

van den Elsen, G. A. H., Ahmed, A. I. A., Lammers, M., Kramers, C., Verkes, R. J., van der Marck, M. A., & Olde Rikkert, M. G. M. (2014). Efficacy and safety of medical cannabinoids in older subjects: a systematic review. *Ageing Research Reviews*, *14*, 56–64.

van der Stelt, M., Mazzola, C., Esposito, G., Matias, I., Petrosino, S., De Filippis, D., …, Di Marzo, V. (2006). Endocannabinoids and beta-amyloid-induced neurotoxicity in vivo: effect of pharmacological elevation of endocannabinoid levels. *Cellular and Molecular Life Sciences*, *63*(12), 1410–1424.

Volicier, L., Stelly, M., Morris, J., McLaughlin, J., & Volicier, B. J. (1997). Effects of dronabinol on anorexia and disturbed behavior in patients with Alzheimer's disease. *International Journal of Geriatric Psychiatry*, *12*, 913–919.

Walther, S., & Halpern, M. (2010). Cannabinoids and dementia: a review of the clinical and preclinical data. *Pharmaceuticals*, *3*(8), 2689–2708.

Walther, S., Schupbach, B., Seifritz, E., Homan, P., & Strik, W. (2011). Randomized, controlled crossover trial of dronabinol, 2.5 mg, for agitation in 2 patients with dementia. *Journal of Clinical Psychopharmacology*, *31*, 256–258.

Wilson, R. I., & Nicoll, R. A. (2002). Endocannabinoid signaling in the brain. *Science*, *296*(5568), 678–682.

Wu, J., Bie, B., Yang, H., Xu, J. J., Brown, D. L., & Naguib, M. (2013). Activation of the CB2 receptor system reverses amyloid-induced memory deficiency. *Neurobiology of Aging*, *34*(3), 791–804.

Zhuang, S. -Y., Bridges, D., Grigorenko, E., McCloud, S., Boon, A., Hampson, R. E., & Deadwyler, S. A. (2005). Cannabinoids produce neuroprotection by reducing intracellular calcium release from ryanodine-sensitive stores. *Neuropharmacology*, *48*(8), 1086–1096.

CHAPTER

87

Synthetic Cannabinoid Receptor Agonists (Spice) as New Recreational Psychoactive Substances

A. Helander

Department of Laboratory Medicine, Karolinska Institutet, and Karolinska University Laboratory, Stockholm, Sweden

SUMMARY POINTS

- "Spice" is a generic name for herbal smoking blends containing highly potent synthetic cannabinoid receptor agonists.
- Because many synthetic cannabinoids are not classified, they can be sold openly without risk of legal consequences (legal highs).
- A large number of Spice products are easily available over the internet from online retailers of new psychoactive substances (NPS).
- Spice products are increasingly being used, especially by teenagers and young adults.
- When new synthetic cannabinoids are introduced, little is usually known about their potency (ie, receptor affinity), pharmacology, toxicology, and metabolism, implying risks for overdose and serious adverse effects.
- Synthetic cannabinoids are not detected by standard urine drug testing procedures.
- Because many synthetic cannabinoids are not banned, they can be marketed over the internet, in herbal smoking blends as "legal marijuana substitutes."
- Compared with Δ^9-tetrahydrocannabinol (THC), the principal psychoactive constituent of the cannabis plant, which is a partial cannabinoid receptor agonist, many synthetic cannabinoids are highly potent receptor full agonists, increasing the risk for overdose and adverse health consequences.
- Spice products are increasingly being abused, especially by teenagers and young adults.
- When new synthetic cannabinoids are introduced, little is usually known about their pharmacology, toxicology, and metabolism, implying a risk for serious adverse effects.
- Synthetic cannabinoids are not detected by standard urine drug testing procedures.
- The key facts include the cannabinoid receptors, their endogenous and exogenous ligands, and clinical and bioanalytical consequences when synthetic cannabinoids are being used as new recreational drugs in herbal smoking blends ("Spice" products).

KEY FACTS ON SYNTHETIC CANNABINOIDS

- Synthetic cannabinoids are cannabinoid receptor agonists originally developed as research tools or therapeutic drug candidates.
- Today, synthetic cannabinoids are added to herbal smoking blends ("Spice" products), and used as "recreational" drugs.

LIST OF ABBREVIATIONS

CB	Cannabinoid receptor
EMCDDA	European monitoring centre for drugs and drug addiction
NPS	New psychoactive substances
SC	Synthetic cannabinoid
THC	Δ^9-Tetrahydrocannabinol

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00101-0
Copyright © 2017 Elsevier Inc. All rights reserved.

INTRODUCTION

The *Cannabis* plant has an ancient history of medicinal and "recreational" use for its physiological and psychoactive effects. Modern use of cannabis preparations (eg, marijuana and hashish) is primarily as a recreational drug, although occasionally also used for treatment and management of chronic pain (Lucas, 2012). Among the large number of chemical compounds isolated from the cannabis plant (phytocannabinoids) (Russo, 2011), Δ^9-tetrahydrocannabinol (THC) was identified in 1964 as the principal psychoactive constituent (Gaoni & Mechoulam, 1964). Subsequent identification of the cannabinoid type 1 receptor (CB_1; mainly centrally located and associated with psychoactive effects) (Devane, Dysarz, Johnson, Melvin, & Howlett, 1988) and type 2 receptor (CB_2; mainly peripheral and associated with immunomodulatory effects) (Munro, Thomas, & Abu-Shaar, 1993) around 1990, and, in the following years, identification of endogenous cannabinoid receptor agonists (endocannabinoids) such as anandamide (arachidonoylethanolamide) (Devane et al., 1992) and 2-arachidonoylglycerol (Mechoulam et al., 1995; Sugiura et al., 1995), led to an intensive research to exploit the endocannabinoid system for treatment of a broad range of diseases (Di Marzo, Bifulco, & De Petrocellis, 2004; Bahr, Karanian, Makanji, & Makriyannis, 2006; Karanian & Bahr, 2006; Vemuri, Janero, & Makriyannis, 2008; Pacher & Kunos, 2013).

Since the discovery of the human endocannabinoid system, hundreds of cannabinoid receptor agonists (synthetic cannabinoids) with variable binding affinities for the CB_1 and CB_2 receptors have been synthesized as research tools, and patented as therapeutic drug candidates by researchers and pharmaceutical companies (Huffman & Padgett, 2005). However, only few have eventually become available as prescription drugs, one example being nabilone that was approved to treat chemotherapy-induced nausea (Davis, 2008). A main reason is that the therapeutic use of synthetic cannabinoids is limited by the psychoactive effects associated with central CB_1 receptor agonism. Accordingly, there has been a search for selective agonists that enhance CB_2 receptor analgesic and antiemetic effects over CB_1 psychoactive effects (Pertwee, 2006).

HERBAL SMOKING MIXTURES

In mid-2006, discussions were started on internet chat forums for psychoactive substances regarding a herbal smoking mixture sold under the brand name "Spice" (Fig. 87.1) that had been available for some years as a "recreational" drug. Smoking Spice was reported to generate a cannabis-like relaxing effect, despite apparently not containing cannabis. Although labeled with the warning text "not for human consumption," the intended use was obvious and the product was generally considered a "legal" alternative to cannabis. Furthermore, use of Spice was reported not to be detected by standard urine drug testing.

FIGURE 87.1 Spice, the brand name of one of the first herbal smoking blends. A bag of Spice Diamond, one of the early herbal smoking mixtures introduced as natural highs. The "Spice" products were eventually demonstrated to consist of plant materials sprayed with synthetic cannabinoids.

Several of the listed plant-based ingredients, such as bay bean (*Canavalia sp.*), blue lotus (*Nymphaea sp.*), honeyweed (*Leonurus sibiricus*), Indian warrior (*Pedicularis densiflora*), lion's tail (*Leonotis leonurus*), and maconha brava (*Zornia latifolia*), had traditionally been used as "marijuana substitutes" in different parts of the world (EMCDDA, 2009), and were thus proposed to be accountable for the psychoactive effects of Spice. Although not indicated on the label, others rather suggested that the dried plant material in Spice had been spiked with synthetic cannabinoids; one tentative substance mentioned was HU-210, an analgesic drug candidate synthesized by Raphael Mechoulam at the Hebrew University (hence named "HU") that is structurally similar to THC (Fig. 87.2) but considerably (100-fold) more potent, and with an extended duration of action (Mechoulam, Lander, Breuer, & Zahalka, 1990). However, it is uncertain whether Spice and alternative herbal smoking blends ("Spice" has become a generic name for this type of products) actually contained all or any of the listed plants (Ogata, Uchiyama, Kikura-Hanajiri, & Goda, 2013), or if they were listed because of the alleged psychoactive effects, simply in order to confuse and mask the real content, and to present the product as "natural" (Cornara et al., 2013).

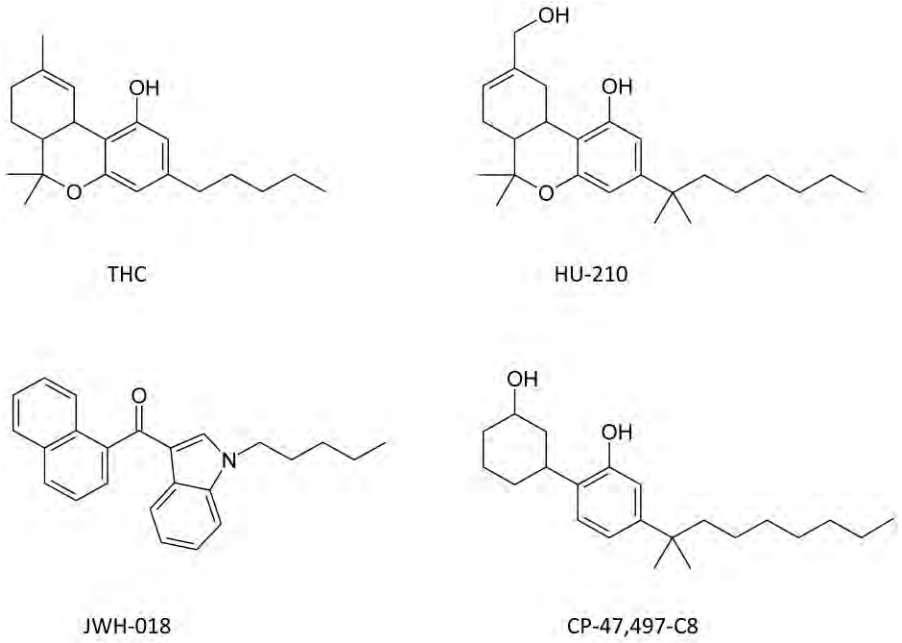


FIGURE 87.2 Chemical structures of THC and the first synthetic cannabinoids used in Spice products. Chemical structure relationships of THC, the principal psychoactive constituent of cannabis, and some "first generation" synthetic cannabinoid receptor agonists (synthetic cannabinoids) added in herbal smoking blends collectively referred to as "Spice" products.

FIRST IDENTIFICATION OF THE PSYCHOACTIVE COMPONENTS IN "SPICE"

The suggestion that the herbal smoking blends had actually been supplemented with synthetic cannabinoids was further supported when new and seemingly stronger Spice variants (ie, Spice Gold and Spice Diamond) (Fig. 87.1) were introduced on the recreational drugs market, despite no apparent change of plant ingredients. Following an increased awareness and associated rapid rise in the popularity of Spice and similar products in many European countries, in 2007–2008, it was eventually reported by Austrian and German national focal points at the end of 2008 that the synthetic cannabinoid receptor agonist JWH-018 (Fig. 87.2) had been identified as the true psychoactive component (EMCDDA, 2009). JWH-018 is one among several hundred analgesic drug candidates synthesized by John W. Huffman (ie,"JWH") at Clemson University, USA (Huffman, 2000). The identification of the naphthoylindole JWH-018, and of the CP-47,497 C8 homolog (Fig. 87.2), a cyclohexylphenol (CP) synthetic cannabinoid developed at Pfizer (Weissman, Milne, & Melvin, 1982), in different Spice products, was soon confirmed in scientific publications (Auwarter et al., 2009; Uchiyama, Kikura-Hanajiri, Kawahara, Haishima, & Goda, 2009).

EFFECT OF LEGISLATION OF SYNTHETIC CANNABINOIDS

Already by the end of Jan. 2009, JWH-018 and CP-47,497-C8 were banned in Germany, due to reports of intoxication, abuse, and health problems associated with chronic use of Spice (Zimmermann et al., 2009). Nevertheless, already a few months later, it was demonstrated that JWH-018 had been replaced in Spice products by one of its "legal" structural analogs, JWH-073 (Lindigkeit et al., 2009).

This represented the start of an ongoing "cat-and-mouse game," where the psychoactive additives in herbal smoking mixtures made illegal are rapidly removed and replaced with novel, similar synthetic cannabinoids (ie, "designer drugs") that are ready in the pipeline, to maintain the legal standing of the products (ie, "legal highs") (Helander, Bäckberg, Hultén, Al-Saffar, & Beck, 2014). Consequently, partly due to legislative actions undertaken in the European countries, the number of "new psychoactive substances" (NPS) noted by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) has increased dramatically in the last years (EMCDDA, 2014). Of all NPS identified, synthetic cannabinoids make up an essential part (ie, 27–47% each year since 2009), totally counting to more than 100 during the period 2008–2013 (Fig. 87.3).

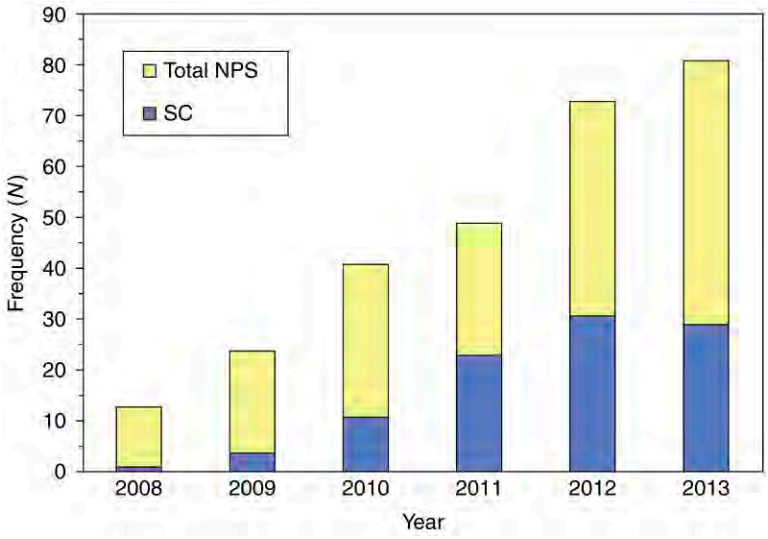


FIGURE 87.3 **NPS reported to the EMCDDA in 2008–2013.** Total number of NPS and the proportion of synthetic cannabinoids (SC) reported to the EMCDDA per year, between 2008–2013 (EMCDDA, 2014).

Since this Spice drug phenomenon first attracted a more widespread attention in the period 2008–2009, different "generations" of synthetic cannabinoids have been used in the herbal smoking products (Table 87.1). JWH-018, JWH-073, JWH-200, CP-47,497, and HU-210 are among the substances usually referred to as the "first generation" Spice substances (Fig. 87.2). Subsequent Spice products have contained a wide variety of synthetic cannabinoids with variable structural design and variable potency, that is, affinity for the CB_1 and CB_2 receptors (Wiley, Marusich, & Huffman, 2014). These include several other JWH variants, fluorinated substances such as AM-694 and AM-2201 (the latter being the fluorinated analog of JWH-018), two in a large series of substances examined by Alexandros Makriyannis (AM) at Northeastern University, USA (Moosmann et al., 2012), RCS-4 and RCS-8 (Kavanagh, Grigoryev, Melnik, & Simonov, 2012), and UR-144 and XLR-11 (ie, the fluorinated analog of UR-144) (Fig. 87.4). AKB48 (also known as APINACA), AB-FUBINACA, AB-CHMINACA, JWH-071, MN-18, and NM-2201 are among the more recently introduced synthetic cannabinoids (Fig. 87.4). In addition, even compounds binding allosterically to the CB1 receptor and thereby eliciting a conformational change that increases agonist affinity (eg, Org 27569) have also become used in Spice products (Price et al., 2005). A list of the synthetic cannabinoids or related substances with CB receptor activity that have been reported to the EMCDDA, by year, until Sep. 2014 are presented in Table 87.1 (EMCDDA-Europol, 2009–2014).

Over the years, a multitude of Spice product and alternative herbal smoking blends with different brand names (eg, Spice Gold/Silver/Diamond, K2, K3, Bonzai, Kronic, Krypton) have been introduced on the recreational drugs market at a rising speed (Fig. 87.5). They have contained variable amounts and/or combinations of synthetic cannabinoids (Table 87.1), to hinder or avoid legal consequences. The Spice products are easily obtainable, initially in "head shops," but since 2009 mainly over the internet from the many online retailers specialized in this and other types of NPS (Vardakou, Pistos, & Spiliopoulou, 2010; Spaderna, Addy, & D'Souza, 2013). They are also sold directly on the illicit drug market.

CLINICAL CONSEQUENCES OF SYNTHETIC CANNABINOIDS

Like smoking cannabis (THC), the desired psychoactive effects of products containing synthetic cannabinoids include euphoria, calmness, relaxation, and a feeling of wellbeing. However, there is limited objective information on possible short-term and long-term effects and health consequences of using Spice products. Because they are marketed as "not for human consumption," this issue is obviously not recognized on the NPS websites. Instead most of the information is anecdotal and obtained from internet chat forums. When novel synthetic cannabinoids are introduced, little is usually known initially about their pharmacology, toxicology, and metabolism, and if so mostly only from studies on laboratory animals (Lewis et al., 2012). Some may actually have been rejected as potential therapeutic drugs due to adverse effects, but the information generated is now used to develop these compounds for illicit use as "marijuana substitutes." As for other types of NPS, synthetic cannabinoids are typically manufactured in unregulated laboratories, implying risks for toxic contaminants, then diluted in a solvent

TABLE 87.1 New Synthetic Cannabinoids Appearing in Europe in 2008–2014

2008	AM-1220	JWH-122 pentenyl 2-methylindole derivative	AM-6527 5-fluoropentyl derivative
JWH-018	AM 1-1220 azepane derivative	JWH-122 pentenyl derivative	AM-2201 indazole analog
2009	MAM-2201/JWH-l22 f1uoropentyl derivative	MAM-2201 chloropentvl derivative	ADB-FUBINACA
CP 47,497		JWH-018 *N*-(5-chloropentyl) derivative	ADB-PINACA
CP 47,497-C6	RCS-4-C4	JWH-018 *N*-(5-bromopentyl) derivative	*N*,*N*-diethyl-2-(1-pentyl-1H-indol-3-yl)-4-thiazol-methanamine
CP 47,497-C8	JWH-387	AM-1248	
CP 47,497-C9	JWH-412	5F-APINACA/5F-AKB48	*N*-(2-methoxyethyl)-*N*-(1-methylethyl)-2-(1-pentyl-lH-indol-3-yl)-4-thiazol-methanamine
JWH-073	JWH-307	AM-2201 indazolecarboxamide analog	
HU-210	AM-2233	PB-22/JWH-018 quinolinecarboxylate analog	1-(Cyc1ohexylmethyl)-2-[(4-ethoxyphenyl) methyl]-*N*,*N*-diethyl-1H-benzimidazol-5-carboxamide
JWH-398	Org 27569	AB-005	
JWH-250	Org 27759	AB-005 azepane isomer	
JWH-200	Org 29647	4-HTMPIPO	A-796,260 isomer
2010	JWH-022	UR-144 *N*-(5-chloropentyl) analog	SDB-006
RCS-4	AM-2232	JWH-302	5F-SDB-006
JWH-081	AM-694 chloro derivative	***2013***	FUB-PB-22
JWH-073 methyl derivative	***2012***	BB-22/Quinolin-8-yl 1-(cyclohexylmethyl)-lH-indole-3-carboxylate	***2014***
AM-694	HU-331		PB-22 indazole analog
JWH-122	AM-679	A-834,735	5F-PB-22 indazole analog
JWH-015	WIN 55212-2	JWH-368	FDU-PB-22
CP47,497-C8+C2	UR-144	EAM-2201	JWH-018 indazole analog
JWH-210	JWH-370	5F-PB-22	Mepirapim
JWH-203	3-(*p*-Methoxbenzoyl)-*N*-methylindole	JWH-307 bromine analog	AM-2201 benzimidazole analog/FUBIMINA
JWH-019	*trans*-Diastereomer of CP 47,497-C8 homolog	JWH-030	AB-CHMINACA
3-(4-Hydroxymethylbenzoyl)-1-pentylindole		JWH-145	5F-AMBICA
2011	URB-754	UR-144 heptyl derivative	5F-AMB
CRA-13	5F-UR-144/XLR-11	URB-597	JWH-071
AM-2201	A-769,260	AB-PINACA	EG-018
JWH-251	APINACA/AKB48	A-836,339	MIN-18
JWH-018 adamantyl derivative	UR-144 (-2H)	JTE-907	FUB-AKB48
JWH-182	STS-135	AE-FUBINACA	AB-FUBINACA 2-fluorobenzyl isomer
JWH-250 derivative	APICA	5F-AB-PINACA	NM-2201
RCS-4 *ortho*	JWH-018 carboxamide	LY2183240	5F-SDB-005
JWH-007	AM-694 ethyl substituted for iodine	AM-1248 azepane isomer	ADB-CHMINACA
WIN 48,098/Pravadoline	AM-694 methyl substituted for iodine	ADBICA	MDMB-CHMICA

A list of all synthetic cannabinoid receptor agonists and related substances (eg, cannabinoid receptor allosteric modulators) reported to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) per year, from 2008 until Sep. 2014 (EMCDDA-Europol, 2009–2014).

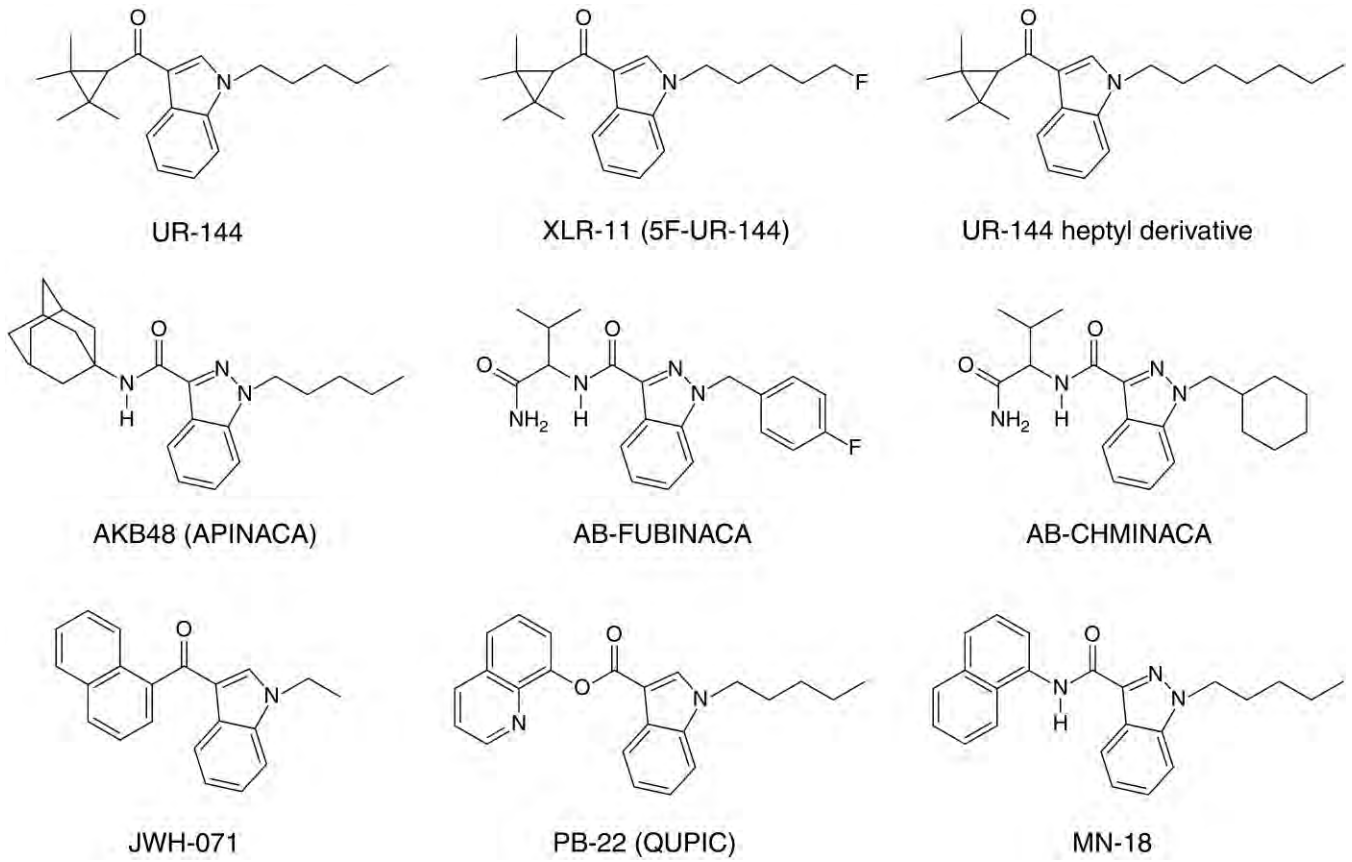


FIGURE 87.4 Chemical structures of a number of novel synthetic cannabinoids appearing in recent Spice products. Chemical structures of several synthetic cannabinoids included in herbal smoking blends ("Spice" products) in 2012–2014 (EMCDDA, 2014).

FIGURE 87.5 Examples of Spice products, and other types of NPS available online as recreational drugs. Over the years, a multitude of products containing synthetic cannabinoids and other types of NPS have been available over the internet from online retailers specialized in this and other types of products.

(eg, acetone), and finally sprayed onto dried plant materials (eg, tobacco, knaster). Hence, in addition to the variability in composition and amounts of synthetic cannabinoids between Spice products, there may also be significant variation between different batches.

Because most synthetic cannabinoids used in Spice products have never been tested for human consumption, and many are considerably more potent CB receptor full agonists, compared with the partial agonist THC, this makes dosing difficult, and increases the risk of accidental overdose and health problems. Furthermore, due to the high lipid-solubility of cannabinoids, they likely persist in the body for long periods of time. As a result, adverse events necessitating contact with poison information centers and treatment at hospital emergency departments are increasing (Centers for Disease, Control, & Prevention, 2013b; Helander et al., 2014), as are the number of published case reports describing serious adverse neurologic and sympathomimetic effects associated with both acute and chronic use of Spice products (Fattore & Fratta, 2011; Seely, Lapoint, Moran, & Fattore, 2012; Freeman et al., 2013; Hopkins & Gilchrist, 2013; Patton et al., 2013; Behonick et al., 2014; Gugelmann et al., 2014; Ibrahim, Al-Saffar, & Wannenburg, 2014; Mckeever et al., 2014). Notably, one specific synthetic cannabinoid,

XLR-11, which is the fluorinated analog of UR-144 (Fig. 87.4), has recently been implicated in several cases of acute kidney injury requiring dialysis (Centers for Disease, Control, & Prevention, 2013a; Thornton, Wood, Friesen, & Gerona, 2013; Buser et al., 2014).

DRUG TESTING FOR SYNTHETIC CANNABINOIDS

In addition to the desired psychoactive effects of synthetic cannabinoids associated with CB_1 receptor agonism, a common reason for using Spice products over cannabis (marijuana) is to avoid detection of drug use, as synthetic cannabinoids are not detected by standard urine drug testing procedures (Gunderson, Haughey, Ait-Daoud, Joshi, & Hart, 2014). For example, apart from some THC analogs (eg, HU-210) (Fig. 87.2), most of these substances will not be detected in the immunoassays used for cannabis screening, due to the marked differences in drug structure (Fig. 87.4). Recently, specific immunoassays targeting synthetic cannabinoids have been introduced, but a main limitation is that they only cover a small part of all these substances (Barnes et al., 2014; Spinelli et al., 2014). Furthermore, due to the rapid turnover of NPS and the constantly changing composition of Spice products (EMCDDA, 2014), these methods risk soon becoming obsolete. Consequently, using flexible methods based on sensitive and selective liquid chromatography-mass spectrometry has become a standard analytical principle for detection of synthetic cannabinoids and their metabolites, in various biological specimens (urine, blood, saliva, and hair) (Salomone, Gerace, D'urso, Di Corcia, & Vincenti, 2012; Kneisel, Auwärter, & Kempf, 2013; Huppertz, Kneisel, Auwärter, & Kempf, 2014; Scheidweiler & Huestis, 2014). Even so, detecting all novel synthetic cannabinoids appearing in Spice products and including them in analytical methods requires frequent updating, and will thus represent a continuing challenge for the clinical and forensic toxicology laboratories (Meyer & Peters, 2012), and also for the implementation of control measures by public health authorities.

Other problems with drug testing for NPS relate to the identification of suitable target compounds for analysis (eg, major metabolites), and an initial lack of certified reference materials. For synthetic cannabinoids, the parent drugs are typically detectable in blood but not, or only rarely, in urine samples (Helander, Beck, Hägerkvist, & Hulten, 2013). A limitation with analysis of blood is that it will cover only recent drug intake. Accordingly, to obtain a longer detection window, analysis of drug metabolites excreted in urine, or even hair, is a common strategy. However, because little is often known about the metabolic pattern of the new drugs, this possibility is also limited until the optimal target metabolites for drug testing have been identified (Grigoryev et al., 2011; Hutter, Broecker, Kneisel, & Auwärter, 2012; Scheidweiler & Huestis, 2014). It should also be considered that because many synthetic cannabinoids are considerably more potent than THC, they need to be administered in much lower doses, resulting in lower drug concentrations and are, thereby, harder to detect. In addition, because many synthetic cannabinoids are structurally closely related and undergo similar biotransformation, they sometimes share common metabolites, making it difficult to identify the specific parent compound (legal or illegal) involved (Hutter, Moosmann, Kneisel, & Auwärter, 2013).

CONCLUSIONS

The Spice phenomenon represents a new trend in substance abuse, and use of Spice products is increasing, especially among teenagers and young adults (Wood, 2013; Helander et al., 2014). A wide variety of herbal smoking blends spiked with highly potent synthetic cannabinoid receptor agonist are continuously introduced by online sale to the recreational drugs market. Banned substances in the Spice products are rapidly replaced by other, structurally similar psychoactive variants, in order to stay ahead of legislation (legal highs). Many synthetic cannabinoids are highly potent CB receptor full agonists, some showing 100-fold higher affinity than the partial agonist THC, and they have not undergone the standard human safety testing required for pharmaceuticals. This may cause problems with drug dosing, and poses a risk for severe medical and psychiatric complications. Due to lack of reference materials, and because little is known initially about the metabolic patterns of novel synthetic cannabinoids, including them in analytical methods for routine drug testing represents an ongoing challenge for the clinical and forensic toxicology laboratories. Furthermore, the rapid turnover and variability in product composition of synthetic cannabinoids also brings major challenges to the control of new drugs. It can be assumed that further structural analogs with CB receptor agonism activity from different chemical groups will continue to appear in Spice products, and that this new drug phenomenon will be a problem to continue. In addition, synthetic cannabinoids are also being distributed in new formulations, such as in liquid form to be consumed using vaporizers and e-cigarettes.

MINI-DICTIONARY

Cannabinoid receptors (CB) Receptor targets for endocannabinoids, phytocannabinoids, and synthetic cannabinoids, of which type 1 (CB1) is mainly centrally located and associated with psychoactive effects, and type 2 (CB2) is mainly peripheral and associated with immunomodulatory effects.

Designer drugs Synthetic variants of classical illicit drugs that have been structurally modified (designed) to circumvent legislative control.

Endocannabinoids Endogenous cannabinoid receptor agonists such as anandamide (arachidonoylethanolamide) and 2-arachidonoylglycerol.

Legal highs Psychoactive substances that are not controlled as illicit drugs, and hence can be sold openly, for example, over the internet.

New psychoactive substances (NPS) Synthetic substances that have the same biological targets as classical illicit drugs, acting on neurotransmitter receptors and transporter, but the chemical structure differences mean legislative control can be circumvented, and products sold openly.

Phytocannabinoids Chemical compounds isolated from the cannabis plant (Cannabis sativa), of which Δ9-tetrahydrocannabinol (THC) is the principal psychoactive constituent.

Spice A common name for herbal smoking blend products containing synthetic cannabinoids that are sold over the internet as "legal marijuana substitutes."

Synthetic cannabinoids Cannabinoid receptor agonists with variable binding affinity for the CB1 and CB2 receptors that have been synthesized as research tools, or patented as therapeutic drug candidates.

References

Auwärter, V., Dresen, S., Weinmann, W., Muller, M., Putz, M., & Ferreiros, N. (2009). 'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs? *Journal of Mass Spectrometry*, *44*, 832–837.

Bahr, B. A., Karanian, D. A., Makanji, S. S., & Makriyannis, A. (2006). Targeting the endocannabinoid system in treating brain disorders. *Expert Opinion on Investigational Drugs*, *15*, 351–365.

Barnes, A. J., Young, S., Spinelli, E., Martin, T. M., Klette, K. L., & Huestis, M. A. (2014). Evaluation of a homogenous enzyme immunoassay for the detection of synthetic cannabinoids in urine. *Forensic Science International*, *241*, 27–34.

Behonick, G., Shanks, K. G., Firchau, D. J., Mathur, G., Lynch, C. F., Nashelsky, M., Jaskierny, D. J., & Meroueh, C. (2014). Four postmortem case reports with quantitative detection of the synthetic cannabinoid, 5F-PB-22. *Journal of Analytical Toxicology*, *38*, 559–562.

Buser, G. L., Gerona, R. R., Horowitz, B. Z., Vian, K. P., Troxell, M. L., Hendrickson, R. G., Houghton, D. C., Rozansky, D., Su, S. W., & Leman, R. F. (2014). Acute kidney injury associated with smoking synthetic cannabinoid. *Clinical Toxicology (Philadelphia)*, *52*, 664–673.

Centers for Disease, Control, & Prevention. (2013a). Acute kidney injury associated with synthetic cannabinoid use—multiple states, 2012. *Morbidity and Mortality Weekly Report*, *62*, 93–98.

Centers for Disease, Control, & Prevention. (2013b). Notes from the field: severe illness associated with reported use of synthetic marijuana—Colorado, August—September 2013. *Morbidity and Mortality Weekly Report*, *62*, 1016–1017.

Cornara, L., Borghesi, B., Canali, C., Andrenacci, M., Basso, M., Federici, S., & Labra, M. (2013). Smart drugs: green shuttle or real drug? *International Journal of Legal Medicine*, *127*, 1109–1123.

Davis, M. P. (2008). Oral nabilone capsules in the treatment of chemotherapy-induced nausea and vomiting and pain. *Expert Opinion on Investigational Drugs*, *17*, 85–95.

Devane, W. A., Dysarz, F. A., III, Johnson, M. R., Melvin, L. S., & Howlett, A. C. (1988). Determination and characterization of a cannabinoid receptor in rat brain. *Molecular Pharmacology*, *34*, 605–613.

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., & Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*, *258*, 1946–1949.

Di Marzo, V., Bifulco, M., & De Petrocellis, L. (2004). The endocannabinoid system and its therapeutic exploitation. *Nature Reviews Drug Discovery*, *3*, 771–784.

EMCDDA-Europol. (2009–2014). *Annual reports on the implementation of Council Decision 2005/387/JHA*. Lisbon, Portugal and The Hague, the Netherlands: EMCDDA and Europol, http://www.emcdda.europa.eu/publications/searchresults?action=list&type=PUBLICATIONS&SERIES_PUB=a104

EMCDDA. (2009). *Understanding the 'Spice' phenomenon*. Lisbon, Portugal: EMCDDA, http://www.emcdda.europa.eu/publications/thematic-papers/spice

EMCDDA. (2014). *European Drug Report 2014: Trends and developments*. Lisbon, Portugal: EMCDDA, http://www.emcdda.europa.eu/attachements.cfm/att_228272_EN_TDAT14001ENN.pdf

Fattore, L., & Fratta, W. (2011). Beyond THC: the new generation of cannabinoid designer drugs. *Frontiers in Behavioral Neuroscience*, *5*, 60.

Freeman, M. J., Rose, D. Z., Myers, M. A., Gooch, C. L., Bozeman, A. C., & Burgin, W. S. (2013). Ischemic stroke after use of the synthetic marijuana "spice". *Neurology*, *81*, 2090–2093.

Gaoni, Y., & Mechoulam, R. (1964). Isolation, structure and partial synthesis of an active constituent of hashish. *Journal of the American Chemical Society*, *86*, 1646–1647.

Grigoryev, A., Savchuk, S., Melnik, A., Moskaleva, N., Dzhurko, J., Ershov, M., Nosyrev, A., Vedenin, A., Izotov, B., Zabirova, I., & Rozhanets, V. (2011). Chromatography-mass spectrometry studies on the metabolism of synthetic cannabinoids JWH-018 and JWH-073, psychoactive components of smoking mixtures. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *879*, 1126–1136.

Gugelmann, H., Gerona, R., Li, C., Tsutaoka, B., Olson, K. R., & Lung, D. (2014). 'Crazy Monkey' poisons man and dog: human and canine seizures due to PB-22, a novel synthetic cannabinoid. *Clinical Toxicology (Philadelphia)*, *52*, 635–638.

Gunderson, E. W., Haughey, H. M., Ait-Daoud, N., Joshi, A. S., & Hart, C. L. (2014). A survey of synthetic cannabinoid consumption by current cannabis users. *Substance Abuse*, *35*, 184–189.

Helander, A., Bäckberg, M., Hultén, P., Al-Saffar, Y., & Beck, O. (2014). Detection of new psychoactive substance use among emergency room patients: results from the Swedish STRIDA project. *Forensic Science International*, *52*, 618–624.

Helander, A., Beck, O., Hägerkvist, R., & Hulten, P. (2013). Identification of novel psychoactive drug use in Sweden based on laboratory analysis—initial experiences from the STRIDA project. *Scandinavian Journal of Clinical and Laboratory Investigation*, *73*, 400–406.

Hopkins, C. Y., & Gilchrist, B. L. (2013). A case of cannabinoid hyperemesis syndrome caused by synthetic cannabinoids. *Journal of Emergency Medicine*, *45*, 544–546.

Huffman, J. W. (2000). The search for selective ligands for the CB2 receptor. *Current Pharmaceutical Design*, *6*, 1323–1337.

Huffman, J. W., & Padgett, L. W. (2005). Recent developments in the medicinal chemistry of cannabimimetic indoles, pyrroles and indenes. *Current Medicinal Chemistry*, *12*, 1395–1411.

Huppertz, L. M., Kneisel, S., Auwärter, V., & Kempf, J. (2014). A comprehensive library-based, automated screening procedure for 46 synthetic cannabinoids in serum employing liquid chromatography-quadrupole ion trap mass spectrometry with high-temperature electrospray ionization. *Journal of Mass Spectrometry*, *49*, 117–127.

Hutter, M., Broecker, S., Kneisel, S., & Auwärter, V. (2012). Identification of the major urinary metabolites in man of seven synthetic cannabinoids of the aminoalkylindole type present as adulterants in 'herbal mixtures' using LC-MS/MS techniques. *Journal of Mass Spectrometry*, *47*, 54–65.

Hutter, M., Moosmann, B., Kneisel, S., & Auwärter, V. (2013). Characteristics of the designer drug and synthetic cannabinoid receptor agonist AM-2201 regarding its chemistry and metabolism. *Journal of Mass Spectrometry*, *48*, 885–894.

Ibrahim, S., Al-Saffar, F., & Wannenburg, T. (2014). A unique case of cardiac arrest following K2 abuse. *Case Reports in Cardiology*, *2014*, 120607.

Karanian, D. A., & Bahr, B. A. (2006). Cannabinoid drugs and enhancement of endocannabinoid responses: strategies for a wide array of disease states. *Current Molecular Medicine*, *6*, 677–684.

Kavanagh, P., Grigoryev, A., Melnik, A., & Simonov, A. (2012). The identification of the urinary metabolites of 3-(4-methoxybenzoyl)-1-pentylindole (RCS-4), a novel cannabimimetic, by gas chromatography-mass spectrometry. *Journal of Analytical Toxicology*, *36*, 303–311.

Kneisel, S., Auwärter, V., & Kempf, J. (2013). Analysis of 30 synthetic cannabinoids in oral fluid using liquid chromatography-electrospray ionization tandem mass spectrometry. *Drug Testing and Analysis*, *5*, 657–669.

Lewis, S. E., Paro, R., Borriello, L., Simon, L., Robinson, L., Dincer, Z., Riedel, G., Battista, N., & Maccarrone, M. (2012). Long-term use of HU210 adversely affects spermatogenesis in rats by modulating the endocannabinoid system. *International Journal of Andrology*, *35*, 731–740.

Lindigkeit, R., Boehme, A., Eiserloh, I., Luebbecke, M., Wiggermann, M., Ernst, L., & Beuerle, T. (2009). Spice: a never ending story? *Forensic Science International*, *191*, 58–63.

Lucas, P. (2012). Cannabis as an adjunct to or substitute for opiates in the treatment of chronic pain. *Journal of Psychoactive Drugs*, *44*, 125–133.

McKeever, R. G., Vearrier, D., Jacobs, D., Lasala, G., Okaneku, J., & Greenberg, M. I. (2014). K2—Not the Spice of life; synthetic cannabinoids and ST elevation myocardial infarction: a case report. *Journal of Medical Toxicology*, *11*, 129–131.

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N. E., Schatz, A. R., Gopher, A., Almog, S., Martin, B. R., Compton, D. R., et al. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochemical Pharmacology*, *50*, 83–90.

Mechoulam, R., Lander, N., Breuer, A., & Zahalka, J. (1990). Synthesis of the individual, pharmacologically distinct, enantiomers of a tetrahydrocannabinol derivative. *Tetrahedron: Asymmetry*, *1*, 315–319.

Meyer, M. R., & Peters, F. T. (2012). Analytical toxicology of emerging drugs of abuse—an update. *Therapeutic Drug Monitoring*, *34*, 615–621.

Moosmann, B., Kneisel, S., Girreser, U., Brecht, V., Westphal, F., & Auwärter, V. (2012). Separation and structural characterization of the synthetic cannabinoids JWH-412 and 1-[(5-fluoropentyl)-1H-indol-3yl]-(4-methylnaphthalen-1-yl)methanone using GC-MS, NMR analysis and a flash chromatography system. *Forensic Science International*, *220*, e17–e22.

Munro, S., Thomas, K. L., & Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, *365*, 61–65.

Ogata, J., Uchiyama, N., Kikura-Hanajiri, R., & Goda, Y. (2013). DNA sequence analyses of blended herbal products including synthetic cannabinoids as designer drugs. *Forensic Science International*, *227*, 33–41.

Pacher, P., & Kunos, G. (2013). Modulating the endocannabinoid system in human health and disease—successes and failures. *FEBS Journal*, *280*, 1918–1943.

Patton, A. L., Chimalakonda, K. C., Moran, C. L., Mccain, K. R., Radominska-Pandya, A., James, L. P., Kokes, C., & Moran, J. H. (2013). K2 toxicity: fatal case of psychiatric complications following AM2201 exposure. *Journal of Forensic Science*, *58*, 1676–1680.

Pertwee, R. G. (2006). The pharmacology of cannabinoid receptors and their ligands: an overview. *International Journal of Obesity (London)*, *30*(Suppl. 1), S13–S18.

Price, M. R., Baillie, G. L., Thomas, A., Stevenson, L. A., Easson, M., Goodwin, R., Mclean, A., Mcintosh, L., Goodwin, G., Walker, G., Westwood, P., Marrs, J., Thomson, F., Cowley, P., Christopoulos, A., Pertwee, R. G., & Ross, R. A. (2005). Allosteric modulation of the cannabinoid CB1 receptor. *Molecular Pharmacology*, *68*, 1484–1495.

Russo, E. B. (2011). Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. *British Journal of Pharmacology*, *163*, 1344–1364.

Salomone, A., Gerace, E., D'urso, F., Di Corcia, D., & Vincenti, M. (2012). Simultaneous analysis of several synthetic cannabinoids, THC, CBD and CBN, in hair by ultra-high performance liquid chromatography tandem mass spectrometry. Method validation and application to real samples. *Journal of Mass Spectrometry*, *47*, 604–610.

Scheidweiler, K. B., & Huestis, M. A. (2014). Simultaneous quantification of 20 synthetic cannabinoids and 21 metabolites, and semi-quantification of 12 alkyl hydroxy metabolites in human urine by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography, A*, *1327*, 105–117.

Seely, K. A., Lapoint, J., Moran, J. H., & Fattore, L. (2012). Spice drugs are more than harmless herbal blends: a review of the pharmacology and toxicology of synthetic cannabinoids. *Progress in Neuropsychopharmacoly and Biological Psychiatry*, *39*, 234–243.

Spaderna, M., Addy, P. H., & D'Souza, D. C. (2013). Spicing things up: synthetic cannabinoids. *Psychopharmacology (Berlin)*, *228*, 525–540.

Spinelli, E., Barnes, A. J., Young, S., Castaneto, M. S., Martin, T. M., Klette, K. L., & Huestis, M. A. (2014). Performance characteristics of an ELISA screening assay for urinary synthetic cannabinoids. *Drug Testing and Analysis*, *7*, 467–474.

Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A., & Waku, K. (1995). 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochemical and Biophysical Research Communications*, *215*, 89–97.

Thornton, S. L., Wood, C., Friesen, M. W., & Gerona, R. R. (2013). Synthetic cannabinoid use associated with acute kidney injury. *Clinical Toxicology (Philadelphia)*, *51*, 189–190.

Uchiyama, N., Kikura-Hanajiri, R., Kawahara, N., Haishima, Y., & Goda, Y. (2009). Identification of a cannabinoid analog as a new type of designer drug in a herbal product. *Chemical and Pharmaceutical Bulletin (Tokyo)*, *57*, 439–441.

Vardakou, I., Pistos, C., & Spiliopoulou, Ch. (2010). Spice drugs as a new trend: mode of action, identification and legislation. *Toxicology Letters*, *197*, 157–162.

Vemuri, V. K., Janero, D. R., & Makriyannis, A. (2008). Pharmacotherapeutic targeting of the endocannabinoid signaling system: drugs for obesity and the metabolic syndrome. *Physiology and Behavior*, *93*, 671–686.

Weissman, A., Milne, G. M., & Melvin, L. S., Jr. (1982). Cannabimimetic activity from CP-47,497, a derivative of 3-phenylcyclohexanol. *Journal of Pharmacology and Experimental Therapeutics*, *223*, 516–523.

Wiley, J. L., Marusich, J. A., & Huffman, J. W. (2014). Moving around the molecule: relationship between chemical structure and in vivo activity of synthetic cannabinoids. *Life Sciences*, *97*, 55–63.

Wood, K. E. (2013). Exposure to bath salts and synthetic tetrahydrocannabinol from 2009 to 2012 in the United States. *Journal of Pediatrics*, *163*, 213–216.

Zimmermann, U. S., Winkelmann, P. R., Pilhatsch, M., Nees, J. A., Spanagel, R., & Schulz, K. (2009). Withdrawal phenomena and dependence syndrome after the consumption of "Spice Gold". *Deutsches Arzteblatt International*, *106*, 464–467.

CHAPTER

88

Accidents and Synthetic Cannabinoids in Blood of Drivers

S.S. Tuv, V. Auwärter**, V. Vindenes**

***Department of Drug Abuse Research, Division of Forensic Sciences, Norwegian Institute of Public Health, Oslo, Norway**

****Forensic Toxicology Department, Medical Center, University of Freiburg, Institute of Forensic Medicine, Freiburg, Germany**

SUMMARY POINTS

- Synthetic cannabinoids are potent drugs that produce cannabis-like effects.
- The prevalence of synthetic cannabinoids among drivers in normal traffic and accidents is not known.
- Analyzing these substances is challenging due to low concentrations and different variants emerging on the drug market.
- Impairment recognized by clinical tests for impairment can reveal the use of synthetic cannabinoids, and should be used as a guideline in cases of driving under the influence.
- Concentrations of synthetic cannabinoids in the concentration range below 1 ng/mL may cause impairment significant for operating a vehicle.

KEY FACTS OF CANNABINOID RECEPTORS

- There are currently two known subtypes of cannabinoid receptors: the cannabinoid CB-1 receptor, and the cannabinoid CB-2 receptor.
- The CB-1 receptor is mainly located in the brain, and the CB-2 receptor is mainly expressed in the immune system.
- Cannabinoid receptors are transmembrane receptors in the G protein-coupled receptor superfamily.
- The CB-1 receptor is thought to be one of the most widely expressed G-protein coupled receptors in the brain, and is believed to play a significant role in the modulation of GABA and glutamate neurotransmission.
- Agonists to the cannabinoid receptors include cannabinoids naturally occurring in the human body (endocannabinoids), cannabinoids from plants, and synthetic cannabinoids.

KEY FACTS OF CLINICAL TEST OF IMPAIRMENT

- Drivers suspected of driving under the influence (DUI) of drugs in Norway are usually examined by a physician to assess drug impairment.
- The test battery consists of 25 tests and observations related to common signs of impairment.
- It includes testing of specific psychomotor and cognitive tasks.
- The physician makes a conclusion regarding the overall degree of impairment using a four-step scale: not impaired, mildly impaired, moderately impaired, or greatly impaired.
- The clinical test of impairment (CTI) was originally designed to assess traffic related impairment due to alcohol, but has been revised to reveal impairment due to other substances as well.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00103-4
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

CB-1R/CB-2R	Cannabinoid receptors 1 and 2
CTI	Clinical test of impairment
DUI	Driving under the influence
DRE	Drug recognition expert
THC	Δ^9-Tetrahydrocannabinol

INTRODUCTION

Driving under the influence (DUI) of drugs is an issue of growing concern, particularly since it is an important cause of road traffic accidents (Blomberg, Peck, Moskowitz, Burns, & Fiorentino, 2009; Gonzalez-Wilhelm, 2007). Among young adults, road injuries have been found to be the number one risk factor for disease in Western Europe (Lim et al., 2012). Worldwide, around 1.24 million people die every year in road traffic deaths (WHO, 2013), and drugs are involved in a large number of these fatal accidents. The most prevalent illicit drug found in DUI cases in the European Union is Δ^9-Tetrahydrocannabinol (THC) (EU, 2005).

Synthetic cannabinoids have been on the illicit drug market since early 2000, also known as "Spice" in Europe and "K2" in the United States. Initially, these products were believed to be herbal blends producing cannabis-like effects, but synthetic cannabinoids were detected for the first time in 2008 from these blends (Auwärter et al., 2009). Today, more than 50 different synthetic cannabinoids were identified in such products, and new variants appear monthly. The availability through the Internet, legal aspects, the low price, and poor detectability in rapid drug tests have been important factors contributing to the popularity among specific subgroups of drug users.

The main psychoactive component of the plant *Cannabis sativa* is THC. Synthetic cannabinoid products do not usually contain THC. Synthetic cannabinoids are a chemically diverse group, but they all bind to and activate the same receptors as THC (CB-1 and CB-2 receptors), and produce cannabis-like effects when consumed in moderate doses. CB-1 receptors are widely distributed throughout the nervous system, and agonist binding to CB-1 receptors can lead to altered mood and perception, reduced motor-function, impaired memory, altered visual and auditory impressions, severe anxiety, and panic attacks. These effects lead to reduced driving skills. Synthetic cannabinoids have higher binding affinities to CB-1 receptors than THC, and many of them are full agonists at the CB-1 receptor, while THC acts as a partial agonist. Both these conditions contribute to the unfavorable risk profile of the synthetic cannabinoids, and enhance the risk of strong adverse effects. Potent metabolites holding agonistic activity at CB-1 receptors have been described for some of the compounds, further complicating the pharmacodynamic properties.

The effects on driving ability related to recreational use of cannabis are well known, and epidemiologic studies have shown an increased risk of being involved in car accidents after consumption of cannabis (Drummer et al., 2004; Mura et al., 2003). There are no such studies investigating the risk of traffic accidents caused by synthetic cannabinoids, but given the pharmacological activity with binding to the same receptors as THC, and a higher potency and efficacy, it is reasonable to assume similar or even stronger impairing effects on driving ability.

PUBLISHED CASES OF DRIVING UNDER THE INFLUENCE OF SYNTHETIC CANNABINOIDS

Five studies have reported detection of synthetic cannabinoids among impaired drivers. Only 11 different synthetic cannabinoids have been proven in samples from these 84 drivers. The whole blood/serum concentrations are low, and the median concentrations for the different drugs vary between 0.17 and 0.69 ng/mL (Table 88.1).

Yeakel and Logan (2013) describe twelve cases of suspected impaired driving testing positive for synthetic cannabinoids. The concentrations in whole blood ranged from 0.1 to 9.9 ng/mL (LOQ 0.1 ng/mL), and more than one synthetic cannabinoid was found in half of the cases. The detected synthetic cannabinoids included JWH-018, JWH-081, JWH-122, JWH-210, JWH-250, and AM-2201. Drivers with positive findings for alcohol or other drugs were excluded from the study, and results from psychophysical assessment were attributed to the use of synthetic cannabinoids. The subjects were evaluated applying the drug recognition expert (DRE) protocol, and the impairment was categorized in terms of the observed signs. Cannabis was suspected to be the drug responsible for impairment in all cases evaluated by the DRE. Common observations were poor coordination, slow and slurred speech, injected conjunctivae, lack of convergence, and muscle tremors. The pupil size varied, and nystagmus was not observed, pulse rate and systolic blood pressure were generally elevated. Yeakel and Logan found no correlation between the blood concentrations of the synthetic cannabinoids and the degree of impairment. Information regarding the accidents was obtained from seven cases, and four of these had been involved in a car accident. All were considered as the culpable driver. The mean age was 22.4 (range 18–31) years.

Musshoff et al. present six DUI cases, and one 14 year-old girl cycling in a wavy way. Analyses confirmed synthetic cannabinoids where impairment was observed by the police or by a physician (Musshoff et al., 2014).

TABLE 88.1 Blood/Serum Concentrations of Synthetic Cannabinoids Detected in Cases of Driving Under the Influence

Synthetic cannabinoid	Number of DUI cases	Involvement in traffic accident	Blood or serum concentrations in ng/mL, median (range)	References
AM-2201	18	4	0.69 (<0.05–5.5)	Dziadosz, Klintschar, and Teske (2014); Musshoff et al. (2014); Tuv et al. (2014); Yeakel and Logan (2013)
JWH-122	16	3	0.33 (<0.05–28)	Dziadosz et al. (2014); Musshoff et al. (2014); Tuv et al. (2014); Yeakel and Logan (2013)
JWH-210	15	2	0.38 (<0.05–6.2)	Dziadosz et al. (2014); Musshoff et al. (2014); Yeakel and Logan (2013)
JWH-018	17	1	0.17 (<0.05–9.9)	Musshoff et al. (2014); Tuv et al. (2014); Yeakel and Logan (2013)
Other (7 different variants)	12	3		
Unknown	6	5		Chase et al. (2013)

Four of the drivers were checked during general road traffic controls, one had driven his car into a ditch, and the police apprehended another after witness observation. In all cases, more than one synthetic cannabinoid were detected, and serum concentrations ranged from <0.1 ng/mL to 28 ng/mL. Drugs detected included JWH-018, AM-2201, JWH-210, and JWH-122. The impairment registered in these cases was similar to the findings of Yeakel and Logan, and the centrally sedating effects and reduction in fine motor skills were assumed to be of great importance. The authors concluded that the consumption of synthetic cannabinoids could lead to impairment similar to typical performance deficits caused by cannabis use, which are not compatible with safe driving. The mean age of the apprehended drivers was 20.7 (range 14–29) years.

Chase et al. (2013) retrospectively compared drivers suspected of DUI of synthetic cannabinoids ($n = 13$) with a similar group suspected of impairment due to marijuana use ($n = 25$). Thirty-eight percent of the synthetic cannabinoid suspects were involved in a car accident, while only 4% of the marijuana suspects were involved in a car accident. Based on the experiences from the DREs, they concluded that intake of synthetic cannabinoids leads to more pronounced confusion and disorientation, and more frequent involvement in car accidents than intake of marijuana. Blood samples were only provided in seven of the synthetic cannabinoid cases, six of these proved positive for synthetic cannabinoids. No further information is available regarding these findings, nor are blood concentrations given.

Tuv et al. (2014) found a prevalence of synthetic cannabinoids in DUI suspects in Norway of 2.2% (16 out of 726 cases). Four of the cases were collected from drivers involved in a traffic accident; eleven of the cases were due to suspicion of DUI by the police and one after suspicion of drug use. AM-2201 and JWH-018 were the most frequently detected synthetic cannabinoids. Concentrations varied between 0.07 and 1.67 µg/L. All of the cases had concomitant findings of other potent psychoactive drugs, and it was not possible to find a correlation between the concentrations of the detected synthetic cannabinoids and the degree of impairment judged by a physician. Only one synthetic cannabinoid was found in each case. In 15 out of the 16 cases, the drivers were males, and the mean age was 29.6 (range 17–49) years.

Dziadosz et al. present a study where DUI cases with THC or THC metabolites in serum were further analyzed for a selection of synthetic cannabinoids. The results revealed a 0.5% prevalence of synthetic cannabinoids among the THC-positive drivers. There is no information regarding the prevalence of cannabis among the DUI drivers, and the prevalence of the synthetic cannabinoids can thus not be estimated. A total of 12 cases were positive with respect to synthetic cannabinoids, and the repertoire tested included JWH-018, JWH-122, JWH-210, and AM-2201. Almost half of the cases revealed more than one synthetic cannabinoid in combination with THC. Serum concentrations ranged from <0.05 to 0.75 ng/mL, and most of the cases were in the lower concentration area.

CLINICAL PRESENTATION OF INTOXICATION DUE TO SYNTHETIC CANNABINOIDS

The recreational use of synthetic cannabinoids has led to numerous intoxications, and also at least three deaths (Gay, 2012; Saito et al., 2013) in the last few years. Physiological effects of synthetic cannabinoids can be wide-ranging, from mild to serious symptoms which require

TABLE 88.2 Effects of Synthetic Cannabinoids That Might Interfere With Safe Driving

Type of effect	Symptom
Cognitive	Memory impairment
	Reduced concentration
	Attention deficit
	Disorientation, confusion
Neurological	Agitation/panic
	Loss of consciousness/coma
	Anxiety/paranoia
	Psychosis/hallucination
	Changes in mood and perception
	Seizures
	Reduced fine motor skills
Cardiovascular	Chest pain/myocardial infarction
	Arrhythmia
Gastrointestinal	Nausea/emesis

TABLE 88.3 Findings Among Apprehended Drivers

Findings among apprehended drivers
Poor coordination
Slow and slurred speech
Injected conjunctivae
Lack of convergence
Lack of nystagmus
Pupil size varied
Muscle tremors
Increased pulse rate
Elevated systolic blood pressure
Reduction in fine motor skills
Confusion
Disorientation

Some common findings among suspects of driving under the influence due to synthetic cannabinoids.

treatment in an intensive care unit. A review of the literature from 2010 to mid-2013 suggests that synthetic cannabinoids can have more severe side effects than marijuana (Gurney, Scott, Kacinko, Presley, & Logan, 2014; Hermanns-Clausen, Kneisel, Szabo, & Auwärter, 2013), and it is reasonable to assume that effects of synthetic cannabinoids might be of importance regarding skills needed to drive safely. Several of the symptoms are similar to cannabis intoxication, but more severe symptoms not usually associated with the use of cannabis have also been noted. An increasing number of papers describes the clinical presentation of intoxication due to synthetic cannabinoids, and the most common symptoms include severe agitation, hallucinations, vomiting, drowsiness, and altered mood and perception (Forrester, Kleinschmidt, Schwarz, & Young, 2011; Rosenbaum, Carreiro, & Babu, 2012). Effects on cognitive function and the nervous system might be of great concern with respect to driving ability, but also cardiovascular and gastrointestinal effects which are commonly observed might be incompatible with safe driving. Table 88.2 gives an overview of effects of synthetic cannabinoids that may be of importance with regard to driving-related skills.

FINDINGS AMONG APPREHENDED DRIVERS

In several of the DUI cases described in the literature, a DRE evaluation, an examination by a physician or a clinical test of impairment (CTI) has been performed (Bramness, Skurtveit, & Morland, 2003). All these examinations are performed by trained personnel looking for specific signs of drug impairment. Some observations or failures of tests seem to recur often, and Table 88.3 shows some common findings among suspects of DUI due to synthetic cannabinoid use.

CONCENTRATIONS OF SYNTHETIC CANNABINOIDS IN BLOOD FROM SUSPECTED DRIVERS

Concentrations ranged from as low as <0.05 to as high as 28 ng/mL for all the synthetic cannabinoids detected, but the mean concentrations from all the studies were 0.69 ng/mL or below. The highest concentration was seen in a DUI case where six additionally different synthetic cannabinoids were detected. The police noted that the suspect showed retarded movements, was apathetic, nervous, and inert, and had delayed reaction of pupils to light (Musshoff et al., 2014). A majority of the cases was in the lower concentration range, but also high concentrations were present in some cases. Several of the suspects with small amounts of synthetic cannabinoids in blood were described as impaired. In one case, the concentration of JWH-018 in whole blood was only 0.24 ng/mL, however, observations of the driver included raised arms and improper turn during walk-and-turn (WAT) test, raised arms during one-leg stand (OLS) test, and eye flutters during Romberg's test (Yeakel & Logan, 2013). In this study, it could not be excluded that other drugs not included in the analytical repertoire were present in the samples; and since there might be drugs with high potency, low concentrations could thus cause heavy impairment.

No direct link between the degree of impairment and blood or serum concentrations of synthetic cannabinoids has been established, as seen for THC, where higher concentrations of THC are associated with increasing impairment and increased risk of traffic accidents (Ramaekers, Berghaus, van Laar, & Drummer, 2004). From the perspective of these findings, it seems plausible, however, that concentrations of synthetic cannabinoids in the range below 1 ng/mL may cause significant impairment.

VARIANTS OF SYNTHETIC CANNABINOIDS DETECTED AMONG APPREHENDED DRIVERS

Despite the enormous number of available synthetic cannabinoids, the different variants of synthetic cannabinoids detected in published DUI cases are relatively limited. This could be due to analytical limitations (you only find what you are looking for), and the fact that the presented cases fall into a narrow time frame (the spectrum of analytes that is present in the samples will changes over time). The most frequently detected synthetic cannabinoids in the presented DUI cases so far include JWH-122, JWH-018, AM-2201, and JWH-210. The different variants detected may also mirror different trends of abuse in different countries and regions, given that different laboratories have the same repertoire of synthetic cannabinoids. In DUI cases referred to in this paper, JWH-122 was one of the most frequently detected synthetic cannabinoids in Germany and the United States, but this variant was not at all detected in the Norwegian material, although included in the analytical method. AM-2201 was the most frequently detected synthetic cannabinoid among apprehended drivers involved in a traffic accident. One-third of all published DUI cases with detection of AM-2201 were involved in traffic accidents. Few of the drivers who tested positive for the other most popular variants were involved in accidents. The different variants detected in the available literature are given in Table 88.4. However, it must be kept in mind that the synthetic cannabinoids available in "herbal mixtures" change rapidly over time.

THE PREVALENCE OF SYNTHETIC CANNABINOIDS IN TRAFFIC ACCIDENTS

The reasons for a traffic accident might be complex, and it is often difficult to distinguish the different contributing factors. Various types of studies are available for studying the effects of drugs on driving ability. For

TABLE 88.4 Variants of Synthetic Cannabinoids and Involvement in Accidents Detected in Driving Under the Influence Cases

	Tuv et al.	Musshoff et al.	Yeakel et al.	Dziadosz et al.	Chase et al.
Number of cases	16	7	12	12	6
Mean age	29.6 (17–49)	20.7 (14–29)	22.4 (18–31)	?	?
Accidents	4 of 16	1 of 7	4 of 7	Unknown	5 of 6
Impairment investigated by	Physician	Police/physician	DRE	?	DRE
Synthetic cannabinoids					
JWH-081	1		2		
JWH-250	1		2		
JWH-018	5	3	6	3	
AM-2201	5	3	7		
RCS-4	1				
JWH-122	3	5	3	8	
JWH-210		6	4	5	
JWH-019		1			
JWH-307		2			
MAM-2201		1			
UR-144		1			
Maximum SC(s) in one case	1	6	4	4	?

well-studied substances, the risk for traffic accidents have been estimated, but the small number of traffic accidents involving analytically confirmed synthetic cannabinoid use sets up limitations. Also, culpability does not exist in all these cases, making it even more difficult to estimate the risk of traffic accidents.

We do not have information about the circumstances in each of the published DUI cases, and only nine traffic accidents are featured in detail in the literature. Other DUI cases with analytically confirmed synthetic cannabinoid use were typically captured in police routine controls. In several of the cases, other psychoactive drugs were detected concomitantly, making it more difficult to assess the contribution of the detected synthetic cannabinoid.

Chase et al. (2013) additionally described five more accidents related to the intake of synthetic cannabinoids, but the different synthetic cannabinoids are not disclosed. From the small number of observations by Chase et al. (2013), it was estimated that among the "Spice" suspects, 38% of the drivers were involved in motor vehicle crashes. However, blood was only available from 7 of the 13 drivers. Nevertheless, the results indicate that DREs in a large part of the cases were able to recognize impairment caused by synthetic cannabinoids.

Dziadosz et al. analyzed samples collected during police traffic control that tested positive for THC and/or metabolites for synthetic cannabinoids. Among these samples, they reported 0.5% to be positive for at least one synthetic cannabinoid in addition. The number of participants in this study is not known. In the Norwegian study, among drugged drivers the prevalence of synthetic cannabinoids was 2.2%.

Table 88.4 gives an overview of DUI cases published, and the different synthetic cannabinoids involved. The prevalence of synthetic cannabinoids in traffic accidents is difficult to estimate from these sparse data. The number of traffic accidents in Table 88.4 exceeds the total number of published accidents because in several of the cases more than one synthetic cannabinoid was detected. In one case, as much as seven different synthetic cannabinoids were analytically confirmed (Musshoff et al., 2014).

It is important to note that the synthetic cannabinoids have been measured in whole blood in some studies, and serum in others. The ratio between concentrations of synthetic cannabinoids in serum and whole blood is not known, and this must be kept in mind when concentrations from different studies are compared. Since the synthetic cannabinoids might have similar properties as THC, it seems likely that the concentrations measured in serum is significantly higher compared to whole blood concentrations. For THC, the ratio between serum and whole blood is around 1.8–2 (Baselt, 2009).

ANALYTICAL CHALLENGES

Only in the last few years analytical methods have been available to detect these substances in blood or serum, and many of the methods are not "up to date" due to quick changes in the composition of synthetic cannabinoid containing products. Knowledge of driving under the influence of these substances is therefore scarce. Many of the synthetic cannabinoids are highly potent substances, and doses in the low or even submilligram range produce the typical effects. This leads to low concentrations in blood or serum samples which are challenging for analytical method development.

Due to the frequent appearance of new variants of synthetic cannabinoids on the drug market, it is a major challenge for analytical laboratories to keep track of the substances to look for. Seizures in the different countries provide some important information. For urine analysis, information regarding metabolism of new substances must be obtained before an analytical method is developed because for most of the synthetic cannabinoids no parent compound is excreted renally. As several of these metabolites act as potent CB-1 agonists, it may be reasonable to include them into methods for blood analyses. However, most forensic laboratories only search for the parent compounds in blood so far (Fantegrossi, Moran, Radominska-Pandya, & Prather, 2014).

The detection rate of synthetic cannabinoids using immunoassays seems to be limited due to the quick structural changes over time (Hutter, Ippisch, Schultis, & Auwärter, 2012).

Most laboratories analyze forensic samples using a standard repertoire where the most common drugs of abuse are included. If there is a suspicion of drug impairment without any findings in the standard drug panel, further analyses are performed. Concomitant consumption of other psychoactive drugs can thus cover the use of synthetic cannabinoids, leading to an underestimation of these drugs in DUI cases. A mixed consumption of synthetic cannabinoids and cannabis has been detected in DUI cases as reported by Dziadosz et al. (2014).

TOLERANCE

Little is known regarding tolerance to the effects of synthetic cannabinoids. From Internet fora, users have described the phenomenon of tolerance after using synthetic cannabinoids. The findings among regular users where high concentrations of synthetic cannabinoids in serum do often not produce signs of intoxication support these statements. Reduced activation of the CB-1 receptor has been observed in rats that had been given HU-201 for a longer period of time (Dalton & Zavitsanou, 2010). In another study, it was shown that synthetic cannabinoids

lead to rapid and robust internalization of CB-1 receptors (Atwood, Huffman, Straiker, & Mackie, 2010; Atwood, Lee, Straiker, Widlanski, & Mackie, 2011). Based on these findings, it is reasonable to assume capacity to produce tolerance to at least some of the effects of synthetic cannabinoids. Regarding well-studied substances, such as the benzodiazepines, it is known that development of tolerance is mostly linked to subjective effects of the drugs, and does not so much affect reduced skills needed to be a safe driver. Users have also described crosstolerance between THC and synthetic cannabinoids with reduced effects of THC after the intake of synthetic cannabinoids, and vice versa. The effect of regular use of synthetic cannabinoids on driving skills is unknown so far.

LEGISLATION

Different countries have different approaches concerning driving under the influence. Two main principles are used: per se law—with fixed concentration limits defining the threshold for punishability (limits of detection (LOD) can be used, but also higher cut-off levels); or impairment-based laws where an individual evaluation of impairment is done in each case, and more severe sanctions are provided with increasing impairment.

Although sometimes referred to as "legal highs," an increasing number of countries have introduced legislation to control synthetic cannabinoids. Due to a constantly changing market of synthetic cannabinoids, different approaches were tried to allow for a rapid and dynamic update of drugs scheduled. Scheduling these substances timely may support prevention work of traffic accidents, as noncontrolled products may mislead users to believe that driving after consumption is legal and safe.

DISCUSSION AND CONCLUSIONS

The prevalence of DUI of synthetic cannabinoids is not known, and neither the prevalence of such drugs in traffic injuries nor in fatal car crashes. Synthetic cannabinoids are not detected using regular rapid tests. Even with chromatographic methods, only a limited number of the available substances can be revealed, and not all laboratories provide analytical methods for the detection of these drugs. Impairment recognized by clinical tests for impairment by physicians, the police, or drug recognition experts can reveal such use, however, and the awareness of such drugs among these people is thus of major importance. The legal approach concerning driving under the influence of synthetic drugs vary between different countries, and, as the name "legal highs" indicates, in some countries there is no legislation effectively regulating these substances. From studies describing the clinical effects of synthetic cannabinoids, and also the reports from apprehended drivers where these drugs have been detected, synthetic cannabinoid use is not compatible with safe driving, and should therefore be regulated in the driving legislation. It is thus of major importance to increase the general knowledge in society of the risk of driving under the influence of synthetic cannabinoids, and further to provide comprehensive analytical methods to be able to reveal such use, and also appropriate legislation.

MINI-DICTIONARY

Spice/K2 Brands of synthetic cannabinoid products often used as an umbrella term for herbal products containing synthetic cannabinoids.

Synthetic cannabinoids A chemically diverse group of substances functionally similar to THC in binding to the same receptors, resulting in a cannabis-like high.

Clinical test of impairment (CTI) A test battery consisting of 25 tests and observations related to common signs of drug impairment.

Drug recognition expert (DRE) A drug recognition expert is a police officer trained to recognize impairment in drivers due to drugs other than alcohol.

Chromatographic analysis Technique for separating the components, or solutes, of a mixture on the basis of the relative amounts of each solute distributed between a moving fluid stream, called the mobile phase, and a stationary phase.

References

Atwood, B. K., Huffman, J., Straiker, A., & Mackie, K. (2010). JWH018, a common constituent of 'Spice' herbal blends, is a potent and efficacious cannabinoid CB receptor agonist. *British Journal of Pharmacology*, *160*(3), 585–593.

Atwood, B. K., Lee, D., Straiker, A., Widlanski, T. S., & Mackie, K. (2011). CP47,497-C8 and JWH073, commonly found in 'Spice' herbal blends, are potent and efficacious CB(1) cannabinoid receptor agonists. *European Journal of Pharmacology*, *659*(2–3), 139–145.

Auwärter, V., Dresen, S., Weinmann, W., Müller, M., Pütz, M., & Ferreiros, N. (2009). 'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs? *Journal of Mass Spectrometry*, *44*(5), 832–837.

Baselt, C. R. (2009). *Disposition of toxic drugs and chemicals in man* (8th ed.). Seal Beach, CA: Biomedical Publications.

Blomberg, R. D., Peck, R. C., Moskowitz, H., Burns, M., & Fiorentino, D. (2009). The Long Beach/Fort Lauderdale relative risk study. *Journal of Safety Research*, *40*(4), 285–292.

Bramness, J. G., Skurtveit, S., & Morland, J. (2003). Testing for benzodiazepine inebriation—relationship between benzodiazepine concentration and simple clinical tests for impairment in a sample of drugged drivers. *European Journal of Clinical Pharmacology*, *59*(8–9), 593–601.

Chase, P. B., Hawkins, J., Mosier, J., Boesen, K., Jimenez, E., Logan, B., & Walter, F. (2013). Signs of synthetic cannabinoid vs. marijuana intoxication as determined by police drug recognition experts: a restrospective study in the State of Arizona. *Clinical Toxicology*, *51*(7), 667.

Dalton, V. S., & Zavitsanou, K. (2010). Cannabinoid effects on CB1 receptor density in the adolescent brain: an autoradiographic study using the synthetic cannabinoid HU210. *Synapse*, *64*(11), 845–854.

Drummer, O. H., Gerostamoulos, J., Batziris, H., Chu, M., Caplehorn, J., Robertson, M. D., & Swann, P. (2004). The involvement of drugs in drivers of motor vehicles killed in Australian road traffic crashes. *Accident Analysis and Prevention*, *36*(2), 239–248.

Dziadosz, M., Klintschar, M., & Teske, J. (2014). Mixed consumption of cannabis and "Spice". *Forensic Science International*, *235*, e1–e2.

EU. (2005). The Rosita Project. European Union.

Fantegrossi, W. E., Moran, J. H., Radominska-Pandya, A., & Prather, P. L. (2014). Distinct pharmacology and metabolism of K2 synthetic cannabinoids compared to Δ9-THC: mechanism underlying greater toxicity? *Life Sciences*, *97*(1), 45–54.

Forrester, M. B., Kleinschmidt, K., Schwarz, E., & Young, A. (2011). Synthetic cannabinoid exposures reported to Texas poison centers. *Journal of Addictive Diseases*, *30*(4), 351–358.

Gay, M. (2012). Synthetic marijuana spurs state bans, The New York Times, July 10, 2012.

Gonzalez-Wilhelm, L. (2007). Prevalence of alcohol and illicit drugs in blood specimens from drivers involved in traffic law offenses. Systematic review of cross-sectional studies. *Traffic Injury Prevention*, *8*(2), 189–198.

Gurney, S. M. R., Scott, K. S., Kacinko, S. L., Presley, B. C., & Logan, B. K. (2014). Pharmacology, toxicology, and adverse effects of synthetic cannabinoid drugs. *Forensic Science Review*, *26*, 53–78.

Hermanns-Clausen, M., Kneisel, S., Szabo, B., & Auwärter, V. (2013). Acute toxicity due to the confirmed consumption of synthetic cannabinoids: clinical and laboratory findings. *Addiction*, *108*(3), 534–544.

Hutter, M., Ippisch, J., Schultis, H. -W., & Auwärter, V. (2012). Evaluation of an ELISA for synthetic cannabinoids applied to urine samples by comparison with a comprehensive and highly sensitive LC-MS/MS method. *Rechtsmedizin*, *22*(4), 286–287.

Lim, S. S., Vos, T., Flaxman, A. D., Danaei, G., Shibuya, K., Adair-Rohani, H., …, Memish, Z. A. (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, *380*(9859), 2224–2260.

Mura, P., Kintz, P., Ludes, B., Gaulier, J. M., Marquet, P., Martin-Dupont, S., …, Pourrat, O. (2003). Comparison of the prevalence of alcohol, cannabis and other drugs between 900 injured drivers and 900 control subjects: results of a French collaborative study. *Forensic Science International*, *133*(1–2), 79–85.

Musshoff, F., Madea, B., Kernbach-Wighton, G., Bicker, W., Kneisel, S., Hutter, M., & Auwärter, V. (2014). Driving under the influence of synthetic cannabinoids ("Spice"): a case series. *International Journal of Legal Medicine*, *128*(1), 59–64.

Ramaekers, J. G., Berghaus, G., van Laar, M., & Drummer, O. H. (2004). Dose related risk of motor vehicle crashes after cannabis use. *Drug and Alcohol Dependence*, *73*(2), 109–119.

Rosenbaum, C. D., Carreiro, S. P., & Babu, K. M. (2012). Here today, gone tomorrow... and back again? A review of herbal marijuana alternatives (K2, Spice), synthetic cathinones (bath salts), kratom, Salvia divinorum, methoxetamine, and piperazines. *Journal of Medical Toxicology*, *8*(1), 15–32.

Saito, T., Namera, A., Miura, N., Ohta, S., Miyazaki, S., Osawa, M., & Inokuchi, S. (2013). A fatal case of MAM-2201 poisoning. *Forensic Toxicology*, *31*(2), 333–337.

Tuv, S. S., Krabseth, H., Karinen, R., Olsen, K. M., Oiestad, E. L., & Vindenes, V. (2014). Prevalence of synthetic cannabinoids in blood samples from Norwegian drivers suspected of impaired driving during a seven weeks period. *Accident Analysis and Prevention*, *62*, 26–31.

WHO. (2013). Available from http://www.who.int/violence_injury_prevention/road_safety_status/2013/en/

Yeakel, J. K., & Logan, B. K. (2013). Blood synthetic cannabinoid concentrations in cases of suspected impaired driving. *Journal of Analytical Toxicology*, *37*(8), 547–551.

Page left intentionally blank

CHAPTER

e12

Cannabidiol as an Antioxidant

*R.S. Borges**, *A.B.F. da Silva***

***Faculty of Pharmacy, Institute of Health Sciences, Federal University of Pará, Belém, Para, Brazil**
****Institute of Chemistry of São Carlos, University of São Paulo, São Carlos, Sao Paulo, Brazil**

SUMMARY POINTS

- This chapter focuses on the theoretical antioxidant capacity of cannabidiol (CBD), using density functional theory calculations.
- Structural properties of CBD are compared with related derivatives.
- Antioxidant properties are compared with trolox, a simplified model of α-tocopherol.
- Theoretical properties of CBD and derivatives are associated to electron or hydrogen transfer.
- The antioxidant property of CBD may be related to its electron and hydrogen abstraction at the phenol position.
- Phenol and alkyl moieties increase the highest occupied molecular orbital (HOMO) energies, and decrease the ionization potential (IP) values.
- The lowest O—H bond dissociation energies (BDE_{OH}) are related to the phenol position.
- The limonene moiety has a slight higher relevance than phenyl-pentane.
- Antioxidant mechanisms in biological system and their applications are discussed.

KEY FACTS OF ANTIOXIDANT CAPACITY

- The antioxidant capacity can be determined using experimental and theoretical methods.
- Quantum mechanical methods can provide descriptors to be related with the antioxidant capacity of molecules.
- Density functional theory calculations have been employed to evaluate electron or hydrogen transfer of antioxidant compounds.
- Antioxidant mechanisms can be determined using structure–activity relationship.
- In general, the electron transfer is related to the ionization potential, and the bond dissociation energy to the hydrogen transfer.
- A high antioxidant capacity depends on the number and position of hydroxyl groups or electron donating groups.
- The antioxidant capacity of any molecule can be compared to every structural fragment of parent molecule.

LIST OF ABBREVIATIONS

6–311++G(2d,2p)	Largest basis sets
ArO$^{\bullet}$	Phenoxyl free radical
ArO^{-}	Phenoxyl anion radical
ArOH$^{\bullet+}$	Phenol cation free radical
ArOH	Neutral phenol
B3LYP	Hybrid functional Becke and three-parameter Lee–Yang–Parr
CBD	Cannabidiol
CBD-O$^{\bullet}$	Cannabidiol semiquinone radical
CBD-OH	Neutral cannabidiol
CBD-OH$^{\bullet+}$	Cannabidiol cation free radical
DFT	Density functional theory
E	Energy
E_{GAP}	Energy gap or bandgap
ΔEiso	Stabilization energy

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00093-4
Copyright © 2017 Elsevier Inc. All rights reserved.

Eq.	Equation
FES$^{\bullet}$	Free radical species
FESH	Free radical species
HOMO	Highest occupied molecular orbital
IP	Ionization potential
LUMO	Lowest unoccupied molecular orbital
P450	Cytochrome P450
PPARγ	Peroxisome proliferator-activated receptor gamma
R$^{\bullet}$	Alkyl free radical
R^{-}	Alkyl anion radical
RH	Alkyl radical
SAR	Structure–activity relationship
TLX	Trolox
THC	(−)Δ^9-Tetrahydrocannabinol

INTRODUCTION

The use of cannabidiol (CBD) or 2-[(1R,6R)-6-isopropenyl-3-methylcyclohex-2-en-1-yl]-5-pentylbenzene-1,3-diol (Fig. e12.1) as an antioxidant and neuroprotective agent is well known in the literature (Moldzio et al., 2012; Hayakawa et al., 2007; Di Marzo, Bifulco, & de Petrocellis, 2004). The CBD is a very low endogenous ligand of cannabinoid receptors CB1 and CB2, however, its metabolism by fatty acid amide hydrolase and monoacylglycerol lipase induces antinociceptive effects (Hohmann & Suplita, 2006; Walker & Huang, 2002).

Currently, its clinical recommendation as analgesic is growing (Fox & Bevan, 2005; Meng, Manning, Martin, & Fields, 1998). In fact, this compound has several biological effects and potential uses, such as antiinflammatory (Naftali, Mechulam, Lev, & Konikoff, 2014), anticancer (Murase et al., 2014), ischemia (Gonca & Darıcı, 2014), antimicrobial (Appendino et al., 2008), and anxiolytic (Almeida et al., 2013). Nevertheless, it has poor solubility in water, and good solubility in organic solvents (Mannila, Järvinen, Järvinen, & Jarho, 2007).

Unlike other cannabinoids, CBD contains two phenolic groups. It has antioxidant activity sufficient to protect the liver against binge alcohol-induced steatosis, by a mechanism including inhibition of oxidative stress (Yang et al., 2014). It has a cardioprotective effect in rats exposed to doxorubicin toxicity by free radical scavenging (Fouad, Albuali, Al-Mulhim, & Jresat, 2013). Actually, tests in mouse during hepatic microsomal metabolism indicate that CBD prevents the generation of reactive oxygen species (Usami, Yamamoto, & Watanabe, 2008). Some CBD properties were observed in previews in an in vivo study, where this compound attenuates cisplatin-induced nephrotoxicity by decreasing oxidative stress through oxygen or nitrogen free radical species, inflammation, and cell death (Pan et al., 2009). Other studies using colon cancer lines showed that CBD has a chemopreventive effect (Aviello et al., 2012).

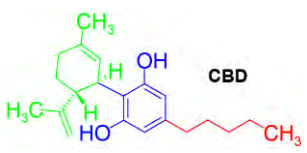


FIGURE e12.1 **Chemical structure of CBD.** Molecular structure of CBD has three different chemical moieties: limonene *(green)*, phenol *(blue)*, and aliphatic group *(red)*.

An experimental study showed that cannabinoids can prevent Alzheimer disease in an animal model by preventing the inflammation caused by microglia cells which are activated by the binding of amyloid protein (Steffens et al., 2005). Nevertheless, the evaluation of the neuroprotective effect of cannabinoids in a rat model of Parkinson disease has confirmed the importance of the antioxidant mechanism, and the cannabinoid receptor-independent properties (García-Arencibia et al., 2007).

In addition, pain and inflammation are other major clinical signs that can be triggered by a free radical mechanism. Nonetheless, CBD exerts an orally effective therapeutic agent in rat models of chronic inflammatory and neuropathic pain (Costa, Trovato, Comelli, Giagnoni, & Colleoni, 2007). CBD has been shown to be extensively metabolized by experimental animals and humans, in the liver, predominantly by cytochrome P450 enzymes from oxidation reactions (Stout & Cimino, 2014). The hydroxylation reactions on limonene and *n*-pentyl moieties were the main metabolites (Jiang, Yamaori, Takeda, Yamamoto, & Watanabe, 2011; Yamaori, Ebisawa, Okushima, Yamamoto, & Watanabe, 2011). In fact, cytochrome P450 enzymes catalyze a variety of reactions (mainly oxidation), converting chemicals to potentially reactive products, as well as producing less toxic compounds. The majority of these reactions can be intermediated by electron or hydrogen abstraction mechanisms (Guengerich, 2001).

In accordance with the literature, two main mechanisms are proposed to explain the protective role for antioxidants for phenol derivatives (Leopoldini, Chiodo, Russo, & Toscano, 2011a; Galano, Macías-Ruvalcaba, Campos, & Pedraza-Chaverri, 2010; Galano, 2011; Iuga, Alvarez-Idaboy, & Russo, 2012; Chiodo, Leopoldini, Russo, & Toscano, 2010; Leopoldini, Marino, Russo, & Toscano, 2004; Leopoldini, Russo, & Toscano, 2006; Leopoldini, Russo, & Toscano, 2007; Leopoldini, Russo, & Toscano, 2011b). One is the H-atom transfer mechanism, in which a free radical R$^{\bullet}$ removes a hydrogen atom from the antioxidant (ArOH) (Eq. e12.1), and the other one is the one-electron transfer mechanism, where the antioxidant compound can give an electron to the radical (Eqs. e12.2 and e12.3). In addition to these mechanisms, the radicals arising from both reactions (ArO$^{\bullet}$ and ArOH$^{\bullet+}$) must be stable, in order to prevent chain radical reactions.

$$R^{\bullet} + ArOH \rightarrow RH + ArO^{\bullet} \quad \text{(e12.1)}$$

$$R^{\bullet} + ArOH \rightarrow R^{-} + ArOH^{\bullet +} \quad \text{(e12.2)}$$

$$R^{\bullet} + ArO^{-} \rightarrow R^{-} + ArO^{\bullet} \quad \text{(e12.3)}$$

An understanding about its mechanism and the role played by the different structural features of CBD, defining how these functional moieties are responsible for its antioxidant properties, can be elucidated using molecular modeling (Borges et al., 2013). Thus, the relationship among electronic properties, antioxidant capacity of CBD, and its possible antioxidant mechanism can be determined using theoretical methods.

Here, a systematic study of the influence of the phenol, limonene, and *n*-pentyl-benzene groups on the antioxidant properties of CBD is reviewed (Borges et al., 2013) (Fig. e12.2). In addition, the properties of CBD and related derivatives were compared with a simplified model of α-tocopherol (trolox, (±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxilic acid) (Huang, Ou, & Prior, 2005). The structural and electronic characteristics of CBD obtained using the Density Functional Theory (DFT) methodology (Frisch et al., 2004) is reviewed with the objective of shedding light on the possible oxidation mechanism of CBD, and its relationship with its putative antioxidant properties. The studies performed here assessed the energies of the highest occupied molecular orbital (HOMO), and the lowest unoccupied molecular orbital (LUMO), ionization potential (IP), stabilization energy (ΔEiso), and hydroxyl bond dissociation energy (BDE_{OH}) (Queiroz, Gomes, Moraes, & Borges, 2009).

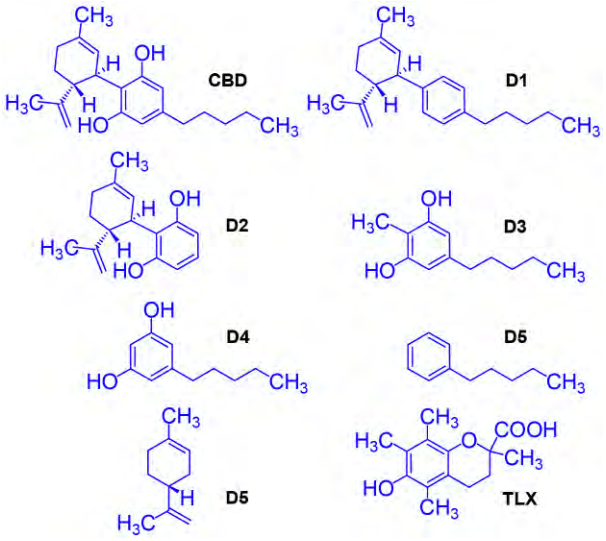


FIGURE e12.2 The structure of CBD and related derivatives. Molecular fragmentation of chemical structure of CBD.

The adiabatic IP were calculated as the energy difference between the neutral molecule and its respective cation free radical (Eq. e12.4). On the other hand, the semiquinone radical stability is usually calculated by the hydroxyl bond dissociation energies (BDE_{OH}). The BDE_{OH} values were calculated as the energy difference between the neutral molecule and its respective semiquinone, plus the hydrogen radical (Eq. e12.5).

$$IP = [E ArOH_{+}] - [E ArOH] \quad \text{(e12.4)}$$

$$BDE_{OH} = [E ArO^{\bullet} + E H^{\bullet}] - [E ArOH] \quad \text{(e12.5)}$$

CONFORMATIONAL STUDIES

The final molecular geometry of CBD and related derivatives (Fig. e12.3) can be obtained by using the DFT methods and B3LYP functional (Kohn, Becke, & Parr, 1996; Parr & Pearson, 1983) with the 6–311++G(2d,2p) basis sets (Parr, Szentpaly, & Liu, 1999).

From the optimized geometries, it was possible to observe that the structure of CBD does not have intermolecular hydrogen bond from the oxygen atom of both phenolic moieties. In this case, the hydrogen of two phenolic groups have a great electronic repulsion with the 3-methyl-cyclohexene and 6-isopropene-cyclohexene rings, due to the difference of polarity (Borges et al., 2013; Borges, 2015).

The geometry of CBD is not planar, having strong repulsion between hydroxyl groups and the limonene ring. The CBD has two rings (phenol and cyclohexene) and five radicals (*n*-penthyl, two hydroxyl phenolic, methyl, and isopropene). In fact, the transverse positions between resorcionol and limonene rings are responsible for the semirigid characteristic of CBD. Thus, when hydroxyl groups are removed (D1), there is a decrease in the molecular rigidity.

FRONTIER MOLECULAR ORBITALS ON ANTIOXIDANT CAPACITY OF CANNABIDIOL

The final geometries were used for the calculations of the antioxidant properties for CBD and related derivatives. Table e12.1 presents the frontier orbital energies, IP, and the O—H bond dissociation energies of the molecules studied (Borges, 2015).

Comparing the HOMO properties from CBD and other hydroxylated derivatives (D2, D3, and D4) with TLX, a more similar value with the nonphenolic derivatives (D1, D5, and D6) was observed. The TLX and CBD molecules showed HOMO values of –5.42 and –6.02 eV, respectively. This indicates that the phenol moiety increases the HOMO values. In fact, the hydroxyl group has great influence on the HOMO contributions, as shown in Fig. e12.4.

The electronic effect in the phenolic compound is mainly influenced by other functional groups, such as

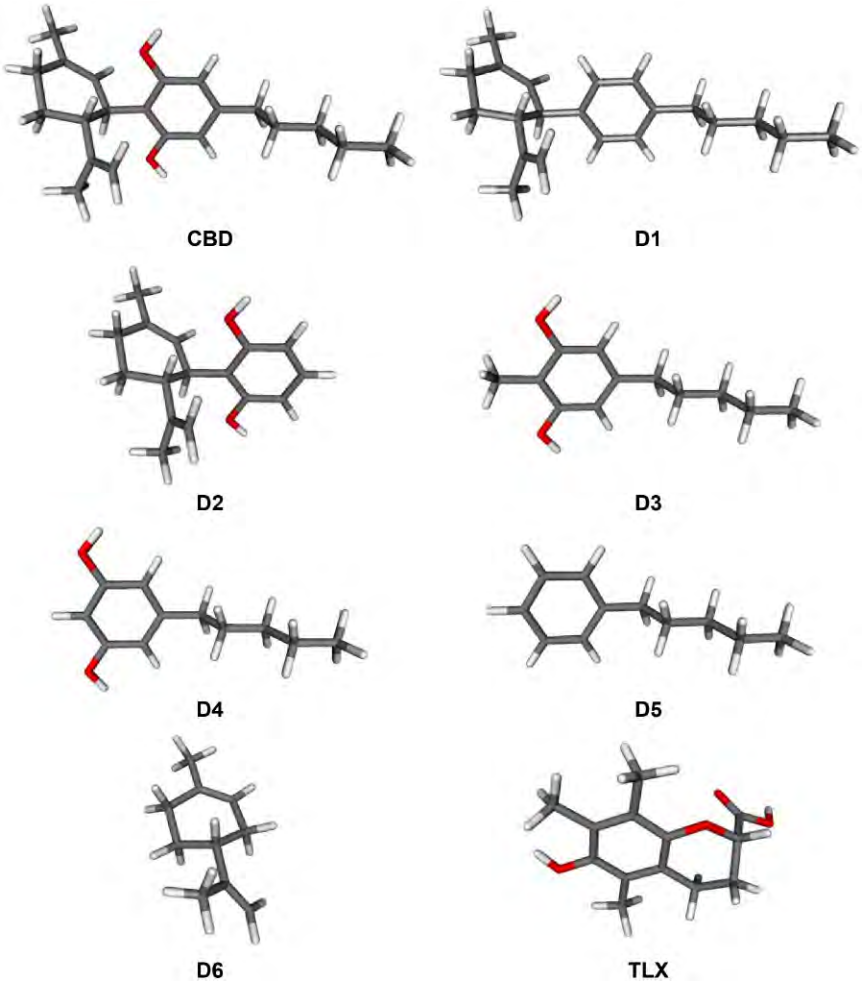


FIGURE e12.3 **The optimized structures of the neutral molecules studied.** The molecular structures of CBD and related derivatives 1-(6-isopropenyl-3-methyl-cyclohex-2-enyl)-4-pentyl-benzene (D1), 2-(6-isopropenyl-3-methyl-cyclohex-2-enyl)-benzene-1,3-diol (D2), 2-methyl-5-pentyl-benzene-1,3-diol (D3), 5-pentyl-benzene-1,3-diol (D4), pentyl-benzene (D5), limonene (D6), and trolox (TLX), show three different atoms: carbon *(gray)*, hydrogen *(white)*, and oxygen *(red)*.

the limonene ring and the *n*-penthyl moiety that have a participation in the inductive or resonance effects, and can indicate a reactive point of scavenging free radicals due to the H-abstraction after electron transfer. The number of resonance structures or electron donating groups, such as alkyl groups (limonene ring and *n*-penthyl moiety) linked to the *ortho*- or *meta*-positions of the phenol moiety, can be related to the highest nucleophilicity of CBD and its hydroxylated derivatives.

The HOMO energy is an important electronic parameter for describing the antioxidant ability of a molecule, since this property can be related to electron transfer reactions. Nevertheless, sometimes its application is very limited. In fact, a molecule with low values of HOMO energy has a weak electron donating ability. On the contrary, a higher HOMO energy implies that the molecule is a good electron donor (Queiroz et al., 2009; Borges et al., 2013). However, it is interesting to note that CBD and its hydroxylated derivatives (D2, D3, and D4) had the lowest values of GAP energy, indicating their low electron transition energy between HOMO and LUMO, when compared with the highest GAP values of nonphenolic derivatives (D1, D5, and D6).

In Fig. e12.4, the most nucleophilic positions were determined by the main HOMO contributions of the phenol moieties. Additional contributions from the alkyl groups (limonene ring and *n*-penthyl moiety) linked to the phenol moieties can be also observed. However, the most important electron donating groups for TLX are ether and methyl moieties.

ANTIOXIDANT CAPACITY BY ELECTRON TRANSFER USING IONIZATION POTENTIAL

The nucleophilicity of CBD and its related derivatives can be expressed by the ionization potential (IP) value, calculated as the necessary energy for the abstraction of an electron of a molecule. The IP value is another

TABLE e12.1 Calculated Electronic Properties for CBD and Related Derivatives (Borges, 2015)

Compound	HOMO (eV)	LUMO (eV)	GAP (eV)	IP (kcal mol^{-1})	ΔEiso (kcal mol^{-1})	BDE_{OH} (kcal mol^{-1})
CBD	−6.02	−0.46	5.56	168.34	3.65	100.83
D1	−6.24	−0.35	5.89	174.35	9.66	—
D2	−6.12	−0.46	5.66	176.11	11.42	
D3	−6.06	−0.45	5.61	178.01	13.31	
D4	−6.14	−0.46	5.68	185.88	21.19	—
D5	−6.71	−0.39	6.32	193.62	28.93	—
D6	−6.42	−0.29	6.12	186.73	22.03	—
TLX	−5.42	−0.56	4.85	164.69	0	92.11

Orbital energies (HOMO, LUMO, and GAP), ionization potential (IP), stabilization energy (ΔEiso), and bond dissociation energy of hydroxyl groups (BDE_{OH}) for cannabidiol (CBD) and related derivatives 1-(6-isopropenyl-3-methyl-cyclohex-2-enyl)-4-pentyl-benzene (D1), 2-(6-isopropenyl-3-methyl-cyclohex-2-enyl)-benzene-1,3-diol (D2), 2-methyl-5-pentyl-benzene-1,3-diol (D3), 5-pentyl-benzene-1,3-diol (D4), pentyl-benzene (D5), limonene (D6), and trolox (TLX).

important electronic parameter used to evaluate the antioxidant ability of molecules. In fact, IP value represents the ease of an electron transfer, and it is the first studied antioxidant mechanism that represents the electron donation capacity (Queiroz et al., 2009).

Thus, a molecule with a low IP value can easily suffer an oxidation process, such as CBD and TLX that have IP values of 168.34 and 164.69 kcal mol^{-1}, respectively. CBD and TLX have an IP value of 168.34 and 164.69 kcal mol^{-1}, respectively. This difference indicates that the number of

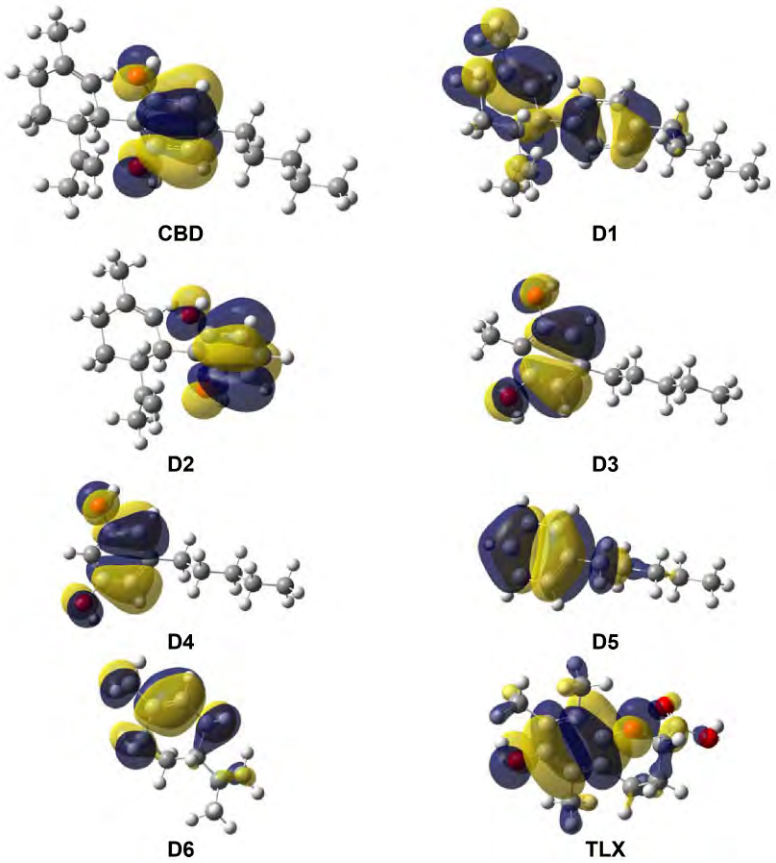


FIGURE e12.4 The HOMO for CBD and its related derivatives. HOMO superficies *(blue and yellow)* show all groups involved in inductive or resonance effects for molecular stabilization, after an electron or hydrogen transfer for CBD and related derivatives 1-(6-isopropenyl-3-methyl-cyclohex-2-enyl)-4-pentyl-benzene (D1), 2-(6-isopropenyl-3-methyl-cyclohex-2-enyl)-benzene-1,3-diol (D2), 2-methyl-5-pentyl-benzene-1,3-diol (D3), 5-pentyl-benzene-1,3-diol (D4), pentyl-benzene (D5), limonene (D6), and trolox (TLX) (Borges, 2015).

alkyl and ether groups at *ortho*- and *para*-position of the phenol ring in the TLX decreases its IP value, and also increases the electron-donating capacity.

Actually, the lowest IP values for the hydroxylated derivatives (D2, D3, and D4), when compared with the highest IP values for the nonphenolic derivatives (D1, D5, and D6), show that the TLX and CBD molecules have a more electron donation capacity due to their electron donating groups.

The stabilization energy (ΔEiso) of CBD and related derivatives is compared with TLX. The determination of ΔEiso is frequently used as a simple method to predict the antioxidant ability to trap free radicals or scavenging effects of molecules. Here, it is related with the experimental method trolox equivalent antioxidant capacity (TEAC). These results show that the lowest ΔEiso values are for CBD and its hydroxylated derivatives (D2, D3, and D4). The electronic capacity for the cation free radical stabilization of these compounds can be considered high, when compared with the nonphenolic derivatives (D1, D5, and D6) (Borges, 2015).

ANTIOXIDANT CAPACITY BY HYDROGEN TRANSFER USING BOND DISSOCIATION ENERGY

In addition, the hydroxyl bond dissociation energies (BDE_{OH}) for the molecules represent the ease of hydrogen donation presented by the CBD and TLX to give semiquinone derivatives. The BDE_{OH} represents the ease of the hydrogen transfer, and it is the second antioxidant mechanism. The BDE_{OH} values for CBD and TLX are 100.83 and 92.11 kcal mol^{-1}, respectively. These properties indicate a great influence of the ether and alkyl moieties on the *ortho*- and *para*- positions, than the limonene ring and the *n*-penthyl moiety of CBD on the BDE_{OH} values. The energy difference between these two molecules is 8.72 kcal mol^{-1} (Borges, 2015).

In fact, a good agreement between HOMO and IP values was observed (Borges, 2015). However, the antioxidant capacity of CBD can be determined by free radical stability generated after the electron or hydrogen abstractions, where the highest energy may be found after a hydrogen abstraction due to the π-delocalized system between the phenol, cyclohexene, and *n*-penthyl moieties (Borges et al., 2013).

These theoretical properties are important in several oxidation processes by electron or hydrogen transfer. Thus, the oxidation in biological system can be inhibited by diverse molecules, in accordance to their antioxidant capacity, which can act on different pathways (Leopoldini et al., 2011a,b; Galano et al., 2010; Galano, 2011; Iuga et al., 2012; Chiodo et al., 2010; Leopoldini et al., 2004, 2006, 2007; Borges et al., 2013). Hence, the elimination of free radical species ($FES^{\bullet}$) using CBD as an antioxidant can occur through two reactions, that is, electron (Eq. e12.6) or hydrogen (Eq. e12.7) transfer, converting them in long-lived and less reactive radicals, mainly at the propagation and termination steps.

$$FES^{\bullet} + \text{CBD-OH} \rightarrow FES^{-} + \text{CBD-OH}^{\bullet+} \quad \text{(e12.6)}$$

$$FES^{\bullet} + \text{CBD-OH} \rightarrow \text{FESH} + \text{CBD-O}^{\bullet} \quad \text{(e12.7)}$$

Therefore, the electron or hydrogen transfer of CBD is all from the phenolic group. In fact, when compared to the TLX molecule, CBD has a good antioxidant activity, and none of their derivatives is more active than itself.

ANTIOXIDANT MECHANISMS IN BIOLOGICAL SYSTEM, AND THEIR APPLICATIONS

A neuroprotective effect was observed for CBD and (−)Δ^9-tetrahydrocannabinol (THC), independent of the cannabinoid receptor. These compounds demonstrated their antioxidant potential by cyclic voltammetry. They have also prevented hydroperoxide-induced oxidative damage much better than other antioxidant compounds. CBD has more protective effect against glutamate neurotoxicity than either ascorbic acid (ASC) or α-tocopherol (TOC), indicating that it is a potent antioxidant compound (Hampson, Grimaldi, Axelrod, & Wink, 1998) (see Fig. e12.5). Among the molecular mechanisms of neuroprotection presented by THC and CBD, one can highlight the attenuation of peroxynitrite on retinal neurotoxicity induced by *N*-methyl-D-aspartate (El-Remessy et al., 2003). Other antioxidant mechanism of CBD is observed by the reduction of neuroinflammation and promotion of hippocampal neurogenesis through PPARγ involvement (Esposito et al., 2011). In addition, the neuroprotective effects might be due to the antioxidant properties of cannabinoids (Lastres-Becker, Molina-Holgado, Ramos, Mechoulam, & Fernández-Ruiz, 2005).

CBD has an excellent safety and tolerability profile in humans, and a great therapeutic potential for the treatment of diabetic complications and cardiovascular

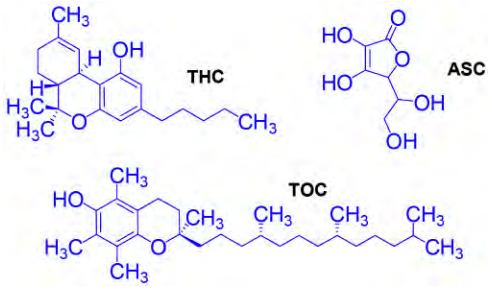


FIGURE e12.5 Structure of THC and vitamins C and E. Molecular structure of THC, ASC, and TOC.

disorders, by attenuating oxidative/nitrative stress, inflammation, cell death, and fibrosis (Rajesh et al., 2010). In fact, the treatment with CBD decreases doxorubicin toxicity by reducing cardiac dysfunction, oxidative/nitrative stress, and cell death. It reduces damage on cardiac mitochondrial function and biogenesis (Fouad et al., 2013; Hao et al., 2015), and has also protected mouse liver from acute alcohol-induced steatosis through multiple mechanisms, including attenuation of alcohol-mediated oxidative stress (Yang et al., 2014). Its efficacy in vivo as a neuroprotective agent, preventing binge ethanol-induced brain injury, can be understood due to its lipophilic properties (Hamelink, Hampson, Wink, Eiden, & Eskay, 2005). CBD also presented a stronger antioxidative power than THC in an in vitro study using the 1,1-diphenyl-2-picryhydrazyl (DPPH) radical (Hayakawa et al., 2007). In fact, the scavenging activity using DPPH assay showed that cannabinoid derivatives exhibited in vitro antioxidative action ranging from 669 ± 11.1 (THC) to 356 ± 29.5 (CBD) μg trolox (a vitamin E derivative)/mg (Moldzio et al., 2012).

CONCLUSIONS

The antioxidant mechanism of CBD was reviewed by a Quantum Chemical approach. Its antioxidant potential can be related to electron and hydrogen abstraction. The limonene ring and the *n*-penthyl moiety are responsible for the stabilization of the cation free radical, or its semiquinone forms. Several resonance structures are attributed for these alkyl groups. These groups contribute to increase the HOMO value, and to decrease the IP and the BDE_{OH} values. The calculated properties showed that the CBD molecule has a good antioxidant profile. None of the CBD derivatives was more antioxidant than the CBD. The CBD antioxidant mechanisms in biological system, and their applications as neuroprotective, cardioprotective, and cytoprotective effects were related with other antioxidant molecules.

MINI-DICTIONARY

Antioxidant Antioxidants are natural or synthetic derivatives that prevent free radical reactions, inhibiting the formation of these radicals, scavenging them, or by promoting their decomposition and then reducing tissue damages.
Antioxidant and cancer Free radicals are highly reactive, and have the potential to cause damage to cells, mainly of DNA and proteins, including damage that may lead to cancer. In general, many antioxidants are used as chemopreventive effects on carcinogenesis.
Antioxidant and inflammation In general, antioxidant molecules have potent antiinflammatory properties, and exert chemopreventive effects on carcinogenesis.
Antioxidant and neuroprotection Antioxidant decreases the lipid peroxidation of membrane, and oxidative damage of proteins; these biological properties are associated with a variety of chronic pathological problems, such as the neurodegenerative diseases.
Antioxidant capacity A powerful antioxidant molecule delays or prevents oxidation of a substrate significantly, by chemical or physical quenching. Its antioxidant capacity is only related to other similar analogs or parents.
Antioxidant mechanisms The antioxidant mechanisms are related to balance concentrations between free radicals and antioxidant defense systems.
Antioxidant theoretical parameters The main physicochemical parameters related to antioxidant properties are the ionization potential and the bond-dissociation energy that involves electron or hydrogen atom transfer.
Density functional theory (DFT) A quantum mechanical and computational method used in physics, chemistry, and materials science. It investigates the electronic and conformational structure of atoms and molecules.
Free radical species Reactive oxygen, nitrogen, or carbon species have high chemical reactivity, when compared with sugar, proteins, nucleic acids, and lipids. They are responsible for cellular aging, mutagenesis, carcinogenesis, inflammation, coronary heart and neurodegenerative diseases.
Molecular modeling Molecular modeling is a method developed by the association between theoretical methods and computational techniques. It can be used as an artificial model of molecular behavior. It is used in computational chemistry, drug design, computational biology, and materials science for studying molecular structures, or theoretical predictions.
Structure–activity relationship Orbital energies and electron or hydrogen abstraction energies are the main quantum chemical descriptors that can be considered in structure–antioxidant activity relationship. They can also be related with chemical stability and nucleophilicity. These values are correlated with the experimental antioxidant activity obtained by using physical, chemical, or biological methods.

Acknowledgment

The authors are grateful for the financial support given by the Brazilian agency CNPq.

References

Almeida, V., Levin, R., Peres, F. F., Niigaki, S. T., Calzavara, M. B., Zuardi, A. W., Hallak, J. E., Crippa, J. A., & Abílio, V. C. (2013). Cannabidiol exhibits anxiolytic but not antipsychotic property evaluated in the social interaction test. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *41*, 30–35.

Appendino, G., Gibbons, S., Giana, A., Pagani, A., Grassi, G., Stavri, M., Smith, E., & Rahman, M. M. (2008). Antibacterial cannabinoids from *Cannabis sativa*: a structure–activity study. *Journal of Natural Products*, *71*(8), 1427–1430.

Aviello, G., Romano, B., Borrelli, F., Capasso, R., Gallo, L., Piscitelli, F., Di Marzo, V., & Izzo, A. A. (2012). Chemopreventive effect of the non-psychotropic phytocannabinoid-cannabidiol on experimental colon cancer. *Journal of Molecular Medicine*, *90*(8), 925–934.

Borges, R. S. (2015). *Theoretical study of cannabidiol as antioxidant. Post-Doctoral Fellowship Report*. Belém-PA, Brazil: UFPA.

Borges, R. S., Batista, J., Jr., Viana, R. B., Baetas, A. C., Orestes, E., Andrade, M. A., Honório, K. M., & da Silva, A. B. (2013). Understanding the molecular aspects of tetrahydrocannabinol and cannabidiol as antioxidants. *Molecules*, *18*(10), 12663–12674.

Chiodo, S. G., Leopoldini, M., Russo, N., & Toscano, M. (2010). The inactivation of lipid peroxide radical by quercetin. A theoretical insight. *Physical Chemistry Chemical Physics*, *12*, 7662–7670.

Costa, B., Trovato, A. E., Comelli, F., Giagnoni, G., & Colleoni, M. (2007). The non-psychoactive cannabis constituent cannabidiol is an orally effective therapeutic agent in rat chronic inflammatory and neuropathic pain. *European Journal of Pharmacology*, *556*(1–3), 75–83.

Di Marzo, V., Bifulco, M., & De Petrocellis, L. (2004). The endocannabinoid system and its therapeutic exploitation. *Nature Reviews Drug Discovery*, *3*(9), 771–784.

El-Remessy, A. B., Khalil, I. E., Matragoon, S., Abou-Mohamed, G., Tsai, N. J., Roon, P., Caldwell, R. B., Caldwell, R. W., Green, K., & Liou, G. I. (2003). Neuroprotective effect of (−)-Delta9-tetrahydrocannabinol and cannabidiol in *N*-methyl-D-aspartate-induced retinal neurotoxicity: involvement of peroxynitrite. *The American Journal of Pathology*, *163*(5), 1997–2008.

Esposito, G., Scuderi, C., Valenza, M., Togna, G. I., Latina, V., De Filippis, D., Cipriano, M., Carratù, M. R., Iuvone, T., & Steardo, L. (2011). Cannabidiol reduces Aβ-induced neuroinflammation and promotes hippocampal neurogenesis through PPARγ involvement. *PLoS One*, *6*(12), e28668.

Fouad, A. A., Albuali, W. H., Al-Mulhim, A. S., & Jresat, I. (2013). Cardioprotective effect of cannabidiol in rats exposed to doxorubicin toxicity. *Environmental Toxicology and Pharmacology*, *36*(2), 347–357.

Fox, A., & Bevan, S. (2005). Therapeutic potential of cannabinoid receptor agonists as analgesic agents. *Expert Opinion on Investigational Drugs*, *14*(6), 695–703.

Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Zakrzewski, V. G., Montgomery, J. A., Stratmann, R. E., Burant, J. C., et al. (2004). *Gaussian 09, Revision C.02*. Wallingford, CT: Gaussian, Inc..

Galano, A. (2011). On the direct scavenging activity of melatonin towards hydroxyl and a series of peroxyl radicals. *Physical Chemistry Chemical Physics*, *13*, 7147–7157.

Galano, A., Macías-Ruvalcaba, N. A., Campos, O. N. M., & Pedraza-Chaverri, J. (2010). Mechanism of the OH radical scavenging activity of nordihydroguaiaretic acid: a combined theoretical and experimental study. *The Journal of Physical Chemistry B*, *114*(19), 6625–6635.

García-Arencibia, M., González, S., de Lago, E., Ramos, J. A., Mechoulam, R., & Fernández-Ruiz, J. (2007). Evaluation of the neuroprotective effect of cannabinoids in a rat model of Parkinson's disease: importance of antioxidant and cannabinoid receptor-independent properties. *Brain Research*, *1134*(1), 162–170.

Gonca, E., & Darıcı, F. (2014). The effect of cannabidiol on ischemia/reperfusion-induced ventricular arrhythmias: the role of adenosine a1 receptors. *Journal of Cardiovascular Pharmacology and Therapeutics*, *20*(1), 76–83.

Guengerich, F. P. (2001). Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity. *Chemical Research in Toxicology*, *14*(6), 611–650.

Hamelink, C., Hampson, A., Wink, D. A., Eiden, L. E., & Eskay, R. L. (2005). Comparison of cannabidiol, antioxidants, and diuretics in reversing binge ethanol-induced neurotoxicity. *Journal of Pharmacology and Experimental Therapeutics*, *314*(2), 780–788.

Hampson, A. J., Grimaldi, M., Axelrod, J., & Wink, D. (1998). Cannabidiol and (−)Delta^9-tetrahydrocannabinol are neuroprotective antioxidants. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(14), 8268–8273.

Hao, E., Mukhopadhyay, P., Cao, Z., Erdélyi, K., Holovac, E., Liaudet, L., Lee, W. S., Haskó, G., Mechoulam, R., & Pacher, P. (2015). Cannabidiol protects against doxorubicin-induced cardiomyopathy by modulating mitochondrial function and biogenesis. *Molecular Medicine*, *21*, 38–45.

Hayakawa, K., Mishima, K., Nozako, M., Ogata, A., Hazekawa, M., Liu, A. X., Fujioka, M., Abe, K., Hasebe, N., Egashira, N., Iwasaki, K., & Fujiwara, M. (2007). Repeated treatment with cannabidiol but not Delta9-tetrahydrocannabinol has a neuroprotective effect without the development of tolerance. *Neuropharmacology*, *52*(4), 1079–1087.

Hohmann, A. G., & Suplita, R. L. (2006). Endocannabinoid mechanisms of pain modulation. *AAPS Journal*, *8*(4), E693–E708.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, *53*(6), 1841–1856.

Iuga, C., Alvarez-Idaboy, J. R., & Russo, N. (2012). Antioxidant activity of trans-resveratrol toward hydroxyl and hydroperoxyl radicals: a quantum chemical and computational kinetics study. *The Journal of Organic Chemistry*, *77*(8), 3868–3877.

Jiang, R., Yamaori, S., Takeda, S., Yamamoto, I., & Watanabe, K. (2011). Identification of cytochrome P450 enzymes responsible for metabolism of cannabidiol by human liver microsomes. *Life Sciences*, *89*(5–6), 165–170.

Kohn, W., Becke, A. D., & Parr, R. G. (1996). Density functional theory of electronic structure. *The Journal of Physical Chemistry*, *100*(31), 12974–12980.

Lastres-Becker, I., Molina-Holgado, F., Ramos, J. A., Mechoulam, R., & Fernández-Ruiz, J. (2005). Cannabinoids provide neuroprotection against 6-hydroxydopamine toxicity in vivo and in vitro: relevance to Parkinson's disease. *Neurobiology of Disease*, *19*(1–2), 96–107.

Leopoldini, M., Chiodo, S. G., Russo, N., & Toscano, M. (2011a). Detailed investigation of the OH radical quenching by natural antioxidant caffeic acid studied by quantum mechanical models. *Journal of Chemical Theory and Computational*, *7*(12), 4218–4233.

Leopoldini, M., Marino, T., Russo, N., & Toscano, M. (2004). Antioxidant properties of phenolic compounds: h-atom versus electron transfer mechanism. *The Journal of Physical Chemistry A*, *108*(22), 4916–4922.

Leopoldini, M., Russo, N., & Toscano, M. (2006). Gas and liquid phase acidity of natural antioxidants. *Journal of Agricultural and Food Chemistry*, *54*(8), 3078–3085.

Leopoldini, M., Russo, N., & Toscano, M. (2007). A comparative study of the antioxidant power of flavonoid catechin and its planar analogue. *Journal of Agricultural and Food Chemistry*, *55*(19), 7944–7949.

Leopoldini, M., Russo, N., & Toscano, M. (2011b). The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chemistry*, *125*(2), 288–306.

Mannila, J., Järvinen, T., Järvinen, K., & Jarho, P. (2007). Precipitation complexation method produces cannabidiol/beta-cyclodextrin inclusion complex suitable for sublingual administration of cannabidiol. *Journal of Pharmaceutical Sciences*, *96*(2), 312–319.

Meng, I. D., Manning, B. H., Martin, W. J., & Fields, H. L. (1998). An analgesia circuit activated by cannabinoids. *Nature*, *395*, 381–383.

Moldzio, R., Pacher, T., Krewenka, C., Kranner, B., Novak, J., Duvigneau, J. C., & Rausch, W. D. (2012). Effects of cannabinoids Δ(9)-tetrahydrocannabinol, Δ(9)-tetrahydrocannabinolic acid and cannabidiol in MPP+ affected murine mesencephalic cultures. *Phytomedicine*, *19*(8–9), 819–824.

Murase, R., Kawamura, R., Singer, E., Pakdel, A., Sarma, P., Judkins, J., Elwakeel, E., Dayal, S., Martinez-Martinez, E., Amere, M., Gujjar, R., Mahadevan, A., Desprez, P. Y., & McAllister, S. D. (2014). Targeting multiple cannabinoid antitumor pathways with a resorcinol derivative leads to inhibition of advanced stages of breast cancer. *British Journal of Pharmacology*, *171*(19), 4464–4477.

Naftali, T., Mechulam, R., Lev, L. B., & Konikoff, F. M. (2014). Cannabis for inflammatory bowel disease. *Digestive Diseases*, *32*(4), 468–474.

Pan, H., Mukhopadhyay, P., Rajesh, M., Patel, V., Mukhopadhyay, B., Gao, B., Haskó, G., & Pacher, P. (2009). Cannabidiol attenuates cisplatin-induced nephrotoxicity by decreasing oxidative/nitrosative stress, inflammation, and cell death. *The Journal of Pharmacology and Experimental Therapeutics*, *328*(3), 708–714.

Parr, R. G., & Pearson, R. G. (1983). Absolute hardness: companion parameter to absolute electronegativity. *Journal of American Chemistry Society*, *105*(26), 7512–7516.

Parr, R. G., Szentpaly, L. V., & Liu, S. (1999). Electrophilicity index. *Journal of American Chemistry Society*, *121*(9), 1922–1924.

Queiroz, A. N., Gomes, B. A. Q., Moraes, W. M., Jr., & Borges, R. S. (2009). A theoretical antioxidant pharmacophore for resveratrol. *European Journal of Medicinal Chemistry*, *44*(4), 1644–1649.

Rajesh, M., Mukhopadhyay, P., Bátkai, S., Patel, V., Saito, K., Matsumoto, S., Kashiwaya, Y., Horváth, B., Mukhopadhyay, B., Becker, L., Haskó, G., Liaudet, L., Wink, D. A., Veves, A., Mechoulam, R., & Pacher, P. (2010). Cannabidiol attenuates cardiac dysfunction, oxidative stress, fibrosis, and inflammatory and cell death signaling pathways in diabetic cardiomyopathy. *Journal of the American College of Cardiology*, *56*(25), 2115–2125.

Steffens, S., Veillard, N. R., Arnaud, C., Pelli, G., Burger, F., Staub, C., Karsak, M., Zimmer, A., Frossard, J. L., & Mach, F. (2005). Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. *Nature*, *434*, 782–786.

Stout, S. M., & Cimino, N. M. (2014). Exogenous cannabinoids as substrates, inhibitors, and inducers of human drug metabolizing enzymes: a systematic review. *Drug Metabolism Reviews*, *46*(1), 86–95.

Usami, N., Yamamoto, I., & Watanabe, K. (2008). Generation of reactive oxygen species during mouse hepatic microsomal metabolism of cannabidiol and cannabidiol hydroxy-quinone. *Life Sciences*, *83*(21–22), 717–724.

Walker, J. M., & Huang, S. M. (2002). Endocannabinoids in pain modulation. *Prostaglandins Leukotrienes and Essential Fatty Acids*, *66*(2–3), 235–242.

Yamaori, S., Ebisawa, J., Okushima, Y., Yamamoto, I., & Watanabe, K. (2011). Potent inhibition of human cytochrome P450 3A isoforms by cannabidiol: role of phenolic hydroxyl groups in the resorcinol moiety. *Life Sciences*, *88*(15–16), 730–736.

Yang, L., Rozenfeld, R., Wu, D., Devi, L. A., Zhang, Z., & Cederbaum, A. (2014). Cannabidiol protects liver from binge alcohol-induced steatosis by mechanisms including inhibition of oxidative stress and increase in autophagy. *Free Radical Biology and Medicine*, *68*, 260–267.

CHAPTER

e13

The Anxiolytic Effects of Cannabidiol (CBD)

*A.W. Zuardi**, *J.A. de Souza Crippa**, *J.E.C. Hallak**, *A.C. Campos***, *F.S. Guimarães****

Department of Neurosciences and Behavior, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

****Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil**

SUMMARY POINTS

- The present chapter focused on evidences related to the antianxiety effects of cannabidiol (CBD).
- CBD anxiolytic effects are dose-dependent, in a bell shaped dose–response curves manner.
- CBD antianxiety effects were demonstrated and replicated in several animal models of anxiety.
- CBD also promotes antianxiety effects in humans.
- CBD may affect the function of brain structures related to the control of anxiety-like states in laboratory animals and humans.
- The specific pharmacological mechanism that would explain CBD antianxiety effects remains to be determined, but may involve the modulation of CB1 and 5HT1A receptors functions.

KEY FACTS OF CANNABIDIOL ANTIANXIETY PROPERTIES

- Cannabidiol was isolated from *Cannabis sativa* in 1940, but its chemical structure was elucidated in 1963.
- Cannabidiol can be obtained as extract from the plant, by standard chemical techniques, or as a pure synthetic compound.
- In the early 1980s, the first evidence of CBD's anxiolytic effect in laboratory animals emerged, and independent groups replicated this effect several times.
- Elevated plus maze is based on the innate avoidance of rodents to unprotected and elevated places.
- Social anxiety disorder is characterized by the intense anxiety and fear of humiliation or embarrassment in social situations.

LIST OF ABBREVIATIONS

AM251	*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide
BNST	Bed nucleus stria terminalis
CB_1	Cannabinoid receptor 1
CB_2	Cannabinoid receptor 2
CBD	Cannabidiol
CFC	Contextual fear conditioning
DPAG	Dorsal periaqueductal gray
eCB	Endocannabinoid
EPM	Elevated plus maze
ETM	Elevated T-maze
GPR55	Orphan cannabinoid receptor
GTPγS	Guanosine-50-O-(3-thiotriphosphate)
$5\text{-}HT_{1A}$	Serotonergic receptor 1A

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00097-1
Copyright © 2017 Elsevier Inc. All rights reserved.

OCD	Obsessive-compulsive disorder
PL	Prelimbic frontal cortex
PTSD	Posttraumatic stress disorder
rCBF	Regional blood flow
SAD	Social anxiety disorder
SPECT	Single-photon emission computed tomography
SPST	Simulation of the public speaking test
THC	Δ^9-Tetrahydrocannabinol
TRP	Transient receptor potential
$TRPV_1$	Transient receptor potential vanilloid 1 (vanilloid receptor 1)
$TRPV_2$	Transient receptor potential vanilloid 2 (vanilloid receptor 2)
VAMS	Visual analog mood scales

INTRODUCTION

The literature on the relationship between *Cannabis* and anxiety is contradictory. While users report decreased anxiety as motivation for their use, the most common adverse effect of acute cannabis use is the reaction of intense anxiety or panic (Crippa et al., 2009). The dose-dependent biphasic effect of THC on anxiety (Viveros, Marco, & File, 2005) could partially justify this observation, but another possibility is the effect of other cannabinoids present in the plant. In the early 1970s, a Brazilian group gave the first evidences that other cannabinoids interfere with the effects of Δ^9-tetrahydrocannabinol (THC) in animals (Karniol & Carlini, 1972), particularly cannabidiol (CBD), a compound until them considered inactive (Karniol & Carlini, 1973).

These results led to studies of interaction between THC and CBD, in humans. In the first of these studies, healthy volunteers were submitted to cognitive tests, physiologic measures, and interview. Strong feelings of anxiety were reported after the ingestion of 30 mg of THC, while the subjects that received this THC dose with 60 mg of CBD showed less anxiety (Karniol, Shirakawa, Kasinski, Pfeferman, & Carlini, 1974). Confirming this result, a study with appropriate rating scales detected a statistically significant reduction in the elevation of anxiety scores induced by THC (0.5 mg/kg), when associated with CBD (1 mg/kg) (Zuardi, Shirakawa, Finkelfarb, & Karniol, 1982). A possible anxiolytic effect of CBD, suggested by these interaction studies, started a line of research in animals and humans.

CBD EFFECTS IN ANIMAL MODELS OF ANXIETY

The antianxiety properties of CBD have been extensively studied in the last 3 decades. However, the initial studies in animal models found controversial results. In the early 1980s, Silveira Filho and Tufik (1981), using a model based on conflict responses, failed to demonstrate any anxiolytic-like effect of CBD, at a dose of 100 mg/kg i.p. On the other hand, lower doses of CBD (10 mg/kg) were able to attenuate conditioned emotional responses in rats (Zuardi & Karniol, 1983).

Guimarães, Chiaretti, Graeff, and Zuardi (1990), subsequently explained these apparent contradictory results. Using the elevated plus maze (EPM), an ethologically based animal model of anxiety, and testing a large range of CBD doses (2.5–20 mg/kg), they showed that CBD does produce antianxiety effects in laboratory animals in a dose-depend manner, but with an inverted U-shaped dose–response curve (Guimarães et al., 1990). These findings were later replicated by Guimarães and coworkers, as well as other groups, not only in ethologically based tests, but also in conflict and aversive conditioning models of anxiety (Table e13.1).

More recently, the investigation of the antianxiety properties of CBD were extended to animal models related to specific types of anxiety disorders. For instance, some studies suggest that CBD may be useful in trauma-related anxiety disorders (Bitencourt et al., 2008; Campos et al., 2013a; Stern et al., 2012). CBD repeated treatment attenuated the long-lasting behavioral consequences evoked by predator threats, in a proposed animal model of posttraumatic stress disorder (PTSD; Campos et al., 2013a). Moreover, CBD accelerates extinction, and impairs fear memory reconsolidation, two processes that have been associated with PTSD. Recently, a putative antipanic action of CBD was also described. CBD acute and chronic treatment reduce the defensive responses in two different models of panic disorder, the elevated T-maze (ETM) and the electrical stimulation of the dorsal portions of the periaqueductal gray matter (Campos et al., 2013b; Soares et al., 2010). Chronic treatment with a low dose of CBD (5 mg/kg) decreases the escape latency in the ETM, similar to the antidepressant Fluoxetine (Campos et al., 2013b). In addition, CBD reduces marble-burying behaviors, suggesting that this compound could be effective in obsessive-compulsive disorder (OCD; Casarotto et al., 2010; Nardo et al., 2014).

Considering the close relationship between stressful experiences and the precipitation of anxiety disorders symptoms, the effects of CBD during stress responses have also been studied. CBD prevents the autonomic and behavioral consequences of inescapable stress (Resstel et al., 2006). Moreover, Campos et al. (2013a) showed that chronic administration of CBD (30 mg/kg/daily for 14 days) prevented the anxiogenic effect of chronic unpredictable stress in mice tested in the elevated plus maze, and novelty suppressed feeding tests.

TABLE e13.1 Animal and Human Studies of Cannabidiol on Anxiety

Study	Model	Route	Dose	Anxiolytic effect
Animals				
Silveira Filho and Tufik (1981)	Geller–Seifter conflict test	Intraperitoneal	100 mg/kg	No
Zuardi and Karniol (1983)	Conditioned emotional response paradigm	Intraperitoneal	10 mg/kg	+
Onaivi, Green, and Martin (1990)	Elevated plus maze test	Intraperitoneal	0.01, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 50.0, and 100.0	+
Guimarães et al. (1990)	Elevated plus maze test	Intraperitoneal	2.5, 5.0, 10.0, and 20.0 mg/kg	+
Moreira, Aguiar, and Guimarães (2006)	Vogel's conflict test	Intraperitoneal	2.5, 5.0, and 10.0 mg/kg	+
Resstel, Joca, Moreira, Correa, and Guimarães (2006)	Contextual fear conditioning	Intraperitoneal	10 mg/kg	+
	Elevated plus maze	Intraperitoneal	?	+
Campos and Guimarães (2008)	Elevated plus maze test and Vogel's conflict test	Intradorsal periaqueductal gray	15–60 nmol/0.2 µL	+
Bitencourt, Pamplona, and Takahashi (2008)	Contextual fear conditioning	i.c.v.	2.0 µg/µL	+
Campos and Guimarães (2009)	Elevated plus maze test	Intradorsal periaqueductal gray	30 or 60 nmol	+
Resstel et al. (2009)	Restraint stress	Intraperitoneal	1, 10, and 20 mg/kg	+
Soares et al. (2010)	Elevated T maze	Intradorsal periaqueductal gray	15, 30, or 60 nmol	+
Lemos, Resstel, and Guimarães (2010)	Contextual fear conditioning	Intraperitoneal and direct microinjection into the PL and IL prefrontal cortex	10 mg/kg (i.p.) and 15–60 nmol	+
Casarotto, Gomes, Resstel, and Guimarães (2010)	Marble-burying test	Intraperitoneal	15, 30, and 60 mg/kg	+
Gomes et al. (2011)	Elevated plus maze/ Vogel's conflict test	Intrabed nucleus of the stria terminalis	15, 30, and 60 nmol	+
Gomes, Resstel, and Guimarães (2011)	Contextual fear conditioning	Intrabed nucleus of the stria terminalis	15, 30, and 60 nmol	+
Deiana et al. (2012)	Marble-burying test	Intraperitoneal and oral	120 mg/kg	+
Uribe-Mariño et al. (2012)	Prey–predator paradigm	Intraperitoneal	0.3, 3.0, and 30 mg/kg	+
Hsiao, Yi, Li, and Chang (2012)	Open field/elevated plus maze	Intracentral amygdala	0.5–1 µg/µL	+
Stern, Gazarini, Takahashi, Guimarães, and Bertoglio (2012)	Contextual fear conditioning	Intraperitoneal	10 mg/kg	+
Campos, Moreira, Gomes, Del Bel, and Guimarães (2012)	Prey–pradator paradigm	Intraperitoneal	5 mg/kg	+
Do Monte, Souza, Bitencourt, Kroon, and Takahashi (2013)	Contextual fear conditioning	Intra-PL prefrontal cortex	0.1–0.4 µg/0.2 µL	+
Campos et al. (2013a)	Elevated T maze	Intraperitoneal	5–20 mg/kg	+
Campos et al. (2013b)	Chronic unpredictable stress/novelty suppressed feeding/ elevated plus maze	Intraperitoneal	30 mg/kg	+

(*Continued*)

TABLE e13.1 Animal and Human Studies of Cannabidiol on Anxiety (*cont.*)

Study	Model	Route	Dose	Anxiolytic effect
Nardo, Casarotto, Gomes, and Guimarães (2014)	Marble-burying test	Intraperitoneal	15 mg/kg	+
Fogaça, Reis, Campos, and Guimarães (2014)	Elevated plus maze/ contextual fear conditioning/restraint stress + elevated plus maze	Intra-PL prefrontal cortex	15–30 nmol/0.2 μL	+/− Depending on stressful experience
Humans				
Zuardi et al. (1982)	Decreased STAI scores elevation induced by THC (healthy volunteers)	Oral	1 mg/kg	+
Zuardi, Cosme, Graeff, and Guimarães (1993)	Decreased VAS factor anxiety scores after public speaking (healthy volunteers)	Oral	300 mg	+
Crippa et al. (2004)	Decreased VAS factor anxiety scores before SPECT procedure (healthy volunteers)	Oral	400 mg	+
Fusar-Poli et al. (2009)	Decreased skin conductance fluctuation in task with fearful faces during an fMRI procedure (healthy volunteers)	Oral	600 mg	+
Crippa et al. (2011)	Decreased VAS factor anxiety scores before SPECT procedure (social phobia patients)	Oral	400 mg	+
Bergamaschi et al. (2011)	Decreased VAS factor anxiety scores after public speaking (social phobia patients)	Oral	600 mg	+

+, Anxiolytic effect; −, anxiogenic effect; no, no effects on anxiety.
Adapted from Schier et al. (2012).

ANXIOLYTIC EFFECTS OF CBD IN HUMANS

The first study evaluating the anxiolytic effect of CBD in humans was conducted on healthy volunteers submitted to a simulation of the public speaking test (SPST) (Zuardi et al., 1993). In a double-blind protocol, CBD (300 mg), ipsapirone (5 mg), diazepam (10 mg), or placebo were administered per os, 1 h and 30 min before the SPST. CBD, ipsapirone, and diazepam reduced significantly the anxiety induced by SPST.

Another double-blind, crossover trial study with healthy volunteers investigated CBD effects on regional blood flow (rCBF), using single-photon emission computed tomography (SPECT). It was observed that CBD (400 mg per os) attenuated the anxiety induced by the procedure related to the exam (insertion of the venous cannula, tracer injection, and scanning procedure) (Crippa et al., 2004). Using a similar protocol to study the effect of CBD on rCBF of patients with social anxiety disorder (SAD), the same reductions of anxiety induced by the protocol was observed (Crippa et al., 2011).

The effects of one dose of CBD was studied in patients with SAD submitted on SPST, since the fear of speaking in public is one of the most prevalent symptoms of this disorder. As expected, the SAD group presented significantly higher anxiety level than health volunteers, during the SPST. Previous administration of CBD (600 mg, per os) reduced significantly the anxiety level of SAD group, during the public speaking (Bergamaschi et al., 2011).

These studies suggest that the acute administration of CBD in humans has an anxiolytic action in normal volunteers and patients with SAD. Studies of chronic administration of CBD in anxiety disorder are needed.

MECHANISMS OF CBD EFFECTS

Human Studies

In a study in which we investigated the central effects of CBD on rCBF in healthy volunteers, using SPECT, the anxiety induced by the experiment was reduced in the subjects who received CBD (Crippa et al., 2004). The volunteers received either CBD (400 mg) or placebo, in a crossed double-blind design, in two experimental sessions, one week apart. CBD significantly decreased subjective anxiety, as measured by rating scales (VAMS), while brain activity was changed in limbic and paralimbic areas, specifically increased in the left parahippocampal gyrus, and decreased in the left amygdala-hippocampus complex, including the fusiform gyrus (Fig. e13.1A). Interestingly, the pattern of SPECT results of this study was consistent with an anxiolytic action, as observed in other functional imaging studies using classical anxiolytic drugs such as benzodiazepines. The SPECT neuroimaging technique was also used later to investigate the neural anxiolytic correlates of CBD in a sample of drug-naïve patients with SAD (Crippa et al., 2011). A single dose of CBD 400 mg was able to reduce subjective anxiety measures, and SPECT showed changes in the same regions previously identified in healthy volunteers, but mainly in the opposite direction of activation (Fig. e13.1B). fMRI (functional magnetic resonance imaging), a neuroimaging method that allows the acquisition of larger series of images with a much better temporal and spatial resolution, was used to investigate the neural correlates of the anxiolytic effects of CBD in 15 healthy volunteers (Fusar-Poli et al., 2009). This study showed that 600 mg of CBD attenuated fMRI responses during the recognition of fearful facial expressions, in the amygdala and the anterior cingulate, which are brain areas related to anxiety and emotion. Moreover, the authors found that this attenuation pattern correlated with skin conductance responses to the stimuli. The same group further reported that the antianxiety action of CBD is mediated by altering the subcortical prefrontal connectivity via amygdala and anterior cingulated (Fusar-Poli et al., 2010).

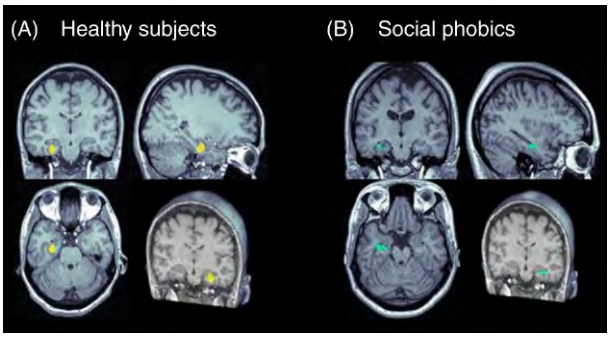


FIGURE e13.1 (A) The brain focus (yellow) of significantly increased rCBF in healthy subjects (n = 10) during CBD (400 mg) versus placebo on sagittal, coronal, axial sections (left side), and coronal sections (right side) located in the left hippocampal area; (B) the brain focus (blue) of significantly decreased rCBF in social anxiety disorder subjects (n = 10) during CBD (400 mg) versus placebo on sagittal, coronal, axial sections (left side) and coronal sections (right side) located in the left hippocampal area. *Part A, adapted from Crippa et al. (2004); part B, adapted from Crippa et al. (2011).*

Animal Studies (Intracerebral Injection)

Although the anxiolytic effects of systemically administered CBD have been extensively reported since the beginning of the 1980s, the neurobiological substrates involved in behavioral actions of this phytocannabinoid only began to be investigated in the last decade (Table e13.1). In 2008, Campos and Guimarães showed for the first time that direct brain administration of CBD into the dorsal portions of the periaqueductal gray (DPAG) promotes anxiolytic-like effects in the EPM, and in Vogel's conflict test (Campos & Guimarães, 2008, 2009). These findings were later replicated by Soares et al. (2010), using two other different models of anxiety-like behaviors, the ETM and the electrical stimulation of the DPAG.

Investigating the effects of systemic administration of CBD (10 mg/kg) in animals exposed to a fear-conditioned environment, Lemos et al. (2010) showed that this drug decreases cFos expression, a marker of neuronal activation, in brain areas related to conditioned fear responses, the ventral medial prefrontal cortex and bed nucleus stria terminalis (BNST). Following these results, we showed that CBD direct injection into BNST produces antianxiety effect in rats tested in EPM, in Vogel's conflict, and contextual fear conditioning (CFC) models (Gomes et al., 2011, 2012). A more complex panorama, however, was found in the ventral medial prefrontal cortex. Whereas direct injections of CBD (30 nmol) into the prelimbic frontal cortex (PL) decreased freezing response in the contextual fear conditioning model (an antianxiety effect), it facilitated these responses when injected into the infralimbic cortex (IL) (Lemos et al., 2010). Recently, Fogaça et al. (2014) confirmed the anxiolytic-like effect of intra-PL administration of CBD. However, the same treatment was anxiogenic in animals tested in the EPM. To explain these contradictory results, the authors suggested that in the PL region of the prefrontal cortex the antianxiety effects of CBD depend on the level of previous stressful experiences. Corroborating this possibility, CBD produces anxiolytic effects in the EPM when

injected into the PL of animals that have been previously (24 h) exposed to a stressful event (2 h restraint) (Fogaça et al., 2014). Intracerebral ventricular administration of CBD also facilitated extinction in a contextual aversive conditioning model (Bitencourt et al., 2008). Moreover, repeated microinjections of CBD into the IL subregion of the prefrontal cortex also facilitated fear extinction (Do Monte et al., 2013). In the amygdala, a brain structure similarly related to the control of anxiety-like behaviors, inconsistent effects have been found in the basolateral as well as in the medial amygdala nuclei (Campos et al., and Lisboa and Guimarães, unpublished results). There is a report, however, of anxiolytic-like effects of CBD after injection into the central nucleus in rats tested in the EPM and open field (Hsiao et al., 2012).

PHARMACOLOGICAL MECHANISMS OF THE ANXIOLYTIC EFFECTS OF CBD

While several pieces of the evidence reviewed earlier indicate that CBD could be a promising new anti-anxiety treatment, its specific mechanism of action remains to be fully determined. CBD seems to exert its anxiolytic effects by interacting with several systems in a wide range of drug concentrations (Campos, Ferreira, & Guimarães, 2012a; Izzo, Borrelli, Capasso, Di Marzo, & Mechoulam, 2009). Moreover, the common bell-shaped dose–response curves produced by this compound further complicate the elucidation of its mechanisms (Campos and Guimarães, 2008, 2013a; Guimarães et al., 1990; Izzo et al., 2009).

CBD is proposed to directly interact with several receptors, including CB_1, CB_2, GPR55, TRPV1, and 5-HT1A (Bisogno et al., 2001; Campos et al., 2008; Russo, Burnett, Hall, & Parker, 2005; Ryberg et al., 2007; Thomas et al., 2007). It also interferes with the uptake and degradation system of endocannabinoids signaling (Bisogno et al., 2001; Campos et al., 2013b). However, the relevance of each of these mechanisms for the anti-anxiety effects of CBD still remains unclear.

Of particular importance, several studies have now established that the complex interaction between CBD and 5-HT1A receptors may explain some of its anxiolytic actions. Russo et al. (2005) published the first report that proposed this interaction. Using Chinese hamster ovary cultured cells overexpressing 5-HT1A, they observed that CBD, at micromolar range, displaces OH-DPAT, a selective 5-HT1A receptor agonist (Russo et al., 2005). In the same year, Mishima et al. (2005) suggested that the pharmacological mechanism of CBD-induced neuroprotection during cerebral infarction in rats involved 5-HT1A receptors activation. Regarding CBD's antianxiety effects, Campos and Guimarães (2008) were the first to demonstrate that the anxiolytic effects of CBD injected into the DPAG depends on facilitation of 5-HT1A receptor signaling, rather than CB_1 receptors activation. The ability of systemically injected CBD in attenuating acute autonomic responses evoked by restraint stress is also mediated by 5-HT1A receptors. Moreover, the 5-HT1A receptor antagonist WAY-100635 also prevented the panicolytic-like effects of intra-DPAG injections of CBD, in rats tested in the DPAG-electrically induced escape responses (Campos & Guimarães, 2008; Soares et al., 2010). Activation of 5-HT1A receptors by CBD in other brain structures related to the control of anxiety-like behaviors, such as the BNST and PL, also decreases anxiety-related behaviors (Gomes et al., 2011, 2012; Fogaça et al., 2014). Although the involvement of 5-HT1A receptors in the clinical effects of CBD has not been directly tested, the study by Zuardi et al. (1993) showed that CBD induces anxiolytic effects in a simulated public speaking model that closely resemble those induced by the 5-HT1A receptor partial agonist ipsapirone.

Despite these pieces of evidence associating acute CBD anxiolytic effects with facilitation of 5-HT1A-mediated neurotransmission, the molecular mechanism of this interaction is still unclear. It does not seem to involve blockade of 5-HT reuptake, as recently demonstrated, after acute and repeated CBD treatment, by Campos et al. (2013b). This same study failed to show any changes in 5-HT1A mRNA expression in the DPAG, after chronic CBD administration. It was recently shown, however, that CBD, although failing to displace the binding of 8-OH-DPAT, even at high concentrations, could enhance the ability of this 5-HT1A agonist to stimulate [^{35}S] GTPγS binding at relatively low concentrations (100 nM) (Rock et al., 2012). Although these results have not been fully explained, they suggest that CBD modulation of 5-HT1A receptors is complex, and could involve allosteric interactions.

Not all CBD effects in anxiety-related behaviors, however, can be explained by interaction with 5-HT1A receptors. Another important mechanism that might contribute to CBD anxiolytic effects involves a complex action of this cannabinoid on the endocannabinoid (eCB) system. CBD could facilitate eCB signaling by blocking the metabolism and uptake of anandamide (Bisogno et al., 2001; Campos et al., 2013b). Although, in the DPAG, AM251 (a CB1 receptor antagonist) failed to interfere in the anxiolytic effects of locally injected CBD, this drug was able to prevent CBD effects on processes related to aversive memories, such as extinction and reconsolidation (Bitencourt et al., 2008; Do Monte et al., 2013; Stern et al., 2012). In addition, AM251, but not a 5-HT1A-receptor antagonist, prevented CBD effects in the marble-burying model (Casarotto et al., 2010). Endocannabinoids can reduce the release of several neurotransmitters, including glutamate, a neurotransmitter closely involved in anxiety responses (Guimarães, Carobrez, De Aguiar, &

Graeff, 1991). An indirect antiglutamatergic action via increased eCB neurotransmission could contribute to the antianxiety effects of CBD.

Another effect of CBD that seems to involve the eCB system is its ability to increase hippocampal adult neurogenesis. Impairment in adult hippocampal neurogenesis has been associated to the pathogenesis of anxiety disorders and depression, and at least some of the behavior effects of antidepressant drugs, such as fluoxetine, depend on facilitation of this process (Samuels & Hen, 2011). CBD facilitation of adult hippocampus has been demonstrated by at least three independent works (Campos et al., 2013b; Esposito et al., 2011; Wolf et al., 2010). Wolf et al. (2010) showed that the proneurogenic effect of CBD was absent in CB_1-knockout mice. CBD has very low affinity for CB_1-receptor, thus this result might be reflecting its ability to inhibit anandamide metabolism/uptake (Bisogno et al., 2001). Corroborating this latter possibility, our group has recently shown that CBD increases in vivo and in vitro cell proliferation by activation of CB_1 and CB_2 receptors (Campos et al., 2013b). In this study, CBD effects were prevented by the overexpression of the fatty acid amide hydrolase, the enzyme responsible for anandamide degradation. Moreover, the anxiolytic effect of repeated administered CBD (30 mg/daily for 14 days) in mice submitted to a chronic unpredictable stress model was abolished in the absence of hippocampal neurogenesis. This behavior profile and neurogenic is similar to that observed with classic antidepressant drugs, and suggests a causal link between the proneurogenic and anxiolytic CBD effects.

Other mechanisms could also be involved in CBD effects on adult hippocampal neurogenesis, for instance the activation of peroxisome proliferator-activated receptors. This particular mechanism seems to be important during neuroinflammation and neurodegenerative processes related to β-amyloid protein deposits in the central nervous system (Esposito et al., 2011).

CBD can also act as an agonist of transient receptor potential (TRP) channels. These channels include over 50 members, and are present in different species, including yeast, worms, insects, fish, and mammals. The vanilloid receptor 1 or TRPV1 is one of the first identified members of the family, and are expressed in the brain, where anandamide has been proposed as an endogenous agonist, or endovanilloid. These receptors facilitate the release of glutamate, a neurotransmitter that, as described earlier, facilitates anxiety-like behaviors (Guimarães et al., 1991). Campos and Guimarães (2009) suggested that TRPV1 activation could explain, at least partially, the inverted U-shaped dose–response curves commonly observed in experiments conducted with CBD. They showed that intra-DPAG injections of an ineffective dose of the TRPV1 antagonist capsazepine turned a higher, ineffective dose (60 nmol) of CBD, into an anxiolytic one (Campos & Guimarães, 2009). TRPV1 blocked is also involved in the bell-shaped dose–responses curves produced by anandamide analogues (Casarotto et al., 2012).

In addition to TRPV1, CBD could also interfere with other members of the TR family, activating TRPV2 and ankyrin type 1 (TRPA1) channels, and antagonizing melastatin type 8 (TRPM8) channels (Qin et al., 2008). The contribution of these mechanisms to CBD's anxiolytic effects has not been tested yet.

In addition to the activation of a classic pharmacological receptor, CBD pharmacological mechanisms could also involve intracellular pathways. The role played by these mechanisms on CBD antianxiety effects also remains to be further investigated.

CONCLUSIONS

Taken together, the results from laboratory animals, healthy volunteers, and patients with anxiety disorders support the idea that CBD clearly presents antianxiety properties. This cannabinoid does not present psychoactive effects, does not affect cognition, has an adequate safety profile (Bergamaschi et al., 2011), good tolerability, positive results in trials with humans, and a broad spectrum of pharmacological actions. Moreover, since this compound does not induce dependence, tolerance, and abstinence symptoms, it may be a good alternative to the benzodiazepinics. Therefore, CBD appears to be the cannabinoid compound that is closer to have its preliminary findings in anxiety translated into clinical practice. However, future double-blind controlled studies should test this possibility in clinical trials, including patients with the diverse anxiety disorders, especially panic, obsessive-compulsive, social anxiety, and posttraumatic stress disorder. Additionally, as the actions of CBD usually present a bell-shaped pattern, the adequate therapeutic dose window for each anxiety disorder should be determined. In respect to the mechanism underlying its antianxiety effects, the most consistent evidence points to the involvement of the serotonergic system, probably through direct action on 5-HT1A receptors, although other systems, such as the eCB itself, may also be involved. Thus, further investigation is necessary to clarify these issues, especially if we consider that CBD is a drug with a wide spectrum of effects in the nervous system.

MINI-DICTIONARY

Bell-shape dose–response-curve Drug-response curve produced by drugs that have the ability of promoting different or opposite responses at low or high concentrations.

Cannabinoids A term that refers a heterogeneous group of compounds that modulates cannabinoid receptors functions, classified into three main groups: endogenous, synthetic, and phytocannabinoids.

Chronic unpredictable stress A model applied to produce sustained stress response in rodents that aims to simulate the variability of stressful stimulus to which humans are exposed in daily life.

Elevated plus maze A wide applied animal model of anxiety, composed by two open arms opposed to two enclosed arms elevated to the floor.

Fear conditioning Behavioral paradigm where animals exposed to an aversive stimulus (eg, foot shocks) learn to predict an unpleasant event.

Functional magnetic resonance imaging A noninvasive technique largely used to detect brain activity by detecting changes in blood flow, and oxygen consumption in the brain tissue.

Intracerebral injection Technique that involves stereotaxic surgery to inject a drug directly into a specific brain structure, in laboratory animals.

Simulated public speaking A model with clinical validity that induces anxiety by exposing healthy volunteers to a simulation of a public speech.

Single photon emission computed tomography A clinical image technique that uses gamma rays emission, and a radioactive material as a tracer, to analyze blood flow in target tissues and organs.

Vogel's conflict test A model used for the screening of antianxiety drugs in which water-deprived rodents are exposed to a conflict: decide to drink water from the spout of a bottle that is connected to a shock generator.

References

Bergamaschi, M. M., Queiroz, R. H., Chagas, M. H., de Oliveira, D. C., De Martinis, B. S., Kapczinski, F., Quevedo, J., Roesler, R., Schröder, N., Nardi, A. E., Martín-Santos, R., Hallak, J. E., Zuardi, A. W., & Crippa, J. A. (2011). Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naïve social phobia patients. *Neuropsychopharmacology*, *36*(6), 1219–1226.

Bisogno, T., Hanus, L., De Petrocellis, L., Tchilibon, S., Ponde, D. E., Brandi, I., et al. (2001). Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *British Journal of Pharmacology*, *134*(4), 845–852.

Bitencourt, R. M., Pamplona, F. A., & Takahashi, R. N. (2008). Facilitation of contextual fear memory extinction and anti-anxiogenic effects of AM404 and cannabidiol in conditioned rats. *European Neuropsychopharmacology*, *18*(12), 849–859.

Campos, A. C., & Guimarães, F. S. (2008). Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. *Psychopharmacology*, *199*(2), 223–230.

Campos, A. C., & Guimarães, F. S. (2009). Evidence for a potential role for TRPV1 receptors in the dorsolateral periaqueductal gray in the attenuation of the anxiolytic effects of cannabinoids. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *33*(8), 1517–1521.

Campos, A. C., Ferreira, F. R., & Guimarães, F. S. (2012a). Cannabidiol blocks long-lasting behavioral consequences of predator threat stress: possible involvement of 5HT1A receptors. *Journal of Psychiatry Research*, *46*, 1501–1510.

Campos, A. C., Moreira, F. A., Gomes, F. V., Del Bel, E. A., & Guimarães, F. S. (2012b). Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. *Philosophical Transactions of the Royal Society of London. Series B*, *367*, 3364–3378.

Campos, A. C., Ortega, Z., Palazuelos, J., Fogaca, M. V., Aguiar, D. C., Diaz-Alonso, J., Ortega-Gutierrez, S., Vazquez-Villa, H., Moreira, F. A., Guzman, M., Galve-Roperh, I., & Guimarães, F. S. (2013a). The anxiolytic effect of cannabidiol on chronically stressed mice depends on hippocampal neurogenesis: involvement of the endocannabinoid system. *International Journal of Neuropsychopharmacology*, *16*, 1407–1419.

Campos, A. C., Soares, de P. de V., Carvalho, M. C., Ferreira, F. R., Vicente, M. A., Brandao, M. L., Zuardi, A. W., Zangrossi, H., Jr., & Guimarães, F. S. (2013b). Involvement of serotonin-mediated neurotransmission in the dorsal periaqueductal gray matter on cannabidiol chronic effects in panic-like responses in rats. *Psychopharmacology*, *226*, 13–24.

Casarotto, P. C., Gomes, F. V., Resstel, L. B., & Guimarães, F. S. (2010). Cannabidiol inhibitory effect on marble-burying behaviour: involvement of CB1 receptors. *Behavioural Pharmacology*, *21*(4), 353–358.

Casarotto, P. C., Terzian, A. L., Aguiar, D. C., Zangrossi, H., Guimarães, F. S., Wotjak, C. T., & Moreira, F. A. (2012). Opposing roles for cannabinoid receptor type-1 (CB(1)) and transient receptor potential vanilloid type-1 channel (TRPV1) on the modulation of panic-like responses in rats. *Neuropsychopharmacology*, *37*, 478–486.

Crippa, J. A., Zuardi, A. W., Garrido, G. E., Wichert-Ana, L., Guarnieri, R., Ferrari, L., Azevedo-Marques, P. M., Hallak, J. E., McGuire, P. K., & Filho Busatto, G. (2004). Effects of cannabidiol (CBD) on regional cerebral blood flow. *Neuropsychopharmacology*, *29*(2), 417–426.

Crippa, J. A., Zuardi, A. W., Martín-Santos, R., Bhattacharyya, S., Atakan, Z., McGuire, P., & Fusar-Poli, P. (2009). Cannabis and anxiety: a critical review of the evidence. *Human Psychopharmacology*, *24*(7), 515–523.

Crippa, J. A., Derenusson, G. N., Ferrari, T. B., Wichert-Ana, L., Duran, F. L., Martin-Santos, R., Simões, M. V., Bhattacharyya, S., Fusar-Poli, P., Atakan, Z., Santos Filho, A., Freitas-Ferrari, M. C., McGuire, P. K., Zuardi, A. W., Busatto, G. F., & Hallak, J. E. (2011). Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. *Journal of Psychopharmacology*, *25*(1), 121–130.

Deiana, S., Watanabe, A., Yamasaki, Y., Amada, N., Arthur, M., Fleming, S., Woodcock, H., Dorward, P., Pigliacampo, B., Close, S., Platt, B., & Riedel, G. (2012). Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Δ^9-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive-compulsive behaviour. *Psychopharmacology (Berl)*, *219*(3), 859–873.

Do Monte, F. H., Souza, R. R., Bitencourt, R. M., Kroon, J. A., & Takahashi, R. N. (2013). Infusion of cannabidiol into infralimbic cortex facilitates fear extinction via CB1 receptors. *Behavioural Brain Research*, *250*, 23–27.

Esposito, G., Scuderi, C., Valenza, M., Togna, G. I., Latina, V., De Filippis, D., et al. (2011). Cannabidiol reduces Abeta-induced neuroinflammation and promotes hippocampal neurogenesis through PPARgamma involvement. *PLoS One*, *6*(12), e28668.

Fogaça, M. V., Reis, F. M., Campos, A. C., & Guimarães, F. S. (2014). Effects of intra-prelimbic prefrontal cortex injection of cannabidiol on anxiety-like behavior: involvement of 5HT1A receptors and previous stressful experience. *European Neuropsychopharmacology*, *24*, 410–419.

Fusar-Poli, P., Allen, P., Bhattacharyya, S., Crippa, J. A., Mechelli, A., Borgwardt, S., Martin-Santos, R., Seal, M., O'Carrol, C., Atakan, Z., Zuardi, A. W., & McGuire, P. K. (2010). Modulation of effective connectivity during emotional processing by delta 9-tetrahydrocannabinol and cannabidiol. *International Journal of Neuropsychopharmacology*, *13*(4), 421–432.

Fusar-Poli, P., Crippa, J. A., Bhattacharyya, S., Borgwardt, S. J., Allen, P., Martin-Santos, R., Seal, M., Surguladze, S. A., O'Carrol, C., Atakan, Z., Zuardi, A. W., & McGuire, P. K. (2009). Distinct effects of delta9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Archives of General Psychiatry*, *66*(1), 95–105.

Gomes, F. V., Reis, D. G., Alves, F. H., Correa, F. M., Guimarães, F. S., & Resstel, L. B. (2012). Cannabidiol injected into the bed nucleus of the stria terminalis reduces the expression of contextual fear conditioning via 5-HT1A receptors. *Journal of Psychopharmacology*, *26*(1), 104–113.

Gomes, F. V., Resstel, L. B., & Guimarães, F. S. (2011). The anxiolytic-like effects of cannabidiol injected into the bed nucleus of the stria terminalis are mediated by 5-HT1A receptors. *Psychopharmacology*, *213*(2–3), 465–473.

Guimarães, F. S., Carobrez, A. P., De Aguiar, J. C., & Graeff, F. G. (1991). Anxiolytic effect in the elevated plus-maze of the NMDA receptor antagonist AP7 microinjected into the dorsal periaqueductal grey. *Psychopharmacology*, *103*, 91–94.

Guimarães, F. S., Chiaretti, T. M., Graeff, F. G., & Zuardi, A. W. (1990). Antianxiety effect of cannabidiol in the elevated plus-maze. *Psychopharmacology*, *100*, 558–559.

Hsiao, Y. T., Yi, P. L., Li, C. L., & Chang, F. C. (2012). Effect of cannabidiol on sleep disruption induced by the repeated combination tests consisting of open field and elevated plus-maze in rats. *Neuropharmacology*, *62*(1), 373–384.

Izzo, A. A., Borrelli, F., Capasso, R., Di Marzo, V., & Mechoulam, R. (2009). Non-psychotropic plant cannabinoids: new therapeutic opportunities from na ancient herb. *Trends in Pharmacological Sciences*, *30*, 515–527.

Karniol, I. G., & Carlini, E. A. (1972). The content of (−)9-*trans*-tetrahydrocannabinol (9-thc) does not explain all biological activity of some Brazilian marihuana samples. *Journal of Pharmaceutics and Pharmacology*, *24*(10), 833–834.

Karniol, I. G., & Carlini, E. A. (1973). Pharmacological interaction between cannabidiol and delta 9-tetrahydrocannabinol. *Psychopharmacologia*, *33*(1), 53–70.

Karniol, I. G., Shirakawa, I., Kasinski, N., Pfeferman, A., & Carlini, E. A. (1974). Cannabidiol interferes with the effects of delta 9-tetrahydrocannabinol in man. *European Journal of Pharmacology*, *28*(1), 172–177.

Lemos, J. I., Resstel, L. B., & Guimarães, F. S. (2010). Involvement of the prelimbic prefrontal cortex on cannabidiol-induced attenuation of contextual conditioned fear in rats. *Behavioural Brain Research*, *207*(1), 105–111.

Mishima, K., Hayakawa, K., Abe, K., Ikeda, T., Egashira, N., Iwasaki, K., et al. (2005). Cannabidiol prevents cerebral infarction via a serotonergic 5-hydroxytryptamine1A receptor-dependent mechanism. *Stroke*, *36*(5), 1077–1082.

Moreira, F. A., Aguiar, D. C., & Guimarães, F. S. (2006). Anxiolytic-like effect of cannabidiol in the rat Vogel conflict test. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *30*, 1466–1471.

Nardo, M., Casarotto, P. C., Gomes, F. V., & Guimarães, F. S. (2014). Cannabidiol reverses the mCPP-induced increase in marble-burying behavior. *Fundamental and Clinical Pharmacology*, *28*, 544–550.

Onaivi, E. S., Green, M. R., & Martin, B. R. (1990). Pharmacological characterization of cannabinoids in the elevated plus maze. *Journal of Pharmacology and Experimental Therapeutics*, *253*, 1002–1009.

Qin, N., Neeper, M. P., Liu, Y., Hutchinson, T. L., Lubin, M. L., & Flores, C. M. (2008). TRPV2 is activated by cannabidiol and mediates CGRP release in cultured rat dorsal root ganglion neurons. *Journal of Neuroscience*, *28*, 6231–6238.

Resstel, L. B., Joca, S. R., Moreira, F. A., Correa, F. M., & Guimarães, F. S. (2006). Effects of cannabidiol and diazepam on behavioural and cardiovascular responses induced by contextual conditioned fear in rats. *Behavioural Brain Research*, *172*, 294–298.

Resstel, L. B., Tavares, R. F., Lisboa, S. F., Joca, S. R., Correa, F. M., & Guimaraes, F. S. (2009). 5-HT1A receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats. *British Journal of Pharmacology*, *156*, 181–188.

Rock, E. M., Bolognini, D., Limebeer, C. L., Cascio, M. G., Anavi-Goffer, S., Fletcher, P. J., et al. (2012). Cannabidiol, a non-psychotropic component of cannabis, attenuates vomiting and nausea-like behaviour via indirect agonism of 5-HT(1A) somatodendritic: autoreceptors in the dorsal raphe nucleus. *British Journal of Pharmacology*, *165*, 2620–2634.

Russo, E. B., Burnett, A., Hall, B., & Parker, K. K. (2005). Agonistic properties of cannabidiol at 5-HT1a receptors. *Neurochemical Research*, *30*(8), 1037–1043.

Ryberg, E., Larsson, N., Sjogren, S., Hjorth, S., Hermansson, N. O., Leonova, J., et al. (2007). The orphan receptor GPR55 is a novel cannabinoid receptor. *British Journal of Pharmacology*, *152*(7), 1092–1101.

Samuels, B. A., & Hen, R. (2011). Neurogenesis and affective disorders. *European Journal of Neuroscience*, *33*(6), 1152–1159.

Schier, A. R., Ribeiro, N. P., Silva, A. C., Hallak, J. E., Crippa, J. A., Nardi, A. E., & Zuardi, A. W. (2012). Cannabidiol, a Cannabis sativa constituent, as an anxiolytic drug. *Rev Bras Psiquiatr*, *34*(Suppl. 1), S104–S110.

Silveira Filho, N. G., & Tufik, S. (1981). Comparative effects between cannabidiol and diazepam on neo-phobia, food intake and conflict behavior. *Research Communications in Psychology, Psychiatry and Behavior*, *6*, 251–266.

Soares, P. de V., Campos, A. C., Bortoli, V. C., Zangrossi, H., Jr., Guimaraes, F. S., & Zuardi, A. W. (2010). Intra-dorsal periaqueductal gray administration of cannabidiol blocks panic-like response by activating 5-HT1A receptors. *Behavioural Brain Research*, *213*(2), 225–229.

Stern, C. A., Gazarini, L., Takahashi, R. N., Guimarães, F. S., & Bertoglio, L. J. (2012). On disruption of fear memory by reconsolidation blockade: evidence from cannabidiol treatment. *Neuropsychopharmacology*, *37*, 2132–2142.

Thomas, A., Baillie, G. L., Phillips, A. M., Razdan, R. K., Ross, R. A., & Pertwee, R. G. (2007). Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. *British Journal of Pharmacology*, *150*(5), 613–623.

Uribe-Mariño, A., Francisco, A., et al. (2012). Anti-aversive effects of cannabidiol on innate fear-induced behaviors evoked by an ethological model of panic attacks based on a prey vs the wild snake *Epicrates cenchria* crassus confrontation paradigm. *Neuropsychopharmacology*, *37*(2), 412–421.

Viveros, M. P., Marco, E. M., & File, S. E. (2005). Endocannabinoid system and stress and anxiety responses. *Pharmacology Biochemistry and Behavior*, *81*, 331–342.

Wolf, S. A., Bick-Sander, A., Fabel, K., Leal-Galicia, P., Tauber, S., Ramirez-Rodriguez, G., et al. (2010). Cannabinoid receptor CB1 mediates baseline and activity-induced survival of new neurons in adult hippocampal neurogenesis. *Cell Communication and Signalling*, *8*, 12.

Zuardi, A. W., Cosme, R. A., Graeff, F. G., & Guimarães, F. S. (1993). Effects of ipsapirone and cannabidiol on human experimental anxiety. *Journal of Psychopharmacology*, *7*, 82–88.

Zuardi, A. W., & Karniol, I. G. (1983). Changes in the conditioned emotional response of rats induced by Δ9-THC, CBD and mixture of the two cannabinoids. *Brazilian Archives of Biology and Technology*, *26*, 391–397.

Zuardi, A. W., Shirakawa, I., Finkelfarb, E., & Karniol, I. G. (1982). Action of cannabidiol on the anxiety and other effects produced by delta 9-THC in normal subjects. Psychopharmacology, 76(3), 245–250.

CHAPTER

e14

New Ethological and Morphological Perspectives for the Investigation of Panicolytic-Like Effects of Cannabidiol

N.C. Coimbra[*,**], *J. Mendes-Gomes*[*], *J.A. da Silva*[*], *T. dos Anjos-Garcia*[*], *F. Ullah*[*], *R.C. Almada*[*]

*Laboratory of Neuroanatomy & Neuropsychobiology, Department of Pharmacology, Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP), Ribeirão Preto, São Paulo, Brazil
**Neurobiology of Emotions Research Centre (NAP-USP-NuPNE), Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP), Ribeirão Preto, São Paulo, Brazil

SUMMARY POINTS

- This chapter focuses on the antiaversive effects of cannabidiol (CBD).
- The putative antiaversive effect of CBD was studied in threatened rodents.
- We discuss the viability of a new ethological model of panic attacks to address the panicolytic-like effect of CBD.
- The treatment of the ventral midbrain with CBD caused CB1-receptor-signaled antipanic-like effect.
- The neocortico-neostriatal disinhibitory pathway and the nigro-tectal GABAergic inhibitory connections are discussed as new morphological targets for the panicolytic effect of CBD.

KEY FACTS FOR CANNABIDIOL TREATMENT OF MIDBRAIN STRUCTURES

- The stimulation of dorsal midbrain in humans elicited fear (Nashold, Wilson, & Slaughter, 1969).
- The neostriatum-nigral disinhibitory pathway, and the nigro-tectal GABAergic inhibitory connections modulate the activity of dorsal midbrain structures (Coimbra & Brandão, 1993).
- CBD is a *Cannabis sativa* plant compound with psychoactive medicinal effects.
- The brain produces endogenous agonists that bind to the same receptors than CBD.
- The panicolytic-like effect of CBD, microinjected in the ventral midbrain, depends on CB1-cannabinoid receptor activation.

LIST OF ABBREVIATIONS

AC	Amygdaloid complex
AEA	Anandamide
2-AG	2-Arachidonoylglycerol
CB1	Cannabinoid receptor type 1
CB2	Cannabinoid receptor type 2
CBD	Cannabidiol
dlSC	Deep layers of the superior colliculus
DM	Dorsal midbrain
DPAG	Dorsal columns of the periaqueductal gray matter
EAS	Encephalic aversion system
GABA	γ-aminobutyric acid
5-HT$_{1A}$	5-Hydroxytryptamine (serotonin) 5-HT$_{1A}$ receptor
IC	Inferior colliculus
ilSC	Intermediate layers of the superior colliculus

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00098-3
Copyright © 2017 Elsevier Inc. All rights reserved.

MT	Midbrain tectum
PAG	Periaqueductal gray matter
SNpr	Substantia nigra, pars reticulata
Δ^9-THC	Δ^9-Tetrahydrocannabinol
TRPV1	Transient receptor potential cation channel subfamily V member 1
VM	Ventral midbrain

INTRODUCTION

Cannabinoid is a term that designates compounds of the *Cannabis sativa* plant. The main psychoactive compounds are Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD), which may produce opposite effects on human brain function (Bhattacharyya et al., 2010). Cannabinoids may produce their effects via CB1 and CB2 receptors (Devane et al., 1992). Generally, the CB1 receptor is found in cerebellum, hippocampus, striatum, cortex, substantia nigra, and hypothalamus (Herkenham et al., 1991; Tsou, Brown, Sanudo-Pena, Mackie, & Walker, 1998). CB2 receptors are primarily found in cells and immune system tissue (Pertwee, 1997).

After the discovery of CB1 and CB2 receptors, studies demonstrated that mammals produce endogenous agonists, which are endocannabinoids binding to these receptors (Di Marzo, Melck, Bisogno, & De Petrocellis, 1998; Mechoulam, Fride, & Di Marzo, 1998). The main identified endocannabinoids are N-arachidonoyl ethanolamide, known as anandamide (AEA), 2-arachidonoylglycerol (2-AG), and O-arachidonoylethanolamine (Devane et al., 1992; Sugiura et al., 1995).

Schlicker and Kathmann (2001) and Alger (2002) reported that many axon terminals of the central nervous system express CB1 receptors, but its functions seem to inhibit the release of inhibitory neurotransmitters, such as γ-aminobutyric acid (GABA) (Freund, Katona, & Piomelli, 2003). However, CBD has a low affinity for CB1 and CB2 receptors. CBD may enhance endocannabinoid neurotransmission through an indirect mechanism, such as blockade of AEA uptake and inhibition of its enzymatic hydrolysis (Bisogno et al., 2001). In vitro assays also demonstrated some antagonistic actions of CBD at CB1 and CB2 receptors, which illustrates the complex actions of CBD on the endocannabinoid system (Thomas et al., 2007). CBD may also act as an agonist on other receptors, such as 5-HT_{1A} receptors, according to in vitro (Russo, Burnett, Hall, & Parker, 2005) and in vivo (Campos & Guimarães, 2008; Twardowschy et al., 2013) studies, and transient receptor potential cation channel subfamily V member 1 (TRPV1) (De Petrocellis et al., 2008).

The use and therapeutic efficacy of CBD is controversial, but the literature describes a wide range of possible therapeutic effects of CBD on several medical conditions, including cerebral ischemia, diabetes, rheumatoid arthritis, and other inflammatory diseases, nausea, cancer, motor diseases, schizophrenia, Parkinson's disease, Alzheimer's disease, epilepsy, depression, and anxiety disorders (for a review, see Zuardi, 2008). Notably, CBD promoted anxiolytic-like effects in rats exposed to the elevated plus maze (Campos & Guimarães, 2008; Onaivi, Green, & Martin, 1990), the social interaction test (Almeida et al., 2013), and the Vogel conflict test (Campos & Guimarães, 2008). Anxiolytic properties of CBD were also described in humans (Bergamaschi et al., 2011). CBD also promotes an antiaversive-like effect in rats exposed to the elevated T-maze, or in cases of dPAG electrical stimulation (Soares et al., 2010). However, some results are contradictory because low doses of CBD (10 mg/kg) decrease conditioned emotional responses in rats (Zuardi & Karniol, 1983), and high doses fail to produce anxiolytic-like effects in a conflict paradigm-based model (Silveira Filho & Tufik, 1981). Guimarães, Chiaretti, Graeff, & Zuardi (1990) showed that these opposite results likely occurred because CBD produces an inverted U-shaped dose–response curve, also recruiting noncannabinoid receptors.

The use of animal models to study human behaviors is based on Charles Darwin's studies that support that human beings share basic emotions with other animals, in an evolutionary perspective. Therefore, the root of anxiety and fear may be defensive reactions, expressed by animals submitted to a stimulus that elicits fear or a threat to the survival, well-being, and physical integrity. Notable, these emotions play an important role in our survival, but they are pathological and not useful when they disturb a person's normal activities. Reactions in this situation are classified as anxiety disorders, which are the consequence of dysfunctions in the brain systems that modulate defensive reactions (Zangrossi & Graeff, 2004).

Notably, fear and anxiety are distinct emotions, but they are correlated. The differentiation can be characterized by the stimuli or the situations that trigger them. Fear may be trigged by evident situations of threat and danger, whereas anxiety may be experienced in situations where the dangers are potential, vague, and uncertain (Blanchard, Yudko, Rodgers, & Blanchard, 1993). Gray and McNaughton (2000) suggest that fear can be contrasted with anxiety, because the tendency to avoid (based on fear) in the latter case is combined with a tendency to approach.

Some authors suggest that defensive responses are organized by encephalic structures that are responsible for the integration of aversive states, such as the hypothalamus, amygdaloid complex (AC), periaqueductal gray matter (PAG), deep layers of the superior colliculus (dlSC), and the inferior colliculus (IC) (Castellan-Baldan et al., 2006; Graeff, 1990), which comprise the encephalic aversion system (EAS), that contains potential sites of action for the therapeutic effects of CBD.

NEW ETHOLOGICAL MODELS FOR THE STUDY OF ANTIPANIC-LIKE EFFECTS OF CANNABIDIOL

Polygonal arenas without burrows (Lobão-Soares et al., 2008; Maurin, 2008) or enriched with shelters and escalators with escape platforms (Almada, Roncon, Elias-Filho, & Coimbra, 2014; Twardowschy et al., 2013; Uribe-Mariño et al., 2012) and complex labyrinths (Guimarães-Costa et al., 2007) were used as new experimental models for the study of the panic attack-like behaviors displayed by small rodents confronted with wild venomous Viperidae snakes (Fig. e14.1) or wild constrictor Boidae serpents (Fig. e14.2).

The polygonal arena consists of a semitransparent acrylic quadrangular enclosure (154 cm × 72 cm × 64 cm) with the inner wall surface covered with a light-reflector film that provides 80% light reflection to minimize the visual contact of the predator with the surrounding experimental area, and focus its attention toward the prey. The floor of the quadrangular arena was made of a transparent acrylic platform placed on a rectangular stainless steel plaque below the arena, which was divided into 20 equal rectangles using a green fluorescent line (4.2 mm width; Pritt mark-it). The entire apparatus was placed on a granite rock surface (170 cm × 85 cm × 2 cm) elevated 83 cm above the floor of the laboratory to minimize vibratory stimuli. In one corner of the arena, a shelter in the form of box (36 cm × 26 cm × 12.5 cm) with black acrylic walls was provided.

The complex labyrinth consisted of a transparent acrylic enclosure that was partially a quadrangular arena (small "open-field") contiguous to a complex maze composed of black acrylic walls. The arena totaled 0.42 m^2, and the remaining labyrinth measured 15 cm in height and 6.92 m in length. The entire apparatus containing the complex labyrinth and arena measured 140 × 70 × 15 cm. The top of the labyrinth and the arena contained 84 circular holes (1.5 cm in diameter). The floor of the complex labyrinth, made of a transparent crystal acrylic plaque (140 × 70 cm), was placed on another metallic plaque of the same dimensions. The

FIGURE e14.1 Behavior of threatened rodents in the polygonal arena. Innate fear-induced defensive responses evoked in Swiss mice confronted with *Crotalus durissus terrificus* (A–C) and *Bothrops alternatus* (D–F) for 5 min in a polygonal arena with shelter. Anxiety-related responses: alertness (a1); and flat back approach (E). Panic attack-like responses: freezing (b1); oriented escape behavior (C); and explosive escape behavior (F). An interaction between prey and predator is shown in D.

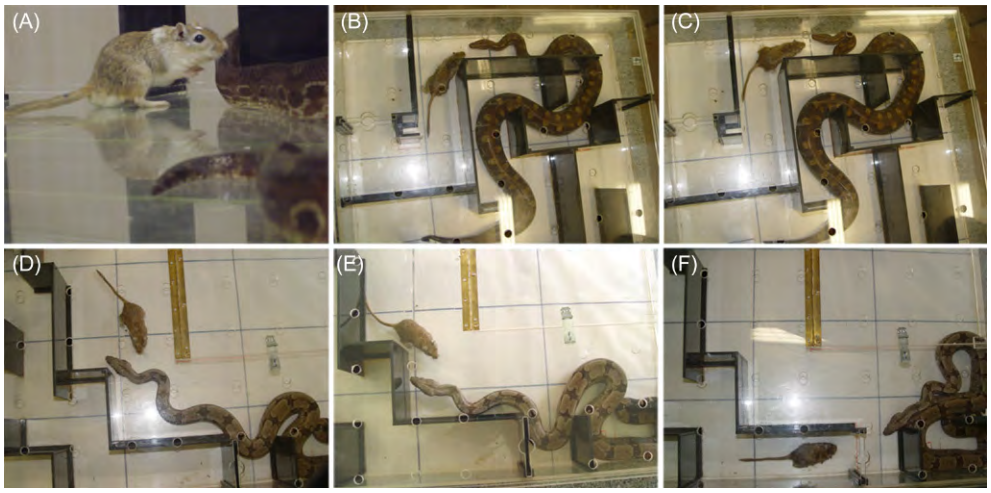


FIGURE e14.2 Behavior of threatened rodents in the complex labyrinth. Innate fear-induced defensive responses evoked in Mongolian gerbils confronted with *Epicrates cenchria crassus* (A–C) and *Boa constrictor amarali* (D–F) for 15 min in Coimbra's complex labyrinth. Anxiety-related responses: alertness (A); and flat back approach inside galleries (B), and in the arena (D). Panic attack-like responses: freezing inside galleries (C) and in the arena (E); inhibitory avoidance and freezing inside galleries (F).

metallic plaque was made of 1-mm-wide stainless steel lamina, divided by 0.4 cm green fluorescent lines in 20 equal rectangles (27.7 cm × 17.2 cm each).

Associations between defensive reactions and predatory attacks were proposed to study the neural bases of innate fear and panic attack (Blanchard et al., 1993). These associations were based on experimental models involving prey and their predators. They include observations of defense strategies, which differ according to some variables, such as the characteristics in which the stimulus is presented, the proximity of the aversive stimulus, previous experiences of the animal, and the social position occupied by the animal in its social group (Blanchard et al., 1993). Other studies (Guimarães-Costa et al., 2007; Uribe-Mariño et al., 2012; Weltson et al., 2002) used experimental models of prey–predator interactions to evaluate the effects of human anxiety-modulating drugs, and drugs that interfere with the endocannabinoid and serotonergic systems (which we believe underlie the action of CBD) in a series of anxiety/defense and fear/defense batteries. These models were also used to evaluate activation of the central nervous system in dangerous situations (Maurin, 2008), which validates the model proposed in these experiments, and presents interesting results of defensive responses evoked in the presence of predators.

The previously cited experiments allowed correlations of defensive responses with integrated functions of encephalic structures (Maurin, 2008; Weltson et al., 2002). The main structures involved in the antipredatory defensive repertoire in the prey versus predator paradigm are the dorsomedial division of the ventromedial hypothalamic nucleus, the anterior hypothalamus, and the premammillary hypothalamic nucleus (Canteras, 2002).

Panicolytic-like effects of CBD were demonstrated in mice submitted to the prey versus predator paradigm using polygonal areas for snakes (Twardowschy et al., 2013; Uribe-Mariño et al., 2012). Notably, Uribe-Mariño et al. (2012) demonstrated that CBD caused a significant decrease in panic-attack-related responses, such as the behavioral index and the duration of defensive immobility (freezing) and explosive escape behavior. However, no significant action on anxiety-related responses, such as defensive attention and risk assessment (flat back approach), or oriented escape behavior, were observed during confrontations with rainbow Boidae snake in a polygonal arena for snakes and rodents. These antipanic-like effects decreased after WAY100635 pretreatment in mice, which suggests that cannabidiol recruited 5-HT_{1A} receptors to exert at least part of its panicolytic effects (Twardowschy et al., 2013).

ANATOMICAL TARGETS FOR THE ANTIPANIC-LIKE EFFECTS OF CANNABIDIOL

Several studies showed that midbrain tectum (MT) structures, such the dPAG, dlSC, and IC are important in the organization of nonoriented/explosive panic-like defensive reactions (Almada et al., 2015; Borelli, Nobre, Brandão, & Coimbra, 2004; Coimbra & Brandão, 1993; Castellan-Baldan et al., 2006; Nashold et al., 1969). There is also evidence that the medial hypothalamus (Biagioni, Silva, & Coimbra, 2012) elaborates more oriented escape reactions when stimulated. Chronic GABAergic input dysfunction of this diencephalic structure is used as a model of panic syndrome in laboratory animals (Shekhar, Keim, Simon, & McBride, 1996). Evidence also

suggests that GABA receptors in the medial and posterior hypothalamic nuclei regulate anxiety (Shekhar, Hingtgen, & DiMicco, 1990) and panic-related responses (Biagioni et al., 2012).

The substantia nigra pars reticulata (SNpr) is a crucial structure in the modulation of instinctive fear-related behaviors elicited by electrical and chemical stimulation of the midbrain tectum (Castellan-Baldan et al., 2006; Coimbra & Brandão, 1993). Coimbra and Brandão (1993) demonstrated that microinjections of the $GABA_A$ receptor selective agonist muscimol into the SNpr increase the sensitivity of the dlSC and dPAG to electrical and chemical stimulation, similar to the effect of neurochemical lesions of the nigro-collicular GABAergic pathways. Microinjections of bicuculline into the continuum comprised of dlSC and dlPAG, following the same procedure in the SNpr, produced the opposite effect (Coimbra & Brandão, 1993). These results highlight the relevance of GABAergic inputs to the SNpr and dorsal midbrain in the modulation of instinctive fear-induced responses produced by dorsal mesencephalic structures.

GABAergic neurons from the reticulate division of the substantia nigra send inputs to the intermediate layers of the superior colliculus (ilSC) and dlSC, and the dorsomedial and dorsolateral columns of the PAG (Di Chiara, Porceddu, Morelli, Mulas, & Gessa, 1979; Eichenberger et al., 2002; Faull & Mehler, 1978; Grofová, Ottersen, & Rinvik, 1978), and exert a strong inhibitory influence on the MT in cranial (Coimbra et al., 2006; Coimbra & Brandão, 1993) and more caudal (Castellan-Baldan et al., 2006) midbrain levels. This GABAergic pathway is under an inhibitory influence exerted by another GABAergic pathway stemming from the neostriatum and pallidum (neostriatum-pallidum-nigral disinhibitory pathway) (Castellan-Baldan et al., 2006; Faull & Mehler, 1978), which modulates the activity of nigrotectal inhibitory projecting neurons also utilizing GABA as a neurotransmitter (Castellan-Baldan et al., 2006; Coimbra & Brandão, 1993). The projections sent by the SNpr to the ilSC and dlSC/dPAG act as a sensorial channel of MT neurons, which organizes defensive reactions (Eichenberger et al., 2002; Redgrave, Marrow, & Dean, 1992). These connections are critical to the modulation of innate fear, and they may be under pre- and postsynaptic endogenous opioid peptide-mediated control (Castellan-Baldan et al., 2006; Eichenberger et al., 2002).

The modulatory mechanisms of aversive states elaborated by the MT are widely associated with GABAergic control (Di Scala, Schmitt, & Karli, 1983). Dorsal midbrain tectum electrical stimulation and intramesencephalic microinjections of the $GABA_A$ receptor selective antagonist bicuculline elicit a clear panic attack-like response (Coimbra & Brandão, 1993; Coimbra et al., 2006; Di Scala et al., 1983) that is expressed by defensive immobility, explosive escape behavior, and neurovegetative responses, such as increased heart rate and frequency (Brandão, Coimbra, & Leão Borges, 1990), defecation, urination, and exophthalmos. This set of responses are similar to innate fear characteristics that are provoked by threatening situations (Twardowschy et al., 2013; Uribe-Mariño et al., 2012).

Electrical (Bovier, Broekkamp, & Lloyd, 1982) and chemical (Coimbra et al., 2006; Eichenberger et al., 2002) stimulation of the MT are used as a model of instinctive fear in rodents. However, more ethological models of innate fear, based on the interaction between prey and predator, are also used as a noninvasive approach to investigate emotional reaction related to innate fear (Guimarães-Costa et al., 2007; McNaughton & Corr, 2004; Twardowschy et al., 2013; Uribe-Mariño et al., 2012). The panic attack model based on the confrontation between prey and predator using small rodents and venomous snakes was validated ethologically, pharmacologically, and morphologically in our laboratory beginning in 2002 (Maurin, 2008; Uribe-Mariño et al., 2012; Weltson et al., 2002). These experimental models of generalized anxiety and panic attacks are used to investigate intense fear-related emotions in rodents submitted to the presence of wild snakes (Lobão-Soares et al., 2008; Uribe-Mariño et al., 2012), with or without artificial burrows and platforms.

The endogenous opioid system and endocannabinoid signaling mechanisms during neuromodulation also play important roles in intramesencephalic GABAergic connections, and the organization of instinctive fear and panic (Castellan-Baldan et al., 2006; Eichenberger et al., 2002). Investigations using morphological techniques demonstrated the presence of μ- and κ-opioid receptors and CB1- and CB2-cannabinoid receptors in the SNpr (Gong, 2006; Herkenham et al., 1991; Mansour, Khachaturian, Lewis, Akil, & Watson, 1988). The presence of μ-opioid receptors and moderate concentrations of δ- and κ-opioid receptors were demonstrated in the SNpr (Mansour et al., 1988). Several axosomatic and axodendritic synapses of tecto-nigral opioid pathways likely use these opioid receptors to exert their modulatory influence on nigro-tectal GABAergic pathways. CB1 cannabinoid receptors are widely distributed in the SNpr (Fig. e14.3), in pre- and postsynaptic membranes (Herkenham et al., 1991). These findings suggest that endogenous cannabinoids act as neuromodulators of opioid inputs and GABAergic outputs in the ventral mesencephalon. Therefore, the role of these receptors during the control of panic attacks deserves attention for the development of new drugs with putative effects on this neural substrate. Pharmacological evidence demonstrated interactions between the endogenous opioid peptide-mediated system and endocannabinoid-signaled mechanisms (López-Moreno, López-Jiménez, Gorriti, & De Fonseca, 2010; Manzanares et al., 1999) in

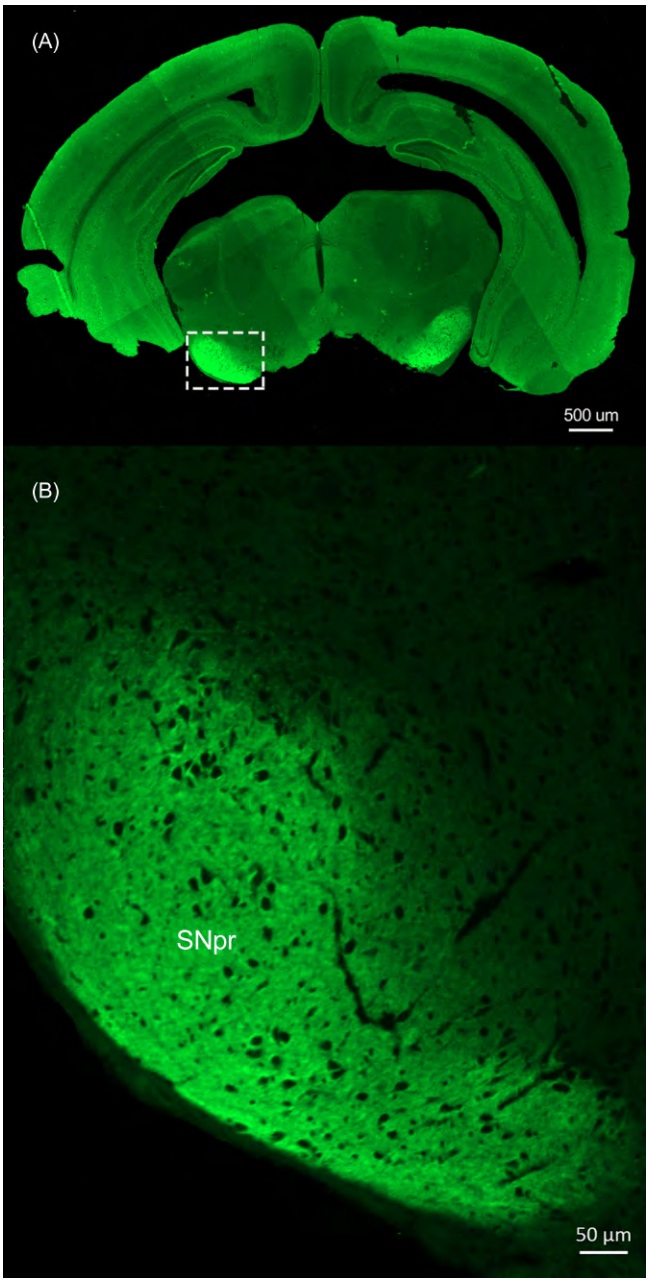


FIGURE e14.3 Morphological localization of CB1 receptors in the ventral mesencephalon. Photomicrographs of transverse sections of *Mus musculus* midbrain showing a panoramic view of the substantia nigra in which CB1-cannabinoid receptors were immunolabeled using monoclonal antibodies labeled with Alexa Fluor-488. The *dashed square* in (A) was amplified in (B). Images were obtained under black-field photomicroscopy (AxioImager Z1with APOTOME, Zeiss, Germany). Calibration bar: 500 µm in A; 50 µm in B.

the brain. Exogenous cannabinoids and endocannabinoids also exert an inhibitory influence on GABA release in the central nervous system, and this effect is mediated by CB1- and CB2-cannabinoid receptor-mediated neurochemical mechanisms (Andó, Bíró, Csölle, Ledent, & Sperlágh, 2012). Cannabinoids also decrease the activity of neostriatum-nigral GABAergic pathways in rodents (da Silva et al., 2015; Wallmichrath & Szabo, 2002).

These findings motivated us to investigate the role of GABAergic mechanisms of the striatum-nigral inputs on nigro-tectal pathway activity during the elaboration of defensive behaviors elicited by microinjections of the $GABA_A$ receptor selective antagonist bicuculline into the MT of mice confronted with the Brazilian *urutu cruzeiro* venomous snake (*Bothrops alternatus*; Reptilia; Viperidae). The decrease in neostriatum-nigral input activity was investigated using SNpr pretreatment with the synaptic blocker cobalt chloride, followed by bicuculline microinjection into the MT of mice confronted with a venomous snake. The decrease in activity of the nigro-tectal GABAergic pathway was studied using lidocaine microinjections into the SNpr. Lidocaine and cobalt chloride are widely used in several brain regions to produce functional and reversible inactivation of neural activity (Kretz, 1984; Sandkühler, Maisch, & Zimmermann, 1987). Pretreatment of the SNpr with physiological saline, followed by $GABA_A$ receptor blockade in the MT in a threatening situation, elicited a clear defensive behavior that was expressed by increased panic attack-like behaviors, such as increased duration of defensive immobility, and increased frequency and duration of nonoriented (explosive) escape behaviors during the venomous snake confrontation. Pretreatment of mouse SNpr with cobalt chloride followed by bicuculline microinjections in the MT decreased the panic attack-like responses elicited by the presence of the wild snake. In contrast, lidocaine microinjections in the SNpr increased panic-like responses. Finally, intranigral injections of AEA produced a clear antiaversive effect in mice exposed to a wild snake in a polygonal arena with burrows (Almada et al., 2014, 2015). These findings suggest that a decrease in striatum-nigral pathway activity causes panicolytic-like effects, and that the inactivation of the nigro-tectal pathway produces panic attack facilitation in threatening situations. This neural substrate of the cranial mesencephalon, including ventral and dorsal midbrain structures, should be explored as a pharmacological target for novel medicines, such as CBD. Da Silva (2011) investigated the effects of cannabidiol microinjections into the SNpr of Wistar rats submitted to bicuculline microinjections in the dorsal midbrain (Fig. e14.4A). $GABA_A$ receptor blockade in the MT increased the frequency [$F_{(7,56)} = 69.23$; $p < 0.001$] and duration [$F_{(7,56)} = 17.39$; $p < 0.001$] of panic attack-like explosive escape reactions. Pretreatment of the SNpr with CBD decreased the frequency and duration ($p < 0.001$ in both cases) of panic attack-like escape behaviors elicited by MT microinjections of bicuculline (Fig. e14.4B and C). The effect of CBD on the frequency of escape was reversed by SNpr pretreatment with the CB1-cannabinoid receptor antagonist AM251, but not after intranigral microinjections of the $5\text{-}HT_{1A}$ receptor selective antagonist

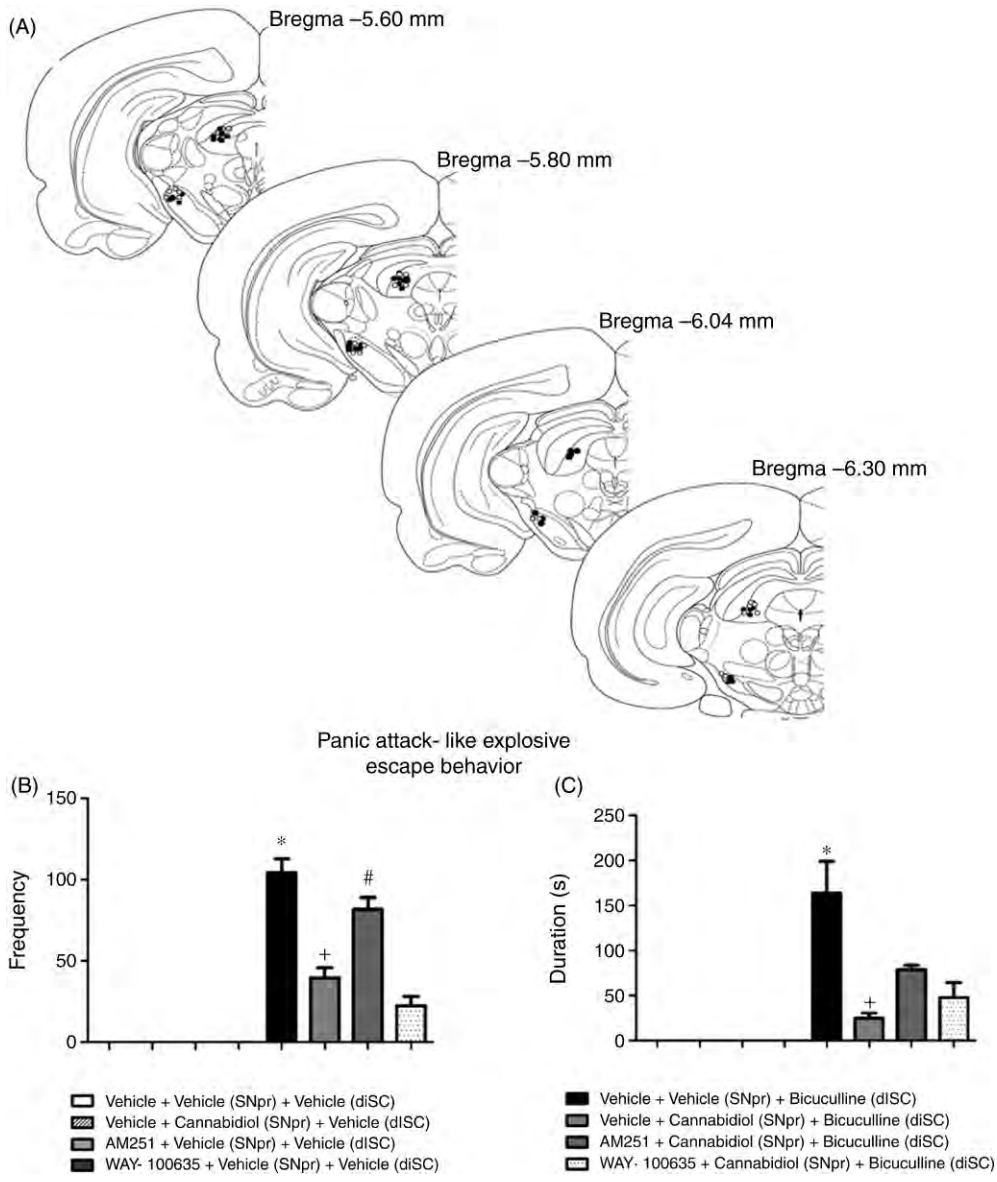


FIGURE e14.4 Panicolytic effects of cannabidiol. Effects of central administration of vehicle, CBD, AM251, or WAY100135 (n = 8 per group) into the substantia nigra pars reticulata (SNpr) on panic attack-like explosive escape behaviors elicited by GABA receptor blockade in the dorsal midbrain (DM), using microinjections of bicuculline (40 ng/200 nL) into the deep layers of the superior colliculus (dlSC). (A): hemitransverse section of the rat midbrain showing histologically confirmed sites of drug microinjections in the SNpr and DM, depicted in modified diagrams of rat brain stereotaxic atlas by Paxinos and Watson (2007). The columns represent the means, and the bars represent the standard errors of the means. $^{*}p < 0.001$, compared to vehicle + vehicle (SNpr) + vehicle (dlSC)-treated group; $^{+}p < 0.001$, compared to vehicle + vehicle (SNpr) + bicuculline (dlSC)-treated group; $^{\#}p < 0.001$, compared to vehicle + CBD (SNpr) + bicuculline (dlSC)-treated group, according to one-way ANOVA followed by Tukey's posthoc test.

WAY100135. These results suggest the involvement of SNpr CB1-cannabinoid receptors, but not serotonergic 5-HT_{1A} receptors, in the panicolytic-effects of intranigral microinjections of CBD (Da Silva et al., 2015).

A summary of the cortico-neostriatum-nigral disinhibitory GABAergic pathways and the nigro-tectal inhibitory GABAergic connections as possible morphological targets to partially explain the panicolytic effects of CBD is provided in Fig. e14.5.

CONCLUSIONS

In conclusion, the prey versus wild snake paradigm is a novel neuropsychobiological tool to investigate new drugs with potential anxiolytic and panicolytic effects. Peripheral CBD treatment of mice confronted with rainbow Boidae snakes in a polygonal arena produced a clear antipanic effect mediated by 5-HT_{1A} receptors. Dorsal and ventral mesencephalic neurons are the putative

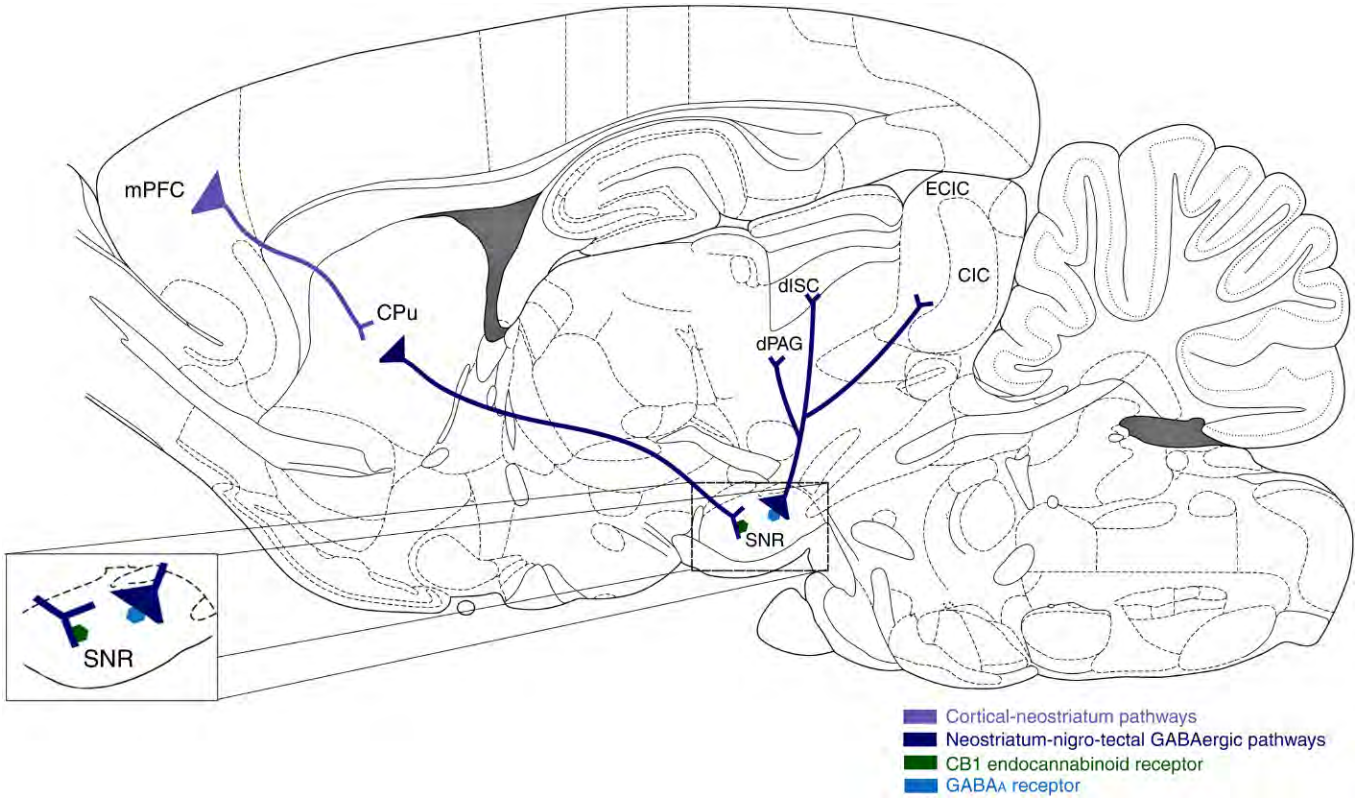


FIGURE e14.5 **New morphological targets for the panicolytic effect of CBD.** Schematic of a parasagittal section of *Rattus norvegicus* central nervous system showing *(in purple)* the cortico-neostriatal pathways, and *(in blue)* the striatum-nigral disinhibitory and nigro-tectal inhibitory GABAergic neural pathways involved in the modulation of innate fear-induced defensive behaviors organized by dorsal midbrain structures. *CPu*, caudatum-putamen; *mPFC*, medial prefrontal cortex; *SNR*, substantia nigra pars reticulata; *dPAG*, dorsal columns of the periaqueductal gray matter; *dlSC*, deep layers of the superior colliculus; *CIC*, central nucleus of the inferior colliculus; *ECIC*, external cortex of the inferior colliculus. *Modified drawing from the rat brain stereotaxic atlas of Paxinos and Watson (2007).*

pharmacological targets for these antiaversive drugs. The neostriatum-nigral disinhibitory and nigro-tectal inhibitory GABAergic pathways are possible neural substrates for the antipanic-like effect of CBD microinjections in the reticulate division of the substantia nigra. Microinjections of CBD into the SNpr produced a clear panicolytic effect. The GABAergic inputs to ventral mesencephalic structures are likely the central neural substrate of the panic attack attenuation effects of CBD in threatening situations.

Acknowledgments

We thank Daoud Hibrahim Elias Filho and Dr Tatiana Paschoalin Maurin for their technical assistance with Figs. e14.1 and e14.2. The authors declare no conflicts of interest with the data presented herein.

MINI-DICTIONARY

γ-Aminobutyric acid (GABA) Inhibitory neurotransmitter widely distributed in the brain.
Amygdaloid complex Prosencephalic (cerebral) limbic structure.
Cannabidiol One of the main compounds of the *Cannabis sativa* plant with medicinal properties.
Endocannabinoids Neuromodulators released on demand in the brain.
Hypothalamus Caudal division of the diencephalon.
Inferior colliculus Caudal division of the mesencephalic tectum.
Midbrain Also known as the mesencephalon, the midbrain consists as an anatomical division of the brainstem situated between the pons and medulla oblongata.
Neostriatum More phylogenetically recent part of the corpus striatum (basal nuclei).
Periaqueductal gray matter Set of neuronal nuclei surrounding the aquaeductus mesencephali (aqueduct of Sylvius).
Serotonin Neurotransmitter involved in several brain functions, found in some brainstem structures.
Substantia nigra A ventral mesencephalic structure divided into lateral, compacta, and reticulata regions.
Superior colliculus Rostral division of the mesencephalic tectum.

References

Adams, D. B. (2006). Brain mechanisms of aggressive behavior: an updated review. *Neuroscience and Biobehavioral Reviews*, *30*(3), 304–318.
Alger, B. E. (2002). Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. *Progress in Neurobiology*, *68*(4), 247–286.
Almada, R. C., Roncon, C. M., Elias-Filho, D. H., & Coimbra, N. C. (2014). Substantia nigra inputs modulates nigro-tectal pathways during the organisation of defensive behaviour in a dangerous environment. *European Neuropsychopharmacology*, *24*(2), S227–S228.

Almada, R. C., Roncon, C. M., Elias-Filho, D. H., & Coimbra, N. C. (2015). Endocannabinoid signaling mechanisms in the substantia nigra pars reticulata modulate GABAergic nigrotectal pathways in mice threatened by urutu-cruzeiro venomous pit viper. *Neuroscience*, *303*, 503–514.

Almeida, V., Levin, R., Peres, F. F., Niigaki, S. T., Calzavara, M. B., Zuardi, A. W., Hallak, J. E., Crippa, J. A., & Abílio, V. C. (2013). Cannabidiol exhibits anxiolytic but not antipsychotic property evaluated in the social interaction test. *Progress in Neuropsychopharmacology and Biological Psychiatry*, *41*, 30–35.

Andó, R. D., Bíró, J., Csölle, C., Ledent, C., & Sperlágh, B. (2012). The inhibitory action of exo- and endocannabinoids on [3H] GABA release are mediated by both CB1 and CB2 receptors in the mouse hippocampus. *Neurochemistry International*, *60*(2), 145–152.

Bergamaschi, M. M., Queiroz, R. H., Chagas, M. H., de Oliveira, D. C., De Martinis, B. S., Kapczinski, F., Quevedo, J., Roesler, R., Schröder, N., Nardi, A. E., Martín-Santos, R., Hallak, J. E., Zuardi, A. W., & Crippa, J. A. (2011). Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naïve social phobia patients. *Neuropsychopharmacology*, *36*(6), 1219–1226.

Bhattacharyya, S., Morrison, P. D., Fusar-Poli, P., Martin-Santos, R., Borgwardt, S., Winton-Brown, T., Nosarti, C., O'Carroll, C. M., Seal, M., Allen, P., Mehta, M. A., Stone, J. M., Tunstall, N., Giampietro, V., Kapur, S., Murray, R. M., Zuardi, A. W., Crippa, J. A., Atakan, Z., & McGuire, P. K. (2010). Opposite effects of [delta]-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology*, *35*(3), 764–774.

Biagioni, A. F., Silva, J. A., & Coimbra, N. C. (2012). Panic-like defensive behaviour but not fear-induced antinociception is differently organised by dorsomedial and posterior hypothalamic nuclei of *Rattus norvegicus* (Rodentia, Muridae). *Brazilian Journal of Medical and Biological Research*, *45*(4), 328–336.

Bisogno, T., Hanus, L., De Petrocellis, L., Tchilibon, S., Ponde, D. E., Brandi, I., Moriello, A. S., Davis, J. B., Mechoulam, R., & Di Marzo, V. (2001). Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *British Journal of Pharmacology*, *134*(4), 845–852.

Blanchard, R. J., Yudko, E. B., Rodgers, R. J., & Blanchard, D. C. (1993). Defense system psychopharmacology: an ethological approach to the pharmacology of fear and anxiety. *Behavioral Brain Research*, *58*(1–2), 155–165.

Borelli, K. G., Nobre, M. J., Brandão, M. L., & Coimbra, N. C. (2004). Effects of acute and chronic fluoxetine and diazepam on freezing behavior induced by electrical stimulation of dorsolateral and lateral columns of the periaqueductal gray matter. *Pharmacology Biochemistry and Behavior*, *77*(3), 557–566.

Bovier, P., Broekkamp, C. L., & Lloyd, K. G. (1982). Enhancing GABAergic transmission reverses the aversive state in rats induced by electrical stimulation of the periaqueductal grey region. *Brain Research*, *248*(2), 313–320.

Brandão, M. L., Coimbra, N. C., & Leão Borges, P. C. (1990). Effects of morphine and midazolam on reactivity to peripheral noxious and central aversive stimuli. *Neuroscience and Biobehavioral Reviews*, *14*, 495–499.

Campos, A. C., & Guimarães, F. S. (2008). Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. *Psychopharmacology*, *199*(2), 223–230.

Canteras, N. S. (2002). The medial hypothalamic defensive system: hodological organization and functional implications. *Pharmacology Biochemistry and Behavior*, *71*(3), 481–491.

Castellan-Baldan, L., Da Costa Kawasaki, M., Ribeiro, S. J., Calvo, F., Corrêa, V. M. A., & Coimbra, N. C. (2006). Topographic and functional neuroanatomical study of GABAergic disinhibitory striatum-nigral inputs and inhibitory nigrocollicular pathways: neural hodology recruiting the substantia nigra, pars reticulata, for the modulation of the neural activity in the inferior colliculus involved with panic-like emotions. *Journal of Chemical Neuroanatomy*, *32*(1), 1–27.

Coimbra, N. C., & Brandão, M. L. (1993). GABAergic nigro-collicular pathways modulate the defensive behaviour elicited by midbrain tectum stimulation. *Behavioural Brain Research*, *59*(1–2), 131–139.

Coimbra, N. C., De Oliveira, R., Freitas, R. L., Ribeiro, S. J., Borelli, K. G., Pacagnella, R. C., Moreira, J. E., Da Silva, L. A., Melo, L. L., Lunardi, L. O., & Brandão, M. L. (2006). Neuroanatomical approaches of the tectum-reticular pathways and immunohistochemical evidence for serotonin-positive perikarya on neuronal substrates of the superior colliculus and periaqueductal gray matter involved in the elaboration of the defensive behavior and fear-induced analgesia. *Experimental Neurology*, *197*(1), 93–112.

Da Silva, J.A. (2011). Neuropharmacological study of the interactions between opioid and endocannabinoid-signalled circuits of the substantia nigra, pars reticulata, and its role on the modulation of innate fear-induced analgesia. Scientiae Doctor Thesis. Ribeirão Preto, São Paulo: Ribeirão Preto Medical School of the University of São Paulo.

Da Silva, J. A., Biagioni, A. F., Almada, R. C., de Souza Crippa, J. A., Hallak, J. E. C., Zuardi, A. W., & Coimbra, N. C. (2015). Dissociation between the panicolytic effect of cannabidiol microinjected into the substantia nigra, pars reticulata, and fear-induced antinociception elicited by bicuculline administration in deep layers of the superior colliculus: the role of CB1-cannabinoid receptor in the ventral mesencephalon. *European Journal of Pharmacology*, *758*, 153–163.

De Petrocellis, L., Vellani, V., Schiano-Moriello, A., Marini, P., Magherini, P. C., Orlando, P., & Di Marzo, V. (2008). Plant-derived cannabinoids modulate the activity of transient receptor potential channels of ankyrin type-1 and melastatin type-8. *Journal of Pharmacology and Experimental Therapeutics*, *325*(3), 1007–1015.

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., & Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*, *258*(5990), 1946–1949.

Di Chiara, G., Porceddu, M. L., Morelli, M., Mulas, M. L., & Gessa, G. L. (1979). Evidence for a GABAergic projection from the substantia nigra to the ventromedial thalamus and to the superior colliculus of the rat. *Brain Research*, *176*(2), 273–284.

Di Marzo, V., Melck, D., Bisogno, T., & De Petrocellis, L. (1998). Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. *Trends in Neuroscience*, *21*(12), 521–528.

Di Scala, G., Schmitt, P., & Karli, P. (1983). Unilateral injection of GABA agonists in the superior colliculus: asymmetry to tactile stimulation. *Pharmacology Biochemistry and Behaviour*, *19*(2), 281–285.

Eichenberger, G. C. D., Ribeiro, S. J., Osaki, M. Y., Maruoka, R. Y., Resende, G. C. C., Castellan-Baldan, L., Corrêa, S. A. L., Da Silva, L. A., & Coimbra, N. C. (2002). Neuroanatomical and psychopharmacological evidence for interaction between opioid and GABAergic neuronal pathways in the modulation of fear and defense elicited by electrical and chemical stimulation of deep layers of the superior colliculus and dorsal periaqueductal gray matter. *Neuropharmacology*, *42*(1), 48–59.

Faull, R. L., & Mehler, W. R. (1978). The cells of origin of nigrotectal, nigrothalamic and nigrostriatal projections in the rat. *Neuroscience*, *3*(11), 989–1002.

Freund, T. F., Katona, I., & Piomelli, D. (2003). Role of endogenous cannabinoids in synaptic signaling. *Physiological Reviews*, *83*(3), 1017–1066.

Gong, J. P. (2006). Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Research*, *1071*(1), 10–23.

Graeff, F. G. (1990). Brain defense systems and anxiety. In M. Roth, G. D. Burrows, & R. Noyes, Jr. (Eds.), *Handbook of anxiety* (pp. 307–354). Amsterdam: Elsevier.

Gray, J. A., & McNaughton, N. (2000). *The neuropsychology of anxiety: an enquiry into the functions of the septo-hippocampal system* (2nd ed.). London: Oxford University Press (Chapter 2).

Grofová, I., Ottersen, O. P., & Rinvik, E. (1978). Mesencephalic and diencephalic afferents to the superior colliculus and periaqueductal gray substance demonstrated by retrograde axonal transport of horseradish peroxidase in the cat. *Brain Research*, *146*(2), 205–220.

Guimarães, F. S., Chiaretti, T. M., Graeff, F. G., & Zuardi, A. W. (1990). Antianxiety effect of cannabidiol in the elevated plus-maze. *Psychopharmacology*, *100*(4), 558–559.

Guimarães-Costa, R., Guimarães-Costa, M. B., Pippa-Gadioli, L., Weltson, A., Ubiali, W. A., Paschoalin-Maurin, T., Felippotti, T. T., Elias-Filho, D. H., Laure, C. J., & Coimbra, N. C. (2007). Innate defensive behaviour and panic-like reactions evoked by rodents during aggressive encounters with Brazilian constrictor snakes in a complex labyrinth: behavioural validation of a new model to study affective and agonistic reactions in a prey versus. *Journal of Neuroscience Methods*, *165*(1), 25–37.

Herkenham, M., Lynn, A. B., Johnson, M. R., Melvin, L. S., de Costa, B. R., & Rice, K. C. (1991). Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *Journal of Neuroscience*, *11*(2), 563–583.

Kretz, R. (1984). Local cobalt injection: a method to discriminate presynaptic axonal from postsynaptic neuronal activity. *Journal of Neuroscience Methods*, *11*(2), 129–135.

Lobão-Soares, B., Walz, R., Prediger, R. D. S., Freitas, R. L., Calvo, F., Bianchin, M., Leite, J. P., Landemberger, M. C., & Coimbra, N. C. (2008). Cellular prion protein modulates defensive attention and innate fear-induced behaviour evoked in transgenic mice submitted to an agonistic encounter with the tropical coral snake *Oxyrhopus guibei*. *Behavioral Brain Research*, *194*(2), 129–137.

López-Moreno, J. A., López-Jiménez, A., Gorriti, M. A., & De Fonseca, F. R. (2010). Functional interactions between endogenous cannabinoid and opioid systems: focus on alcohol, genetics and drug-addicted behaviors. *Current Drug Targets*, *11*(4), 406–428.

Mansour, A., Khachaturian, H., Lewis, M. E., Akil, H., & Watson, S. J. (1988). Anatomy of CNS opioid receptors. *Trends in Neuroscience*, *11*(7), 308–314.

Manzanares, J., Corchero, J., Romero, J., Fernández-Ruiz, J. J., Ramos, J. A., & Fuentes, J. A. (1999). Pharmacological and biochemical interactions between opioids and cannabinoids. *Trends in Pharmacological Sciences*, *20*(7), 287–294.

Maurin, T. P. (2008). Neuromorphologic and behavioural study of the effect of chronic treatment with paroxetine or alprazolam on defensive behaviours evoked during confrontation between *Mesocricetus auratus* and the venomous coral snake *Micrurus frontalis*. Scientiae Doctor Thesis. Ribeirão Preto, São Paulo: Medical School of the University of São Paulo.

McNaughton, N., & Corr, P. J. (2004). A two-dimensional neuropsychology of defense: fear/anxiety and defensive distance. *Neuroscience and Biobehavioral Reviews*, *28*(3), 285–305.

Mechoulam, R., Fride, E., & Di Marzo, V. (1998). Endocannabinoids. *European Journal of Pharmacology*, *359*(1), 1–18.

Nashold, B. S., Jr., Wilson, W. P., & Slaughter, D. G. (1969). Sensations evoked by stimulation in the midbrain of man. *Journal of Neurosurgery*, *30*(1), 14–24.

Onaivi, E. S., Green, M. R., & Martin, B. R. (1990). Pharmacological characterization of cannabinoids in the elevated plus maze. *Journal of Pharmacology and Experimental Therapeutics*, *253*(3), 1002–1009.

Paxinos, G., & Watson, C. (2007). *The rat brain in stereotaxic coordinates* (6th ed.). Sydney: Academic Press.

Pertwee, R. G. (1997). Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacology and Therapeutics*, *74*(2), 129–180.

Redgrave, P., Marrow, L., & Dean, P. (1992). Topographical organization of the nigrotectal projection in rat: evidence for segregated channels. *Neuroscience*, *50*(3), 571–595.

Russo, E. B., Burnett, A., Hall, B., & Parker, K. K. (2005). Agonistic properties of cannabidiol at 5-HT_{1A} receptors. *Neurochemical Research*, *30*(8), 1037–1043.

Sandkühler, J., Maisch, B., & Zimmermann, M. (1987). The use of local anaesthetic microinjections to identify central pathways: a quantitative evaluation of the time course and extent of the neuronal block. *Experimental Brain Research*, *68*(1), 168–178.

Schlicker, E., & Kathmann, M. (2001). Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends in Pharmacological Sciences*, *22*(11), 565–572.

Shekhar, A., Hingtgen, J. N., & DiMicco, J. A. (1990). GABA receptors in the posterior hypothalamus regulate experimental anxiety in rats. *Brain Research*, *512*(1), 81–88.

Shekhar, A., Keim, S. R., Simon, J. R., & McBride, W. J. (1996). Dorsomedial hypothalamic GABA dysfunction produces physiological arousal following sodium lactate infusions. *Pharmacology Biochemistry and Behaviour*, *55*(2), 249–256.

Silveira Filho, N. G., & Tufik, S. (1981). Comparative effects between cannabidiol and diazepam on neophobia, food intake and conflict behavior. *Research Communications in Psychology, Psychiatry and Behavior*, *6*(3), 251–266.

Soares, V. de P., Campos, A. C., Bortoli, V. C., Zangrossi, H., Jr., Guimarães, F. S., & Zuardi, A. W. (2010). Intra-dorsal periaqueductal gray administration of cannabidiol blocks panic-like response by activating 5-HT1A receptors. *Behavioral Brain Research*, *213*(2), 225–229.

Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A., & Waku, K. (1995). 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochemical and Biophysical Research Communications*, *215*(1), 89–97.

Thomas, A., Baillie, G. L., Phillips, A. M., Razdan, R. K., Ross, R. A., & Pertwee, R. G. (2007). Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. *British Journal of Pharmacology*, *150*(5), 613–623.

Tsou, K., Brown, S., Sanudo-Pena, M. C., Mackie, K., & Walker, J. M. (1998). Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience*, *83*(2), 393–411.

Twardowschy, A., Castiblanco-Urbina, M. A., Uribe-Mariño, A., Biagioni, A. F., Salgado-Rohner, C. J., de Souza Crippa, J. A., & Coimbra, N. C. (2013). The role of 5-HT_{1A} receptors in the anti-aversive effects of cannabidiol on panic attack-like behaviors evoked in the presence of the wild snake *Epicrates cenchria crassus* (Reptilia, Boidae). *Journal of Psychopharmacology*, *27*(12), 1149–1159.

Uribe-Mariño, A., Francisco, A., Castiblanco-Urbina, M. A., Twardowschy, A., Salgado-Rohner, C. J., Crippa, J. A. S., Hallak, J. E. C., Zuardi, A. W., & Coimbra, N. C. (2012). Anti-aversive effects of cannabidiol on innate fear-induced behaviors evoked by an ethological model of panic attacks based on a prey vs the wild snake *Epicrates cenchria crassus* confrontation paradigm. *Neuropsychopharmacology*, *37*(2), 412–421.

Wallmichrath, I., & Szabo, B. (2002). Cannabinoids inhibit striatonigral GABAergic neurotransmission in the mouse. *Neuroscience*, *113*(3), 671–682.

Weltson, A., Pippa-Gadioli, L., Koji-Narazaki, R., Paschoalin-Maurin, T., Del Bel, E. A., & Coimbra, N. C. (2002). Neuromorphologic evidence for c-Fos-immunoreactive neurons in forebrain, diencephalon and brainstem structures involved in the elaboration of panic and fear in golden hamster after aggressive encounter with South American coral snake. *International Journal of Psychophysiology*, *45*(1–2), 156–157.

Zangrossi, H., Jr., & Graeff, F. G. (2004). Modelos animais. In L. A. B. Hetem, & F. G. Graeff (Eds.), *Transtornos de ansiedade* (pp. 55–73). São Paulo: Atheneu.

Zuardi, A. W. (2008). Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. *Revista Brasileira de Psiquiatria*, *30*(3), 271–280.

Zuardi, A. W., & Karniol, I. G. (1983). Effects on variable-interval performance in rats of delta 9-tetrahydrocannabinol and cannabidiol, separately and in combination. *Brazilian Journal of Medical and Biological Research*, *16*(2), 141–146.

CHAPTER

e15

Spice Use Among United States Military Personnel

H.A. Morris, J.M. Stogner

Department of Criminal Justice and Criminology, University of North Carolina at Charlotte, Charlotte, NC, United States

SUMMARY POINTS

- Because of its "legal" state, synthetic cannabinoids were introduced to the military for those who would otherwise use illegal drugs.
- In addition to its legal status, synthetic cannabinoids are perceived as safer than other psychoactive drugs; hence, why many more individuals, including service members, are abusing synthetic cannabinoids.
- Use of synthetic cannabinoids is likely higher among military personnel than comparable populations, due to the frequency of drug testing for marijuana.
- According to the Navy.mil website, synthetic marijuana (or synthetic cannabinoids) is being added to the current drug test screening for naval officers.
- Because of the rigors and stresses that being on active duty produce, military personnel feel the need to alleviate their stress through the use of drugs, more recently synthetic cannabinoids.
- Policy that allows service members to seek alternative ways to deal with the stress of being active, such as psychiatric help, needs to be implemented.

KEY FACTS ABOUT SYNTHETIC CANNABINOIDS AND DRUG USE IN THE MILITARY

- There is a long history of substance use among active duty and former military personnel.
- Military personnel are tested for use of illicit substances more frequently than individuals in other careers, suggesting that marijuana use is more risky for military personnel.
- Synthetic cannabinoids are more commonly referred to as synthetic cannabis or synthetic marijuana, and their effects are similar to that of cannabis.
- Synthetic cannabinoids have been used as an alternative for marijuana for over a decade, but use became increasingly prevalent starting around 2008.
- Only limited information exists about synthetic cannabinoid use in the military.

LIST OF ABBREVIATIONS

AM	Alexandros Makriyannis
CP	Charles Pfizer
CSA	Controlled Substances Act (1970)
DoD	Department of Defense
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
HU	Hebrew University (Raphael Mechoulam)
JWH	John W. Huffman
SCs	Synthetic cannabinoids
THC	Tetrahydrocannabinol

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00102-2
Copyright © 2017 Elsevier Inc. All rights reserved.

INTRODUCTION

Psychoactive drugs are often used as a means of "escaping" from dreadful moments and stressful life events. Many of those who abuse drugs recreationally are not content with their lifestyle and/or seek alternative mental states to alleviate stress, establish a sense of control, or avoid thinking about problematic issues. Active-duty military personnel experience great deals of stress as a result of their commitment to the armed forces, both due to the rigors of the job and the threat of bodily harm (Ballweg & Li, 1991; Berry-Cabán, Kleinschmidt, Rao, & Jenkins, 2012). These men and women are oftentimes placed in situations where they cannot afford to show any signs of weakness to their peers or superiors. In fact, training may emphasize the need to reduce or eliminate weakness, doubt, and fear. For some, this task becomes one of internalization and compartmentalization. Others confront their emotions and deal with them successfully either by addressing and accepting their situation independently, or with the aid of family and peers. For others, a less healthy approach may be selected—one that involves coping through drug and alcohol use. Alcohol, tobacco, and illicit drug use are all prominent among the United States military (Ballweg & Li, 1991; Berry-Cabán et al., 2012). This is partially due to substances' short-term effects, which may alleviate stress (or the awareness of it).

In the past, the US military provided drugs and cigarettes to their soldiers to help them with their duties and maintain morale. Amphetamines were used to keep soldiers awake for long missions, while cigarettes and alcohol were available to help them relax after the missions were completed (Bray, Kroutil, & Marsden, 1995; Ballweg & Li, 1991; Bray et al., 1986; Bachman, Freedman-Doan, O'Malley, Johnston, & Segal, 1999). This provision of drugs and tobacco to soldiers led to dependences (Bachman et al., 1999), and accidents due to intoxication (Berry-Cabán et al., 2012), which subsequently led the military to cease providing drugs and create drug-testing policies (Borack, 1998). However, even rigorous testing policies are not uniformly successful, particularly within a population predisposed to substance use (young males) and under a great deal of stress. Soldiers developed a "clean urine" black market in which urine from nonusers was sold to other soldiers so that urinalyses would yield negative results. This initial loophole was eventually combated by stricter guidelines in testing (ie, medical personnel watched the personnel provide urine). However, this did not diminish the desire to use psychoactive substances, and only led potential substance users to adapt and become more creative. The soldiers, who would otherwise abuse well-understood drugs, and likely those with limited health consequences (eg, marijuana), but had to pass urinalysis testing, sought alternative substances that were "legal highs," not included in standard drug screens. Synthetic cannabinoids and synthetic cathinones, also known as "designer drugs," are "produced clandestinely and contain modified molecular structures of illegal or controlled substances" (Loeffler, Hurst, Penn, & Yung, 2012, p. 1041). These designer drugs are chemically structured in a way which allows users to experience a high similar to that of the analogous street drug (eg, marijuana for synthetic cannabinoids, methamphetamine or cocaine for synthetic cathinones), but that does not result in failed drug tests. The US military was proactive in dealing with these so-called "legal highs," establishing bans, requiring testing, and developing educational materials. The potential use of designer drugs among military personnel has led to extensive research involving these (formerly) legal highs by military medical services. While this line of inquiry and policy development is critical and efficient, we also stress that the armed forces may similarly reduce substance use by improving the resources available to soldiers for dealing with psychological issues (ie, counseling, psychiatric help, etc.). The current chapter focuses on drug abuse trends among US military personnel, more specifically designer drug use among US active-duty soldiers.

HISTORY

Drug abuse has been common among soldiers in the US military, and in other armies, throughout history. Substance use was commonly viewed as acceptable, and even as recommended due to the harsh realities of combat and combat preparation (Bray et al., 1986, 1995; Ballweg & Li, 1991; Bachman et al., 1999). From morphine and opiate use by American Civil War soldiers to designer drug use today, drugs have continually been utilized as a means of coping "with the challenges of conflict in a setting of dislocation and devastation" (Golub & Bennett, 2013, p. 2). Alcohol, cigarettes, and common illicit drugs such as marijuana were the most prevalent in the preceding decades (Bray, Marsden, Herbold, & Peterson, 1992), but new synthetic drugs may be gaining a foothold.

Today, the Department of Defense (DoD) focuses on maintaining a healthy and combat-ready military through attempting to minimize alcohol, tobacco, and illicit drug use among the soldiers. This has not always been the case—it was once considered the norm for an American soldier to abuse alcohol, tobacco, and other drugs while on serving their country. Alcohol rations and other drugs were given to soldiers by their authorities as "rewards for extra service" (Ballweg & Li, 1991, p. 601). Bray et al. (1995) noted "that within the predominantly male military population, heavy drinking and being able to 'hold one's liquor' have served as tests 'of

suitability for the demanding masculine military role' (pp. 271–272). The perceived need to hypermasculinize one's self is considered a reason that drug use of many sorts is prevalent within the military. Ballweg and Li (1991) note that there are three main motivations of alcohol consumption by soldiers: "relief of boredom, relaxation from tension and anxieties, and reinforcement of the masculine mystique" (pp. 601–602).

Not only was alcohol use acceptable and encouraged within the US military, but other substances, now illicit street drugs, have been provided to soldiers by the United States and other countries. Amphetamine and other stimulants were provided to soldiers in order to help them work harder and longer hours (Kuzmarov, 2009a; Golub & Bennett, 2013). The military would circulate synthetic derivatives of amphetamine, known as "pep pills" to soldiers during the Vietnam War, in order to stay awake while on long missions. Stimulants were also the most prevalent during the period before and during WWII (1935–45); soldiers would oftentimes become dependent, continuing abuse when they left the service (Golub & Bennett, 2013). During the Vietnam War (1955–75), heroin was also popular, and the United States saw the highest documented prevalence of military drug use (Kuzmarov, 2009a). In addition to pep pills, and other amphetamines, soldiers would take drugs to help them sleep in order to "come down" from and relax (Golub & Bennett, 2013). Because marijuana was so readily available, grew naturally, had high potency, and was very cheap for the soldiers, estimates of the prevalence of recreational marijuana use among US soldiers were as high as 90% during that time (Kuzmarov, 2009a).

The government generally took a relatively relaxed position on drug abuse within the military (Kuzmarov, 2009a). From drinking alcohol and caffeinated drinks, such as coffee, in the American Revolution, to amphetamine provisions during WWII (Golub & Bennett, 2013), there has always been a close, oft ignored, relationship between military service and substance abuse. The historical amphetamine provision, recreational drug abuse among the soldiers, and the prevalence of self-medication within the US military today should draw attention to the issue of military substance use and testing. Some scholars have identified inconsistencies in the military's treatment of drug use among US soldiers (Donovan, 2010; Bray et al., 1992; Bray et al., 2010). Drug use was encouraged and very prevalent historically, but is now frowned upon. In fact, pharmaceutical scientists made efforts to extract the THC and "synthesize cannabinoid receptor agonists" for soldiers to reduce symptoms such as nausea and pain, in 1964 (Loeffler et al., 2012, p. 1041). The recent punitive treatment of substance use and/or possession has done little to deter substance use today (Heath, 1992; Berry-Cabán et al., 2012), but more research is needed.

Because the views of drug use of key military leaders have changed—moving from pushing and providing them to extensive drug screening—aspects of soldier lifestyles have also changed. During WWII, soldiers were actively abusing various types of amphetamines, often provided by the military, and becoming dependent (Bachman et al., 1999). Many eventually took larger doses to get quicker and more enhanced effects, some returned to the United States as a dependent user, and several accidents while on duty were attributed to intoxication (Kuzmarov, 2009a). Once again, these issues raised concerns and led the US military to change its views on drug use and begin wide scale drug testing on the soldiers. Policies were developed to force users to desist, and deter potential users from administering illicit substances. Intense drug testing began in the early 1970s, when accidents from intoxication of amphetamines and heroin were becoming more regular (Kuzmarov, 2009a). According to Dr Jerome H. Jaffe, the physician in charge of the urinalysis testing at this time, low numbers of heroin-positive tests were reported: "[of departing soldiers] 3.6 percent were found to have heroin in their urine, and 5.5 percent were thought to have previously used the drug. Only 1 percent of 13,190 US Air Force personnel tested positive, and 0.3 percent of the 5,754 from the US Navy, Coast Guard, and Marine Corps" (Kuzmarov, 2009a, p. 19). This system did have unintended consequences. A "black market" for "clean urine" developed among the soldiers. Dr Jaffe jokingly claimed the soldiers were using anything, from "beer to their grandmother's urine," in order to have a clean test (p. 19). There were no apparent measures for ensuring to the military that the soldiers were in fact giving their own urine. After recognition of this loophole, military personnel were forced to watch each soldier urinate into a collection cup to ensure accurate urinalysis results (*DOD Urinalysis Program, 2014*). Additionally, the US military provides a "chain-of-custody" document for every specimen which records everyone involved in the collection or transportation of that sample. For example, "the observer who watched the person collect the sample, the person who puts it into the box, or the person who takes it out of the box" can be identified (*DOD Urinalysis Program, 2014,* para. 3). Regulations on drug testing such as this has aided the US military in collecting accurate samples, but new policies have failed to fully resolve the issue of substance use within the military.

Although urinalysis testing for known drugs of abuse has become more rigorous, drug use is still prevalent among US soldiers and other military personnel today. However, as remaining active-duty requires passing these tests, and sidestepping analytic techniques is challenging, soldiers sought new ways to alter their mental state without violating military policy. They sought alternatives of illegal drugs (for which they were being

screened) that would not affect their test results. Soldiers would use these novel synthetic substances to experience the same euphoria, stimulation, or relaxation as their illicit counterparts, while still avoiding positive drug tests (Kuzmarov, 2009a; Loeffler et al., 2012). This form of replacement would eventually lead to essentially the same problems that led to the development of the rigorous testing protocols in the first place: accidents, overdoses, dependency, and unhealthy and irrational soldiers (Kuzmarov, 2009a; Khey, Stogner, & Miller, 2014). Many of these alternative "legal highs" were eventually banned by most of branches of the US military in 2010, ahead of federal regulation (Vardakou, Pistos, & Spiliopoulou, 2010). Of these novel synthetic substances, the most commonly used forms fall under the umbrella of "synthetic cannabinoids," colloquially referred to as "spice" after one of the more popular brand names, and mimic the effect of cannabis.

TABLE e15.1 Known Brand Names of Synthetic Cannabinoids

Aroma	Hard Core	Spark
Blaze	Highdi's Almadrohner	Spice Arctic Synergy
Blue Lotus	K2	Spice Diamond
ChillX	Magma	Spice Egypt
Deliverance	Moon Rocks	Spice Gold
Dream	PEP Spice	Spice Gold Spirit
Earth Impact	PEPpourri	Spice Silver
Ex-ses	Scope	Spice Tropical Synergy
Fusion	Sence	Spike 99
Galaxy Gold	Skunk	Truckin
Genie	Smoke	Yucatan Fire
Gorillaz	Solar Flare	

SYNTHETIC CANNABINOIDS OR "SPICE"

Legal drugs that produce a similar euphoric state to cannabis are considered "synthetic cannabinoids," a form of "designer drugs" or "novel highs," by pharmaceutical companies and other various drug administrations. Designer drugs are those "that are produced clandestinely and contain modified molecular structures of illegal or controlled substances" (Loeffler et al., 2012, p. 1041). Synthetic cannabinoids are one of the broader categories of new designer drugs, and initially allowed users to achieve a level of euphoria similar to that of cannabis, without violating the law, thus the term, "legal high." Synthetic cannabinoids can come in many various forms, but the most common brands were "Spice" and "K2," and included the chemicals such as JWH-200 and JWH-018. The JWH chemical compounds, the largest label of synthetic cannabinoids, are named after an organic chemist at Clemson University, John W. Huffman, credited for their initial synthesis (Loeffler et al., 2012). Other synthetic cannabinoids are often preceded by CP, AM, or HU [standing for Charles Pfizer, Alexandros Makriyannis, and Hebrew University (Raphael Mechoulam), respectively]. CP-47,497 was often found in "spice," as was HU-210, which is noted to be anywhere between 100 and 800 times stronger than THC (Loeffler et al., 2012). Though initially a brand name of one specific synthetic cannabinoid product, spice may now refer to any substance that can be smoked or inhaled, and that reacts on the body similarly to that of cannabis. It can be thought of as the street label for all synthetic cannabinoids (much like how "Band-Aid" brand is used to describe any type of simple bandage). "Bath salts" refers to synthetic cathinone-like chemicals sold as novel highs, but are very different in terms of effect and user profile from synthetic cannabinoids (Berry-Cabán et al., 2012).

As mentioned previously, synthetic cannabinoids come in various forms; many are packaged in small foil packets with colorful cartoon logos. They may carry a label of potpourri or other suggested function, but the packaging, name, and price all allude to its intended recreational purpose. Synthetic cannabinoids consisting "of psycho-actively inert dry plant material sprayed with synthetic cannabinoid receptor agonists" were first introduced in Europe in 2004 under the product names "Spice," "Arctic Spice," "Dream," and "K2" (Loeffler et al., 2012, p. 1041). Other products marketed as spice include Spike 99, Blaze, Genie, and Spice Diamond (Flor, 2010; Sedefov et al., 2009). For a lengthier list of synthetic product names, see Table e15.1.

Spice may create a euphoric state for the user, much like cannabis, although it can be between 4 and 100 times stronger than regular THC (Berry-Cabán et al., 2012). The Controlled Substances Act of 1970 controlled substances that could potentially be abused, and gave many substances "abuse liability" profiles, which placed them within certain classes or "schedules" of drugs (Khey et al., 2014), but synthetic cannabinoids were not assigned a schedule until recently. In order to sidestep the CSA and the Controlled Substances Analogue Enforcement Act of 1986 in the United States, spice products are typically labeled with the "not for human consumption." This regulation only bans sale or possession of analogues of illicit substances if they are intended for human use. This made spice technically legal, so long as it was labeled, advertised, and sold as a product with another purpose (potpourri). The Synthetic Drug Abuse Prevention Act (2012) eventually scheduled several cannabinoids, allowed for the

TABLE e15.2 Drugs on the Navy Drug Screen

Marijuana
Cocaine
Amphetamine/methamphetamine
Designer amphetamines (MDMA, MDA)
Heroin
Opiates (codeine, morphine, oxycodone, oxymorphone, hydrocodone, hydromorphone)
Benzodiazepines (α-OH-alprazolam, lorazepam, nordiazepam, oxazepam, temazepam)

federal government to more easily place substances on an emergency scheduling list, in which the Attorney General feels the substance has an "imminent hazard to public safety" and must take into concern: "(i) the history of the drug or substance and its current pattern of abuse; (ii) the scope, duration, and significance of the drug or substance's abuse; (iii) the risk to public health; (iv) diversion of the drug or substance from legitimate channels; and (v) the drug or substance's 'clandestine importation, manufacture, or distribution' (quoted in Khey et al., 2014). This new act made it much easier for the government to quickly place synthetic cannabinoids into a scheduled tier, once discovered and extended the amount of time that a substance may be "emergently scheduled" prior to reevaluation.

Additionally, spice was not initially detected on routine drug tests, making it easier for soldiers or other individuals undergoing routine drug testing to be able to use a drug while remaining "legal." Drug testing by urinalysis assessments began in 1981 with the Navy's "zero-tolerance" policy on drug use (Borack, 1998), but the Navy is not yet uniformly testing for synthetic cannabinoids (Navy Drug Screening Labs, 2015). For a list of all drugs for which the Navy screens, see Table e15.2. Sweeps of the soldiers' bunks were completed, in addition to soldiers being required to provide urine, however, once soldiers learned how and what they were being tested for, analogues were used to regulations. Thus, synthetic cannabinoids were particularly appealing to enlisted personnel between their introduction and their eventual inclusion on urine screens.

MEDIA COVERAGE OF MILITARY SUBSTANCE USE

Popular media and political rhetoric in the past several decades has painted drug abuse as a substantial problem. As a result, the United States government authorized a "War on Drugs" and enacted legislation that harshly punished drug users. Since the Nixon and Reagan administrations, punitive responses have intensified, even for minor drug offenses that may have otherwise resulted in a citation or fine (Kuzmarov, 2009b). The "War on Drugs" is largely responsible for the substantial increase in prison population, but that does not seem to be a major issue in the eyes of the media. The issue of drug dependence is a substantial one, and needs to be addressed increasingly through intervention and rehabilitation, and less by lengthy mandatory sentences. This, unfortunately, has not been the way the mass media portrays this issue, or the overwhelming public sentiment. A national poll in Sep. 1989 indicated that almost 65% of the nation agreed that drug use is "the major issue facing this country" after President Bush Sr proclaimed the country needed to take a "tough on crime" stance (Heath, 1992, p. 270). Around the early 2000s, drug abuse was still considered a major issue in our society, and costs were increasing at an exponential rate, costing US citizens around $484 billion (National Institute of Health, National Institute on Drug Abuse, 2001). Today, fewer US citizens are using and abusing drugs. According to the National Crime Justice Reference Service, America's drug abuse profile was indicating less drug abuse. Around 12.8 million Americans aged 12 and older abuse illicit drugs—a massive 50% decrease of illicit drug use from earlier extremes.

Not only was the issue of drug use considered a substantial issue in the United States, but drug use among the US military was a specific issue of emphasis. While drug use has been and remains prevalent among military personnel, the media may have sensationalized the relationship between military service and substance use in several instances. Regardless of media coverage, drug use among the military should be, and is, an issue that needs to be addressed, but the "war on drugs" and "tough on crime" philosophies prevalent within today's polity may themselves be dangerous, as would be inaccurate and sensationalized coverage. According to Kuzmarov (2009b), media rumors in the 1960s and 1970s "created the perception that drug use was endemic in Vietnam and linked to a breakdown in military discipline and fighting efficiency," as well as "promoted widespread fear that addicted soldiers returning to the United States would exacerbate domestic crime rates and disorder" (p. 189). These perceptions by the media fueled a nationwide panic that drug use was rampant within the US military.

To further provoke the moral panic of drug use among US military personnel, General Barry R. McCaffrey made an inflammatory statement while under President Clinton's administration:

> The US Army that I was a part of and so loved was almost destroyed by illegal drugs in the 1970s. Up to two thirds of our battalion was using drugs all the time or some of the time, and the impact on our discipline, on rape, violence, spiritual loss of focus, physical health was just atrocious. (*as cited in* Kuzmarov, 2009b, p. 191)

Recently, federal court cases involving military drug use have been on the rise. Many are related to newly emergent substances such as synthetic cannabinoids. In *United States* v. *Larry* (2010) a defendant was convicted of possession and intent to distribute spice, and given a 6-month sentence (Flor, 2010). This case was appealed because there were questions addressing if "possession with intent to distribute the substance is a violation of Article 134," but the original conviction remained (Flor, 2010, p. 24). Although Article 134 did not include language specific to synthetic cannabinoids, the court ruled, someone may still be convicted under Article 134, given its military language, for possession with intent to distribute spice products. The ruling confirmed that unspecified behaviors "such as introduction or simple possession of Spice, may qualify as chargeable offenses under Article 134 if the Government can prove that the conduct was prejudicial to good order and discipline in the Armed Forces" (Flor, 2010, p. 25). Nevertheless, the majority of reports are on *illicit* drug use, leaving questions regarding the extent of the misuse of synthetic cannabinoids and cathinones in the military.

With credible statements such as the one by Gen. McCaffrey (Kuzmarov, 2009b), the general population, as well as the media, has had reason to believe drug abuse among the military was a substantial problem. Continuing the "war on drugs" and "zero-tolerance" approaches, President George W. Bush wanted to ensure the public that our troops are not, by any means, abusing illegal drugs while on duty. He maintained the policies from the original drug abuse acts of the late 20th century, such as the Anti-Drug Abuse Act of 1986, Drug Abuse Prevention and Control Act of 1970, the Controlled Substances Act (CSA) of 1970, as well as funding military troops to travel to Colombia for combating drug traffickers (Khey et al., 2014; Geffray, 2001), and pushed to ensure that illegal drugs were not making their way into the US military. His policies and those already in existence may have led military personnel to seek alternative means of achieving a high, facilitating the development of a black market for "legal highs" among the troops.

Due to the media's emphasis on drug abuse within the US military, our society tends to believe that our military forces might be frequently "high" while active on duty. The resulting moral panic has in turn led politicians to enforce strict "zero-tolerance" policies and restrictions on active US military personnel. Research has shown that this has decreased the use of *illegal* drug use substantially (Kuzmarov, 2009a). However, active duty soldiers are continuing to achieve the "highs" they desire without disobeying the new policies. How? With the introduction of "legal highs" or synthetic cannabinoids as an alternative to illegal drug use, soldiers are sidestepping the system. New policies that address the use of Spice and other synthetic cannabinoids within the US military have been adopted in order to continue the "tough" approaches to keeping our troops sober. However, a major issue with acknowledging synthetic cannabinoids as a scheduled drug is the complications with detecting them (Khey et al., 2014). Further, as new drugs are added to drug screens, still newer variants replace them which may not be specifically regulated or lead to a positive test.

POLICY

The Controlled Substances Act (CSA), enacted in 1970, categorized substances according to "schedules" linked to a certain levels of regulation (Khey et al., 2014). For example, drugs such as marijuana, cocaine, and heroin were categorized as Schedule I drugs, but legal highs often remain unscheduled for some time after their initial introduction. The list of scheduled drugs did not include Spice or similar synthetic cannabinoids until recently, even though the option to place a substance as "temporary" or "emergency" was put in place in 1984. The Federal Analogue Act does not provide clear definitions of similarly designed drugs, and is sometimes challenged in court for vagueness (Khey et al., 2014). This analogue provision does ban compounds chemically similar to specifically scheduled substances, but the legislation is easily rendered ineffective so long as analogues are labeled "not for human consumption." Consequently, the government passed the "Synthetic Drug Abuse Prevention Act" in 2012. This Act clarified the process for temporary or emergency scheduling, and further banned several synthetic cannabinoids.

Drug testing can also be useful in reducing the amount of drug use in any given environment by two ways: (1) terminating the careers of those who do not pass their drug screening, and (2) deterring individuals from taking illicit drugs initially in order to pass their drug tests. This can directly be applied to the abuse of legal highs within the military. In a study to evaluate the deterrence potential of drug use policy among Navy service members in 1998, Jules I. Borack compared the impact of drug testing in the Navy with drug testing among nonactive duty civilians. This allowed Borack to assess whether the "threat" of drug testing does have its intended deterrent qualities within the Navy. Findings of the study indicate that utilizing regular monthly drug tests deters approximately 60% of potential users. Additionally, positive drug urinalysis fell in the 15 years preceding the study, despite increases in nonmilitary drug use (Borack, 1998). Moreover, self-reported data of drug use in the Navy have mirrored these results as well.

We support the need for drug testing, but stress that harm reduction should be prioritized above punitive and restrictive measures. Military personnel need to educate soldiers on the health issues and health disparities among drug abusers and nonabusers, in order to help them understand that, although being active service member on duty is difficult, consuming drugs or alcohol as a dulling agent is not the best option. Campaigns should stress that abusing drugs is related to other negative outcomes, and on-job accidents due to intoxication may be lethal. By educating all levels and sectors of the military, and implementing policy that covers *every* abused and potentially abused drug (even those unregulated/yet to be regulated substances), personnel can better understand the repercussions of their actions, make informed decisions, and continue to be healthy, functioning soldiers (Bray et al., 1992).

The military has engaged in several sensationalized campaigns in recent years, some of which focus on emerging drugs. Antidrug campaigns such as "Not in My Corps" include antispice and antibath salt messages. They stress health repercussions, and the strict guidelines and regulations on the use of spice and its analogues (Loeffler et al., 2012). "Not in My Corps" and other awareness campaigns "targeting active duty service members are increasingly promulgating the dangers and consequences of designer drug use, and screening for designer drugs is rapidly being integrated into command urinalyses throughout the service branches" (Loeffler et al., 2012, p. 1046). According to Loeffler and coworkers, these campaigns were somewhat effective. Prior to the study, 15% of those tested were positive for designer drug use, but after the campaigning, only 5% were positive (2012).

In conclusion, synthetic cannabinoid use among US military soldiers has likely been higher than in other demographics. Intoxication from these drugs may have led to accidents, damaged the health of military personnel, and facilitated drug dependence among US soldiers. This is particularly of concern because the men and women who are defending their country already face a number of risks. Designer drugs and their effects, whether they be direct or the indirect consequences of a fellow soldier's intoxication, should not threaten the men and women of the armed forces. By understanding legal highs, educating our soldiers on their adverse effects, and providing alternative methods to deal with stress and other anxieties, the military can avoid using psychoactive drugs, and defend their country in the best possible way.

MINI-DICTIONARY

Bath salts Synthetic cathinone-like chemicals sold as novel highs, but are very different in terms of effect and user profile from synthetic cannabinoids.

Designer drug A substance that is a synthetic analogue of a particular illegal, or prohibited drug that is created or formulated to avoid drug laws.

Spice Novel synthetic cannabinoid brand that is also used to refer to other brands of similar substance.

Synthetic cannabinoids A type of designer drug that is designed to mimic the psychoactive effects of cannabis.

Tetrahydrocannabinol (THC) Psychoactive compound in marijuana.

References

Bachman, J. G., Freedman-Doan, P., O'Malley, P. M., Johnston, L. D., & Segal, D. R. (1999). Changing patterns of drug use among US military recruits before and after enlistment. *American Journal of Public Health*, *89*(5), 672–677.

Ballweg, J. A., & Li, L. (1991). Trends in substance use by US military personnel. *Armed Forces and Society*, *17*(4), 601–618.

Berry-Cabán, C. S., Kleinschmidt, P. E., Rao, D. S., & Jenkins, J. (2012). Synthetic cannabinoid and cathinone use among US soldiers. *US Army Medical Department Journal*, *1*, 19–24.

Borack, J. I. (1998). An estimate of the impact of drug testing on the deterrence of drug use. *Military Psychology*, *10*(1), 17.

Bray, R. M., Kroutil, L. A., & Marsden, M. E. (1995). Trends in alcohol, illicit drug, and cigarette use among US military personnel: 1980–1992. *Armed Forces and Society*, *21*(2), 271–293.

Bray, R. M., Marsden, M. E., Guess, L. L., Wheeless, S. C., Pate, D. K., Dunteman, G. H., & Iannacchione, V. G. (1986). Worldwide Survey of Alcohol and Nonmedical Drug Use Among Military Personnel: 1985 (No. RTI/3306/06-02FR). Research Triangle Park, NC: Research Triangle Institute (RTI).

Bray, R. M., Marsden, M. E., Herbold, J. R., & Peterson, M. R. (1992). Progress toward eliminating drug and alcohol abuse among US military personnel. *Armed Forces and Society*, *18*(4), 476–496.

Bray, R. M., Pemberton, M. R., Lane, M. E., Hourani, L. L., Mattiko, M. J., & Babeu, L. A. (2010). Substance use and mental health trends among US military active duty personnel: key findings from the 2008 DoD Health Behavior Survey. *Military Medicine*, *175*(6), 390–399.

Donovan, E. (2010). Propranolol use in the prevention and treatment of posttraumatic stress disorder in military veterans: forgetting therapy revisited. *Perspectives in Biology and Medicine*, *53*(1), 61–74.

Flor, A. (2010). Spice—"I want a new drug". *Army lawyer*, (p. 446). The Judge Advocate General's Legal Center & School (JAGS), U.S. Army, Charlottesville, Virginia.

Geffray, C. (2001). Introduction: drug trafficking and the state. *International Social Science Journal*, *53*(169), 421–426.

Golub, A., & Bennett, A. S. (2013). Introduction to the special issue: drugs, wars, military personnel, and veterans. *Substance Use and Misuse*, *48*(10), 795–798.

Heath, D. B. (1992). US drug control policy: a cultural perspective. *Daedalus*, *121*, 269–291.

Khey, D. N., Stogner, J., & Miller, B. L. (2014). What is being done about emerging drugs? In *Emerging trends in drug use and distribution* (pp. 75–93). Switzerland: Springer International Publishing.

Kuzmarov, J. (2009a). *The myth of the addicted army: Vietnam and the modern war on drugs*. Amherst, MA: University of Massachusetts Press (pp. 15–36).

Kuzmarov, J. (2009b). *The myth of the addicted army: Vietnam and the modern war on drugs*. Amherst, MA: University of Massachusetts Press (pp. 189–193).

Loeffler, G., Hurst, D., Penn, A., & Yung, K. (2012). Spice, bath salts, and the US military: the emergence of synthetic cannabinoid receptor

agonists and cathinones in the US Armed Forces. *Military Medicine, 177*(9), 1041–1048.

Navy Drug Screening Labs. (2015). Navy and Marine Corps Public Health Center. Available from http://www.med.navy.mil/sites/nmcphc/navy-drug-screening-labs/our-process/Pages/default.aspx

Sedefov, R., Gallegos, A., King, L., Lopez, D., Auwärter, V., Hughes, B., & Griffiths, P. (2009). Understanding the "Spice" phenomenon. Thematic papers, European Monitoring Centre for Drugs and Drug Addiction. Available from http://www.emcdda.europa.eu/publications/thematic-papers/spice

Vardakou, I., Pistos, C., & Spiliopoulou, C. (2010). Spice drugs as a new trend: mode of action, identification and legislation. *Toxicology Letters, 197*(3), 157–162.

PART VII

MEDICINAL CANNABIS USE

89 *Cannabis and synthetic cannabinoids for cancer patients: multiple palliative indications together with promising laboratory antineoplastic effects* 859

90 *The use of medical marijuana in the treatment of psychiatric disorders* 869

91 *Beneficial effects of cannabis and related compounds on sleep* 877

92 *Cannabinoid-based medicines for the treatment of gilles de la tourette syndrome* 883

93 *Cannabidiol and multiple sclerosis* 893

94 *Cannabinoids and their effects on painful neuropathy* 905

95 *Cannabis for basal ganglia disorders (parkinson disease and huntington disease)* 917

96 *Medical cannabis for the treatment of inflammatory bowel disease* 931

97 *Cannabidiol for the treatment of drug use disorders* 939

98 *Cannabinoids and effects on the gastrointestinal tract: a focus on motility* 947

99 *Potential medical uses of cannabigerol: a brief overview* 958

Page left intentionally blank

CHAPTER

89

Cannabis and Synthetic Cannabinoids for Cancer Patients: Multiple Palliative Indications Together With Promising Laboratory Antineoplastic Effects

D. Zalman, G. Bar-Sela

Division of Oncology, Rambam Health Care Campus and Faculty of Medicine, Technion—Israel Institute of Technology, Haifa, Israel

SUMMARY POINTS

- Cannabinoids are reported to exert various palliative effects in cancer patients.
- Dronabinol and Cesamet were the first approved cannabinoids to relieve chemotherapy-induced nausea and vomiting in cancer patients.
- Cannabinoids may be helpful for appetite stimulation, pain control, mainly of the neuropathic type, and possibly for mood and psychological symptoms relief.
- In laboratory and animal models, cannabinoids were found to have antitumor activity.
- Cannabinoids might have a future role in cancer therapy through their ability to modulate important signaling pathways in the cell cycle, to induce cell arrest and apoptosis, and to inhibit angiogenesis.
- Cannabinoids are psychoactive drugs, and nearly half the patient dropout during trials is due to central nervous system effects, such as dizziness, confusion, hallucinations, etc.
- The newer oromucosal cannabinoid, Sativex THC/CBD 1:1, tries to maintain efficacy with less central side effects, by adding the nonpsychoactive derivative cannabidiol.
- The medical use of cannabinoids is increasing, but still without proven efficacy or safety according to medical standards.

KEY FACTS OF THE USE OF CANNABINOIDS IN CANCER

- The longevity of metastatic cancer patients has nearly doubled in recent decades.
- A major problem for metastatic cancer patients is maintaining a reasonable quality of life, due to chronic multisystem and undertreated symptoms.
- In 2003, ESMO (European Society for Medical Oncology) designated an accreditation program for medical centers which integrates Oncology and Palliative Care at high selected standards.
- Although cannabinoids are prescribed for many medical conditions and symptoms, only chemotherapy-induced nausea and vomiting, and cancer pain are formally indicated and supported by evidence in cancer patients.
- The activity of medically synthetic tetrahydrocannabinol extracts may be limited by central nervous system side effects, mostly hallucinations, confusion, and dysphoria.
- Balancing the activities of the central cannabinoid receptors type 1 with the peripheral cannabinoid receptors type 2 seems to exert the maximal clinical effect with fewer complications.
- The basic data on cannabinoids is large. Nevertheless, only a selected number of medications have been tested in clinical trials, and few have been approved for clinical indications.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00104-6
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

AEA	Anandamide
2-AG	2-Arachidonoylglycerol
CB1 and CB2	Cannabinoid receptors type 1 and 2
CBD	Cannabidiol
CINV	Chemotherapy-induced nausea and vomiting
CTZ	Chemoreceptor trigger zone
DA	Dopamine
ERK1 and 2	Extracellular regulating kinases
GBM	Glioblastoma multiforme
IL1	Interleukin 1
MAPK	Mitogen activated protein kinase
NTS	Nucleus of the solitary tract
THC	Δ^9-Tetrahydrocannabinol
TNF	Tumor necrosis alpha

INTRODUCTION

During the last decade, medications and technologies in oncology treatment have been greatly improved, leading to prolonged survival of cancer patients. Nevertheless, the symptoms of incurable disease last for long periods. Early palliative intervention has improved quality of life, as well as prolonging survival (Temel et al., 2010). The World Health Organization's definition for palliative care indicates that it should relieve all types of suffering in multidisciplinary ways, without influencing life longevity (Becker, 2009).

The patient with advanced cancer can suffer from multiple symptoms, usually in clusters, either physical and/or psychological. The most commonly described symptoms are: pain, fatigue/lack of energy, anorexia/early satiety, nausea/vomiting, dry mouth, constipation, and dyspnea (Shoemaker, Estfan, Induru, & Walsh, 2011).

Cannabis sativa, one of the most ancient psychotropic drugs known to man, gained new medicinal interest in the last 10 years and was approved by many western countries for symptom control, either in cancer or in nonmalignant illnesses, based on its highly unique and diverse pathophysiology.

Endocannabinoids are retrograde transmitters, meaning that they are released from postsynaptic cells, and act on receptors in the axonal terminals of presynaptic cells to release substantial neurotransmitters, such as glutamate, GABA, and also NE, 5HT, etc. Cannabinoids may exert their diverse effects through direct stimulation of both central and peripheral receptors (CB1, CB2), and also through indirect interaction with different neurotransmitters (Manzanares & Carrascosa, 2006).

Table 89.1 summarizes the pharmacokinetic and pharmacodynamic of cannabis and of the synthetic cannabinoid drugs. These unique mechanisms, which activate different organs, systems, and neurotransmitters simultaneously, increase interest in the efficacy of the cannabis extract to relieve symptoms in complicated palliative cancer patients, such as intractable pain, decreased appetite, nausea, and vomiting, and also to improve psychological symptoms, by ameliorating mood, insomnia, and depression. These positive effects should be considered along with the possible side effects of cannabis use. Fig. 89.1 demonstrates the possible effects of cannabinoids in relation to the dispersion of anatomic CB1 receptors in the brain.

TABLE 89.1 Pharmacokinetics and Pharmacodynamics: Values in Commonly Used Cannabinoids

Smoking cannabis	2–56%	10–25 min	2 h
Dronabinol–Marinol	5–20%	1–5 h	24–36 h
Cesamet–Nabilone	20%	2 h	2 h (35 h metabolites)
Levonantradol–IM	Variable	1–2 h	2 h
Ajulemic acid	Unknown	1–2 h (up to 5 h)	Several days
Nabidiolex–Sativex	>90%	2–4 h	24–36 h

Table 89.2 summarizes the possible biochemical pathways of cannabinoids in moderating clinical symptoms. Preclinical data suggest that cannabis may possess antineoplastic mechanisms which merit exploration.

In a prospective observational study published recently (Bar-Sela et al., 2013), cancer patients were evaluated for the supportive and/or palliative benefits which medicinal licensed cannabis might offer. Patients were interviewed and completed questionnaires regarding symptom control and side effects. Nearly half of the 211 patients included in the study dropped out for various reasons, including 12% who dropped out early due to cannabinoid side effects. The remaining 106 patients showed significant symptomatic improvement in cancer and therapy-related symptoms, such as chemotherapy-induced nausea and vomiting (CINV), fatigue, mood disorder, pain, sleep disorder, and anorexia ($p < 0.001$). Memory loss was noted in patients with prolonged cannabis use ($p = 0.002$), and the study had limitations of small size, heterogeneity, subjectivity of symptom scaling, and bias of the results, as those who dropped out were not included.

Only a very small number of synthetic cannabinoids have been approved by the regulatory authorities for symptom relief in cancer patients (Table 89.3).

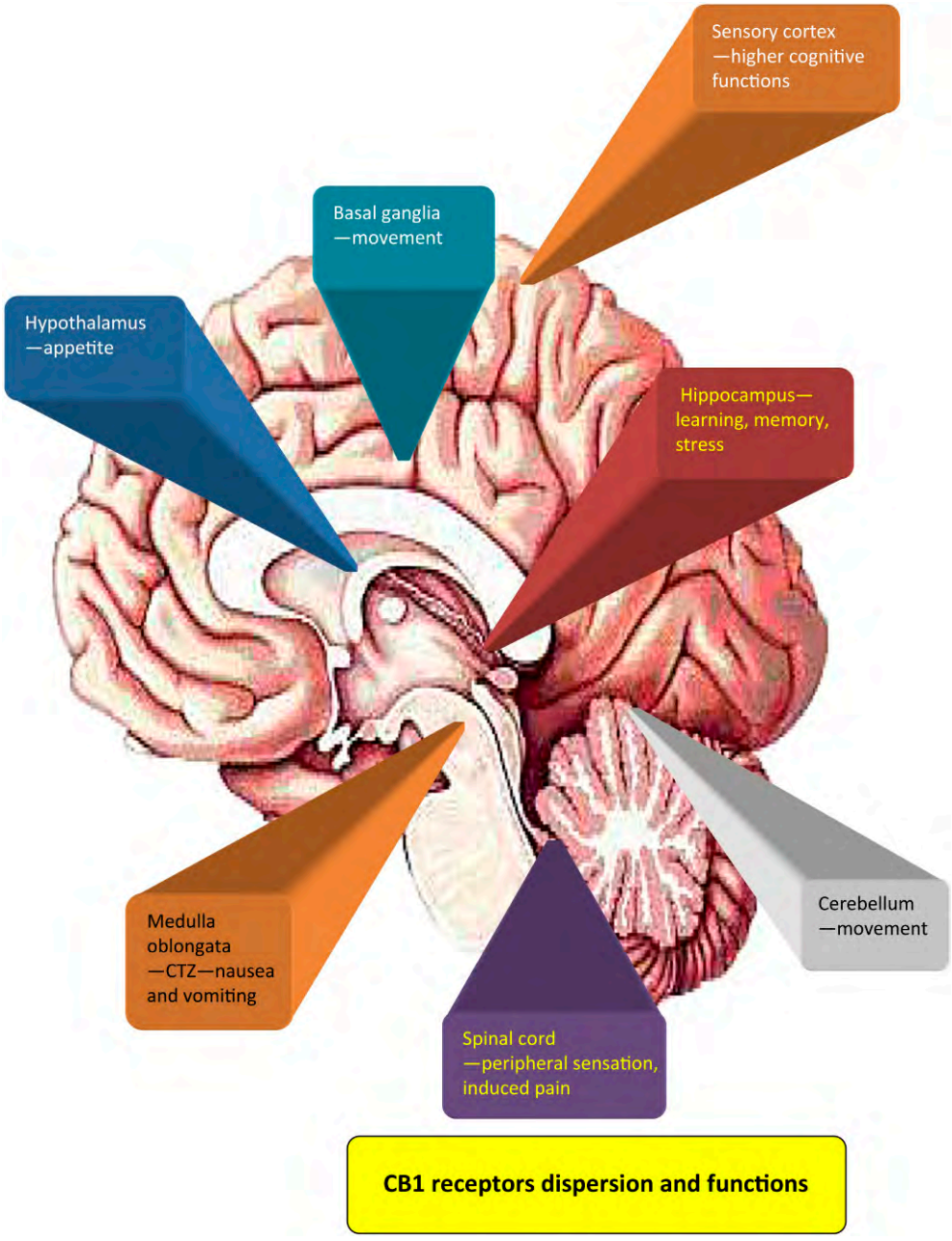


FIGURE 89.1 **CB1 receptors, dispersion in brain and related functions.**

TABLE 89.2 Commonly Treated Symptoms With Cannabinoids and Suggested Mechanisms

Symptoms	Anatomy	Neurotransmitter
CINV	Vagus nerve	Muscarine, CB2
	CTZ	Dopamine, 5HT, CB1
	Vestibular cerebellum	Histamine, muscarine
	Limbic region	CB1
	NTS	5HT, muscarine, histamine, CB1
	Upper GI	5HT, dopamine, CB2
Cancer pain	Neuropathic pain—CB1 activation	Inhibition of GABA, L-glutamate, serotonine, NA, Ach, and DA
Anorexia–cachexia	Hypothalamus	Leptin
	Anabolic factors decrease	IGF, GH, thyroid hormone
	Catabolic factors increase	Cortisol, glucagon, TNF-alpha, IL1, IL6, IFN-gamma
Depression	Cortex	5HT1/2/3, NA
	Hypothalamus-pituitary-axis	5HT, NA
	CBD	5HT1-anxiolytic

The table summarizes the main cannabinoids responsive symptoms and the possible anatomical and pathophysiological mechanisms (Kotler, 2000; Slatkin, 2007; Crippa et al., 2009).

TABLE 89.3 Currently Approved Cannabinoids for Palliative Cancer Symptoms Relief

Symptom	Approved drug	Chemical compound	Approval	References
CINV—chemotherapy-induced nausea and vomiting	Dronabinol–Marinol	Synthetic THC extract (tabs.)	USA (1985), Canada (1998)	Plasse et al. (1991)
	Cesamet–Nabilone	Synthetic THC-alike extract (caps.)	United Kingdom, Canada, Mexico, Australia, USA	Einhorn, Nagy, Furnas, and Williams (1981), Herman et al. (1979)
PAIN—persistent cancer pain	Nabidiolex–Sativex	Combined 1:1 THC/CBD submucosal extract (spray)	USA (2011)	Portenoy et al. (2012)

CHEMOTHERAPY-INDUCED NAUSEA AND VOMITING

The pathogenesis of nausea and vomiting is highly complicated (Grunberg & Hesketh, 1993). It involves the central and peripheral nervous systems, and is mediated by the vagus nerve. Other parts of the nervous system that play a role in the mechanism of nausea and vomiting include the chemoreceptor trigger zone (CTZ) located in the medulla oblongata, the vestibular cerebellum, and the peripheral nerves in the upper GI tract.

It is assumed that the center substantially eliciting nausea and vomiting lies at the medullary nucleus of the solitary tract (NTS), which may represent the site where different inputs from the vagal nerve, the vestibular cerebellum, the postrema area, and the limbic lobe area converge (Miller, 1998).

CINV pathogenesis involves a variety of neurotransmitters, such as dopamine, histamine, muscarine, and serotonin, each responding to a variety of stimuli that may improve or deteriorate nausea and vomiting status. It is not surprising that drug combinations act better than a single medication to overcome CINV.

It is assumed that most of the antiemetic effect of cannabinoids is derived from direct activity on CB1 receptors in the brain, especially in the NTS. Nevertheless, activity in higher cortical regions and in the limbic lobe was also reported to have some antiemetic effects. The NTS nucleus receives information regarding emetogenic substances in two different ways: through the blood stream in the brain stem and CTZ, and neurologically through the vagus nerve. At the limbic system, cannabinoids may have an antianxiety effect which might contribute to reducing anticipatory emesis, and delayed emesis. Through peripheral stimulation of CB2 receptors, cannabinoids are thought to stabilize enterochromaffin cells in the intestine and to reduce vagal stimulation. Also, the indirect effect of cannabinoids on emesis mechanism has been recognized through its effect on dopamine and serotonin receptors (Slatkin, 2007).

Only two trials have been published in which cannabis was given by smoking for CINV. The first trial included 15 patients diagnosed with osteogenic sarcoma, and was published in 1979 (Chang et al., 1979). Patients were treated with high-dose methotrexate, and randomized to smoked marijuana, oral synthetic cannabinoid, or placebo. The patients served as their own controls. Correlation between cannabinoid blood levels and response to treatment was evaluated, as well. Cannabinoid treatment by smoking marijuana or synthetic cannabinoids reduced the level of nausea and vomiting. Efficacy was correlated to blood levels as follows: only 6% suffered from CINV when blood levels of THC were >10 ng/mL, 21% for 5–10 ng/mL, and 44% for <5 ng/mL, compared to 72% in the placebo group.

The second trial published by the same authors in 1981 (Chang et al., 1981) included only eight sarcoma patients who received the adriamycin/cyclophosphamide combination. Only three of eight patients had fair symptom relief. However, only two of eight patients reached what seems to be the therapeutic blood levels of THC > 5 ng/mL.

In 2001, a systematic review was published (Tramèr et al., 2001) which included 30 trials with a total of 1366 patients. The review included studies with the common cannabinoid drugs available at that time: oral medications—Marinol (Dronabinol, Unimed Pharmaceutical by Banner), Nabilone (Cesamet, Valent Pharmaceuticals by MEDA), and a parenteral drug—Levonantradol IM (by Pfizer). These cannabinoids were compared to common antiemesis medications available at that time, for example, metochlopramide, haloperidol, chlorpromazine, and domeperidone, in a variety of malignancies and chemotherapy medications. The antiemetic effect was measured only during the first 24 h after chemotherapy. Therapy with cannabinoids significantly reduced CINV in the low-to-moderate emetogenic treatments, compared with regular medications. The nausea rate was reduced from 59% to 43%, and vomiting from 57% to 45%. In 2008, a metaanalysis was published regarding the therapeutic use of cannabinoids in CINV (Machado Rocha, Stéfano, De Cássia Haiek, Rosa Oliveira, & Da

Silveira, 2008). Only randomized, double-blind controlled trials with high methodology (13 trials) were included. The same three cannabinoids listed in the 2001 review by Tramèr et al. (2001) were analyzed according to each drug. Only Marinol showed significant advantage as an antiemetic. The main reason for dropping out from cannabinoid therapy according to the systematic reviews was cannabinoid toxicity. The most serious side effects described were: 5% paranoia delusions, 6% hallucinations, 13% dysphoria/depression. Additionally, 20–25% hypotension, and dizziness in nearly half the patients was reported.

It seems that cannabis use may result in some CINV relief. However, these results should be interpreted with caution, as the group size in each trial was small, and the cannabinoids were compared to either placebo (which is unethical nowadays) or to old and unpresentable antiemesis medications. None of the newer medications which are the standard of care for CINV in cancer patients, like 5HT3 antagonist or NK1 antagonist, were tested in cannabinoids trials.

In a small recent trial which included 61 patients, cannabinoids were tested against a newer generation of antiemesis medication, 5HT3 antagonist Ondasetron. Patients were divided into four groups. All received 20 mg of dexamethasone and 2.5 mg of Dronabinol before and after therapy, or 16 mg of Ondasetron IV prior to chemotherapy, or both therapies, or none. Both Dronabinol and Ondasetron were also given orally on days 2–5 for delayed emesis prevention. After 5 days, absence of nausea was demonstrated in 71% of patients in the Dronabinol group, 64% in the Ondasetron group, 53% in the combined group, and 15% in the placebo group. No significant difference was observed between the differently treated groups (Meiri et al., 2007). Although small, this is the only trial that may demonstrate a Dronabinol (THC) noninferiority effect in preventing acute and delayed type CINV in comparison to the new generation of antiemetic medications.

Although evidence on cannabinoids efficacy to relieve CINV is inconclusive, their unique ability to interact with a few key receptors involved in the pathogenesis of emesis support adding cannabinoids to the antiemetic combination offered to cancer patients suffering from intractable CINV.

ANOREXIA–CACHEXIA SYNDROME

Cancer anorexia–cachexia syndrome is a complicated metabolic syndrome of continuous process of skeletal muscle breakdown and wasting, together with caloric intake reduction due to loss of appetite (MacDonald, Easson, Mazurak, Dunn, & Baracos, 2003). The process is attributed to decreased levels of various anabolic factors, such as insulin growth factor, thyroid hormone, or growth hormone, and the elevation of catabolic factors and proinflammatory factors, such as cortisol, glucagon, and mainly cytokines, especially TNFα, IL1, IL6, and INF-γ (Kotler, 2000).

Cannabinoids, like many other therapies in this syndrome, may relieve the anorexia part, but they do not have any impact on muscle wasting. To influence the muscle breakdown, an anabolic medication must be provided. Cannabinoids may have some influence on a few cytokines (IL10, IL12) (Smith, Terminelli, & Denhardt, 2000). However, these cytokines do not belong to the major proinflammatory cytokines which play the most important role in the anorexia–cachexia syndrome, for example, IL1, IL6, and TNF.

The exact mechanism by which cannabinoids exert their effect on the anorexia–cachexia syndrome has yet to be found. A study of an animal model (Di Marzo et al., 2001) succeeded to provide evidence of the relationship between the leptin (hormone regulating food intake and body weight) regulation system, the CB1 receptors at the hypothalamus, and cannabinoids. Activation of endocannabinoids in that area may activate the CB1 receptors to maintain food intake through the regulatory leptin system (Cota et al., 2003).

In the early 1990s, a small phase II trial tested the use of oral cannabinoid to improve appetite in advanced cancer patients. The trial included 18 patients, and the cannabinoid therapy improved appetite in nearly two-thirds (Nelson, Walsh, Deeter, & Sheehan, 1994). Based on the positive results supporting the use of cannabinoids for cachexia–anorexia symptoms in HIV patients and the early phase II study, a randomized double-blind trial with more than 460 patients was published in 2002, describing the effect of 2.5 mg Dronabinol b.i.d. and/or 800 mg Megestrol acetate daily to cause weight gain in patients with advanced malignancies suffering from anorexia–cachexia syndrome. The primary end-point was a weight gain of ≥10% from baseline. Treatment with Dronabinol failed to show the anticipated results, but appetite improvement was seen in 75% of the Megesterol group versus 49% in the Dronabinol group, weight gain 11% versus 3%. Quality of life was also better with the megesterol acetate therapy. A combination of both drugs showed no advantage (Jatoi et al., 2002).

A randomized, phase III, double-blind study was published in 2006, comparing two types of cannabinoids (THC alone ± CBD) to placebo. A total of 289 patients were included in the study (Strasser et al., 2006). Increase in appetite was observed in 73% of THC/CBD, 58% of THC alone, and 68% in the placebo arm (these results were insignificant). No significant differences were seen in other symptoms, such as mood distress or nausea. It is worthwhile to note the high percentage of clinical improvement attributed to the placebo effect.

The literature today does not support specific activity of cannabinoids in the cancer anorexia–cachexia syndrome. A theory was suggested that cannabinoids might have a clinical effect through the stimulation of chemoreceptors related to the olfactory zone. Many cancer patients describe not only reduced appetite, but also changes in perception of tastes and odors, which complicates the task of feeding, and reduces the enjoyment from eating. In a small study (Brisbois et al., 2011), 46 patients were randomized to THC tablets or placebo. Adding THC significantly improved chemosensory perception that translated to 73% greater appreciation of food in the THC group, compared to only 30% in the placebo group.

PAIN

During the last decade, awareness and knowledge about pain management increased tremendously. Nevertheless, in advanced cancer patients, pain is still a common problem, causing suffering, disability, and anxiety, and reducing quality of life. In animal models, cannabinoids have been shown to have useful analgesic properties (Pertwee, 2001). However, in humans, the limiting factor is related to dose, as most of the action is transmitted through CB1 receptors in the central nervous system (Staquet, Gantt, & Machin, 1978).

According to studies, the analgesic effect of cannabinoids is presumed to be mild to moderate, at best. The estimated equal analgesic dose of 10 mg of THC is comparable to only 60 mg of codeine (Noyes, Brunk, Avery, & Canter, 1975). However, effective therapy is limited due to dose-related side effects, especially dizziness, somnolence, ataxia, and blurred vision, which are more common when the dose increases to 20 mg (Noyes et al., 1975).

The rationale of giving cannabinoids for intractable pain relates to the increasing data of synergistic effects between opioids and cannabinoids (Cichewicz & McCarthy, 2003). In a phase II study, vaporized THC was given to patients with chronic pain who were treated with long-acting opioids. Pain levels were measured along with opioid blood levels. Adding THC resulted in a temporary reduction of pain levels, without affecting opiate blood levels (Abrams, Couey, Shade, Kelly, & Benowitz, 2011).

A rationale for using cannabinoids in neuropathic pain derives from an animal model which demonstrated mechanical and thermal antihyperalgesia and antiallodynia effects. These effects are probably mediated through the central nervous system, as well as the peripheral nervous system (Fox et al., 2001). Cannabinoids can also relieve neuropathic pain by influencing several neurotransmitters, such as inhibition of noradrenaline, dopamine, serotonin, acetylcholine, GABA, and L-glutamate (Manzanares & Carrascosa, 2006). It is well known that the last two mentioned play an important role in the pathogenesis of neuropathic pain. Unfortunately, clinical data to prove these animal models is still pending. Only several small trials have been completed in cancer patients treated with cannabinoids for pain control.

In a multicenter, double-blind, randomized, placebo-controlled, parallel-group trial (Johnson et al., 2010), 177 patients with advanced cancer and intractable pain despite chronic use of opioids were randomized to three therapy groups—THC/CBD extract, THC alone, or placebo. Treatment was given for 2-week periods. Improvement of pain of more than 30% was significantly better with THC/CBD extract, compared to placebo; however, the results for THC alone did not differ from those of the placebo. A follow-up trial (Johnson et al., 2013) did not limit the length of cannabinoid use. The longest time was nearly 2 years and the shortest less than 2 weeks. Those who achieved pain relief sustained it over time, without a need to elevate opioids dose. Tolerability was acceptable, although more than one-quarter of patients withdrew due to side effects, especially dizziness, somnolence, nausea, and confusion.

Another randomized double-blind study (Portenoy et al., 2012) selectively tested the oromucosal–cannabinoid combination THC/CBD, known as Sativex (GW Pharmaceuticals, United Kingdom), in opioid refractory advanced cancer patients with pain. The spray was given to 260 cancer patients in three escalated doses, low (1–4 sprays/day), moderate (6–10 sprays/day), and high (above 11 sprays/day). Pain improvement was reported, especially with lower doses of Sativex. Failure of the higher dose may be related to side effects which are dose-related and cause high withdrawal due to low tolerance.

MOOD DISORDER AND INSOMNIA

Mood disorders are mainly affected by the serotonergic system. Tryptophan is a major precursor for 5HT, and cannabinoids were found to modulate signaling in the serotonergic system by elevating tryptophan levels (Jenny, Santer, Pirich, Schennach, & Fuchs, 2009).

Only a few studies with cancer patients have been conducted to evaluate cannabinoid effects on anxiety, mood swings, and insomnia. In a small double-blind trial of 25 cancer patients suffering from anxiety, Nabilone was compared to placebo over a 28-day treatment period (Fabre & McLendon, 1981). The study showed significant improvement of anxiety in the Nabilone group compared to placebo. Adverse effects were minimal, mostly dry mouth and drowsiness, with no altered states phenomenon.

TABLE 89.4 Summary of Suggested Antitumor Mechanisms of Cannabinoids in Various Malignancies

Antiproliferative mechanisms	Cellular pathogenesis	Malignancy related	References
Induction of apoptosis	Sustain ERK activation	Breast cancer Prostate cancer	Melck et al. (1999) Sanchez, Sanchez, Ruiz, and Diaz-Laviada (2003)
	Sustained AKT inhibition	Brain GBM	Velasco, Galve-Roperh, Sánchez, Blázquez, and Guzmán (2004)
	De novo Ceramide accumulation	GBM	Velasco et al. (2004)
Cell cycle arrest	Growth factors receptors signaling inhibition	General cancer Breast cancer	Blázquez et al. (2003) Caffarel et al. (2010)
	Inhibition of adenylyl cyclase	General cancer Breast cancer	Melck et al. (1999) Bifulco et al. (2001)
	Loss of CdK2 activity and S-phase arrest	Breast cancer	Laezza, Pisanti, Crescenzi, and Bifulco (2006)
Inhibition of angiogenesis and metastases	Inhibition in expression of proangiogenic factors and MMPs	General cancer	Guzmán (2003)
	Inhibition of vascular endothelial cell migration and survival	General cancer	Blázquez et al. (2003)

THC was tested for sleep disorders (Feinberg, Jones, Walker, Cavness, & Floyd, 1976). In normal volunteers, THC caused alterations of the sleep cycle, especially prolongation of stage IV at the expense of the rapid eye movement (REM) sleep length. However, REM rebound, as described with common hypnotics, is rarely seen with cannabinoid withdrawal, unless high doses (more than 70 mg) are given.

The psychological effects of the cannabinoids are highly complicated. In different trials, cannabinoids demonstrated all the possible effects on anxiety: anxiolytic effects, anxiogenic effects, and no effect (Crippa et al., 2009).

Unfortunately, the psychological effects of cannabinoids are still not fully understood. Although some patients experience positive antidepressive, relaxant, euphoric sensations, others may feel exacerbations of their symptoms, from dysthymia to major depression, from restlessness to anxiety, lack of motivation, and withdrawal from society. Negative anxiolytic effects were noticed with THC, and positive or protective ones were more likely to be attributed to CBD (Fusar-Poli et al., 2009).

ANTITUMOR EFFECTS

In cell cultures and mice animal models of lung adenocarcinoma, Munson, Harris, Friedman, Dewey, and Carchman (1975) demonstrated that 10 consecutive days with either Δ^9-THC or Δ^8-THC had a dose-dependent antitumor growth effect, with some survival benefit.

Cannabinoids were found to impact various cellular pathways which are important in cell cycle and survival. For example, by inhibition of c-AMP, they influence Na/K+ channels to cause inhibition of neurotransmitters release (Melck et al., 1999). They also modulate central pathways in apoptosis, like stimulating the MAPKs (mutagen-activated protein kinas) and ERK, the extracellular-signal-regulated kinases that have a role in controlling cell growth and differentiation (Melck et al., 1999). Stimulation of the PIK-AKT survival pathway was reported also (Guzmán, 2003). In a mice model, Blázquez et al. (2003) found two possible mechanisms of cannabinoids' antitumor activity. They demonstrated direct inhibition of vascular endothelial cell migration, and indirect contribution to cell arrest, and apoptosis by activation of cannabinoid receptors in vascular endothelial cells causing inhibition of cell migration and survival. Table 89.4 summarizes the possible mechanisms of antineoplastic activity of cannabis, and a few are illustrated in Fig. 89.2.

Many studies have been done to prove cannabinoids antitumor activity in different malignancies. However, almost all of them were performed in either cell cultures or mice xenograft models. Some of the information from these studies is described briefly regarding several malignancies.

Breast carcinoma is the most common cancer in females. Cell survival in breast cancer is mainly controlled by two pathways: the Ras-Raf/MEK/ERK or the PI3K/AKT. The Src/Ras/Erk pathway is also affected by the hormone system (estrogen, progestin), which is highly important in breast malignancies (Edwards, 2005).

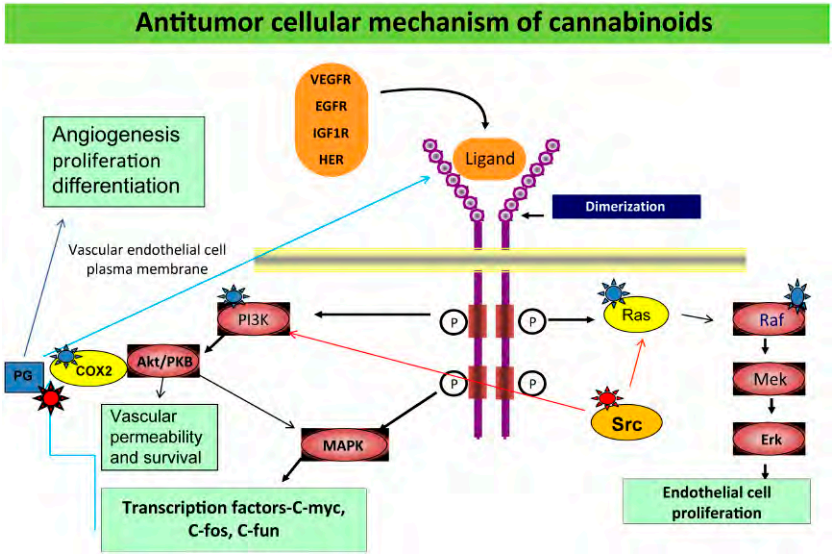


FIGURE 89.2 **Illustration of pathways and connections in a cell which influence proliferation and survival.** The *stars* mark points of possible antitumor cannabinoid effect; *blue star*, stimulatory effect; *red star*, inhibitory effect.

It was shown that cannabinoids prolonged activity of the Raf/Mek/Erk pathway which causes PRL-Rs (prolactin) downregulation in breast cancer cells (Melck et al., 1999).

Qamri et al. (2009) tested CB1 and CB2 agonists on breast cancer cells and in mice models. Mice treated with CB1 and CB2 agonists showed a 40–50% reduction in tumor growth, and a 65–80% reduction in lung metastasis. These effects were reversed by CB1 and CB2 antagonists.

In a mice model, Caffarel et al. (2010) reported that Δ^9-THC can achieve a reduction of tumor growth, and a decrease in the number of lung metastasis. These effects were related to inhibition of cancer cell proliferation, induction of cancer cell apoptosis, and impairment of tumor angiogenesis. It is also worth mentioning that more than 90% of HER2 positive breast tumors express the CB2 receptors, which might open a therapeutic option of combined therapy with anti-HER2 medications.

Prostate carcinoma is the most common cancer in men, and the second leading cause of cancer mortality. Prostate cancer pathogenesis is hormone-dependent, and many tumors, especially in early diagnosis, are androgen-dependent, which means that the tumor may be brought under control by deprivation of androgen. The androgen receptor activity seems to be regulated by the activation of distinct growth factors, such as EGFR, insulin-like growth factor-I (IGF-I), keratinocyte growth factor, IL6 and others (Moschos & Mantzoros, 2002). Some of these growth factors are influenced by the cannabinoid system.

In prostate cancer cell lines, inhibition of CB1/CB2 influences the Erk/PI3k pathways, and causes a decrease of either androgen receptor levels and/or prostatic serum antigen (PSA) levels in androgen-dependent cells (Sanchez et al., 2003).

For brain tumors, Velasco et al. (2004) noticed that, in glioma cell cultures, cannabinoids induce apoptosis and cell death through the accumulation of ceramide, angiogenesis inhibition, and decay of the Akt pathway. Interestingly, cell death is selective to transformed glioma cells only.

Glioblastoma multiforme (GBM) is one of the most devastating malignancies, with a poor prognosis, short survival, and treatments which are mostly palliative in nature (Guzmán et al., 2006). The only clinical trial in humans was conducted in this disease. This phase I trial included nine patients with relapsed or progression of GBM. Cannabinoids were given intracranially directly to the tumor bed, through a specially inserted catheter with a subcutaneous subclavicular reservoir. Median survival was 42 weeks. No correlation was found between CB1/CB2 levels in the tumor tissue and response to THC treatment. Although not statistically significant, the cannabinoids seem to decrease tumor vascularization, demonstrated by CD31 staining. However, this trial is too small to draw real clinical conclusions.

CONCLUSIONS

The results of basic studies regarding the antitumor effects of cannabinoids are complex, and involve different cellular pathways and mechanisms which eventually lead to tumor regression, reduction in blood supply, tumor vascularization, and decreased metastases formation. Still, the clinical application of

the antitumor effects of cannabinoids is unknown and unproven.

MINI-DICTIONARY

Anorexia Loss of appetite.
Anxiolytic An antianxiety medication, a tranquilizer.
Apoptosis A natural genetic determined programmed cell death.
Cachexia Metabolic syndrome of continuous skeletal muscle breakdown and wasting due to unbalanced levels of anabolic and catabolic factors.
Ceramide A combination of fatty acids with sphingosine to form groups of amido-sphingolipids which have a role in cell proliferation and survival.
Chemotherapy Treatment of cancer by using specific chemical agents, or drugs which are selectively destructive to the malignant cells or tissues.
Cytokines A large group of low molecular weight nonantibodies proteins, secreted by various types of cells, which contribute to cell-to-cell communication, intercellular mediators, and immune reactions.
Dysphoria An emotional state marked by anxiety, unhappiness, and/or restlessness.

References

Abrams, D. I., Couey, P., Shade, S. B., Kelly, M. E., & Benowitz, N. L. (2011). Cannabinoid–opioid interaction in chronic pain. *Clinical Pharmacology and Therapeutics*, *90*, 844–851.

Bar-Sela, G., Vorobeichik, M., Drawsheh, S., Omer, A., Goldberg, V., & Muller, E. (2013). The medical necessity of medicinal cannabis: prospective evaluation of treatment in cancer patients on supportive or palliative care. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 510392.

Becker, R. (2009). Palliative care 1: principles of palliative care nursing and end-of-life care. *Nursing Times*, *105*, 14–16.

Bifulco, M., Laezza, C., Portella, G., Vitale, M., Orlando, P., De Petrocellis, L., & Di Marzo, V. (2001). Control by the endogenous cannabinoid system of ras oncogene-dependent tumour growth. *FASEB Journal*, *15*, 2745–2747.

Blázquez, C., Casanova, M. L., Planas, A., Gómez Del Pulgar, T., Villanueva, C., Fernández-Aceñero, M. J., Aragonés, J., Huffman, J. W., Jorcano, J. L., & Guzmán, M. (2003). Inhibition of tumor angiogenesis by cannabinoids. *FASEB Journal*, *17*, 529–531.

Brisbois, T. D., de Kock, I. H., Watanabe, S. M., Mirhosseini, M., Lamoureux, D. C., Chasen, M., MacDonald, N., Baracos, V. E., & Wismer, W. V. (2011). Delta-9-tetrahydrocannabinol may palliate altered chemosensory perception in cancer patients: results of a randomized, double-blind, placebo-controlled pilot trial. *Annals of Oncology*, *22*, 2086–2093.

Caffarel, M. M., Andradas, C., Mira, E., Pérez-Gómez, E., Cerutti, C., Moreno-Bueno, G., Flores, J. M., García-Real, I., Palacios, J., Mañes, S., Guzmán, M., & Sánchez, C. (2010). Cannabinoids reduce ErbB2-driven breast cancer progression through Akt inhibition. *Molecular Cancer*, *9*, 196.

Chang, A. E., Shiling, D. J., Stillman, R. C., Goldberg, N. H., Seipp, C. A., Barofsky, I., Simon, R. M., & Rosenberg, S. A. (1979). Delta-9-tetrahydrocannabinol as an antiemetic in cancer patients receiving high-dose methotrexate: a prospective, randomized evaluation. *Annals of Internal Medicine*, *91*, 819–824.

Chang, A. E., Shiling, D. J., Stillman, R. C., Goldberg, N. H., Seipp, C. A., Barofsky, I., Simon, R. M., & Rosenberg, S. A. (1981). Delta-9-tetrahydrocannabinol as an antiemetic in cancer patients receiving adriamycin and cytoxan chemotherapy. *Cancer*, *47*, 1746–1751.

Cichewicz, D. L., & McCarthy, E. A. (2003). Antinociceptive synergy between delta(9)-tetrahydrocannabinol and opioids after oral administration. *Journal of Pharmacology and Experimental Therapeutics*, *304*, 1010–1015.

Cota, D., Marsicano, G., Tschöp, M., Grübler, Y., Flachskamm, C., Schubert, M., Auer, D., Yassouridis, A., Thöne-Reineke, C., Ortmann, S., Tomassoni, F., Cervino, C., Nisoli, E., Linthorst, A. C., Pasquali, R., Lutz, B., Stalla, G. K., & Pagotto, U. (2003). The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *Journal of Clinical Investigation*, *112*, 423–431.

Crippa, J. A., Zuardi, A. W., Martin-Santos, R., Bhattacharyya, S., Atakan, Z., McGuire, P., & Fusar-Poli, P. (2009). Cannabis and anxiety: a critical review of the evidence. *Human Psychopharmacology*, *24*, 515–523.

Di Marzo, V., Goparaju, S. K., Wang, L., Liu, J., Bátkai, S., Járai, Z., Fezza, F., Miura, G. I., Palmiter, R. D., Sugiura, T., & Kunos, G. (2001). Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature*, *410*, 822–825.

Edwards, D. P. (2005). Regulation of signal transduction pathways by estrogen and progesterone. *Annual Review of Physiology*, *67*, 335–376.

Einhorn, L. H., Nagy, C., Furnas, B., & Williams, S. D. (1981). Nabilone: an effective antiemetic in patients receiving cancer chemotherapy. *Journal of Clinical Pharmacology*, *21*, 64–69.

Fabre, L. I., & McLendon, D. (1981). The efficacy and safety of nabilone (a synthetic cannabinoid) in the treatment of anxiety. *Journal of Clinical Pharmacology*, *21*, 377s–382s.

Feinberg, I., Jones, R., Walker, J., Cavness, C., & Floyd, T. (1976). Effects of marijuana extract and tetrahydrocannabinol on electroencephalographic sleep patterns. *Clinical Pharmacology and Therapeutics*, *19*, 782–794.

Fox, A., Kesingland, A., Gentry, C., McNair, K., Patel, S., Urban, L., & James, I. (2001). The role of central and peripheral cannabinoid1 receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. *Pain*, *92*, 91–100.

Fusar-Poli, P., Crippa, J., Bhattacharyya, S., Borgwardt, S. J., Allen, P., Martin-Santos, R., Seal, M., Surguladze, S. A., O'Carrol, C., Atakan, Z., Zuardi, A. W., & McGuire, P. K. (2009). Distinct effects of D9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Archives of General Psychiatry*, *66*, 95–105.

Grunberg, S. M., & Hesketh, P. J. (1993). Drug therapy: control of chemotherapy-induced emesis. *New England Journal of Medicine*, *329*, 1790–1796.

Guzmán, M. (2003). Cannbinoids: potential anticancer agents nature reviews. *Cancer*, *3*, 745–755.

Guzmán, M., Duarte, M. J., Blázquez, C., Ravina, J., Rosa, M. C., Galve-Roperh, I., Sánchez, C., Velasco, G., & González-Feria, L. (2006). A pilot clinical study of D9-tetrahydrocannabinol in patients with recurrent glioblastoma multiforme. *British Journal of Cancer*, *95*, 197–203.

Herman, T. S., Einhorn, L. H., Jones, S. E., Nagy, C., Chester, A. B., Dean, J. C., Furnas, B., Williams, S. D., Leigh, S. A., Dorr, R. T., & Moon, T. E. (1979). Superiority of nabilone over prochlorperazine as an antiemetic in patients receiving cancer chemotherapy. *New England Journal of Medicine*, *300*, 1295–1297.

Jatoi, A., Windschitl, H. E., Loprinzi, C. L., Sloan, J. A., Dakhil, S. R., Mailliard, J. A., Pundaleeka, S., Kardinal, C. G., Fitch, T. R., Krook, J. E., Novotny, P. J., & Christensen, B. (2002). Dronabinol versus megestrol acetate versus combination therapy for cancer-associated anorexia: a North Central Cancer Treatment Group Study. *Journal of Clinical Oncology*, *20*, 567–573.

Jenny, M., Santer, E., Pirich, E., Schennach, H., & Fuchs, D. (2009). Delta9-tetrahydrocannabinol and cannabidiol modulate mitogen-induced tryptophan degradation and neopterin formation in peripheral

blood mononuclear cells in vitro. *Journal of Neuroimmunology*, *207*, 75–82.

Johnson, J. R., Burnell-Nugent, M., Lossignol, D., Ganae-Motan, E. D., Potts, R., & Fallon, M. T. (2010). Multicenter, double-blind, randomized placebo-controlled, parallel-group study of the efficacy, safety, and tolerability of THC:CBD extract and THC extract in patients with intractable cancer-related pain. *Journal of Pain and Symptom Management*, *39*, 167–179.

Johnson, J. R., Burnell-Nugent, M., Lossignol, D., Ganae-Motan, E. D., Potts, R., & Fallon, M. T. (2013). An open-label extension study to investigate the long-term safety and tolerability of THC/CBD oromucosal spray and oromucosal THC spray in patients with terminal cancer-related pain refractory to strong opioid analgesics. *Journal of Pain and Symptom Management*, *46*, 107–118.

Kotler, D. P. (2000). Cachexia. *Annals of Internal Medicine*, *133*, 622–634.

Laezza, C., Pisanti, S., Crescenzi, E., & Bifulco, M. (2006). Anandamide inhibits Cdk2 and activates Chk1 leading to cell cycle arrest in human breast cancer cells. *FEBS Letters*, *580*, 6076–6082.

MacDonald, N., Easson, A. M., Mazurak, V. C., Dunn, G. P., & Baracos, V. E. (2003). Understanding and managing cancer cachexia. *Journal of the American College of Surgeons*, *197*, 143–161.

Machado Rocha, F. C., Stéfano, S. C., De Cássia Haiek, R., Rosa Oliveira, L. M., & Da Silveira, D. X. (2008). Therapeutic use of *Cannabis sativa* on chemotherapy-induced nausea and vomiting among cancer patients: systematic review and meta-analysis. *European Journal of Cancer Care*, *17*, 431–443.

Manzanares, J., & Carrascosa, A. (2006). Role of the cannabinoid system in pain control and therapeutic implications for the management of acute and chronic pain episodes. *Current Neuropharmacology*, *4*, 239–257.

Meiri, E., Jhangiani, H., Vredenburgh, J. J., Barbato, L. M., Carter, F. J., Yang, H. M., & Baranowski, V. (2007). Efficacy of dronabinol alone and in combination with ondansetron versus ondansetron alone for delayed chemotherapy-induced nausea and vomiting. *Current Medical Research and Opinion*, *23*, 533–543.

Melck, D., Rueda, D., Galve-Roperh, I., De Petrocellis, L., Guzman, M., & Di Marzo, V. (1999). Involvement of the cAMP/protein kinase A pathway and of mitogen-activated protein kinase in the anti-proliferative effects of anandamide in human breast cancer cells. *FEBS Letters*, *463*, 235–240.

Miller, A. D. (1998). Nausea and vomiting: underlying mechanisms and upcoming treatments. *Journal of the Japan Bronchoesophagological Society*, *49*, 57–64.

Moschos, S. J., & Mantzoros, C. S. (2002). The role of the IGF system in cancer: from basic to clinical studies and clinical applications. *Oncology*, *63*, 317–332.

Munson, A. E., Harris, L. S., Friedman, M. A., Dewey, W. L., & Carchman, R. A. (1975). Antineoplastic activity of cannabinoids. *Journal of the National Cancer Institute*, *55*, 597–602.

Nelson, K., Walsh, D., Deeter, P., & Sheehan, F. (1994). A phase II study of delta-9-tetrahydrocannabinol for appetite stimulation in cancer-associated anorexia. *Journal of Palliative Care*, *10*, 14–18.

Noyes, R., Jr., Brunk, S. F., Avery, D. A., & Canter, A. C. (1975). The analgesic properties of delta-9-tetrahydrocannabinol and codeine. *Clinical Pharmacology and Therapeutics*, *18*, 84–89.

Pertwee, R. G. (2001). Cannabinoid receptors and pain. *Progress in Neurobiology*, *63*, 569–611.

Plasse, T. F., Gorter, R. W., Krasnow, S. H., Lane, M., Shepard, K. V., & Wadleigh, R. G. (1991). Recent clinical experiences with dronabinol. *Pharmacology, Biochemistry and Behavior*, *40*, 695–700.

Portenoy, R. K., Ganae-Motan, E. D., Allende, S., Yanagihara, R., Shaiova, L., Weinstein, S., McQuade, R., Wright, S., & Fallon, M. T. (2012). Nabiximols for opioid-treated cancer patients with poorly-controlled chronic pain: a randomized, placebo-controlled, graded-dose trial. *Journal of Pain*, *13*, 438–449.

Qamri, Z., Preet, A., Nasser, M. W., Bass, C. E., Leone, G., Barsky, S. H., & Ganju, R. K. (2009). Synthetic cannabinoid receptor agonists inhibit tumor growth and metastasis of breast cancer. *Molecular Cancer Therapeutics*, *3*, 3117–3129.

Sanchez, M. G., Sanchez, A. M., Ruiz, L. L., & Diaz-Laviada, I. (2003). Enhancement of androgen receptor expression induced by (R)-methandamide in prostate LNCaP cells. *FEBS Letters*, *555*, 561–566.

Shoemaker, L. K., Estfan, B., Induru, R., & Walsh, T. D. (2011). Symptom management: an important part of cancer care. *Cleveland Clinic Journal of Medicine*, *78*, 25–34.

Slatkin, N. E. (2007). Cannabinoids in the treatment of chemotherapy-induced nausea and vomiting: beyond prevention of acute emesis. *Journal of Supportive Oncology*, *5*, 1–9.

Smith, S. R., Terminelli, C., & Denhardt, G. (2000). Effects of cannabinoid receptor agonist and antagonist ligands on production of inflammatory cytokines and anti-inflammatory interleukin-10 in endotoxemic mice. *Journal of Pharmacology and Experimental Therapeutics*, *293*, 136–150.

Staquet, M., Gantt, C., & Machin, D. (1978). Effect of a nitrogen analog of tetrahydrocannabinol on cancer pain. *Clinical Pharmacology and Therapeutics*, *23*, 397–401.

Strasser, F., Luftner, D., Possinger, K., Ernst, G., Ruhstaller, T., Meissner, W., Ko, Y. D., Schnelle, M., Reif, M., & Cerny, T. (2006). Comparison of orally administered cannabis extract and delta-9-tetrahydrocannabinol in treating patients with cancer-related anorexia–cachexia syndrome: a multicenter, phase III, randomized, double-blind, placebo-controlled clinical trial from the Cannabis-In-Cachexia-Study-Group. *Journal of Clinical Oncology*, *24*, 3394–3400.

Temel, J. S., Greer, J. A., Muzikansky, A., Gallagher, E. R., Admane, S., Jackson, V. A., Dahlin, C. M., Blinderman, C. D., Jacobsen, J., Pirl, W. F., Billings, J. A., & Lynch, T. J. (2010). Early palliative care for patients with metastatic non-small-cell lung cancer. *New England Journal of Medicine*, *363*, 733–742.

Tramèr, M. R., Carroll, D., Campbell, F. A., Reynolds, D., John, M., Moore, R. A., & McQuay, H. J. (2001). Cannabinoids for control of chemotherapy induced nausea and vomiting: quantitative systematic review. *British Medical Journal*, *323*, 1–8.

Velasco, G., Galve-Roperh, I., Sánchez, C., Blázquez, C., & Guzmán, M. (2004). Hypothesis: cannabinoid therapy for the treatment of gliomas? *Neuropharmacology*, *47*, 315–323.

CHAPTER

90

The Use of Medical Marijuana in the Treatment of Psychiatric Disorders

T. Tellioğlu, Z. Tellioğlu***

***Brown University, Alpert Medical School, Substance Abuse Division, Rhode Island Hospital, Providence, RI, United States**

****Brown University, Alpert Medical School, Rhode Island Hospital, Providence, RI, United States**

SUMMARY POINTS

- Currently, there is no scientific evidence that marijuana is beneficial for the treatment of any psychiatric disorder. Therefore current treatments should not be altered until sufficient clinical evidence supports such changes.
- If the scientific evidence supports the use of marijuana to treat specific conditions, the medication should be subject to the approval process of the Food and Drug Administration (FDA).
- Further research on the use of marijuana as medicine in psychiatric conditions should be encouraged and facilitated by the federal government in collaboration with the appropriate medical societies, such as the American Psychiatric Association (APA) and/or the American Society of Addiction Medicine (ASAM).

KEY FACTS OF MEDICAL MARIJUANA IN PSYCHIATRIC DISORDERS

- This chapter is aimed to investigate the current evidence in the medical literature about the possible use of medical marijuana in the treatment of psychiatric disorders.
- It appears that the majority of anecdotally reported improvements are related to the effects of heightened mood or euphoria, and relaxation, but not to an evidence-based improvement in psychiatric conditions.
- There are some meaningful and promising research data available for anxiety disorders, especially PTSD, which warrants further, higher quality research.
- Current legal regulations ominously limit the research of marijuana and its components.

LIST OF ABBREVIATIONS

CB_1	Cannabinoid type 1 receptors
CBD	Cannabidiol
Delta-9-THC	Delta-9-tetrahydrocannabinol

MEDICAL MARIJUANA

Medical marijuana is one of the most discussed and highly controversial issues in medical treatment. From ordinary people on the streets to high-rating TV news anchormen; from medical practitioners of the various specialties to the legislators of the several States' Senates, the pros and cons of medical marijuana have been debated extensively. Meanwhile, marijuana use rates have risen by 30% from 2006 to 2010, according to a new report from the White House Office of National Drug Control Policy and RAND Drug Policy Research Center (Kilmer

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00105-8
Copyright © 2017 Elsevier Inc. All rights reserved.

et al., 2014). Marijuana is a Schedule 1 chemical—placed in the same classification as heroin—which states that there is no medicinal use or benefit, and there is a high risk of harm. Therefore under federal law, the use, sale, and possession of marijuana in the United States are still illegal. Some states (Colorado and Washington) have created exemptions for the medical use of cannabis, as well as decriminalized nonmedical/recreational marijuana use; however, the decisions were made by legislators but not by the medical authorities. These legislators are under extensive pressure from the public (their voters and also active users), from occluded judicial system which has been struggling to keep chasing, trying or imprisoning the offenders, or from unstable State budgets which hope to create additional income by taxing marijuana sales. On the contrary, medicinal use of marijuana is not supported by the medical authorities (ASAM, 2010; Zaman, Rosenthal, Renner, Kleber, & Milin, 2013) which have major, realistic concerns of allowing a "street drug" to replace (or, at least, to challenge with) the well-supported, established, and controllable medicinal treatments. Medicine, as an evidence-based science, has been approaching the medical marijuana issue very cautiously due to the lack of sufficient, supportive clinical data, and also due to the legal risk of being penalized by using, researching, or recommending a chemical compound which has been declared a crime under federal law.

Medical marijuana simply refers to the use of marijuana, or cannabis derivates as a medical therapy to treat a medical condition, or to relieve its symptoms. (Of note, since cannabis herb or marijuana is much more prominently cultivated and consumed than cannabis resin, hashish, therefore the focus will be more on marijuana in this chapter.) Similar to being treated with prescription medications, which are recommended or prescribed by a clinician/prescriber, individuals who suffer from certain medical conditions have been using the parts of the cannabis plant by smoking, ingesting, or inhaling the vapor. In 2012, the United Nations Office on Drugs and Crime (UNODC) estimated that there were between 119 million and 224 million cannabis users worldwide, ranging from 2.6% to 5% of the adult population (UNODC, 2012). The data are not very reliable as to what portion of these active users consume marijuana for recreational or for medicinal purposes. In both conditions, marijuana users usually experience a heightened mood or euphoria and relaxation soon after the consumption (inhalation faster than ingestion, as expected). Some people report increased enjoyment of food, taste and smell, an enhanced appreciation for music and comedy, and the subjective impression of long elapsed time. At higher doses, altered body image, auditory and/or visual hallucinations, and dissociative states were reported which are most often considered desirable as it has been used in certain religious rituals. All those self-reported experiences and their intensity may differ among people since those are highly subjective.

In laboratory settings, the most common short-term physical effects are observed as (Galanter & Kleber, 2008):

- increased heart rate,
- lowered blood pressure,
- muscle relaxation,
- decreased intraocular pressure,
- reddening of the eyes.

The central nervous system effects of cannabis may be categorized under four major groups (Galanter & Kleber, 2008):

1. mood (euphoria, unreasonable laughing, and in larger doses feelings of paranoia or anxiety),
2. perception (distortions in the perception of time and space),
3. somatic (weakness, lightheadedness, motor coordination difficulties),
4. cognitive (confusion, concentration problems and impairment of short-term and working memory).

Since the beginning of the written history, people have been using several substances to self-administer treatment for often unmanaged, undiagnosed physical, or psychological conditions (Snelders, Kaplan, & Pieters, 2006). Recreational chemicals, psychoactive drugs, alcohol, and other forms of self-medication are used to alleviate symptoms of mental distress, stress, and anxiety, including mental illnesses and/or psychological trauma. Among those, marijuana is often consumed recreationally for its heightened mood or euphoria, and relaxation; however, historic evidence shows that it was also used as a medicine for insomnia, for pain conditions, and to increase appetite in 19th and 20th century. Although its mechanism is not yet understood, several randomized controlled studies suggest that cannabinoids can create analgesia compared to placebo in chronic noncancer pain patients. Severe nausea caused by chemotherapies may respond to marijuana components. Several debilitating conditions, such as acute immune deficiency syndrome (AIDS), anorexia, may benefit from the effect of appetite increasing and antiemetic properties of marijuana. Besides cancer, AIDS and pain conditions, marijuana has been suggested as a potential effective, safe, and low-cost alternative (or adjunct treatment) in other medical conditions, such as multiple sclerosis (MS) and epilepsy. (More detailed discussions about the medical use of marijuana may be found in the other chapters of this book.) All these exciting news has been getting a lot of attention from public, from lawmakers, and from the scientific community.

While the medicinal use of marijuana has been debated for the treatment of medical conditions, patients who are suffering from mental disorders have also reported some benefit (Tellioğlu & Tellioğlu, 2012). Therefore in this chapter, we aimed to investigate the current evidence in the medical literature about the possible use of medical marijuana in the treatment of psychiatric disorders.

MOOD DISORDERS

With the use of marijuana, some patients with depression report improvement in their mood symptoms: living and enjoying life again, taking walks, riding bicycles, maintaining regular sleep and appetite schedules, and doing things that they would not even consider/or be able to do while they were depressed. Carter, Gieringer and Rosenthal (2008) mentioned in their *Marijuana Medical Handbook* that marijuana has potential to manage and improve depressive conditions. In an Internet survey over 4400 adult Internet users it was reported that those who were using marijuana once per week had less depressed mood, more positive affect, and fewer somatic complaints than nonusers (Denson & Earleywine, 2006).

Several clinical studies have also indirectly observed some improvement in depression with the use of marijuana. Prentiss, Power, Balmas, Tzuang, and Israelski (2004) reported perceived benefit of smoked marijuana on 252 HIV-positive patients in terms of relief of anxiety and/or depression (57%), improved appetite (53%), increased pleasure (33%), and relief of pain (28%). Although the study used subjective experience of improvement assessed with a Likert scale (from "1 = not helpful" to "5 = very helpful"), these results may be encouraging to help patients' compliance with treatment and overall improvement. Similarly, patients with MS report symptomatic improvements from marijuana (either self medicated or cannabinoid treated). While the main improvements in MS are observed particularly for muscle stiffness and spasms, anecdotal improvements were reported in the mood symptoms. However, these improvements were not supported by clinical research. A study in 630 MS patients who were treated with oral THC showed evidence of a small treatment effect on muscle spasticity energy level, and tiredness, but unfortunately not on depression rating scales (Zajicek et al., 2005).

An interesting piece of information came from a recent study (Anderson, Rees, & Sabia, 2014) reporting that suicide rates among men aged 20–39 years fell after medical marijuana legalization compared with those in States that did not legalize. The negative relationship between legalization and suicides among young men was interpreted that marijuana may be used to cope with stressful life events.

On the contrary, acute interruption of heavy use of marijuana (multiple times daily) was reported to cause depression along with other symptoms, including mood swings, irritability, anxiety, aggression, nervousness, restlessness, and a loss of concentration (Budney, Hughes, Moore, & Novy, 2001). Compared to withdrawal symptoms associated with quitting alcohol or other drugs, marijuana withdrawal symptoms are relatively mild, but they are uncomfortable enough to cause many individuals to relapse to relieve those symptoms (Levin et al., 2010). Typically, these symptoms begin to diminish after 2–3 weeks, but can remain longer. Interestingly, studies showed that blockade of cannabinoid receptors are also linked to depressive thoughts and suicidal behavior. In 2008, marketing of rimonabant, a promising, breakthrough weight loss agent which blocks the cannabinoid type 1 receptors (CB_1) receptor, was halted because of psychiatric concerns and ongoing clinical trials were stopped after the observation of serious psychiatric side effects and four suicides in the rimonabant arm, while one patient taking the placebo committed suicide (Topol et al., 2010).

Psychiatric side effects caused by either acute interruption of marijuana use or blockade of cannabis receptors suggest that these receptors may actively play a role in mood regulation. However, current data lack of sufficient evidence to support a medical use of marijuana or its derivates in the treatment of depressive disorders. Anecdotal reports of potential benefits of marijuana is likely a result of the euphoria and relaxation experienced by individuals; well-designed randomized clinical trials have not demonstrated any role of marijuana in the treatment of mood disorders, it would be premature to discuss its therapeutic value.

Marijuana use disorders commonly cooccur in bipolar disorder, too; however, the effects of marijuana abuse on outcome have not been well researched. Case histories indicate that a number of patients find marijuana useful in the treatment of their bipolar disorder (Ashton, Moore, Gallagher, & Young, 2005). A report implied that marijuana may actually be mood stabilizing in patients with bipolar disorder, or as an adjunct to lithium (allowing reduced dose or relief of lithium's side effects) (Grinspoon & Bakalar, 1998). However, this suggestion lacks research support. A clinical trial of CBD in two patients with bipolar I disorder failed to show any substantial improvement for the manic episode of these patients (Zuardi et al., 2010). Another study (Strakowski et al., 2007), which followed 144 patients with bipolar I disorder (manic or mixed) for up to 5 years found that marijuana use was associated with longer affective episodes and with rapid cycling. Therefore aggressive drug abuse treatment might decrease

the rate of recurrence and readmission in the course of bipolar disorder.

ANXIETY DISORDERS

Marijuana is commonly known for its relaxing effect which is the most common reason cited for its use. However, patients with panic and anxiety were found to be particularly likely to experience increased anxiety while smoking marijuana (Szuster, Pontius, & Campos, 1988). As a result, continued marijuana smoking in these individuals was found to be infrequent. Panic attacks may occur during the first use of marijuana, and they may even persist in the absence of continued use of marijuana (Yanık, 2002).

These opposing effects of marijuana are considered to be related to the location and CB_1 receptors which are on either presynaptic glutamatergic or GABAergic neurons, resulting in a respective decrease in either glutamate or GABA release. As a result, limiting glutamate release causes reduced excitation, while limiting GABA release suppresses inhibition (Elphick & Egertova, 2001). Researchers from Vanderbilt University identified recently CB receptors in the central amygdala (CeA) which is a key structure at the limbic-motor interface regulating stress responses (Ramikie et al., 2014). Since activation of the CB_1 receptor suppresses glutamate release in this region, it may help explain why marijuana users say they take the drug mainly to reduce anxiety. On the other hand, animal and humans studies suggest that the two main constituents of the marijuana plant delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) have quite different acute effects. A functional magnetic resonance imaging study demonstrated that THC and CBD differentially modulate prefrontal, striatal, and hippocampal function during attentional salience processing (Bhattacharyya et al., 2010). In a different study (Martin-Santos et al., 2012), individuals were administered oral THC 10 mg or CBD 600 mg or placebo in a randomized, double-blind, cross-over, placebo-controlled trial. While administration of THC was associated with anxiety, dysphoria, positive psychotic symptoms, physical and mental sedation, subjective intoxication, and an increase in heart rate, there were no differences between CBD and placebo on any symptomatic or physiological variable, suggesting that CBD is better tolerated. Therefore it may not be surprising to hear that individuals who use marijuana actively state that either the suppliers do recommend specific marijuana mixtures to be used for certain conditions, (such as for sleep, pain, or relaxation, etc.) mostly due to THC and CBD content in the mixture.

People with posttraumatic stress disorder (PTSD) who use marijuana often report more relief from their symptoms (anxiety, flashbacks, nightmares, and emotional instability) than they do from antidepressants and other psychiatric medications. However, there has been no controlled trial to test how (or, if) marijuana attenuates any symptoms. In 2013, NYU researchers discovered a connection between the quantities of cannabinoid CB_1 receptors in the human brain by demonstrating that PTSD patients had higher levels of CB_1 receptors in the parts of the brain associated with fear and anxiety (Neumeister et al., 2013). They also have markedly lower concentrations of anandamide, an endocannabinoid, which suggested that trauma-related low levels of anandamide may lead to the brain compensation by increasing the number of CB_1 receptors, which eventually cause clinical symptoms. Abnormal CB_1 receptor-mediated anandamide signaling could provide a promising target to develop novel pharmacotherapies for this disorder. Since blockade of CB_1 receptors could lead to depression and suicide (Topol et al., 2010), a chemical treatment that would restore or provide a normal balance of the endocannabinoids in the brains of people with PTSD by activating CB receptors. Recently, the US Department of Health and Human Services approved that researchers at the University of Arizona could move forward with a study which will measure the effects of 5 different potencies of smoked or vaporized marijuana in treating symptoms of PTSD in 50 veterans (Klimas, 2014) which may be a pioneering step for further studies.

A possible anxiolytic action of CBD led to the hypothesis that this cannabinoid could be effective to treat social anxiety disorder (SAD). In a study of 24 patients with SAD who were given CBD 600 mg, the patients performed similarly in terms of the levels of anxiety, somatic symptoms, and negative self-assessment to controls and to the SAD patients who received placebo (Bergamaschi et al., 2011). In another study, a single dose of CBD 400 mg in a sample of patients with SAD was able to reduce subjective anxiety measures and regional changes detected by SPECT, supporting the neural correlates of CBD's anxiolytic effects (Crippa et al., 2011).

CBD appears to be the main marijuana compound that is closer to have its preliminary findings in anxiety translated into clinical practice. Future studies should test this possibility in clinical trials involving patients with different anxiety disorders, especially panic disorder, SAD, and PTSD.

SLEEP DISORDERS

Mental health clinicians are aware that many psychiatric patients use marijuana for sleep. It also appears that many patients suffering from insomnia also use it.

Many swear by its ability to help them relax and fall into a deep, restful sleep. Studies showed that acute administration of marijuana decreases the sleep latency, and appears to facilitate falling asleep (Schierenbeck, Riemann, Berger, & Hornyak, 2008). Researchers conducting clinical trials of marijuana for other chronic pain disorders and MS have reported improved sleep as secondary outcomes (Russo, Guy, & Robson, 2007). A study reported that nabilone, a synthetic marijuana, improved sleep disturbance in fibromyalgia (FM) (Ware, Fitzcharles, Joseph, & Shir, 2010). Its effect was found to be superior to amitriptyline, suggesting that nabilone is effective in improving sleep in patients with FM, and is well tolerated, and was suggested as an alternative to amitriptyline in FM patients.

Improvement in sleep quality in chronic pain syndromes was considered to be due to analgesic, and spasmolytic effects resulting in nocturnal symptom relief, in addition to the hypnotic properties of marijuana. Some tolerance may occur to the sleep-inducing effect in the chronic marijuana administration. On the contrary, abrupt discontinuation of heavy marijuana use is associated with self-reports of sleep difficulty (Budney et al., 2001), and resumption of the marijuana use attenuates sleep disturbances. Disturbed sleep is clinically important because marijuana users experiencing sleep problems may relapse to marijuana use to improve their sleep quality (Bolla et al., 2010). Despite these promising observations of sleep improvement as secondary outcomes on certain populations, current literature lacks the long-term safety and efficacy of marijuana and its derivates to be safely used in sleep disorders. Further studies on the effects of marijuana on sleep architecture and long-term safety and efficacy are warranted.

PSYCHOSIS

Marijuana can produce a full range of transient schizophrenia-like positive, negative, and cognitive symptoms in some healthy individuals. In patients with an established psychotic disorder, marijuana can exacerbate symptoms, trigger relapse, and have negative consequences on the course of the illness. Furthermore, exposure to heavy use of marijuana was considered a premorbid precipitant and a stressor eliciting relapse in patients with schizophrenia and related disorders (Burns, 2013; Linszen, Dingemans, & Lenior, 1994). Despite these negative consequences, marijuana use was observed in every fourth schizophrenia patient which is 2 times more frequent than in the general population (Koskinen, Lohonen, Koponen, Isohanni, & Miettunen, 2010). One common theory is that patients with psychosis use marijuana for the same reasons the general population does, to "get high", relax, and have fun. There is also the "self-medication" hypothesis, which explains the high rates of abuse by an "alleviation of dysphoria" model (Kolliakou, Joseph, Ismail, Atakan, & Murray, 2011). While cannabis use has been reported to increase positive symptoms in schizophrenia, the findings regarding negative symptoms have been contradictory. In a study, patients with a history of marijuana use were found to have better performance on tests of visual and working memory, and executive functioning (Yucel et al., 2012) which suggested a short-term stimulation of prefrontal neurotransmission to enhance cognitive functions (Verrico, Jentsch, & Roth, 2003). However, in the long-term, repeated administration is shown unfavorable (D'Souza et al., 2005). Another important data suggest that CBD was able to prevent psychotic-like symptoms induced by high doses of THC (Zuardi et al., 2012), suggesting antipsychotic-like properties of CBD. In addition, a preliminary report of a controlled clinical trial comparing CBD with an atypical antipsychotic drug (amisulpride) has suggested that CBD may be an alternative treatment for schizophrenia (Zuardi, Crippa, Hallak, Moreira, & Guimaraes, 2006).

SUBSTANCE USE DISORDERS

Marijuana is commonly used in combination with heroin, cocaine, alcohol, and benzodiazepines in individuals with addiction disorders. In a community-based dispensary, many individuals report using marijuana as a substitute for other potentially more dangerous substances, such as alcohol, or illicit drugs, and for pharmaceuticals due to side effects, and better symptom management (Reiman, 2009).

Historical evidence shows that *Cannabis indica* (a type of marijuana plant which has a higher ratio of CBD:THC compared to *Cannabis sativa*) was used by doctors for conditions, such as agitation and delirium tremens, related to drug dependence and withdrawals (Snelders et al., 2006). This beneficial effect may be explained by a protective effect of CB system against the hyperexcitability of the neurons. During an alcohol withdrawal, excitotoxic damages cause neuronal death. In an in vitro experiment, a CB agonist HU-210 was shown to decrease *N*-methyl-D-aspartate (NMDA)-induced neuronal death exclusively in alcohol-withdrawn neurons (Rubio et al., 2011). By contrast, it was shown that the inhibition of the CB_1 receptor by rimonabant during alcohol withdrawal increased death of alcohol-withdrawn neurons. These results suggest that cannabinoids may have neuroprotectant effect which may potentially be beneficial in the prevention of neuron damage in alcohol withdrawal.

On the other side, THC was suggested that it could reduce opioid withdrawal symptoms. The increment of activity of endogenous cannabinoids (via inhibition of the hydrolytic enzymes) was shown to reduce naloxone-precipitated morphine withdrawal symptoms in in vivo and in vitro models of opioid dependence (Ramesh et al., 2011). A clinical study reported that increased marijuana use was found to be associated with lower severity of withdrawal during methadone induction, suggesting a potential role for marijuana in the reduction of withdrawal severity (Scavone, Sterling, Weinstein, & Van Bockstaele, 2013). Similarly, in patients with cocaine dependence and comorbid attention deficit hyperactivity disorder, marijuana users were more successful than other patients in abstaining from cocaine use (Aharonovich et al., 2006). Furthermore, animal studies showed that CB_1 receptor plays an important role in the mechanisms underlying relapse to heroin-seeking behavior, and CB_1 antagonists could be possible therapeutic agents in the prevention of relapse to heroin abuse (Spano et al., 2004).

Despite these promising preclinical and pilot data and the availability of medical marijuana, it was reported that it increased the number of binge drank days in adults by 6–9% suggesting that by legalizing marijuana, alcohol consumption and binge drinking have increased (Wen, Hockenberry, & Cummings, 2014). Also, marijuana use was reportedly not associated with treatment retention, opiate/cocaine use, or any measure of treatment outcome in methadone maintenance treatment (Scavone et al., 2013).

COGNITIVE DISORDERS AND DEMENTIA

Marijuana use among healthy users has been associated with cognitive impairments, including residual deficits in memory and attention on tests measuring memory, executive functioning, psychomotor speed, and manual dexterity (Pope, Gruber, Hudson, Huestis, & Yurgelun-Todd, 2002). It was reported that heavy users had more difficulty than light users in sustaining and shifting attention and, hence, in registering, organizing, and using information (Bolla, Brown, Eldreth, Tate, & Cadet, 2002). In controlled trials, it was demonstrated that acute administration of THC impairs cognitive performance not only in healthy controls but also in patients with schizophrenia (D'Souza et al., 2005).

Furthermore, in individuals with no medical or psychiatric history it was shown that long-term marijuana use is associated with structural brain abnormalities in a dose-dependent manner (Yucel et al., 2008).

In patients with dementia, night time agitation occurs frequently and creates a major burden on caregivers. An open study of THC for the treatment of agitated behavior at night in severe dementia revealed a 59% decrease in nocturnal motor activity without any adverse effects after single and repeated administrations of THC (Walther, Mahlberg, Eichmann, & Kunz, 2006). Since treatment options are limited (antipsychotics have a black box warning of cardiovascular deaths and benzodiazepines are not recommended), alternative treatments are desperately warranted in this quickly growing group of patients. Randomized controlled trials with larger sample sizes and longer treatment periods needed to corroborate these preliminary findings.

CONCLUSIONS

While the functions of the endocannabinoid system is still under investigation, anecdotally reported improvements in psychiatric conditions invite researchers to explore the possible use of medical marijuana and its derivates in the treatment of psychiatric disorders. Although mostly anecdotal, there are several conditions reported for which the active ingredients of the marijuana plant might have showed some therapeutic effect. In 2014, the website of *clinicaltrials.gov* (reached on 5/1/14) showed that there were 395 current studies for the search word "marijuana"(National Institutes of Health, 2014). Despite this high interest, available clinical data are extremely limited, which confine clinicians to suggest marijuana use for any psychiatric disorders currently. It appears like majority of the reported improvements of symptoms are related to the effects of heightened mood or euphoria, and relaxation, but not an evidence-based improvement in psychiatric conditions. While marijuana may be initially used as a self-therapy for certain conditions, the prolonged exposure to this substance in vulnerable individuals may in turn alter or aggravate depressive or anxious manifestations, and may cause patients refractory to standard treatments (Tambaro & Bortolato, 2012). Further, these chemicals have the risk of causing serious detriment to physical and mental health due to the risk of the addiction syndrome (ASAM, 2010). Nevertheless, pharmacologically, there are 483 known compounds present in the marijuana plant, including 85 cannabinoids, such as CBD and THC (Russo, 2013). As discussed in this chapter, some of these chemicals (such as CBD) are shown that they may have potential beneficial effects on anxiety, neuroprotection, and sleep, which warrant further, good quality clinical research under close monitoring of the federal agencies in collaboration with the medical/psychiatric authorities (Table 90.1).

TABLE 90.1 Comparison of Some Effects of Delta-9-THC (Delta-9-Tetrahydrocannabinol) and CBD (Cannabidiol)

Actions	THC	CBD
Agonist action on CB_1 receptors	+	–
Inhibition of anandamide reuptake and hydrolysis	–	+
Anxiolytic	+[a]	+
Psychotropic	+	–
Antipsychotic	–	+[b]
Anticonvulsant	–	+
Antidepressant	(+)[c]	–
Sedative/hypnotic	+	+
Antinociceptive	+	+
Neuroprotective (inhibition of glutamate release)	+	+
Antiemetic	+	–
Appetite stimulant	+	No data
Cardiovascular effects[d]	+	+

[a]*THC is anxiolytic in some doses, but can be anxiogenic in higher doses or in drug-naïve individuals.*
[b]*CBD also antagonizes some psychotropic effects of THC.*
[c]*Shown in one study in cancer patients.*
[d]*THC causes tachycardia and hypotension; CBD can cause bradycardia and hypotension.*
Reprinted from Ashton et al. (2005).

References

Aharonovich, E., Garawi, F., Bisaga, A., Brooks, D., Raby, W. N., Rubin, E, … Levin, F. R. (2006). Concurrent cannabis use during treatment for comorbid ADHD and cocaine dependence: effects on outcome. *The American Journal of Drug and Alcohol Abuse*, *32*(4), 629–635.

Anderson, D. M., Rees, D. I., & Sabia, J. J. (2014). Medical marijuana laws and suicides by gender and age. *American Journal of Public Health*, *104*(12), 2369–2376.

America Society of Addiction Medicine (ASAM). (2010). *Public policy statement on medical marijuana*. Chevy Chase, MD: American Society of Addiction Medicine.

Ashton, C. H., Moore, P. B., Gallagher, P., & Young, A. H. (2005). Cannabinoids in bipolar affective disorder: a review and discussion of their therapeutic potential. *Journal of Psychopharmacology*, *19*(3), 293–300.

Bergamaschi, M. M., Queiroz, R. H., Chagas, M. H., de Oliveira, D. C., De Martinis, B. S., Kapczinski, F., … Crippa, J. A. (2011). Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naive social phobia patients. *Neuropsychopharmacology*, *36*(6), 1219–1226.

Bhattacharyya, S., Morrison, P. D., Fusar-Poli, P., Martin-Santos, R., Borgwardt, S., Winton-Brown, T., … McGuire, P.K. (2010). Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology*, *35*(3), 764–774.

Bolla, K. I., Brown, K., Eldreth, D., Tate, K., & Cadet, J. L. (2002). Dose-related neurocognitive effects of marijuana use. *Neurology*, *59*(9), 1337–1343.

Bolla, K. I., Lesage, S. R., Gamaldo, C. E., Neubauer, D. N., Wang, N. Y., Funderburk, F. R. … Cadet, J. L. (2010). Polysomnogram changes in marijuana users who report sleep disturbances during prior abstinence . *Sleep Med*, *11*(9), 882–889.

Budney, A. J., Hughes, J. R., Moore, B. A., & Novy, P. L. (2001). Marijuana abstinence effects in marijuana smokers maintained in their home environment. *Archives of General Psychiatry*, *58*(10), 917–924.

Burns, J. K. (2013). Pathways from cannabis to psychosis: a review of the evidence. *Frontiers in Psychiatry*, *4*, 128.

Carter, G. T., Gieringer, D, & Rosenthal, Ed. (2008). *Marijuana medical handbook: practical guide to therapeutic uses of marijuana* (revised edition). San Francisco, CA: Quick American Archives.

Crippa J. A., Derenusson, G. N., Ferrari, T. B., Wichert-Ana, L., Duran, F. L., Martin-Santos, R., … Hallak, J. E. (2011). Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. *Journal of Psychopharmacol*, *25*(1), 121–130.

D'Souza, D. C., Abi-Saab, W. M., Madonick, S., Forselius-Bielen, K., Doersch, A., Braley, G., … Krystal, J. H. (2005). Delta-9-tetrahydrocannabinol effects in schizophrenia: implications for cognition, psychosis, and addiction. *Biological Psychiatry*, *57*(6), 594–608.

Denson, T. F., & Earleywine, M. (2006). Decreased depression in marijuana users. *Addiction Behavior*, *31*(4), 738–742.

Elphick, M. R., & Egertova, M. (2001). The neurobiology and evolution of cannabinoid signalling. *Philosophical Transactions of the Royal Society of London B. Biological Science*, *356*(1407), 381–408.

Galanter, M., & Kleber, H. D. (2008). *The American Psychiatric Publishing textbook of substance abuse treatment*. Arlington, VA: American Psychiatric Publishing.

Grinspoon, L., & Bakalar, J. B. (1998). The use of cannabis as a mood stabilizer in bipolar disorder: anecdotal evidence and the need for clinical research. *Journal of Psychoactive Drugs*, *30*(2), 171–177.

Kilmer, B., Everingham, S., Caulkins, J., Midgette, G., Pacula, R., Reuter, P., Burns, R., Han, B., & Lundberg, R. (2014). *What America's Users Spend on Illegal Drugs: 2000–2010*. Washington, DC, and Santa Monica, CA: White House Office of National Drug Control Policy and the RAND Drug Policy Research Center, 46–62.

Klimas, J. Veterans push to test marijuana as a life-saving treatment for crippling PTSD. (2014). Available from http://www.washingtontimes.com/news/2014/apr/21/younger-veterans-push-for-marijuana-test-as-treatm/#ixzz31ecYAPPt

Kolliakou, A., Joseph, C., Ismail, K., Atakan, Z., & Murray, R. M. (2011). Why do patients with psychosis use cannabis and are they ready to change their use? *International Journal of Developmental Neuroscience*, *29*(3), 335–346.

Koskinen, J., Lohonen, J., Koponen, H., Isohanni, M., & Miettunen, J. (2010). Rate of cannabis use disorders in clinical samples of patients with schizophrenia: a meta-analysis. *Schizophrenia Bulletin*, *36*(6), 1115–1130.

Levin, K. H., Copersino, M. L., Heishman, S. J., Liu, F., Kelly, D. L., Boggs, D. L., & Gorelick, D. A. (2010). Cannabis withdrawal symptoms in non-treatment-seeking adult cannabis smokers. *Drug and Alcohol Dependence*, *111*(1–2), 120–127.

Linszen, D. H., Dingemans, P. M., & Lenior, M. E. (1994). Cannabis abuse and the course of recent-onset schizophrenic disorders. *Archives of General Psychiatry*, *51*(4), 273–279.

Martin-Santos, R., Crippa, J. A., Batalla, A., Bhattacharyya, S., Atakan, Z., Borgwardt, S., … McGuire, P. K. (2012). Acute effects of a single, oral dose of d9-tetrahydrocannabinol (THC) and cannabidiol (CBD) administration in healthy volunteers. *Current Pharmaceutical Design*, *18*(32), 4966–4979.

National Institutes of Health. ClinicalTrials.gov. (2014). Available from: www.ClinicalTrials.gov

Neumeister, A., Normandin, M. D., Pietrzak, R. H., Piomelli, D., Zheng, M. Q., Gujarro-Anton, A., … Huang, Y. (2013). Elevated brain cannabinoid CB1 receptor availability in post-traumatic stress

disorder: a positron emission tomography study. *Molecular Psychiatry, 18*(9), 1034–1040.

Pope, H. G., Jr., Gruber, A. J., Hudson, J. I., Huestis, M. A., & Yurgelun-Todd, D. (2002). Cognitive measures in long-term cannabis users. *Journal of Clinical Pharmacology, 42*(11 Suppl.), 41S–47S.

Prentiss, D., Power, R., Balmas, G., Tzuang, G., & Israelski, D. M. (2004). Patterns of marijuana use among patients with HIV/AIDS followed in a public health care setting. *Journal of Acquired Immune Deficiency Syndrome, 35*(1), 38–45.

Ramesh, D., Ross, G. R., Schlosburg, J. E., Owens, R. A., Abdullah, R. A., Kinsey, S. G., … Lichtman, A. H. (2011). Blockade of endocannabinoid hydrolytic enzymes attenuates precipitated opioid withdrawal symptoms in mice. *The Journal of Pharmacology and Experimental Therapeutics, 339*(1), 173–185.

Ramikie, T. S., Nyilas, R., Bluett, R. J., Gamble-George, J. C., Hartley, N. D., Mackie, K., … Patel, S. (2014). Multiple mechanistically distinct modes of endocannabinoid mobilization at central amygdala glutamatergic synapses. *Neuron, 81*(5), 1111–1125.

Reiman, A. (2009). Cannabis as a substitute for alcohol and other drugs. *Harm Reduction Journal, 6*, 35.

Rubio, M., Villain, H., Docagne, F., Roussel, B. D., Ramos, J. A., Vivien, D., … Ali, C. (2011). Pharmacological activation/inhibition of the cannabinoid system affects alcohol withdrawal-induced neuronal hypersensitivity to excitotoxic insults. *PLoS One, 6*(8), e23690.

Russo, E. B. (2013). *Cannabis and cannabinoids: pharmacology, toxicology, and therapeutic potential*. London, UK, and New York, NY: Taylor & Francis.

Russo, E. B., Guy, G. W., & Robson, P. J. (2007). Cannabis, pain, and sleep: lessons from therapeutic clinical trials of Sativex, a cannabis-based medicine. *Chemistry and Biodiversity, 4*(8), 1729–1743.

Scavone, J. L., Sterling, R. C., Weinstein, S. P., & Van Bockstaele, E. J. (2013). Impact of cannabis use during stabilization on methadone maintenance treatment. *American Journal of Addiction, 22*(4), 344–351.

Schierenbeck, T., Riemann, D., Berger, M., & Hornyak, M. (2008). Effect of illicit recreational drugs upon sleep: cocaine, ecstasy and marijuana. *Sleep Medicine Reviews, 12*(5), 381–389.

Snelders, S., Kaplan, C., & Pieters, T. (2006). On cannabis, chloral hydrate, and career cycles of psychotropic drugs in medicine. *Bulletin of the History of Medicine, 80*(1), 95–114.

Spano, M. S., Fattore, L., Cossu, G., Deiana, S., Fadda, P., & Fratta, W. (2004). CB1 receptor agonist and heroin, but not cocaine, reinstate cannabinoid-seeking behaviour in the rat. *British Journal of Pharmacology, 143*(3), 343–350.

Strakowski, S. M., DelBello, M. P., Fleck, D. E., Adler, C. M., Anthenelli, R. M., Keck, P. E., Jr., … Amicone, J. (2007). Effects of co-occurring cannabis use disorders on the course of bipolar disorder after a first hospitalization for mania. *Archives of General Psychiatry, 64*(1), 57–64.

Szuster, R. R., Pontius, E. B., & Campos, P. E. (1988). Marijuana sensitivity and panic anxiety. *Journal of Clinical Psychiatry, 49*(11), 427–429.

Tambaro, S., & Bortolato, M. (2012). Cannabinoid-related agents in the treatment of anxiety disorders: current knowledge and future perspectives. *Recent Patents on CNS Drug Discovery, 7*(1), 25–40.

Tellioğlu, T., & Tellioğlu, Z. (2012). Can medical marijuana be used in the treatment of psychiatric disorders? *Bulletin of Clinical Psychopharmacology, 22*, 98–109.

Topol, E. J., Bousser, M. G., Fox, K. A., Creager, M. A., Despres, J. P., Easton, J. D., … Investigators, C. (2010). Rimonabant for prevention of cardiovascular events (CRESCENDO): a randomised, multicentre, placebo-controlled trial. *Lancet, 376*(9740), 517–523.

United Nations Office on Drugs and Crime. (2012). *World drug report*. New York, NY: United Nations Office on Drugs and Crime.

Verrico, C. D., Jentsch, J. D., & Roth, R. H. (2003). Persistent and anatomically selective reduction in prefrontal cortical dopamine metabolism after repeated, intermittent cannabinoid administration to rats. *Synapse, 49*(1), 61–66.

Walther, S., Mahlberg, R., Eichmann, U., & Kunz, D. (2006). Delta-9-tetrahydrocannabinol for nighttime agitation in severe dementia. *Psychopharmacology (Berlin), 185*(4), 524–528.

Ware, M. A., Fitzcharles, M. A., Joseph, L., & Shir, Y. (2010). The effects of nabilone on sleep in fibromyalgia: results of a randomized controlled trial. *Anesthesia and Analgesia, 110*(2), 604–610.

Wen, H., Hockenberry, J., & Cummings, J. (2014). *The effect of medical marijuana laws on marijuana, alcohol, and hard drug use*. Cambridge, MA: National Bureau of Economic Research (NBER).

Yanık, M. (2002). İlk esrar kullanımında oluşan panik atak. *Bulletin of Clinical Psychopharmacology, 12*(4), 191–193.

Yucel, M., Bora, E., Lubman, D. I., Solowij, N., Brewer, W. J., Cotton, S. M., … Pantelis, C. (2012). The impact of cannabis use on cognitive functioning in patients with schizophrenia: a meta-analysis of existing findings and new data in a first-episode sample. *Schizophrenia Bulletin, 38* (2), 316–330.

Yucel, M., Solowij, N., Respondek, C., Whittle, S., Fornito, A., Pantelis, C., & Lubman, D. I. (2008). Regional brain abnormalities associated with long-term heavy cannabis use. *Archives of General Psychiatry, 65*(6), 694–701.

Zajicek, J. P., Sanders, H. P., Wright, D. E., Vickery, P. J., Ingram, W. M., Reilly, S. M., … Thompson, A. J. (2005). Cannabinoids in multiple sclerosis (CAMS) study: safety and efficacy data for 12-months follow-up. *Journal of Neurology Neurosurgery and Psychiatry, 76*(12), 1664–1669

Zaman, T., Rosenthal, R. N., Renner, J. A., Kleber, H. D., & Milin, R. (2013). *Position statement on marijuana as medicine*. Arlington, VA: American Psychiatric Association.

Zuardi, A., Crippa, J., Dursun, S., Morais, S., Vilela, J., Sanches, R., & Hallak, J. (2010). Cannabidiol was ineffective for manic episode of bipolar affective disorder. *Journal of Psychopharmacology, 24*(1), 135–137.

Zuardi, A. W., Crippa, J. A., Hallak, J. E., Bhattacharyya, S., Atakan, Z., Martin-Santos, R., … Guimaraes, F. S. (2012). A critical review of the antipsychotic effects of cannabidiol: 30 years of a translational investigation. *Current Pharmaceutical Design. 18*(32), 5131–5140.

Zuardi, A. W., Crippa, J. A., Hallak, J. E., Moreira, F. A., & Guimaraes, F. S. (2006). Cannabidiol, a Cannabis sativa constituent, as an antipsychotic drug. *Brazilian Journal of Medical and Biological Research, 39*(4), 421–429.

CHAPTER

91

Beneficial Effects of Cannabis and Related Compounds on Sleep

*I.M.P. Linares**, J.A.S. Crippa**, M.H.N. Chagas**,****

***Department of Neuroscience and Behavior, Faculty of Medicine, Ribeirão Preto, University of São Paulo, Ribeirao Preto, Brazil**

****Barretos School of Health Sciences, Dr. Paulo Prata, Barretos, São Paulo, Brazil**

SUMMARY POINTS

- This chapter described the effects of cannabis and its components on the sleep-wake cycle.
- The action of cannabis and their compounds on the sleep-wake cycle appears to depend on several factors such as dose, route and time of administration.
- CBD seems to be associated with an increased total sleep time and less frequent arousals.
- Lower doses of THC between 10 and 30 mg in healthy volunteers appear to increase sleep latency without other significant effects on the sleep-wake cycle.
- There is a link between the endocannabinoid system and the sleep-wake cycle. However, not enough studies have been conducted to properly explore this association.

KEY FACTS OF CANNABIS

Cannabis consists of more than 60 cannabinoids, which may have different effects, including effects on sleep-wake cycle. These studies suggested that these effects can change various sleep aspects, such as architecture and subjective quality.

KEY FACTS OF CANNABIDIOL

The effects of cannabidiol (CBD) at different doses and routes of administration are still unclear, with contradictory reports ranging from increased wakefulness to sedative and hypnotic effects.

KEY FACTS OF Δ^9-TETRAHYDROCANNABINOL

The action of Δ^9-tetrahydrocannabinol (THC) on the sleep-wake cycle appears to depend on several factors such as dose and time of administration. There has been a lack of basic research studies on this subject.

LIST OF ABBREVIATIONS

CB_1 and CB_2	Cannabinoid receptors type 1 and 2
CBD	Cannabidiol
REM	Rapid eye movement
SWS	Slow wave sleep
THC	Δ^9-Tetrahydrocannabinol
TRPV1	Vanilloid-1 receptor

INTRODUCTION

The plant *Cannabis sativa* has historically been used for medicinal purposes in multiple cultures despite its adverse effects (Zuardi, 2006). For example, there are

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00106-X
Copyright © 2017 Elsevier Inc. All rights reserved.

indications that in China, before the Christian era, cannabis was used to treat several medical conditions, such as constipation, pain, malaria, epilepsy, and tuberculosis, among others (Adams, 1940).

In addition, the use of cannabis for psychiatric symptoms has been described in historical data. In the early 20th century, cannabis extracts were marketed for the treatment of mental disorders to obtain sedative and hypnotic effects (Russo & Guy, 2006). However, there was a decline in its medical use (Zuardi, 2006) for several reasons, including (1) its active compounds had not been identified, (2) restrictions were imposed due to its illegal classification and (3) there was variation in the composition of its extracts.

In the late 1980s and early 1990s, there was renewed interest in the study of cannabis after the description and cloning of cannabinoid-specific receptors in the nervous system and identification of endogenous ligands for these receptors (see Fig. 91.1). Thus, there was a notable increase in research into cannabis and its components (Zuardi, 2008; Zuardi, Crippa, & Hallak, 2010).

Cannabis consists of more than 60 cannabinoids, which may have different effects (Zuardi, 2008). The two main components of the plant are Δ^9-tetrahydrocannabinol (THC), responsible for the psychoactive effects, and cannabidiol (CBD). Other substances present in cannabis are cannabinol, which has an effect somewhat less potent than THC, cannabigerol, which, similarly to CBD, is not psychoactive, and β-caryophyllene.

The aim of this chapter is to describe the effects of cannabis and its components on the sleep-wake cycle.

Endocannabinoid System and Sleep

The endocannabinoid system is composed of receptors, endogenous agonists, and biochemical apparatus responsible for synthesis of endocannabinoid substances. The main receptors are CB_1 and CB_2, and the endogenous agonists of these receptors are derived from arachidonic acid. The arachidonoyl ethanolamine, also known as anandamide, was the first endocannabinoid identified (Howlett et al., 2002). Subsequently, 2-arachidonoylglycerol and *N*-arachidonoyl-dopamine were also identified. Endocannabinoids can bind to other receptors, that is, vanilloid-1 receptor (TRPV1), in addition to CB_1 and CB_2. Within the central nervous system, TRPV1 is expressed in postsynaptic nerve terminals and may be intracellularly activated by anandamide (Saito, Wotjak, & Moreira, 2010).

After the study of endocannabinoid system neurobiology, it was speculated that this system may contribute to various functions, including sleep modulation. Thus, research has been conducted to understand the physiological role of endocannabinoids in sleep (Murillo-Rodriguez, Blanco-Centurion, Sanchez, Piomelli, & Shiromani, 2003; Murillo-Rodriguez, Cabeza, Mendez-Diaz, Navarro, & Prospero-Garcia, 2001; Murillo-Rodriguez et al., 1998; Santucci, Storme, Soubrie, & Le Fur, 1996). One of the earliest studies was conducted by Santucci et al. (1996). Different doses of SR141716A (CB1 antagonist) were injected in mice, and a dose-dependent increase in time awake and a decrease in the slow wave sleep (SWS) and rapid eye movement (REM) phases were verified. Therefore, wakefulness induction by SR141716A may be due to CB1 receptor blockage.

An additional study demonstrated that the endocannabinoid anandamide may contribute to sleep modulation (Murillo-Rodriguez et al., 1998), when administered to rats exposed to light. This exposure induced a significant decrease in the time awake and an increase in SWS and REM.

It is also hypothesized by some researchers that the administration of SR141716A, before injection of anandamide, could block sleep effects (Murillo-Rodriguez et al., 2001). This hypothesis was confirmed when SR141716A administration blocked sleep disorders induced by anandamide.

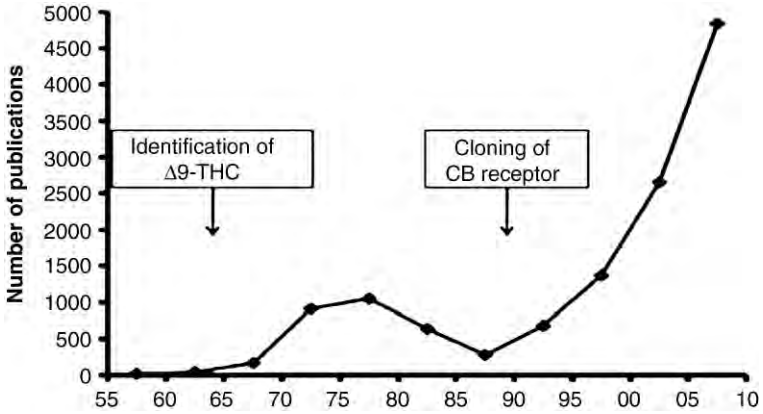


FIGURE 91.1 **Distribution of publication related to *Cannabis sativa*.**

Researchers suggest that anandamide induces sleep by activating a sleep-inducing molecule, such as adenosine. Microdialysis samples were collected in the basal forebrain of rats to measure extracellular levels of adenosine after anandamide injection. Systemic anandamide administration increased extracellular levels of adenosine. Furthermore, it was determined that anandamide injection provoked a decrease in wakefulness, and a significant increase in the SWS phase. Moreover, SR141716A administration significantly reduced adenosine levels. These data support the hypothesis that adenosine is involved in sleep modulation (Murillo-Rodriguez et al., 2003).

Cannabis Sativa

Early research into cannabis and sleep was performed in the 1970s (Mechoulam, 1986). These studies suggested that cannabis can have an effect on various sleep aspects, such as architecture and subjective quality (Babor, Mendelson, & Kuehnle, 1976; Barratt, Beaver, & White, 1974; Chait, 1990; Chait & Perry, 1994; Chait & Zacny, 1992; Ware, Fitzcharles, Joseph, & Shir, 2010). The first study investigated the effect of cannabis administered over 10 days. The researchers noted body movement reduction after 8 days of drug administration. During the same time frame, there was a noted increase in the S2 phase, followed by a reduction after 8 days of drug administration (Barratt et al., 1974). There was no effect on total sleep time and REM.

Subsequently, Karacan et al. (1976) compared the effect of cannabis administered for 8 days between substance users and nonusers. It was noted that the first group had increased sleep latency due to S1 and REM and a reduced number of awakenings during sleep. Another study determined that after 21 days of cannabis use, sleep time was higher in cannabis users when compared with alcohol users (Babor et al., 1976).

Recently, it was demonstrated that there was a decrease in sleep latency for cannabis users when compared with a control group (Chait, 1990; Chait & Perry, 1994). In both studies, cannabis was administered for two nights. Ware et al. (2010) also noted a latency decrease and sleep quality increase in subjects who received higher doses of the drug compared with the control group.

It should be emphasized that methodological aspects of most existing data preclude any definitive conclusions. One example concerns SWS: its time increased in one study (Barratt et al., 1974), whereas it did not change in another study (Karacan et al., 1976).

Although the effects of cannabis on sleep are evident, it is still necessary to understand whether these effects are caused by a specific compound. The effects observed in the sleep-wake cycle following the administration of certain *Cannabis sativa* compounds are described later in the chapter.

THC

Few studies have been performed to evaluate specifically the effects of THC on the sleep-wake cycle.

The first study examining THC was performed by Cousens and DiMascio (1973). In this study, doses of 10, 20, and 30 mg were administered and compared with a placebo. All three doses of THC increased the onset of sleep and had no effect on the amount and duration of awakenings during the night. In the same year, Hosko, Kochar, and Wang (1973) conducted a double-blind placebo controlled trial with higher doses of THC and determined that more than 25% of participants had an increase in SWS and approximately 60% had increased REM sleep in the beginning, followed by a decrease in REM sleep. The total sleep time was similar in both groups.

More recently, Chait and Zacny (1992) observed no difference when compared with the placebo in latency, quality and nocturnal awakenings at doses of 2.5–10 mg/day of THC. Subsequently, Nicholson, Turner, Stone, and Robson (2004) reported increased time awake, increased sleep latency and drowsiness the next day at doses of 15 mg/day, although no differences in sleep efficiency and number of awakenings were reported [see review by Gates, Albertella, and Copeland, 2014].

There has been a lack of basic research studies on this subject, and only one basic research study was found in literature searches. In this study, chronic intraperitoneal administration of THC doses at 5 and 10 mg/kg appears to suppress REM sleep in rats (Moreton & Davis, 1973).

It can be concluded that the action of THC on the sleep-wake cycle appears to depend on several factors such as dose and time of administration. Lower doses between 10 and 30 mg in healthy volunteers appear to increase sleep latency without other significant effects on the sleep-wake cycle.

Cannabidiol

CBD has previously been demonstrated to affect the sleep-wake cycle. Its effects at different doses and routes of administration are still unclear, with contradictory reports ranging from increased wakefulness to sedative and hypnotic effects (Nicholson et al., 2004; Zuardi, 2008).

In healthy volunteers with at least 6 h of sleep, CBD (600 mg) induced sedative effects (Zuardi, Guimaraes, & Moreira, 1993). In volunteers with complaints of insomnia, CBD was associated with an increased total sleep time and less frequent arousals at 160 mg/day (Carlini & Cunha, 1981). This study also reported that CBD reduced dream retrieval and caused no "hangover" at 40,

80, and 160 mg/day (Carlini & Cunha, 1981). However, at low doses (15 mg/day), when coadministered with delta-9-THC (15 mg/day), CBD increased wakefulness (Nicholson et al., 2004).

Similar to CBD sleep clinical trials, studies in animal models are also scarce. The first investigation assessing the effects of systemic CBD administration on sleep demonstrated a decreased SWS latency at 20 mg CBD per kg and increased duration of SWS and decreased wakefulness at 40 mg CBD per kg, with no significant changes in the REM sleep phase (Monti, 1977). During this study, the effects of CBD were recorded for 5 h after CBD administration. Chagas et al. (2013) evaluated the effects of acute CBD administration in rats and demonstrated that CBD appears to increase total sleep time, sleep latency and the percentage of SWS during the light period.

Recently, two studies used intraventricular administration and lateral hypothalamus infusion (Murillo-Rodriguez, Millan-Aldaco, Palomero-Rivero, Mechoulam, & Drucker-Colin, 2006; Murillo-Rodriguez, Millan-Aldaco, Palomero-Rivero, Mechoulam, & Drucker-Colin, 2008) and observed contrasting effects compared with Monti (1977). Intracerebroventricular CBD administration (10 μg/μL) increased wake time 1 and 4 h after CBD administration, and reduced the duration of REM sleep with no changes in SWS (Murillo-Rodriguez et al., 2006). The injection of CBD or placebo in the lateral hypothalamus also increased wake time, reduced REM sleep time, and significantly decreased the SWS (Murillo-Rodriguez et al., 2008). These data suggest that the effects of CBD vary according to the route and dose of administration.

Other Cannabis Compounds

Currently, several cannabinoid substances are known, those present in cannabis and those that are produced synthetically (Table 91.1). Cannabis has two main components: THC, responsible for the psychoactive effects, and CBD, the main component of the plant, accounting for approximately 40% of the total. Other substances present in marijuana are: (1) cannabinol, which has an effect less potent than THC; (2) β-caryophyllene, which acts as an antiinflammatory; and (3) cannabigerol, a nonpsychoactive substance that may be useful for the treatment of glaucoma.

TABLE 91.1 Main Cannabinoid Substances Extracted From Cannabis or Synthetic Compounds

Δ^9-Tetrahydrocannabinol	Sativex (THC + CBD)
Cannabidiol	Canasol
Cannabinol	Nabilone (Cesamet)
β-Caryophyllene	Dronabinol (Marinol)
Cannabigerol	Rimonabant

Nabilone and dronabinol are synthetic CB_1 receptor agonists that have similar characteristics to THC, and their effects on the sleep-wake cycle have been evaluated and will be the subjects of this section.

The studies that evaluated the effects of nabilone on the sleep-wake cycle used volunteers with medical conditions; furthermore, data related to sleep are commonly secondary. The conditions studied were pain, fibromyalgia disorder, and posttraumatic stress (Gates et al., 2014).

At doses ranging from 0.25 to 6 mg daily, two studies observed no changes in sleep patterns in patients with pain (Beaulieu, 2006; Frank, Serpell, Hughes, Matthews, & Kapur, 2008). Moreover, Toth et al. (2012) demonstrated an improved overall quality of sleep, and Bestard and Toth (2011) reported a decrease in pain interference and in sleep disturbances in the sleep-wake cycle.

In a study that evaluated patients with comorbid posttraumatic stress disorder, 72% of patients reported decreased nightmares (Fraser, 2009). In another study that evaluated patients with fibromyalgia and chronic insomnia, the use of 0.5–1 mg of nabilone was associated with decreased insomnia severity and decreased difficulty awakening during sleep, with no effect on the number of awakenings and sleep latency (Ware et al., 2010).

Regarding dronabinol, only four studies have assessed the sleep-wake cycle as secondary outcomes. Haney et al. (2007) observed no effect on objective sleep time. Conversely, Bedi et al. (2010) observed an increase in sleep efficiency during the first week due to the increased time of the S1–S4 stages and decreased time awake. Subjective measures such as sleep satisfaction, "slept well" and "awoke less often" were also improved. These studies were conducted in a small sample, and all participants were HIV positive.

In another sample of patients suffering pain, Narang et al. (2008) observed a decrease in disorders and sleep-related problems; specifically, there was a decrease in the pain interference of sleep at doses between 5 and 60 mg/day. Finally, a study that evaluated patients with amyotrophic lateral sclerosis observed no effect on insomnia symptoms at a dose of 10 mg/day.

Other components or combinations of cannabinoid substances have also been tested, mostly as secondary outcomes and in samples with clinical comorbidities. This fact hampers a specific and detailed assessment of the effects of cannabinoids on the sleep-wake cycle (Fig. 91.2).

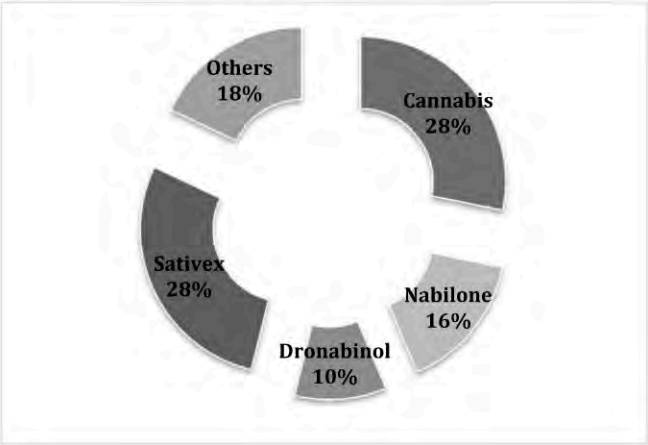


FIGURE 91.2 Percentage distribution of articles relating to the effects of cannabis-based medicine administration on sleep in human protocols. Each point represents the percentage of publications related to *Cannabis sativa* in 5-year periods between 1995 and 2010. *Source: The data were obtained from a search performed on the ISI Web of Science online database using the keywords* cannabis, cannabinoids, marihuana, *and* marijuana. *Adapted from figure published in Zuardi, A. W., Crippa, J. A., & Hallak, J. E. (2010).* Cannabis sativa*: the plant that can induce unwanted effects and also treat them.* Revista Brasileira de Psiquiatria, 32*(Suppl. 1), S1–S2.*

CONCLUSIONS

It can be concluded that there is a link between the endocannabinoid system and the sleep-wake cycle. However, not enough studies have been conducted to properly explore the effects of each cannabinoid compound, and more studies are necessary.

MINI-DICTIONARY

REM Rapid eye movement sleep. Characterized by the active brain waves, flitting motions of the eyes, and weakness of the muscles.

S1 phase A stage of nonrapid eye movement (NREM) sleep occurring after wake. Its criteria consist of a low-voltage EEG with slowing to theta frequencies, alpha activity less than 50%.

S2 phase A stage of NREM sleep characterized by sleep spindles and K complexes against a relatively low-voltage, mixed-frequency EEG background.

Sleep-wake cycle Biological pattern of alternating sleep and wakefulness.

SWS It is represented by slower brain waves called delta activity on EEG. It is a type of NREM sleep: stages 3 and 4.

References

Adams, R. (1940). Marihuana. *Science*, *92*(2380), 115–119.

Babor, T. F., Mendelson, J. H., & Kuehnle, J. (1976). Marihuana and human physical activity. *Psychopharmacology (Berlin)*, *50*(1), 11–19.

Barratt, E. S., Beaver, W., & White, R. (1974). The effects of marijuana on human sleep patterns. *Biological Psychiatry*, *8*(1), 47–54.

Beaulieu, P. (2006). Effects of nabilone, a synthetic cannabinoid, on postoperative pain. *Canadian Journal of Anaesthesia*, *53*(8), 769–775.

Bedi, G., Foltin, R. W., Gunderson, E. W., Rabkin, J., Hart, C. L., Comer, S. D., …, Haney, M. (2010). Efficacy and tolerability of high-dose dronabinol maintenance in HIV-positive marijuana smokers: a controlled laboratory study. *Psychopharmacology (Berlin)*, *212*(4), 675–686.

Bestard, J. A., & Toth, C. C. (2011). An open-label comparison of nabilone and gabapentin as adjuvant therapy or monotherapy in the management of neuropathic pain in patients with peripheral neuropathy. *Pain Practice*, *11*(4), 353–368.

Carlini, E. A., & Cunha, J. M. (1981). Hypnotic and antiepileptic effects of cannabidiol. *Journal of Clinical Pharmacology*, *21*(8–9 Suppl.), 417S–427S.

Chagas, M., Crippa, J., Zuardi, A., Hallak, J., Machado de Sousa, J., Hirotsu, C., …, Andersen, M. (2013). Effects of acute systemic administration of cannabidiol on sleep-wake cycle in rats. *Journal of Psychopharmacology*, *27*(3), 312–316.

Chait, L. D. (1990). Subjective and behavioral effects of marijuana the morning after smoking. *Psychopharmacology (Berlin)*, *100*(3), 328–333.

Chait, L. D., & Perry, J. L. (1994). Acute and residual effects of alcohol and marijuana, alone and in combination, on mood and performance. *Psychopharmacology (Berlin)*, *115*(3), 340–349.

Chait, L. D., & Zacny, J. P. (1992). Reinforcing and subjective effects of oral delta 9-THC and smoked marijuana in humans. *Psychopharmacology (Berlin)*, *107*(2–3), 255–262.

Cousens, K., & DiMascio, A. (1973). (−) Delta 9 THC as an hypnotic. An experimental study of three dose levels. *Psychopharmacologia*, *33*(4), 355–364.

Frank, B., Serpell, M. G., Hughes, J., Matthews, J. N., & Kapur, D. (2008). Comparison of analgesic effects and patient tolerability of nabilone and dihydrocodeine for chronic neuropathic pain: randomised, crossover, double blind study. *British Medical Journal*, *336*(7637), 199–201.

Fraser, G. A. (2009). The use of a synthetic cannabinoid in the management of treatment-resistant nightmares in posttraumatic stress disorder (PTSD). *CNS Neuroscience and Therapeutics*, *15*(1), 84–88.

Gates, P. J., Albertella, L., & Copeland, J. (2014). The effects of cannabinoid administration on sleep: a systematic review of human studies. *Sleep Medicine Reviews*, *18*, 477–487.

Haney, M., Gunderson, E. W., Rabkin, J., Hart, C. L., Vosburg, S. K., Comer, S. D., & Foltin, R. W. (2007). Dronabinol and marijuana in HIV-positive marijuana smokers. Caloric intake, mood, and sleep. *Journal of Acquired Immune Deficiency Syndrome*, *45*(5), 545–554.

Hosko, M. J., Kochar, M. S., & Wang, R. I. (1973). Effects of orally administered delta-9-tetrahydrocannabinol in man. *Clinical Pharmacology and Therapeutics*, *14*(3), 344–352.

Howlett, A. C., Barth, F., Bonner, T. I., Cabral, G., Casellas, P., Devane, W. A., …, Pertwee, R. G. (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacological Reviews*, *54*(2), 161–202.

Karacan, I., Fernandez-Salas, A., Coggins, W. J., Carter, W. E., Williams, R. L., Thornby, J. I., …, Villaume, J. P. (1976). Sleep electroencephalographic-electrooculographic characteristics of chronic marijuana users: part I. *Annals of the New York Academy of Sciences*, *282*, 348–374.

Mechoulam, R. (1986). The pharmacohistory of *Cannabis sativa*. In R. Mechoulam (Ed.), *Cannabinoids as therapeutic agents* (pp. 1–19). Boca Raton, FL: CRC Press.

Monti, J. M. (1977). Hypnotic-like effects of cannabidiol in the rat. *Psychopharmacology (Berlin)*, *55*(3), 263–265.

Moreton, J. E., & Davis, W. M. (1973). Electroencephalographic study of the effects of tetrahydrocannabinols on sleep in the rat. *Neuropharmacology*, *12*(9), 897–907.

Murillo-Rodriguez, E., Blanco-Centurion, C., Sanchez, C., Piomelli, D., & Shiromani, P. J. (2003). Anandamide enhances extracellular levels of adenosine and induces sleep: an in vivo microdialysis study. *Sleep*, *26*(8), 943–947.

Murillo-Rodriguez, E., Cabeza, R., Mendez-Diaz, M., Navarro, L., & Prospero-Garcia, O. (2001). Anandamide-induced sleep is blocked by SR141716A, a CB1 receptor antagonist and by U73122, a phospholipase C inhibitor. *Neuroreport*, *12*(10), 2131–2136.

Murillo-Rodriguez, E., Millan-Aldaco, D., Palomero-Rivero, M., Mechoulam, R., & Drucker-Colin, R. (2006). Cannabidiol, a constituent of *Cannabis sativa*, modulates sleep in rats. *FEBS Letters*, *580*(18), 4337–4345.

Murillo-Rodriguez, E., Millan-Aldaco, D., Palomero-Rivero, M., Mechoulam, R., & Drucker-Colin, R. (2008). The nonpsychoactive Cannabis constituent cannabidiol is a wake-inducing agent. *Behavioral Neuroscience*, *122*(6), 1378–1382.

Murillo-Rodriguez, E., Sanchez-Alavez, M., Navarro, L., Martinez-Gonzalez, D., Drucker-Colin, R., & Prospero-Garcia, O. (1998). Anandamide modulates sleep and memory in rats. *Brain Research*, *812*(1–2), 270–274.

Narang, S., Gibson, D., Wasan, A. D., Ross, E. L., Michna, E., Nedeljkovic, S. S., & Jamison, R. N. (2008). Efficacy of dronabinol as an adjuvant treatment for chronic pain patients on opioid therapy. *Journal of Pain*, *9*(3), 254–264.

Nicholson, A. N., Turner, C., Stone, B. M., & Robson, P. J. (2004). Effect of Delta-9-tetrahydrocannabinol and cannabidiol on nocturnal sleep and early-morning behavior in young adults. *Journal of Clinical Psychopharmacology*, *24*(3), 305–313.

Russo, E., & Guy, G. W. (2006). A tale of two cannabinoids: the therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. *Medical Hypotheses*, *66*(2), 234–246.

Saito, V. M., Wotjak, C. T., & Moreira, F. A. (2010). Pharmacological exploitation of the endocannabinoid system: new perspectives for the treatment of depression and anxiety disorders? *Revista Brasileira de Psiquiatria*, *32*(Suppl. 1), S7–S14.

Santucci, V., Storme, J. J., Soubrie, P., & Le Fur, G. (1996). Arousal-enhancing properties of the CB1 cannabinoid receptor antagonist SR141716A in rats as assessed by electroencephalographic spectral and sleep-waking cycle analysis. *Life Sciences*, *58*(6), PL103–PL110.

Toth, C., Mawani, S., Brady, S., Chan, C., Liu, C., Mehina, E., …, Korngut, L. (2012). An enriched-enrolment, randomized withdrawal, flexible-dose, double-blind, placebo-controlled, parallel assignment efficacy study of nabilone as adjuvant in the treatment of diabetic peripheral neuropathic pain. *Pain*, *153*(10), 2073–2082.

Ware, M. A., Fitzcharles, M. A., Joseph, L., & Shir, Y. (2010). The effects of nabilone on sleep in fibromyalgia: results of a randomized controlled trial. *Anesthesia and Analgesia*, *110*(2), 604–610.

Zuardi, A. W. (2006). History of cannabis as a medicine: a review. *Revista Brasileira de Psiquiatria*, *28*(2), 153–157.

Zuardi, A. W. (2008). Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. *Revista Brasileira de Psiquiatria*, *30*(3), 271–280.

Zuardi, A. W., Crippa, J. A., & Hallak, J. E. (2010). *Cannabis sativa*: the plant that can induce unwanted effects and also treat them. *Revista Brasileira de Psiquiatria*, *32*(Suppl. 1), S1–S2.

Zuardi, A. W., Guimarães, F. S., & Moreira, A. C. (1993). Effect of cannabidiol on plasma prolactin, growth hormone and cortisol in human volunteers. *Brazilian Journal of Medical and Biological Research*, *26*(2), 213–217.

CHAPTER

92

Cannabinoid-Based Medicines for the Treatment of Gilles de la Tourette Syndrome

A.S. Kanaan[*,**], *K.R. Müller-Vahl*[*]

*Clinic of Psychiatry, Social-Psychiatry and Psychotherapy, Hannover Medical School, Hannover, Germany

**Nuclear Magnetic Resonance Unit, Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany

SUMMARY POINTS

- Gilles de la Tourette syndrome (GTS) is a childhood-onset neuropsychiatric movement disorder characterized by motor and vocal tics.
- There is an urgent need in identifying new treatment therapeutic strategies as current forms of treatment are unsatisfactory.
- A substantial number of patients with GTS and other chronic tic disorders use cannabis illegally to reduce their symptoms.
- To date, a limited number of controlled trials have been conducted to explore the utility of cannabinoid based medicines (CBMs) in GTS.
- These studies suggested that cannabinoids, such as Δ^9-tetrahydrocannabinol (THC), are effective and safe in the treatment of tics.
- There is some evidence that CBMs may ameliorate tics, as well as psychiatric comorbidities that include attention deficit/hyperactivity disorder (ADHD), obsessive compulsive disorder (OCD), impulsivity, depression, anxiety, and self-injurious behavior.
- According to European and North American guidelines for the treatment of tics, CBMs should be taken into consideration for the treatment of adult patients with GTS that cannot be treated by standard means.
- Clinical trials with larger sample sizes are necessary to further investigate the effects of cannabinoids in this group of patients.

KEY FACTS OF GILLES DE LA TOURETTE SYNDROME

- GTS is a childhood-onset, neuropsychiatric movement disorder characterized by motor and vocal tics.
- The majority of patients also present with psychiatric comorbidities that most commonly include attention deficit/hyperactivity disorder (ADHD) and obsessive compulsive disorder (OCD).
- Different treatment strategies are currently being used to manage GTS symptoms, including behavioral therapy, pharmacological therapy, deep brain stimulation, and alternative medicine.
- In many cases, treatment options are unsatisfactory due to inefficacy or serious adverse effects.
- Currently, there is no accepted pathophysiological model of GTS; though the relevance of the dopaminergic system and cortico-striato-thalamo-cortical (CSTC) circuitry have been consistently highlighted in the literature.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00107-1
Copyright © 2017 Elsevier Inc. All rights reserved.

KEY FACTS OF CANNABINOID USE IN GILLES DE LA TOURETTE SYNDROME

- Today, many patients with GTS use cannabis as a kind of a self-medication to reduce their tics and improve psychiatric comorbidities.
- A limited number of case reports and small-scale clinical trials have suggested that cannabinoids such as Δ^9-tetrahydrocannabinol (THC) are effective in the treatment of tics without causing significant adverse effects.
- As a result, it has been speculated that the endogenous cannabinoid system is involved in the pathogenesis of GTS.
- According to various national and international treatment guidelines, cannabinoids should be taken into consideration for the treatment of GTS in adult and otherwise treatment resistant patients.
- Large-scale clinical trials using cannabinoids and other agents that stimulate the endogenous cannabinoid system are needed to corroborate the efficacy of these substances in patients with GTS.

LIST OF ABBREVIATIONS

ADHD	Attention deficit/hyperactivity disorder
ANOVA	analysis of variance
CAMs	complementary and alternative medicines
CB1	Cannabinoid receptor type 1
CB2	Cannabinoid receptor type 2
CBD	Cannabidiol
CBMs	Cannabinoid based medicines
CSTC	Cortico-striato-thalamo-cortical circuitry
FAAH	Fatty acid amide hydrolase
GABA	gamma-aminobutyric acid
GTS	Gilles de la Tourette syndrome
GTS-CGI	Tourette's syndrome clinical global impression scale
OCB	Obsessive-compulsive behavior
OCD	obsessive compulsive disorder
SSRIs	selective serotonin-reuptake inhibitors
STSS	Shapiro Tourette-syndrome severity scale
THC	Tetrahydrocannabinol
TSGS	Tourette syndrome global scale
YGTSS	The Yale global tic severity scale

GILLES DE LA TOURETTE SYNDROME

In popular culture, Tourette syndrome is regarded frequently as a disorder in which individuals utter obscene, socially inappropriate, or derogatory statements without any volitional control. This is partly true, as only a minority of patients suffering from Tourette syndrome exhibit this involuntary swearing condition known as coprolalia. In essence, Tourette syndrome—or more appropriately named as Gilles de la Tourette syndrome (GTS)—is a complex genetic, neurodevelopmental disorder that exhibits a varied symptomatology that spans the fields of psychiatry and neurology. GTS is not rare as current estimates have yielded prevalence rates of approximately 0.7–1% of the general population, thus rendering it as one of the most common movement disorders (Knight et al., 2012).

The cardinal features of GTS are the presence of motor and vocal tics, which are defined as rapid, nonrhythmic involuntary movements that are misplaced in context or time (Jankovic, 1997). While most patients report that their tics are temporarily suppressible, tics are often preceded by unpleasant sensory sensations (premonitory urges) which are usually relieved by the tic.

In the clinical setting, GTS is diagnosed if an individual exhibits: (1) multiple motor tics and one or more vocal tics; (2) a waxing and waning course of tic intensity, frequency, and severity; (3) the persistence of tics for at least 1 year following first onset; (4) tic onset before the age of 18; and (5) tics that are not caused by another medical condition or substance (DSM-5, 307.23, ICD-10 F95.2). The typical age of onset of tics is between 6 and 8 years of age, where peak severity is usually reached between ages 10 and 14. While the majority of patients exhibit significant improvements over the course of their adolescent period, a small number of patients do not improve and in most of these cases, the patients take on a negative course of illness in which other comorbid psychiatric conditions surface. In fact, more than 80% of patients with GTS exhibit the presence of comorbid conditions that most commonly include attention deficit/hyperactivity disorder (ADHD) (~50%) and obsessive compulsive behavior (OCB) (40–80%), in addition to self-injurious behavior, depression, anxiety, impulsivity, rage attacks, sleep problems, and learning disorders (Robertson, 2000). Such associated conditions often impair patients' qualify of life more than tics and are usually the main reason why patients seek treatment.

Etiology and Pathophysiology of GTS

Most of the available evidence points to the hypothesis that GTS is a complex genetic disorder that is likely caused by the effect of multiple genetic susceptibility factors (Caspi & Moffitt, 2006; Paschou, 2013). This nonclassical Mendelian pattern of inheritance tied to increasing evidence that environmental factors play a role in the onset and natural course of the disorder, deem its study very difficult.

Pathophysiologically, the abnormality that has been most consistently highlighted in the literature is tied to

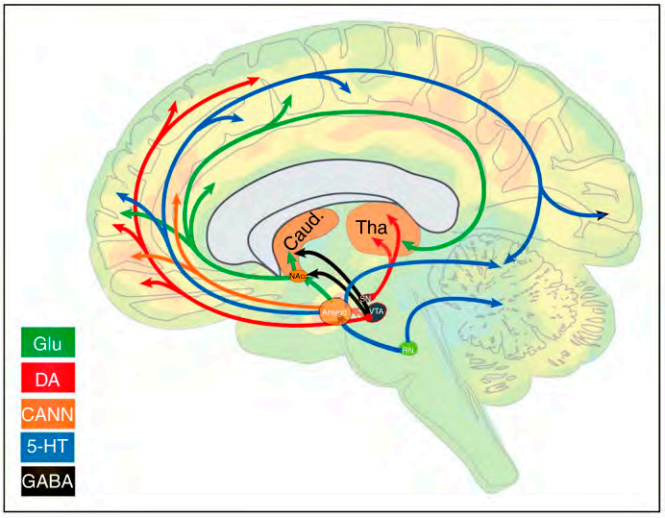


FIGURE 92.1 Schematic illustrating the extent of interaction between the endocannabinoid system and neurotransmitter pathways reported and hypothesized to be involved in Gilles de la Tourette syndrome pathophysiology. The translucent heatmap in the background illustrates the distribution of CB1 receptors. Regions with a high distribution of CB1 receptors *(red/orange)* include the caudate, thalamus, amygdala, prefrontal cortex, and parietal cortex. *Glu*, Glutamatergic; *5-Ht*, seretonergic; *DA*, dopaminergic; *GABA*, gamma-aminobutyric acid; *CANN*, endocannabinoid system. *Source: The figure was adapted based on information from Schumann et al. (2010) and Terry et al. (2010).*

imbalances in the dopaminergic (DA) system within key regions of the cortico-striato-thalamo-cortical (CSTC) circuitry. Nonetheless, given the heterogeneity of clinical phenotypes exhibited by patients with GTS, it is highly likely that more than one neurotransmitter system is affected as the glutamatergic, GABAergic, histaminergic, and cannabinoid receptor systems exhibit various interactions with the dopaminergic system to play a role in the normal mechanics of the CSTC circuitry (Fig. 92.1).

TREATMENT STRATEGIES

Currently, there are different therapeutic strategies that are being used to manage the symptomatology of GTS including: (1) behavioral therapy, (2) pharmacological therapy, (3) deep brain stimulation (DBS), and (4) alternative medicine. The presence of comorbid conditions usually complicates treatment as there are no known treatments to be effective for both tics and associated disorders.

In patients that exhibit tics that interfere with normal functioning, behavioral strategies (eg, habit reversal training or exposure and response prevention training) and pharmacological agents are usually recommended as the first line of treatment (Roessner et al., 2011; Verdellen, Van de Griendt, Hartmann, Murphy, & ESSTS Guidelines Group, 2011). Although a variety of psychotropic drugs have been used to treat tics, only haloperidol and (in some countries) pimozide have been officially licensed for GTS. Nonetheless, due to the severe associated side effects that include sedation, cognitive dulling, dyskinesias and weight gain, haloperidol and pimozide are no longer recommended as first line treatment. Instead newer antipsychotics such as aripiprazole, risperidone as well as tiapride and sulpiride are recommended, although they are also often associated with significant adverse effects (Roessner et al., 2011).

For the treatment of associated obsessive-compulsive disorder (OCD), either behavioral therapy and/or selective serotonin-reuptake inhibitors (SSRIs) are recommended. Psychostimulants such as methylphenidate are the treatment of choice in patients suffering from comorbid ADHD. Treatment of other comorbidities such as depression and anxiety disorders does not differ from recommendations given in patients exhibiting these disorders without GTS. In patients with severe and complex symptoms, combined treatment with several drugs is often inevitable.

Alternative Forms of Treatments

Given the wide range of symptoms and associated conditions that GTS patients might exhibit, many patients are unsatisfied with their treatment strategy. This is possibly due to (1) the low efficacy of the used strategy, (2) complete resistance to treatment, or (3) the emergence of significant adverse effects. Therefore, many patients deem it fit to explore other forms of treatment that have yet to be established scientifically. In fact, two published studies have revealed that an alarming number of GTS patients (64 and 87%, respectively) use at least one other form of complementary or alternative medicine (CAMs) (Kompoliti, Fan, & Leurgans, 2009; Mantel, Meyers, Tran, Rogers, & Jacobson, 2004). For treating physicians, it is important to realize that up 80% of these patients do not inform their doctor that they are pursuing the treatment. Additionally, it is well known that some patients revert to legal and illegal substances such as nicotine, alcohol, and marijuana in bid to assuage their symptoms (Müller-Vahl, Kolbe, & Dengler, 1997b; Müller-Vahl, Kolbe, & Dengler, 1997a).

A RATIONALE FOR THE TREATMENT OF GTS WITH CANNABINOIDS

Despite the fact that the range of treatments for patients with GTS has expanded over the last decade, there remains a need in identifying new substances that might be more effective. With the determination of the chemical structure of the principal psychoactive constituent of the cannabis plant, Δ^9-tetrahydrocannabinol (THC), a new era of cannabinoid based medicines (CBMs) was ushered (Tables 92.1 and 92.2). This discovery was followed by the

TABLE 92.1 Dosage of Cannabinoids in the Treatment of Gilles de la Tourette Syndrome

Substance	Start dose	Increase	Number of doses per day	Usual therapeutic dosages	Maximum dose
THC/dronabinol	2.5 mg	2.5 mg every 3–5 days	Individually	10–20 mg/day	30 mg/day (higher if tolerated and necessary)
Nabiximols	1 spray dose	1 spray dose every 3–5 days	Individually	6–10 spray doses/day	12 spray doses/day (maximum licensed daily dosage)

TABLE 92.2 Studies Employing Cannabinoid-Based Medicines as a Treatment for Gilles de la Tourette Syndrome

References	Number (sex) of patients	Age	Substance	Study design	Main outcome
Sandyk and Awerbuch (1988)	3 (male)	15, 17, 39	*Cannabis sativa* L.	Case report	Reduction of tics, premonitory urges and self-injurious behavior; general relaxation; improvement of attention and hypersexuality.
Hemming and Yellowlees (1993)	1 (male)	36	*C. sativa* L.	Case report	Symptom free
Müller-Vahl, Schneider, Kolbe, and Emrich (1999)	1 (male)	25	THC	Case report	Reduction of tics and premonitory urges, improvement of attention, impulse control, and OCB.
Müller-Vahl et al. (2002)	1 (female)	24	THC (in combination with amisulpride)	Case report	Reductions of tics and premonitor
Brunnauer et al. (2011)	1 (male)	42	THC	Case report	Reduction of tics; improvement of concentration and visual perception.
Hasan et al. (2010)	1 (male)	15	THC (in combination with aripiprazole and risperidone)	Case report	Reduction of tics; improvement of quality of life; treatment with methylphenidate was tolerated without tic increase.
Müller-Vahl et al. (1998)	64 (55 male, 9 female)	15–64	*C. sativa* L.	Case series (standardized interview)	Tic reduction or remission; reduction of premonitory urges; improvement of OCB and ADHD.
Müller-Vahl et al. (2003b)	12 (11 male, 1 female)	18–66	THC	Randomized double-blind placebo-controlled crossover trial	Tic reduction; improvement of OCB.
Müller-Vahl et al. (2002)	24 (19 male, 5 female)	18–68	THC	Randomized double-blind parallel group placebo-controlled trial	Tic reduction; global improvement.

delineation of the endocannabinoid system in the brain, which is a set of endogenous neuromodulatory ligands (eg, anandamide, 2-AG) and their receptors (eg, CB1, CB2) that are understood to have a functional role in movement and behavior. Various studies have demonstrated that (1) endocannabinoid ligands and their receptors are significantly more abundant in basal ganglia nuclei compared to other brain regions (Fig. 92.1) (Herkenham et al., 1990); (2) the endo cannabinoid system plays a major role in the mechanics of the CSTC circuits, both in health and disease; and (3) the manipulation of the endo cannabinoid system is associated with important motor and behavioral responses (Fernandez-Ruiz, 2009).

TREATMENT WITH *CANNABIS SATIVA* L.

Case Reports

There are only two case studies available reporting on the effects of cannabis in patients with GTS. Sandyk and Awerbuch (1988) explored the effect of cannabis on three patients: (1) a 15-year-old male with a tendency for self-injurious behavior exhibited a 50% reduction in motor tics, a reduction in premonitory urge sensations, improvement in self-injurious behavior tendencies, and a generalized feeling of relaxation; (2) a 17-year-old male with severe motor and vocal tics exhibited a 60–70% tic

reduction, and improvement of attention when smoking cannabis; and (3) a 39-year-old male exhibited a reduction of motor tics and hypersexual behavior. In the second report, Hemming and Yellowlees (1993) explored the effects of cannabis on a retired 36-year-old patient with motor and vocal tics that included coprolalia. By taking a constant dose of marijuana via a water pipe, the authors report that the patient was "completely symptom free."

Prospective Surveys

A few years later, Müller-Vahl et al. (1997b) conducted a survey using a standardized questionnaire to further explore the frequency and effect of marijuana use with afflicted patients. 64 patients (55 males and 9 females, mean age 30.3 years, range 15–64 years) were interviewed about marijuana use. Unsurprisingly, 27% of this group reported the use of marijuana with 82% reporting that they felt marijuana improved their symptoms. More specifically, of the 17 patients that reported marijuana use, 9 patients reported moderate or marked reduction of tics, 4 patients felt a complete remission of motor and vocal tics, 4 patients felt an amelioration of premonitory urges, 1 patient reported a remission of their OCB symptoms, and 1 reported improvement of his ADHD symptoms.

Controlled Trials

A very small number of open and controlled studies investigating the effects of CBMs in GTS are available. In all these studies, however, only pure THC has been used. Just recently, a pilot study has been initiated at Yale University investigating the effect of a fatty acid amide hydrolase (FAAH) inhibitor in patients with GTS (personal communication). Additionally, a multicenter clinical trial in Germany has just been granted funding to investigate efficacy and safety of the cannabis extract nabiximols, a plant extract from *C. sativa* L. containing THC and cannabidiol (CBD) at a 1:1 ratio.

TREATMENT WITH THC

Uncontrolled Single Case Studies

There are a few case studies that have explored the effects of THC on the symptomatology of GTS. The first was published by our group showing that a single dose of orally ingested THC drastically improved the symptomatology of a 25-year-old patient with tic onset at age 6 (Müller-Vahl et al., 1999). In this case, the patient suffered from a number of comorbidities that included ADHD, OCB, anxiety disorder, impulsivity, and self-injurious behavior. The patient reported that by smoking 2–3 g of marijuana a day, his tics and associated behavioral conditions markedly improved. This narrative was addressed clinically using established methods after a single 10 mg dose of THC following 3 days of cessation from all forms of treatment. The patient reported improvements in attention, impulse control, OCB, and reductions in premonitory urges. On the Tourette Syndrome global scale (TSGS) (Harcherik, Leckman, Detlor, & Cohen, 1984), the patient's total tic severity score improved by 80%, while neuropsychological tests revealed improvements in signal detection, sustained attention, and reaction time. No adverse effects were encountered in the patient.

In another study, we highlighted the utility of THC in combination with other pharmacological agents for the treatment of a 24-year-old female. The patient's symptoms had been markedly deteriorating culminating in extreme vocal tics at the age of 23. At this point, risperidone, amisulpride, and THC were administered alone or in different combinations leading to the conclusion that the combination of amisulpride and THC is more effective in tic reduction than monotherapy with THC or amisulpride. The authors suggested that THC may augment the positive effects of dopamine receptor blocking drugs such as amisulpride. This hypothesis is in line with findings in animal studies that demonstrated that haloperidol-induced hypokinesia significantly increases after coadministration with THC (Moss, Montgomery, Salo, & Steger, 1984).

More recently, Brunnauer et al. (2011) described various unsuccessful attempts in treating a 42-year-old patient who suffered from multiple motor and vocal tics (including coprolalia) and ruminating obsessive thoughts using different pharmacological agents. Treatment with a 15 mg daily maximal dose of THC resulted in a 75% tic reduction. Given his occupation as a truck driver, his driving ability was investigated using computerized tests that satisfy the German guidelines for road and traffic safety. Interestingly, the patient's concentration and visual abilities where more pronounced in the on-medication state (with THC) than the off-medication state, thus leading the authors to suggest that treatment with cannabinoids may have a beneficial effect on psychomotor functions related to driving performance. Along the same line, another group reported that treatment with cannabinoids had "atypical and even performance-enhancing effects" on a patient with pure ADHD (Strohbeck-Kuhner, Skopp, & Mattern, 2007).

To the best of our knowledge, there is only a single case report in a minor describing the positive effects of THC on the symptomatology of a treatment resistant 15-year-old adolescent with GTS (Hasan et al., 2010). This study echoed previous studies that concluded an

enhanced effect in tic reduction with di-therapeutic strategy using an antipsychotic agent and THC. The patient's symptoms included severe motor and vocal tics including self-injurious behavior, ADHD, and OCB. Treatment with a maximal daily dose of 15 mg of THC was attempted—after the patient reported that his tics and inner tensions were drastically reduced after smoking marijuana—and resulted in a significant improvement of tics (50%) and quality of life. More interestingly, this was the first report indicating that a daily dose of 30 mg of methylphenidate was tolerated without exacerbating his tics. Combining the clinical evidence with the results of a transcranial magnetic stimulation experiment—which demonstrated increased intracortical inhibition in the on-THC state—suggested that THC might counteract deficits of intracortical inhibition in patients with GTS and comorbid ADHD by modulating the release of several neurotransmitters including dopamine and F-aminobutyric acid.

Randomized Controlled Trials

Currently, only two preliminary controlled trials have been conducted to investigate the efficacy and safety of orally administered THC on patients with GTS. In the first study, a randomized double-blind placebo controlled crossover trial was conducted on 12 adult patients who were treated with a single dose of 5, 7.5, or 10 mg of THC (Müller-Vahl et al., 2002). The choice of dosage used was dependent on the patients' weight, gender and prior use of marijuana. Eleven men and one woman participated in the study (mean age = 34 ± 11 years) and were randomly assigned to a THC treatment group or a placebo group, which were then interchanged following a 4 week washout period. The patients in the THC group exhibited a significant improvement when compared to the placebo group using the Tourette Syndrome Symptom List (TSSL) (Leckman, Towbin, Ort, & Cohen, 1988) and the TSGS scales. Results also suggested that higher doses were more effective in improving the symptoms of patients in the THC group. Overall, 83% of patients in the THC group experienced a global improvement, compared to only 25% of patients in the placebo group. There were no detrimental effects detected on short-term verbal and visual memory, recognition, verbal learning, intelligence, information processing, vigilance, reaction time, sustained attention, and divided attention. Nevertheless, it must be noted that five patients in the THC group experienced transient and mild adverse effects (headache, nausea, dizziness, hot flashes, fatigue, concentration difficulties, dry mouth, ataxia) that did not require specific treatments. In clinical practice, treatment with THC is usually up-titrated from 2.5 to 5 mg/day with an incremental increase in dosage until effective values are reached. In this case, the patients might have experienced these side effects since dosages were not up-titrated.

In a second and more elaborate study, a randomized double-blind parallel group procedure was used to investigate the efficacy of THC on 24 adult patients with GTS (19 men, 5 women, mean age = 33 + 11 (SD) years, range, 18–68 years) (Müller-Vahl et al., 2003b). In this study, patients commenced treatment using a daily dose of 2.5 mg which was incrementally increased to a maximum daily dose of 10 mg, unless the dosage was not tolerated. The study consisted of six visits (visit 1 = baseline, visits 2–4 = treatment, visits 5 = immediately after treatment, visit 6 = follow-up 6 weeks after withdrawal) to assess the progress of each patient's treatment. Overall, results demonstrated the efficacy of THC treatment, which was far superior to the placebo (Figs. 92.2–92.4). Specifically, the Tourette's syndrome clinical global impression scale (GTS-CGI) (Leckman et al., 1988) scale revealed a significant difference between the two groups on the third visit (20–22 treatment days). On fourth visit (30–31 treatment days), the significant difference was observed via several rating scales that included the: (1) GTS-CGI; (2) Shapiro Tourette syndrome severity scale (STSS) (Shapiro, 1988); (3) The Yale global tic severity scale (YGTSS) for the subscore "motor global scale" (Leckman et al., 1989); and (4) Rush videotape-based rating scale (Goetz et al., 1999). Moreover, analysis of variance (ANOVA) demonstrated a trend toward an overall significant difference. The TSSL demonstrated a significant difference between the placebo and THC group on 10 different treatment days (between day 16 and 41). Using ANOVA there was an overall significant difference between the two groups. Seven patients dropped out of the study or had to be excluded but only one due to adverse effects. Five patients in the THC group reported mild adverse effects (tiredness, dry mouth, dizziness, and muzziness) and three patients in the placebo group (tiredness, dizziness, anxiety, and depression). One patient in the THC group stopped medication at day 4 (first day at dose 5 mg) due to adverse effects (anxiety, restlessness). All adverse events resolved spontaneously without specific treatment. No serious adverse events occurred.

In parallel, neuropsychological performance and cognitive function were investigated before, during and after the 6-week treatment period with THC using the following tests: (1) German version of the auditory verbal learning test (VLMT) (Helmstaedter, Lendt, & Lux, 2001), (2) Benton-visual-retention-test (BVRT) (Benton, 1945), (3) Divided attention (TAP) (Zimmermann & Fimm, 1989), and (4) multiple choice vocabulary test (Mehrfachwahl-Wortschatztest, MWT-B) (Merz, Lehrl, Galster, & Erzigkeit, 1975). There were no detrimental effects of THC seen on any of these measurements including learning curve, interference, recall and recognition of word lists, immediate visual memory

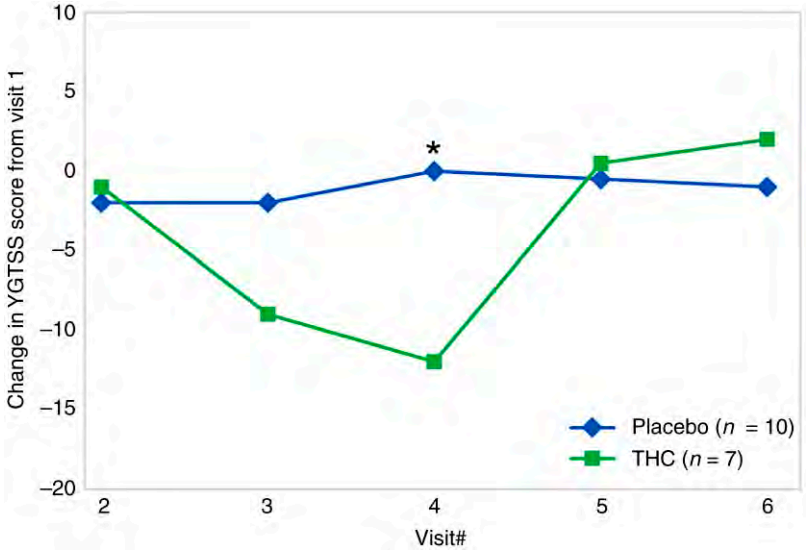


FIGURE 92.2 Changes in YGTSS from visit 1 to visits 2–6 in patients treated with THC, compared with placebo (Müller-Vahl et al., 2003b). Visit 1 = baseline, visit 2 = treatment day 9, visit 3 = treatment days 20–22, visit 4 = treatment days 30–31, visit 5 = 1 day after medication stopped, visit 6 = 5 weeks after medication stopped. ANOVA: $p = 0.077$. *$p < 0.10$. *THC*, Tetrahydrocannabinol; *YGTSS*, Yale Global Tic Severity Scale.

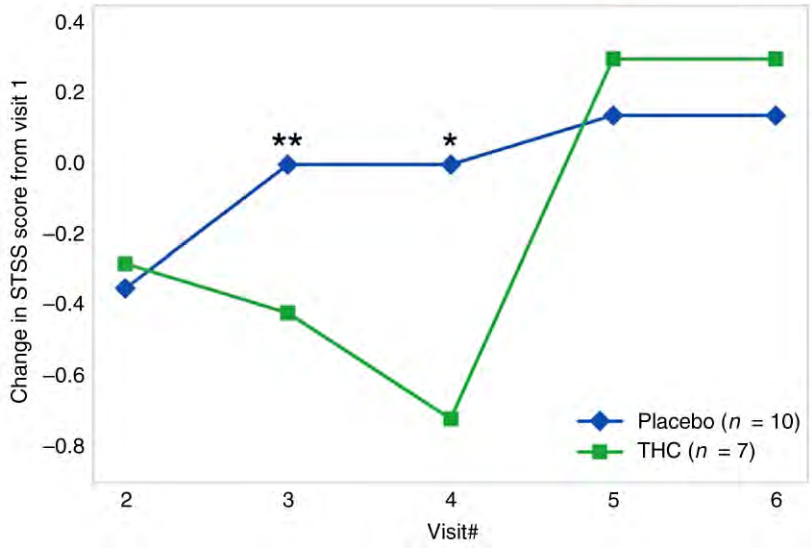


FIGURE 92.3 Changes in STSS from visit 1 to visits 2–6 in patients treated with THC, compared with placebo (Müller-Vahl et al., 2003b). Visit 1 = baseline, visit 2 = treatment day 9, visit 3 = treatment days 20–22, visit 4 = treatment days 30–31, visit 5 = 1 day after medication stopped, visit 6 = 5 weeks after medication stopped. *$p < 0.05$. **$p < 0.10$. *STSS*, Shapiro Tourette syndrome severity scale; *THC*, tetrahydrocannabinol.

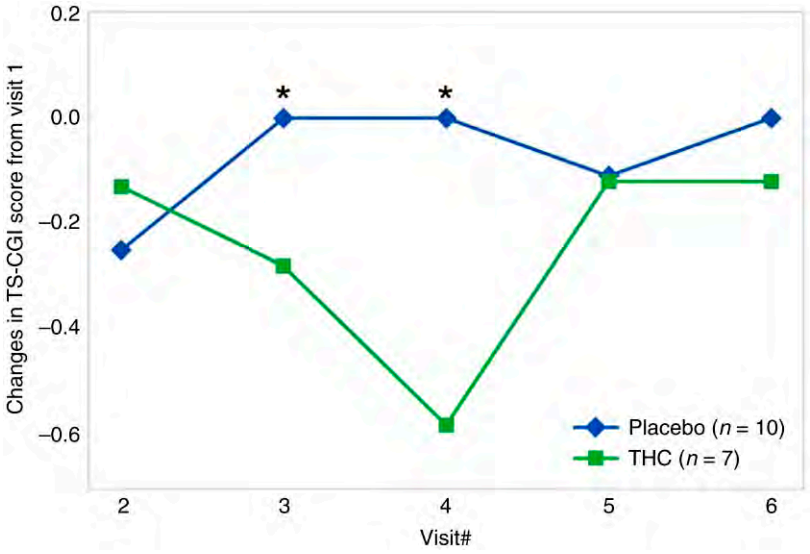


FIGURE 92.4 Changes in TS-CGI scores from visit 1 to visits 2–6 in patients treated with THC, compared with placebo (Müller-Vahl et al., 2003b). Visit 1 = baseline, visit 2 = treatment day 9, visit 3 = treatment days 20–22, visit 4 = treatment days 30–31, visit 5 = 1 day after medication stopped, visit 6 = 5 weeks after medication stopped. ANOVA: $p = 0.079$. *$p < 0.05$. *THC*, Tetrahydrocannabinol; *TS-CGI*, Tourette's syndrome clinical global impressions scale.

span, and divided attention. Measuring immediate verbal memory span, there was a trend toward a significant improvement during and after treatment. Furthermore, no significant influence on OCB, anxiety, depression and "the current emotional state" was found (Müller-Vahl et al., 2003a).

ADVERSE EFFECTS

From our clinical experience and currently published data, we speculate that the psychological and physiological adverse effects encountered by patients with GTS following CBM use are similar to those encountered by other patient groups. There is limited evidence to support any major side effects or negative effects on neuropsychological performance (Müller-Vahl et al., 2001, 2003a). Some work has suggested that in GTS treatment with THC may improve patient's concentration, visual abilities, and psychomotor functions related to driving performance (Brunnauer et al., 2011). However, since it is known that adolescent-onset heavy cannabis use may cause persistent cognitive decline in healthy cannabis users (Meier et al., 2012), it is of seminal importance to carefully consider treating any adolescent patients with CBMs. Nevertheless, these effects need to be further investigated in patients with GTS, since it is not clear whether the same conclusions can be drawn in a population that exhibits putative malfunctions in the dopaminergic, serotonergic, endocannabinoid, and glutamatergic systems. In our view, treatment resistant minors with significant impairment in quality of life, CBMs should be taken into consideration before reverting to DBS as last resort.

PRACTICAL ASPECTS FOR TREATMENT

Despite the limited number of available studies and the lack of well-powered longitudinal studies on the effects of CBMs, cannabinoids have been used by a substantial number of patients—either as a kind of a self-medication or prescribed by the treating clinician—leading to their recommendation as a form of treatment by the European and Canadian Societies for the study of Tourette syndrome (Pringsheim et al., 2012; Roessner et al., 2011). According to these guidelines, CBMs are recommended for the treatment of patients with GTS in cases where the first-line of treatments fail to improve the patient's symptomatology or if the patient exhibits complete resistance to all forms of standard treatments. Different countries have different laws with respect to the use of cannabinoids for the treatment of disorders. For example, synthetic preparations of THC such as dronabinol or nabilone or cannabis extracts such as nabiximols, can be prescribed in several countries. Additionally, some national authorities allow the use of herbal cannabis in medically supervised treatment regimens.

Extrapolating information from the published literature, the recommended strategy for dealing with patients who are either treatment resistant or exhibit marked adverse effects to first-line treatments is to use

an up-titration procedure with slow and incremental increase from 2.5 mg THC per day to a dose between 10 and 20 mg/day while gauging the efficacy and tolerability of the cannabinoid agent at each step. In some patients, however, much higher doses are needed for symptom control. Depending on the route of intake, the individual duration of effect, and patients' desire for treatment, CBMs should be used once or several times daily.

From our experience, most clinical effects caused by *C. sativa* L. seem to be related to THC. Therefore, one might expect similar effects after treatment of GTS patients with *C. sativa* L. extracts (eg, nabiximols) and pure THC. However, our experience is that patients experience stronger and less adverse effects when using marijuana compared to pure THC. This clinical experience is in accordance with reports from other groups of patients (Hazekamp, Ware, Müller-Vahl, Abrams, & Grotenhermen, 2013). We also note that some patients prefer a combined treatment with longer acting orally taken pure THC and immediate but shorter acting inhaled cannabis.

Longer trials including a larger number of patients are urgently needed to further investigate efficacy and safety of CBMs in GTS. As in other groups of patients, it would be of interest to compare the effect of pure THC to drugs containing different cannabinoids. Finally, it can be speculated that substances that bind more selectively to CB1 receptors or inhibit the uptake or the degradation of endogenous cannabinoids might be even more effective and better tolerated than currently available substances.

CONCLUSIONS

Available data from a limited number of case reports and small-scale controlled clinical trials have provided evidence that cannabinoid based medicine do have a beneficial effect on the treatment of tics in patients with GTS. There is also some evidence that cannabinoids help improve conditions associated with GTS including OCB, ADHD, impulsivity, depression, and self-injurious behavior.

Neuropsychological tests demonstrate that THC has no detrimental effects on memory, reaction time, concentration, and attention in patients with GTS. Interestingly, evidence points to improvements in memory, concentration, and visual perception. It can be assumed that beneficial effects of CBMs in GTS are caused by modulations of the CB1 receptor system, rather than unspecific effects such as sedation or decreased general activity. There is some evidence that THC might augment the anti-tic effect of antipsychotics. Although larger-scale clinical trials are urgently needed to further investigate the effect of cannabinoids in the treatment of GTS, available evidence encourages the consideration of CBMs as a form of pharmacological treatment when other substances fail to improve motor or nonmotor symptoms of GTS.

MINI-DICTIONARY

Attention deficit/hyperactivity disorder (ADHD) A common neurodevelopmental psychiatric disorder characterized by inattention, hyperactivity, and impulsivity.

Cannabinoid based medicines (CBMs) A medical therapy consisting of cannabis or its derivatives that is used to treat disease or alleviate symptoms.

Cortico-striato-thalamo-cortical circuitry (CSTC) A set of parallel and segregated cortico-subcortical loops that play a role in the integration of information related to emotional, cognitive, and motor functions.

Fatty acid amide hydrolase (FAAH) An enzyme that serves as the major metabolic regulator of many of the endogenous fatty acid amides.

Gilles de la Tourette syndrome (GTS) A hereditary, childhood-onset and neuropsychiatric movement disorder that is characterized by multiple motor tics and one or more vocal tics.

Obsessive-compulsive behavior (OCB) This term is used, when a person suffers from obsessions (intrusive thoughts) and/or compulsions (repetitive behaviors), but does not fulfill diagnostic criteria for the diagnosis of obsessive-compulsive disorder (OCD) according to DSM-5.

Tetrahydrocannabinol (THC) The principal psychoactive constituent of the cannabis plant.

Yale global tic severity scale (YGTSS) A clinical rating instrument used for the assessment of patients with Tourette's syndrome and other tic disorders. The YGTSS provides an evaluation of the number, frequency, intensity, complexity, and interference of motor and vocal symptoms.

Acknowledgment

ASK is funded by TS-EUROTRAIN (FP7-PEOPLE-2012-ITN, GA no 316978).

References

Benton, A. L. (1945). A visual retention test for clinical use. *Archives of Neurology and Psychiatry*, *54*, 212–216.

Brunnauer, A., Segmiller, F. M., Volkamer, T., Laux, G., Muller, N., & Dehning, S. (2011). Cannabinoids improve driving ability in a Tourette's patient. *Psychiatry Research*, *190*, 382.

Caspi, A., & Moffitt, T. E. (2006). Gene–environment interactions in psychiatry: joining forces with neuroscience. *Nature Reviews Neuroscience*, *7*, 583–590.

Fernandez-Ruiz, J. (2009). The endocannabinoid system as a target for the treatment of motor dysfunction. *British Journal of Pharmacology*, *156*, 1029–1040.

Goetz, C. G., Pappert, E. J., Louis, E. D., Raman, R., & Leurgans, S. (1999). Advantages of a modified scoring method for the Rush video-based tic rating scale. *Movement Disorders*, *14*, 502–506.

Harcherik, D. F., Leckman, J. F., Detlor, J., & Cohen, D. J. (1984). A new instrument for clinical studies of Tourette's syndrome. *Journal of the American Academy of Child Psychiatry*, *23*, 153–160.

Hasan, A., Rothenberger, A., Munchau, A., Wobrock, T., Falkai, P., & Roessner, V. (2010). Oral delta 9-tetrahydrocannabinol improved refractory Gilles de la Tourette syndrome in an adolescent by increasing intracortical inhibition: a case report. *Journal of Clinical Psychopharmacology*, *30*, 190–192.

Hazekamp, A., Ware, M. A., Müller-Vahl, K. R., Abrams, D., & Grotenhermen, F. (2013). The medicinal use of cannabis and cannabinoids—an international cross-sectional survey on administration forms. *Journal of Psychoactive Drugs*, *45*, 199–210.

Helmstaedter, C., Lendt, M., & Lux, S. (2001). *VLMT—verbaler Lern- und Merkfähigkeitstest*. Göttingen: Beltz Test GmbH, Hogrefe Verlag.

Hemming, M., & Yellowlees, P. M. (1993). Effective treatment of Tourette's syndrome with marijuana. *Journal of Psychopharmacology*, *7*, 389–391.

Herkenham, M., Lynn, A. B., Little, M. D., Johnson, M. R., Melvin, L. S., De Costa, B. R., & Rice, K. C. (1990). Cannabinoid receptor localization in brain. *Proceedings of the National Academy of Sciences of the United States of America*, *87*, 1932–1936.

Jankovic, J. (1997). Tourette syndrome. Phenomenology and classification of tics. *Neurology Clinics*, *15*, 267–275.

Knight, T., Steeves, T., Day, L., Lowerison, M., Jette, N., & Pringsheim, T. (2012). Prevalence of tic disorders: a systematic review and meta-analysis. *Pediatric Neurology*, *47*, 77–90.

Kompoliti, K., Fan, W., & Leurgans, S. (2009). Complementary and alternative medicine use in Gilles de la Tourette syndrome. *Movement Disorder*, *24*, 2015–2019.

Leckman, J., Towbin, K., Ort, S., & Cohen, D. (1988). Clinical assessment of tic disorder severity. In D. J. Cohen, R. D. Bruun, & J. F. Leckman (Eds.), *Tourette's syndrome and tic disorders: clinical understanding and treatment*. New York, NY; Chichester, UK: John Wiley & Sons.

Leckman, J. F., Riddle, M. A., Hardin, M. T., Ort, S. I., Swartz, K. L., Stevenson, J., & Cohen, D. J. (1989). The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. *Journal of the American Academy of Child and Adolescent Psychiatry*, *28*, 566–573.

Mantel, B. J., Meyers, A., Tran, Q. Y., Rogers, S., & Jacobson, J. S. (2004). Nutritional supplements and complementary/alternative medicine in Tourette syndrome. *Journal of Child and Adolescent Psychopharmacology*, *14*, 582–589.

Meier, M. H., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R. S. E., et al. (2012). Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proceedings of the National Academy of Sciences*, *109*, E2657–E2664.

Merz, J., Lehrl, S., Galster, V., & Erzigkeit, H. (1975). The multiple selection vocabulary test (MSVT-B)—an accelerated intelligence test. *Psychiatrie, Neurologie und Medizinische Psychologie (Leipzig)*, *27*, 423–428.

Moss, D., Montgomery, S., Salo, A., & Steger, R. (1984). Tetrahydrocannabinol effects on extrapyramidal motor behaviors in an animal model of Parkinson's disease. In S. Agurell, W. L. Dewey, & R. E. Willette (Eds.), *The cannabinoids: chemical, pharmacologic, and therapeutic aspects*. New York, NY: Academic Press.

Müller-Vahl, K. R., Koblenz, A., Jobges, M., Kolbe, H., Emrich, H. M., & Schneider, U. (2001). Influence of treatment of Tourette syndrome with delta9-tetrahydrocannabinol (delta9-THC) on neuropsychological performance. *Pharmacopsychiatry*, *34*, 19–24.

Müller-Vahl, K. R., Kolbe, H., & Dengler, R. (1997a). Alcohol withdrawal and Tourette's syndrome. *Neurology*, *48*, 1478–1479.

Müller-Vahl, K. R., Kolbe, H., & Dengler, R. (1997b). Gilles de la Tourette syndrome. Effect of nicotine, alcohol and marihuana on clinical symptoms. *Nervenarzt*, *68*, 985–989.

Müller-Vahl, K. R., Kolbe, H., Schneider, U., & Emrich, H. M. (1998). Cannabinoids: possible role in patho-physiology and therapy of Gilles de la Tourette syndrome. *Acta Psychiatrica Scandinavica*, *98*, 502–506.

Müller-Vahl, K. R., Prevedel, H., Theloe, K., Kolbe, H., Emrich, H. M., & Schneider, U. (2003a). Treatment of Tourette syndrome with delta-9-tetrahydrocannabinol (delta 9-THC): no influence on neuropsychological performance. *Neuropsychopharmacology*, *28*, 384–388.

Müller-Vahl, K. R., Schneider, U., Koblenz, A., Jobges, M., Kolbe, H., Daldrup, T., & Emrich, H. M. (2002). Treatment of Tourette's syndrome with delta 9-tetrahydrocannabinol (THC): a randomized crossover trial. *Pharmacopsychiatry*, *35*, 57–61.

Müller-Vahl, K. R., Schneider, U., Kolbe, H., & Emrich, H. M. (1999). Treatment of Tourette's syndrome with delta-9-tetrahydrocannabinol. *American Journal of Psychiatry*, *156*, 495.

Müller-Vahl, K. R., Schneider, U., Prevedel, H., Theloe, K., Kolbe, H., Daldrup, T., & Emrich, H. M. (2003b). Delta 9-tetrahydrocannabinol (THC) is effective in the treatment of tics in Tourette syndrome: a 6-week randomized trial. *Journal of Clinical Psychiatry*, *64*, 459–465.

Paschou, P. (2013). The genetic basis of Gilles de la Tourette Syndrome. *Neuroscience and Biobehavioral Reviews*, *37*, 1026–1039.

Pringsheim, T., Doja, A., Gorman, D., Mckinlay, D., Day, L., Billinghurst, L., Carroll, A., Dion, Y., Luscombe, S., Steeves, T., & Sandor, P. (2012). Canadian guidelines for the evidence-based treatment of tic disorders: pharmacotherapy. *Canadian Journal of Psychiatry*, *57*, 133–143.

Robertson, M. M. (2000). Tourette syndrome, associated conditions and the complexities of treatment. *Brain*, *123*(Pt 3), 425–462.

Roessner, V., Plessen, K. J., Rothenberger, A., Ludolph, A. G., Rizzo, R., Skov, L., Strand, G., Stern, J. S., Termine, C., & Hoekstra, P. J. ESST Guidelines Group. (2011). European clinical guidelines for Tourette syndrome and other tic disorders. Part II: pharmacological treatment. *European Child and Adolescent Psychiatry*, *20*, 173–196.

Sandyk, R., & Awerbuch, G. (1988). Marijuana and Tourette's syndrome. *Journal of Clinical Psychopharmacology*, *8*, 444–445.

Schumann, G., Loth, E., Banaschewski, T., Barbot, A., Barker, G., Büchel, C., …, Struve, M. (2010). The IMAGEN study: reinforcement-related behaviour in normal brain function and psychopathology. *Molecular Psychiatry*, *15*(12), 1128–1139.

Shapiro, A. K. (1988). Signs, symptoms, and clinical course. In A. Shapiro, E. Shapiro, J. Young, & T. Feinberg (Eds.), *Gilles de la Tourette syndrome* (2nd ed.). New York, NY: Raven Press.

Strohbeck-Kuhner, P., Skopp, G., & Mattern, R. (2007). Fitness to drive in spite (because) of THC. *Arch für Kriminologie*, *220*, 11–19.

Terry, G. E., Hirvonen, J., Liow, J. -S., Zoghbi, S. S., Gladding, R., Tauscher, J. T., …, Innis, R. B. (2010). Imaging and quantitation of cannabinoid CB1 receptors in human and monkey brains using (18) F-labeled inverse agonist radioligands. *Journal of Nuclear Medicine*, *51*(1), 112–120.

Verdellen, C., Van de Griendt, J., Hartmann, A., & Murphy, T. ESSTS Guidelines Group. (2011). European clinical guidelines for Tourette syndrome and other tic disorders. Part III: behavioural and psychosocial interventions. *European Child and Adolescent Psychiatry*, *20*, 197–207.

Zimmermann, P. A., & Fimm, B. (1989). *Neuropsychologische Testbatterie zur Erfassung von Aufmerksamkeitsdefiziten—Revidierte Fassung*. Freiburg, Germany: Psychologisches Institut der Universität Freiburg.

CHAPTER

93

Cannabidiol and Multiple Sclerosis

M. Mecha, A. Feliú, F.J. Carrillo-Salinas, C. Guaza

Neurobiology and Functional Systems Department, Cajal Institute, CSIC, Madrid, Spain

SUMMARY POINTS

- Multiple sclerosis (MS) is the most frequent chronic neurological disease among young and middle-aged people in the northern industrialized countries.
- MS is pathologically characterized by multifocal inflammation, demyelination and neuronal injury in the central nervous system (CNS).
- The first-line disease modifying therapies for MS patients include immunomodulatory and immunosuppressive medications.
- Cannabidiol (CBD), which constitutes up to 40% of *Cannabis sativa* extract, may represent a promising agent for human therapeutic use due to the lack of psychoactive actions.
- Up to date, there is no evidence about the full binding of CBD to any known receptor site, and the molecular pharmacology of CBD has not been well defined.
- Experimental autoimmune encephalomyelitis (EAE) and Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD) are two experimental models of MS that include classical MS hallmarks like inflammation, neuronal damage, and demyelination.
- CBD has been shown to ameliorate EAE and TMEV-IDD model symptomatology by diminishing inflammation, microglial activity, and leukocyte homing.
- Sativex is used for the treatment of symptoms of MS, particularly spasticity and neuropathic pain.

KEY FACTS OF NEUROLOGY

- Multiple sclerosis is a complex heterogeneous demyelinating autoimmune disease that primarily affects the myelin in the central nervous system (CNS).
- CNS demyelination, the pathological process in which myelin sheaths are lost, is the consequence of a direct insult targeted at the oligodendrocyte.
- Axonal degeneration is accepted as the major cause of irreversible neurological disability in multiple sclerosis (MS) patients.
- Autoantigen-specific Th17 and Th1, B cells and monocytes/macrophages have major pathological roles.
- Pharmacological treatment options approved for MS aim at limiting inflammation and decreasing relapse rate, but, no current therapies can cure the disease.

KEY FACTS OF CNS REPAIR

- Remyelination is the process in which myelin sheaths are restored to demyelinated axons restoring functional deficits.
- Remyelination involves the generation of new mature oligodendrocytes from oligodendrocyte progenitors.
- A key function of remyelination in multiple sclerosis is axon survival.
- The efficiency of remyelination is affected by age, sex, and genetic background regardless of the disease process.
- The most significant approach to enhance remyelination is to target the endogenous regenerative process.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00108-3
Copyright © 2017 Elsevier Inc. All rights reserved.

KEY FACTS ON THE CANNABINOID SYSTEM

- The cannabinoid system is a regulatory system identified following the study of *Cannabis sativa* derivatives involved in the homeostasis control.
- The endocannabinoid signaling system is composed of cannabinoid receptors, their endogenous ligands and the enzymes that produce and inactivate these ligands.
- There are >60 cannabinoid compounds present in extracts of *C. sativa*, and Δ^9-tetrahydrocannabinol (Δ^9-THC) is responsible for the psychoactive effects of cannabis, and for many of the potential medicinal effects.
- The nonpsychoactive cannabidiol (CBD)is the other cannabinoid derived from *C. sativa* of current medical interest in MS.
- Sativex is an oromucosal spray, constituted by an equimolecular combination of Δ^9-THC and CBD-enriched botanical extracts approved for the treatment of spasticity and pain associated to MS.

LIST OF ABBREVIATIONS

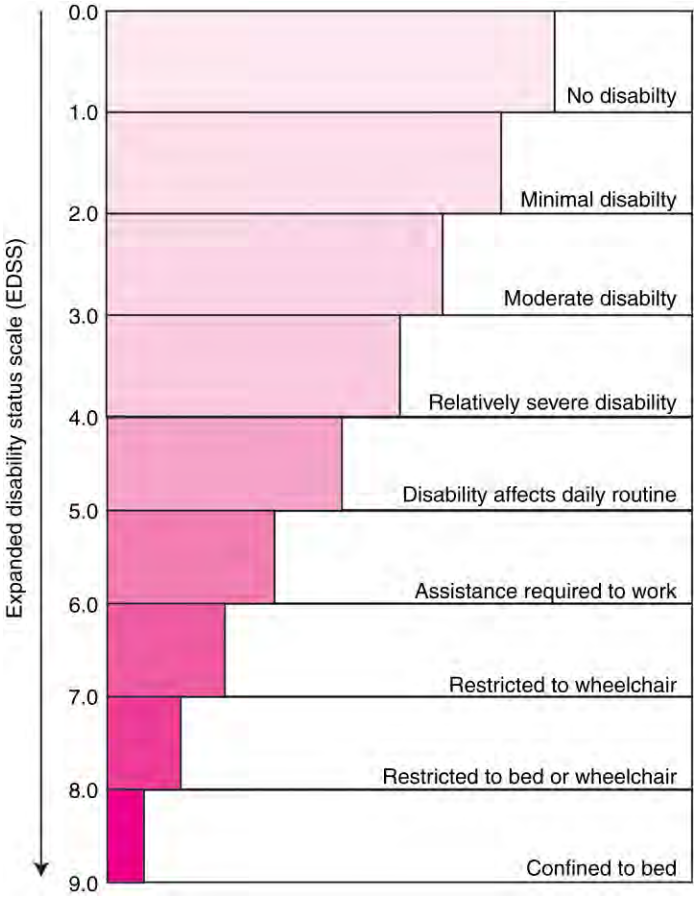
AD	Alzheimer disease
CBD	Cannabidiol
CNS	Central nervous system
EAE	Experimental autoimmune encephalomyelitis
EDSS	Expanded disability status scale
FAAH	Fatty acid amide hydrolase
GPCR	G-protein coupled receptor
IFN-β	Interferon-beta
MCA	Middle-cerebral-artery
MHC	Major histocompatibility complex
MRI	Magnetic resonance image
MS	Multiple sclerosis
PPAR	Peroxisome proliferator activated receptors
PPMS	Primary progressive multiple sclerosis
RRMS	Remittent recurrent multiple sclerosis
SPMS	Secondary progressive multiple sclerosis
THC	Tetrahydrocannabinol
TMEV-IDD	Theiler´s murine encephalomyelitis virus - induced demyelinating disease
TPVR-1	Transient potential vanilloid receptor-1

INTRODUCTION

Multiple sclerosis (MS) is the most frequent chronic neurological disease among young adults in the northern industrialized countries. MS is pathologically characterized by multifocal inflammation, demyelination, and neuronal injury in the optic nerves, brain and spinal cord, and is considered to be an autoimmune disease with a complex pathophysiology (Compston & Coles, 2002). The pathological hallmarks of the disease result in an affectation of white matter tracts and injury to the cortical and deep gray matter, generating neurologic symptoms and disability in patients with MS (Table 93.1).

MS occurs in a female–male ratio of 3 to 1, and has an estimated prevalence of more than 2.1 million people worldwide (Niedziela, Adamczyk-Sowa, & Pierzchala, 2014). It is widely accepted that MS cause is multifactorial, including multiple genetic and environmental risk factors like risk alleles in genes for major histocompatibility complex (MHC), and interleukin-2 and 7 receptors among others (Beecham et al., 2013). Moreover, the geographic location of residence before adolescence may also be predictive of MS risk, as it has been found

TABLE 93.1 Expanded Disability Status Scale (EDSS) in Patients With MS



The Kurtzke's EDSS scores 8 functional systems and the person's ability to walk. From 0.0 to 4.0, people are able to walk without assistance. From 4.0 to 7.5, people can walk but with assistance. Mainly, point 6 on the scale represents walking with a cane, and this point is often used as an endpoint of the progression of disability. From 7.5 to 10, the main determinant of EDSS is the person's ability to transfer from wheelchair to bed and self-care. An EDSS of 10 is not included in this table because it means death of the patient due to MS.

increased rates of the disease in northern and southern latitudes compared with equatorial countries, probably linked to a vitamin D deficiency (Van der Mei et al., 2003). Additionally, the risk to MS may be influenced by the exposure to particular infectious like Epstein–Barr virus (Lassmann, Niedobitek, Aloisi, & Middeldorp, 2011) and Herpesvirus 6 (Tait & Straus, 2008).

The first-line disease modifying therapies for MS patients include immunomodulatory and immunosuppressive medications like interferon-β (IFN-β) or glatiramer acetate among others (Table 93.2), which has been shown to reduce both risk of relapse and new lesion formation on MRI scans. Paradoxically, antiinflammatory treatment might contribute to the failure of repair, as results from studies of patients with MS indicate that the inflammation blockade might trigger counter-regulatory inflammatory mechanisms used to heal the injured tissue (Martino et al., 2002), and suggesting that tissue integrity is restored by the conversion of the inflammatory response from a damaging to a repairing one (Nathan, 2002).

Evidence suggest that neuroinflammation, demyelination, and neurodegeneration may occur in parallel, the combination of antiinflammatory, oligoprotective and neuroprotective strategies arise as an emerging therapy for the symptomatic and therapeutic treatment of MS. In this line, *C. sativa* derivatives have attracted special interest regarding putative therapeutic properties, despite the fact that these compounds have always raised both ethical and practical problems for their potential abuse and unavoidable psychotropic effects. Among marijuana compounds, cannabidiol (CBD), which constitutes up to 40% of cannabis extract, may represent a promising agent for human therapeutic use as it lacks any cognitive and psychoactive actions, and has an excellent tolerability profile in humans (Mechoulam & Hanus, 2002).

A large number of clinical trials have been performed to assess the clinical efficacy of CBD in different pathologies. Most of them has been focused on Sativex compounds (GW Pharmaceuticals; Salisbury, United Kingdom), a commercially available preparation containing CBD and Δ^9-THC under four different formulations that differs in the concentration of CBD and Δ^9-THC (tetrahydrocannabinol). The oromucosal spray administration of Sativex has been agreed in 2005 for the treatment of pain and spasticity in MS (Perras, 2005). In the central nervous system (CNS), CBD has been reported to act as an antiinflammatory compound, thus being useful for neuroinflammatory disorders but also as a neuroprotective agent by normalizing glutamate homeostasis, reducing oxidative stress, and attenuating glial activation and the occurrence of local inflammatory events (reviewed by Fernandez-Ruiz et al., 2013). All these properties point out CBD as a promising therapeutic agent for the treatment of neuroinflammatory disorders like MS.

CBD PHARMACOLOGY AND MECHANISMS OF ACTION

Isolated in across the 1930s and 1940s from *C. sativa*, the structure and configuration of CBD was fully elucidated by Mechoulam et al. in the 1960s (Mechoulam & Shvo, 1963; Mechoulam, Shani, Edery, & Grunfeld, 1970). The first study focusing on CBD pharmacology was published in 1981, regarding hypnotic and anticonvulsant properties (Carlini & Cunha, 1981). Along this time, several studies have been done to identify the mechanisms through which CBD exerts its actions. Up to date, there is no evidence about the full binding of CBD to any known receptor site. The molecular pharmacology of CBD has not been well defined yet, and little is known about a possible CBD-receptor-mediated signaling pathway. It is known that many of the CBD effects are associated with both central and peripheral actions, and numerous studies has been reported trying to elucidate some of its mechanisms of action (Table 93.3), that are detailed later in the chapter.

CBD is a Potent Antioxidant

Pioneer works by Hampson, Grimaldi, Axelrod, and Wink (1998) showed that CBD has antioxidant properties that have been confirmed along several publications. The plant cannabinoids, being monophenols, monophenolic esthers (like THC), or resorcinols (like CBD) are likewise potent antioxidants. Hampson et al. (1998) demonstrated that CBD exerted a potent antioxidant activity that resulted more protective than either ascorbate (vitamin C) or α-tocopherol (vitamin E) against glutamate-mediated neurotoxicity. These observations suggest that CBD may be a potential therapeutic agent for the treatment of oxidative neurological disorders like cerebral ischemia, and for diseases that course with oxidative damage.

CBD Potentiates the Endocannabinoid Signaling System

In the immature damaged brain, CBD has shown direct activity binding to the CB2 receptor, and an indirect activity through an inhibitory effect on the mechanisms of inactivation of endocannabinoids (transporter, FAAH enzyme) (Bisogno et al., 2001; De Filippis et al., 2008). In this line, the enhancement of the endocannabinoid tone may mediate some of the antiinflammatory and neuroprotective effects of CBD.

CBD Binds With Low Affinity to Both CB1 and CB2 Cannabinoid Receptors

At concentrations in the micromolar range, CBD shows weak ability to remove ^{3}[H]CP55940, a not selective ligand for CB receptors from both CB1 and

TABLE 93.2 Currently Available MS Treatments

	Treatment	Year of FDA approval	Type	Route of administration	Implications	Mechanism of action	Side effects
Acute	Methylprednisolone (Solu-Medrol)	1959	Glucocorticoid	Intravenous (3–5 days)	Acute relapse	Stabilizing the BBB, decreasing proinflammatory cytokines, and inducing T cell apoptosis	Disturbance of taste, facial flushing, insomnia, psychiatric disturbance, exacerbation of acne, transient hyperglycemia, and hypertension
	ACTH (H.P. Acthar)	1978	Hormone	Intramuscular or subcutaneous (5–15 days)	Acute relapse	Stimulates the adrenal cortex gland to secrete cortisol, corticosterone, and aldosterone	Vomiting, change in appetite, diarrhea, constipation, restlessness, difficulty sleeping, sweating
	Plasma exchange	/	/	Intravenous	Acute relapse	Exchange of immunoglobulins	Hypocalcemia, hypovolemia, and anaphylactoid reactions
Chronic	IFNβ-1a (Avon ex)	1996	Cytokine (low dose)	Intramuscular injection	RRMS first line	Suppresses the proliferation of myelin-basic protein-specific T cells. Reduces the production of proinflammatory cytokines, and induces antiinflammatory cytokines. Effects on the endothelial cells of the BBB	Production of NAb (IFNβ1A less than IFNβ1B) Influenza-like symptoms. Headache, injection site reaction, asthenia, lymphopenia, depression, hepatic injury, congestive heart failure, anaphylactic shock, and pain
	IFNβ-1a (Rebif)	2002	Cytokine (high dose)	Subcutaneous injection			
	IFNβ-1b (Betaseron)	1993	Cytokine (high dose)	Subcutaneous injection			
	Glatiramer acetate (Copaxone)	1996	Synthetic polymer of random sequences of 4 aa (L-tyr, L-glut L-Ala, L-Lys)	Subcutaneous injection	RRMS first line	Unknown, possible binding to the major MHC-II competing with the other MS putative Ag	Injection site reactions, tightness, anxiety, dyspnea, palpitation, vasodilation
	Mitoxantrone (Novantrone)	2000	Antineoplastic drug	Intravenous infusion	RRMS SPMS	Inhibit B cell, T cell, macrophage proliferation. Impair antigen presentation, and the secretion of IFN-γ, TNFα, and IL-2	Cardiotoxicity, myelogenous leukemia, gonadal dysfunction
	Natalizumab (Tysabri)	2006	Humanized anti-VLA-4 monoclonal antibody	Intravenous infusion	RRMS first line	Targets the α4-chain of α4β1 integrin decreasing the accumulation of activated leukocytes within the CNS	Headache, fatigue, arthralgia, urinary tract infection, lower respiratory infections, gastroenteritis, vaginitis, diarrhea, hypersensitivity reactions, hepatotoxicity, PML
	Dalfampridine (Ampyra)	2010	4-aminopyridine (4-AP)	Oral capsule	Chronic progressive	Potassium channel blocker, potent calcium activator	Dizziness, nervousness, nausea urinary tract infection
	Fingolimod (Gilenya)	2010	Sphingosine-1-phosphate (SIP) receptor modulator	Oral capsule	RRMS second line	SIP receptor antagonist in lymphocytes. It blocks the ability of lymphocytes to leave lymph nodes	Headache, infections, bradycardia bradyarrhythmias, macular edema
	Teriflunomide (Aubagio)	2012	Drug	Oral capsule	RRMS	Binds to DHODH protein inhibiting pyrimidine synthesis in proliferating cells such as T and B lymphocytes (immunomodulatory)	Dyspnea, diarrhea, nausea, alopecia, hepatotoxicity, acute renal failure, hypertension, leukopenia
	Dimethyl fumarate (Tecfidera, BG12)	2013	α,β-unsaturated ester	Oral capsule	RRMS first line	Reduce transendothelial migration of activated leukocytes through the BBB. Neuroprotective effects via activation of antioxidative pathways	Gastrointestinal disorders, lymphopenia, flushing, pruritus, rash, erythema

Acute and chronic treatments available for MS patients (reviewed in Damal, Stoker, & Foley, 2013; Kantarci, Pirko, & Rodriguez, 2014). PML, progressive multifocal leukoencephalopathy; ACTH, adrenocorticotropic hormone; DHODH, dihydroorotate dehydrogenase BBB, blood–brain barrier. Intravenous immunoglobulins (IVIG) and cyclophosphamide for acute relapses are not covered in this table.

TABLE 93.3 CBD Molecular Mechanism of Actions and Effects

Targets	Mechanisms	Affinity/dose	Effect
Receptors and channels			
CB1/CB2	Antagonist	KB 79 nM (CB1); 138 nM (CB2)	Antagonizes cannabinoid induce antispasmodic effect
CB2	Inverse agonist	EC50: 503 nM	Antiinflammatory effect
GPR55	Antagonist	IC50: 350 nM	Antagonistic activity in human osteoclasts
5-HT3A ligand-gated channel	Allosteric inhibition	IC50: 0.6 μM	Modulation of nociception and emesis
TRPM8 cation channel	Antagonist	EC50: 80–140 nM; IC50: 60 nM	Analgesic effects
TRPA1 cation channel	Agonist	EC50: 110 nM; IC50: 160 nM	Analgesic effects
PPARγ nuclear receptor	Agonist	IC50: ±5 μM	Vasorelaxation antiinflammatory effect
T-type Ca^{2+} channel	Inhibitor	IC50: ±1 μM	Nociceptive and antiepileptic effects, sleep regulation
TRPV1 cation channel	Agonist	EC50: 1 μM; IC50: 0.6 μM	Antipsychotic and analgesic effects
TRPV2 cation channel	Agonist	EC50: 1.25 μM; IC50: 4.5 μM	Antiinflammatory/analgesic/antinociceptive effect
5-HT1A receptor	Agonist	80% displacement at 16 μM	Antiischemic and anxiolitic properties. Neuroprotective
μ and δ opioid receptors	Allosteric modulation	EC50 4.38 (μ); 4.10 (δ)	Potentially enhance the effects of opiates
α1 and α1β glycine ligand-gated channels	Positive allosteric modulation	EC50: 12.3 μM (α1); 18.1 (α1β)	Role in chronic pain after inflammation or nerve injury
Abnormal-CBD receptor	Antagonist	Effect at 1 μM	Attenuates the vasodilator response to anandamide
Targets	**Mechanisms**	**Affinity/dose**	**Effect**
Enzymes			
CYP1A1	Competitive inhibitor	IC50: 0.41 μM	Might lead to interaction of drugs or toxicants metabolized by CYP enzymes. Possible adverse effects or intoxication
CYP1A2 and CYP1B1	Competitive inhibitor	IC50: 3.8 μM (A2); 5.96 μM (B1)	
CYP2B6	Inhibitor	K_i: 0.694 μM	
CYP2D6	Competitive inhibitor	IC50: 6.65 μM	
CYP3A5	Inhibitor	IC50: 1.65 μM	
CYP2A6	Inhibitor	K_i: 55 μM	
CYP3A4 and CYP3A7	Inhibitor	IC50: 11.7 μM (A4)23–31 μM (A7)	
FAAH	Inhibitor	IC50: 15.2 μM	Increment of AEA tone. Analgesic, antiinflammatory effects
Mg^{2+} -ATPase	Inhibitor	Effect at 50 μM	Anticonvulsant effect
AANAT	Inhibitor	65% reduction at 10 μM	Reduces melatonin biosynthesis
5-Lipoxygenase	Inhibitor	IC50: 73.73 μM	Antimitotic effect in glioma cells
15-Lipoxygenase	Inhibitor	IC50: 2.56 μM	Involved in atherosclerosis
Phospholipase A2	Activator/inhibitor	EC50: 6.4 μM(+)134 μM(−)	Antiinflammatory effects
Indoleamine-2,3-dioxygenase	Inhibitor	IC50: 2.8 μM/mL	Enhance of tryptophan and therefore serotonin. Improvement of mood disturbances

(*Continued*)

TABLE 93.3 CBD Molecular Mechanism of Actions and Effects (*cont.*)

Targets	Mechanisms	Affinity/dose	Effect
Glutathione peroxidase	Activator	Effect at 10 and 25 μM	ROS production and caspase activation in tumor cells
Glutathion reductase	Activator		
Superoxide dismutase	Inhibitor	Effect at 100 μM	Generates ROS and induces cell toxicity in tumor cells
Catalase	Inhibitor		
NAD(P)H-quinone reductase	Inhibitor		
Progesterone 17α-hydrolase	Inhibitor	Effect at 1 M	Interaction with steroid metabolism. Inhibits the testosterone synthesis in rat testis
Testosterone 6β-hydrolase	Inhibitor		
Testosterone 16α-hydrolase	Inhibitor		
Targets	**Mechanisms**	**Affinity/dose**	**Effect**
Transporters and cellular uptake			
Adenosine uptake	Competitive inhibitor	IC50: 120 nM	Increment of adenosine tone antiinflammatory effect
Intracellular Ca^{2+} uptake	Inhibition	Effect at 1 μM	Neuroprotective and antiepileptic properties
Anandamide reuptake	Inhibitor	IC50: 28 μM	Increment of AEA tone
Choline uptake	Inhibitor	EC50: 1.6 pM	Increment of Ach tone
P-glycoprotein (drug efflux transporter)	Inhibitor	IC50: 39.6 μM	Potentially influence the absorption and disposition of compounds that are P-gp substrates

Molecular targets of CBD, including cannabinoid and noncannabinoid receptors, enzymes, transporters, and cellular uptake proteins (reviewed in Izzo, Borrelli, Capasso, Di Marzo, & Mechoulam, 2009; Mechoulam, Petersa, Murillo-Rodriguez, & Hanus, 2007; Hill, Williams, Whalley, & Stephens, 2012). CYP, Cytochrome P450 enzymes; AANAT, arylalkylamine *N*-acetyltransferase; FAAH, fatty acid amide hydrolase; AEA, *N*-arachidonoylethanolamide; Ach, acetylcholine; ROS, reactive oxygen species.

CB2 receptor sites (Thomas, Gilliam, Burch, Roche, & Seltzman, 1998).

CBD Antagonizes CB1 and CB2 Receptor Agonists, and Can Act as an Inverse Agonist of CB2 Receptor

CBD attenuates the effects of WIN55212 and CP55940 (CB1 agonists) at prejunctional sites in mouse vas deferens (Pertwee, Ross, Craib, & Thomas, 2002). Moreover, at concentration values in low nanomolar range, CBD could work as an inverse agonist of CB2 receptor, as it has been demonstrated in whole-brain membranes and membranes from CHO cells transfected with human CB2 receptors (Thomas et al., 2007). This mechanism may explain some of the pharmacological effects of CBD such as its antiinflammatory properties.

CBD Enhances Adenosine Signaling

CBD binds to the equilibrative nucleoside-transporter-1 with a K_i value below 250 nM, which in turn led to the increase of extracellular adenosine (Carrier, Auchampach, & Hillard, 2006). Neuroprotective effects of CBD in hypoxic–ischemic brain damage also involve adenosine A_2 receptors (Castillo, Tolon, Fernandez-Ruiz, Romero, & Martinez-Orgado, 2010). Moreover, CBD diminishes inflammation in acute models of injury (Ribeiro et al., 2012) and in a viral model of MS through adenosine A_2 receptors (Mecha et al., 2013b).

CBD Interacts With the Transient Potential Vanilloid Receptor Type-1 (TPVR-1), and With 5-HT1$_A$ Serotonin Receptor

Both CBD and its (+) enantiomer interact (EC50 estimated between 3.2 and 3.5 μM) with TPVR-1 receptor, with a maximal effect that is similar to the natural agonist capsaicin. These effects have been confirmed both in vitro (Bisogno et al., 2001) and in a model of acute inflammation in rats (Costa, Giagnoni, Franke, Trovato, & Colleoni, 2004).

In relation to 5HT1$_A$ serotonin receptors, Russo, Burnett, Hall, and Parker (2005) demonstrated that CBD displaces the agonist ([^{3}H]-8-OH-DPAT) from the human cloned receptor in a concentration/dependent manner, and that it increases [^{35}S]GTPgS binding in this G-protein-coupled receptor (GPCR) system decreasing cAMP concentration at similar apparent levels of receptor occupancy. In addition, CBD significantly reduces the infarct

volume induced by middle-cerebral-artery (MCA) occlusion (Mishima et al., 2005), exerting a neuroprotective effect that was inhibited by the serotonin 5-HT1$_A$ receptor antagonist WAY100135, but not by capsazepine, a vanilloid-receptor antagonist.

CBD Allosterically Modulates μ and δ Opioid Receptors

Data from Kathmann, Flau, Redmer, Tränkle, and Schlicker (2006) show that, on rat cerebral cortex membrane homogenates, CBD accelerated the dissociation of both the μ opioid receptor agonist [^{3}H]DAMGO induced by naloxone, and the δ opioid receptor agonist [^{3}H] naltrindole induced by naltrindole. Interestingly, this property was shared by THC, but not by the CB1 cannabinoid-receptor antagonist rimonabant. As this modulation of opioid receptors occurs at very high levels of CBD, it cannot be expected to contribute markedly to the phytocannabinoid actions in vivo.

CBD Affects Nuclear Receptors of the Peroxisome Proliferator-Activated Receptors (PPAR-g), and Antagonizes the Orphan Receptor GPR55

Recently, it has been discovered the ability of different endocannabinoids and phytocannabiniods, including CBD, to display an extra-cannabinoid receptor binding activity by the interaction with peroxisome proliferator-activated receptors (PPARs) (O'Sullivan & Kendall, 2010). PPARs belong to the family of nuclear hormone receptors, and have been reported to control the expression of genes related to inflammatory responses. Esposito et al. (2011) reported that the blockade of PPARγ in a rat model of Alzheimer disease (AD), blunted CBD effects on reactive gliosis and subsequently on β-amyloid-induced neurotoxicity. Moreover, and due to the interaction of CBD with PPARγ, this cannabinoid was observed to stimulate hippocampal neurogenesis.

Additionally, CBD displays GPR55 antagonistic activity in human osteoclasts (Whyte et al., 2009), and has no GPR55 agonistic activity when assayed in β-arrestin recruitment and calcium mobilization assays (Yin et al., 2009; Kapur et al., 2009), demonstrating that CBD can act as an antagonist of the orphan receptor GPR55.

CBD TOXICITY AND PHARMACOKINETIC

In view of the potential therapeutic use of CBD, and encouraged by the lack of its undesired psychotropic effects, several studies have been performed to determine the toxicological profile of this phytocannabinoid.

Very low toxicity of CBD has been found both in human and in other species, with an LD50 of 212 mg/kg when intravenously injected into rhesus monkey (Rosenkrantz, Fleischman, & Grant, 1981). The oral toxicity of CBD had not been clearly established, but Rosenkrantz et al. (1981) showed that an oral dose of 20–50 times larger than the intravenous route of CBD is required to initiate severe intoxication. Additionally, CBD does not cause relevant CNS alterations, and does not display mutagenic or teratogenic activities (Dalterio, Steger, Mayfield, & Bartke, 1984; Matsuyama & Fu, 1981).

In regards of the pharmacokinetic of CBD, reviewed by Grotenhermen (2003), once orally given and due to a marked first-pass effect, CBD bioavailability ranges between values of 13% and 19%, while the systemic bioavailability of inhaled CBD has a range of 11–45% making for this reason the intravenously administration route preferable. With plasma pattern similar to that of THC, after its administration CBD is rapidly distributed, and due to its lipophilic nature can easily pass the blood–brain barrier. The biotransformation routes for CBD are those typically observed for phytocannabinoids, with multiple hydroxilations, oxidations to carboxylic acids, β-oxidation, conjugation and epoxidation (Harvey & Mechoulam, 1990; Samara, Bialer, & Harvey, 1990a). This turns into a prolonged elimination of CBD, with a terminal half-life of about 9 h, being preferentially excreted in the urine as free and its glucuronide compound (Samara, Bialer, & Harvey, 1990b).

CBD AND ANIMAL MODELS OF MS

The use of animal models to study a complex disease like MS has allowed not only to a better understanding about the pathophysiology of the human disease, but also to the development of preclinical testing of disease therapies. There are two main animal models to study MS: experimental autoimmune encephalomyelitis (EAE), induced by immunization against myelin, and experimental viral infection like the one induced with Theiler's murine encephalomyelitis virus (TMEV) in susceptible mice (reviewed in Mecha, Carrillo-Salinas, Mestre, Feliu, & Guaza, 2013a). Both animal models include classical MS hallmarks like inflammation, neuronal damage and demyelination, and it has been described the ability of cannabinoids to exhibit therapeutic potential using these two models.

The enhanced leukocyte trafficking is a key feature in MS, and there are available therapies designed to target CNS inflammation in its early events, such as natalizumab (Tysabri), which interferes with the homing of immune cells to the CNS and is currently prescribed for the treatment of relapsing remitting MS (Krumbholz, Derfuss, Hohlfeld, & Meinl, 2012). In this line, the treatment with cannabinoid agonists has been shown to be effective in the leukocyte rolling and adhesion to endothelial cells in

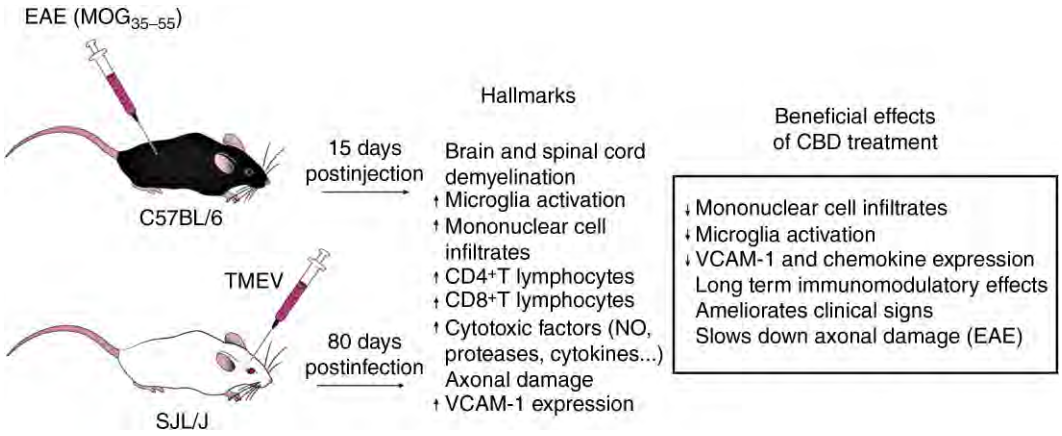


FIGURE 93.1 **CBD and animal models of MS.** Principal hallmarks of the two mouse models of MS, and beneficial effects of CBD (dpi, days postimmunization in the case of EAE; and days postinfection in TMEV-induced demyelinating disease).

EAE (Ni et al., 2004), as well as in the progression of the disease in TMEV infected mice through the regulation of adhesion molecules both in vivo (Mestre et al., 2009) and in vitro (Mestre et al., 2011). The immunoregulatory capacity of cannabinoids in EAE has also been reported (Cabranes et al., 2005; Maresz et al., 2007).

Regarding the effects of CBD in the animal models of MS (Fig. 93.1), only two studies have been published showing the promising therapeutic effect of this *C. sativa* derivative compound. In this line, CBD has been shown to ameliorate EAE symptomatology by diminishing inflammation, microglial activity and leukocyte homing in the spinal cord of immunized animals (Kozela et al., 2011). Lately, Mecha et al. showed in 2013 that CBD also exerts beneficial effects in TMEV-IDD induced demyelinating disease by decreasing the expression of adhesion molecules, the homing of leukocytes to the CNS, and the neuroinflammation in the acute phase of the TMEV-IDD model with long lasting-effects on the clinical course of the disease.

CBD AND MS: PERSPECTIVES

MS, one of the most common autoimmune neurodegenerative diseases, remains with an unclear trigger and still missing therapeutic solutions. Difficulties in clinical trials in patients with MS are due to the diverse and heterogeneous population varying in terms of disease type, severity, variable progression/time course, and with regard to the wide range of presenting symptoms. Beside the currently available MS treatments described in the introduction, there are other potential compounds in clinical phase that involves the immune system as a therapeutic approach (Table 93.4).

Extensive preclinical findings have reinforced the notion deduced from the anecdotal observations that cannabis derivatives may have a role in relieving symptoms in MS patients. The clinical trials focused in Sativex efficacy in the treatment of symptoms of MS, particularly spasticity and neuropathic pain. Beneficial results in placebo-controlled trials were obtained when Sativex was administered as an add-on therapy in these indications, supporting the view that Sativex is efficacious and well tolerated for the treatment of these symptoms (Collin, Davies, Mutiboko, & Ratcliffe, 2007; Wade, Collin, Stott, & Duncombe, 2010; Novotna et al., 2011). Additional trials confirmed that the CBD/THC combination reduce sleep disturbance in patients with MS-related neuropathic pain and that this treatment is mostly well tolerated (Iskedjian, Bereza, Gordon, Piwko, & Einarson, 2007; Rog, Nurmikko, & Young, 2007). Current THC analogs or Sativex trials in MS for symptomatology control have, so far, failed to demonstrate a relevant reduction of relapse, indicative of immunosupressive properties in humans (Zajicek et al., 2005). Evidence from preclinical observations underlines the interest of cannabinoids as neuroprotective agents besides their abilities as immunomodulators. However, there is a lack of preclinical and clinical studies evaluating the Sativex use in progressive MS (primary or secondary) disease as a disease modifying drug. In addition, there is a lack of clinical trials relating the administration of oral CBD alone in MS patients, besides the suggestive profile of CBD as immunomodulator, antioxidant, neuroprotective, and oligoprotective agent as we described above.

Because of the interest of CBD alone as therapeutic agent in other neurodegenerative pathologies (Table 93.5) such as Huntington disease as well as in very diverse CNS pathologies that include epilepsy, schizophrenia or even affective disorders, there are studies administering CBD-alone at different doses to healthy volunteers by oral or IV route (Zhornitsky & Potvin, 2012). On the basis of CBD effects on experimental models of MS and on CNS cell types (Fig. 93.2) future studies should investigate clinical applications of oral CBD for MS, RRMS, and PPMS/SPMS, and should also administer CBD to

TABLE 93.4 MS Therapy Pipeline

Name	Type	Route of administration	Pending indications	Phase	Mechanism of action
Laquinimod	Derivative of linomide	Oral	RRMS	III	Induces antiinflammatory cytokine profile in humans. Inhibits Th1 and Th2 leukocyte migration into de CNS.
Rituximab (Zituxam)	Chimeric human/mouse anti-CD20 monoclonal antibody	Intravenous	RRMS (approved by FDA for lymphoma and arthritis rheumatoid)	III	Depletes $CD20^+$B lymphocytes via cell-mediated and complement-dependent cytotoxic effects, and promotes apoptosis of these cells in the peripheral circulation
Daclizumab (Zenapax)	Humanized anti-CD25 monoclonal antibody	Subcutaneous	RRMS	III	Targets the α subunit of IL2R CD25 on activated T lymphocytes. Blocking the CD25 downregulates the proliferation of B and T lymphocytes via reduction the secretion of proinflammatory cytokines. Production of CD56 positive natural killer cells with regulatory function
Alemtuzumab (Campath-1H)	Humanized anti-CD52 monoclonal antibody	Intravenous	RRMS (approved by FDA for leukemia; better than Rebif)	III	Targets the molecule CD52, expressed on the surface of mature lymphocytes, monocytes, and macrophages. It depletes these cells via complement-mediated lysis antibody-dependent cell toxicity and apoptosis. Induces the production of neurotrophic factors by the reconstituted autoreactive T lymphocytes
Ocrelizumab	Humanized anti-CD20 monoclonal antibody	Intravenous	RRMS	II	Depletes $CD20^+$B lymphocytes

Perspectives for new treatments for MS patients, including the phase of the clinical trial and the expected mechanism of action (reviewed in Minagar, 2013).

TABLE 93.5 Potential Beneficial Effects of CBD in Humans

Ischemia

Huntington disease

Parkinson disease

Multiple sclerosis

Alzheimer disease

Prion diseases

Psychiatric disorders
- Obsessive compulsive behavior
- Depression
- Schizophrenia
- Anxiety
- Psychosis

Epilepsy

Cancer

Diabetes

Rheumatoid arthritis

Amyotrophic lateral sclerosis

Different diseases in which the treatment with CBD can be therapeutic based on the effects on mouse models (reviewed in Fernandez-Ruiz et al., 2013 and Pertwee, 2012).

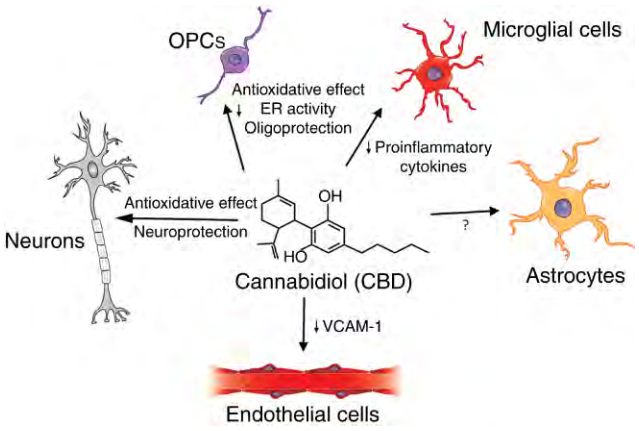


FIGURE 93.2 **Cellular targets of CBD.** Neurons, oligodendrocytes, microglial and endothelial cells can be targets of the effects of CBD in the CNS. The actions on astrocyte cells have not yet been determined.

patients for prolonged period of time in order to simulate the chronic condition of this disease. Among *C. sativa* derived compounds, CBD which lacks any unwanted psychotropic effect may represent a promising agent with the highest prospect for therapeutic use but this hypothesis needs to be proved.

MINI-DICTIONARY

Autoimmune disease An abnormal immune response of the body to tissues and molecules normally present in the body. It compromises the production of autoantibodies from the B-cells of the immune system. The treatment of autoimmunity includes immunosuppresion, to decrease the immune "self" response.
Cannabidiol One of at least 60 active cannabinoids identified in *Cannabis sativa,* counting for up to 40% of the plant's extract, considered to have a wider scope of medical applications than THC with no psychoactive effects.
Demyelination Damage to the myelin sheath that covers nerve fibers in brain and spinal cord. This provokes that nerve impulses slow or even stop, causing neurological alterations.
EAE model An animal model of CNS autoimmune inflammation that courses with inflammation and demyelination, and is used as an experimental model for the human inflammatory demyelinating disease MS. Mainly induced by inoculation with spinal cord homogenates or myelin proteins or peptides, that results in distinct models of disease course depending of the genetic background of mice.
Endocannabinoid system Lipid system of intercellular communication constituted by endogenous ligands, receptors, and the machinery of synthesis and degradation enzymes. It´s involved in a variety of physiological processes including memory, mood, pain-sensation, and appetite. Cannabinoids extracted from *C. sativa* use this endogenous system to exert their central and peripheral actions.
Multiple Sclerosis Chronic neurological disease that is pathologically characterized by multifocal inflammation, demyelination, and neuronal injury in optic nerves, brain, and spinal cord. Mainly considered as an autoimmune disease.
Neurodegeneration A condition that result in progressive loss of neuronal structure and function with neuronal death. Some neurodegenerative disorders include Parkinson, Alzheimer, and Huntington disease.
Neuroinflammation Inflammation of the central or peripheral nervous system. It can be initiated in response to infection, autoimmunity, traumatic brain injury or toxic metabolites among others. Neuroinflammation compromises microglial cells, the resident innate immune cells of the CNS, and circulating peripheral immune cells that infiltrate through the blood brain barrier.
Oligodendrocyte Myelinating cell of the CNS. Myelin acts as an insulator of axonal segments and is a prerequisite for the high velocity of nerve conduction.
Sativex Cannabinoid medicine for the treatment of spasticity due to MS which is also in development for cancer pain and neuropathic pain. Commercialized by GW Pharmaceuticals, the preparation containing CBD and Δ^9-THC is available under four different formulations that differ in the concentration of CBD and Δ^9-THC.
TMEV-IDD model An animal model of primary chronic-progressive disease that courses with inflammation, demyelination, and neurodegeneration in both brain and spinal cord, used as an experimental model for the human disease MS. Induced by the intracerebral inoculation of different strains of the Theiler's virus in susceptible mice.

References

Beecham, A. H., Patsopoulos, N. A., Xifara, D. K., Davis, M. F., Kemppinen, A., Cotsapas, C., International Multiple Sclerosis Genetics Consortium (IMSGC), et al. (2013). Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. *Nature Genetics*, *45*, 1353–1360.

Bisogno, T., Hanus, L., De Petrocellis, L., Tchilibon, S., Ponde, D. E., Brandi, I., Moriello, A. S., Davis, J. B., Mechoulam, R., & Di Marzo, V. (2001). Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *British Journal of Pharmacology*, *134*, 845–852.

Cabranes, A., Venderova, K., de Lago, E., Fezza, F., Sanchez, A., Mestre, L., Valenti, M., Garcia-Merino, A., Ramos, J. A., Di Marzo, V., & Fernandez-Ruiz, A. (2005). Decreased endocannabinoid levels in the brain and beneficial effects of agents activating cannabinoid and/or vanilloid receptors in a rat model of multiple sclerosis. *Neurobiology of Disease*, *20*, 207–217.

Carlini, E. A., & Cunha, J. M. (1981). Hypnotic and antiepileptic effects of cannabidiol. *Journal of Clinical Pharmacology*, *21*, 417S–427S.

Carrier, E. J., Auchampach, J. A., & Hillard, C. J. (2006). Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabidiol immunosuppresion. *Proceedings of the National Academy of Sciences of the United States of America*, *103*, 7895–7900.

Castillo, A., Tolon, M. R., Fernandez-Ruiz, J., Romero, J., & Martinez-Orgado, J. (2010). The neuroprotective effect of cannabidiol in an in vitro model of newborn hypoxic-ischemic brain damage in mice is mediated by CB(2) and adenosin receptors. *Neurobiology of Disease*, *37*, 434–440.

Collin, C., Davies, P., Mutiboko, I. K., & Ratcliffe, S. Sativex Spasticity in MS Study Group. (2007). Randomized controlled trial of cannabis-based medicine in spasticity caused by multiple sclerosis. *European Journal of Neurology*, *14*, 290–296.

Compston, A., & Coles, A. (2002). Multiple sclerosis. *Lancet*, *359*, 1221–1231.

Costa, B., Giagnoni, G., Franke, C., Trovato, A. E., & Colleoni, M. (2004). Vanilloid TRPV1 receptor mediates the antihyperalgesic effect of the nonpsychoactive cannabinoid, cannabidiol, in a rat model of acute inflammation. *British Journal of Pharmacology*, *143*, 247–250.

Dalterio, S., Steger, R., Mayfield, D., & Bartke, A. (1984). Early cannabinoid exposure influences neuroendocrine and reproductive functions in male mice: I. Prenatal exposure. *Pharmacology Biochemistry and Behavior*, *20*, 107–113.

Damal, K., Stoker, E., & Foley, J. F. (2013). Optimizing therapeutics in the management of patients with multiple sclerosis: a review of drug efficacy, dosing, and mechanisms of action. *Biologics: Targets and Therapy*, *7*, 247–258.

De Filippis, D., Iuvone, T., d'Amico, A., Esposito, G., Steardo, L., Herman, A. G., Pelckmans, P. A., de Winter, B. Y., & de Man, J. G. (2008). Effect of cannabidiol on sepsis-induced motility disturbances in mice: involvement of CB receptors and fatty acid amide hydrolase. *Neurogastroenterology and Motility*, *20*, 919–927.

Esposito, G., Scuderi, C., Valenza, M., Togna, G. I., Latina, V., De Filippis, D., Cipriano, M., Carratu, M. R., Iuvone, T., & Steardo, L. (2011). Cannabidiol reduces Ab-induced neuroinflammation and promotes hippocampal neurogenesis through PPARg involvement. *PLoS One*, *6*(12), e28668.

Fernandez-Ruiz, J., Sagredo, O., Pazos, M. R., Garcia, C., Pertwee, R., Mechoulam, R., & Martinez-Orgado, J. (2013). Cannabidiol for neurodegenerative disorders: important new clinical applications for this phytocannabinoid? *British Journal of Clinical Pharmacology*, *75*(2), 323–333.

Grotenhermen, F. (2003). Pharmacokinetics and pharmacodynamics of cannabinoids. *Clinical Pharmacokinetics*, *42*, 327–360.

Hampson, A. J., Grimaldi, M., Axelrod, J., & Wink, D. (1998). Cananbidiol and (−)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(14), 8268–8273.

Harvey, D. J., & Mechoulam, R. (1990). Metabolites of cannabidiol identified in human urine. *Xenobiotica*, *20*, 303–320.

Hill, A. J., Williams, C. M., Whalley, B. J., & Stephens, G. J. (2012). Phytocannabinoids as novel therapeutic agents in CNS disorders. *Pharmacology and Therapeutics*, *133*(1), 79–97.

Iskedjian, M., Bereza, B., Gordon, A., Piwko, C., & Einarson, T. R. (2007). Meta-analysis of *Cannabis*-based treatments for neuropathic and multiple sclerosis-related pain. *Current Medical Research and Opinion*, *23*(1), 17–24.

Izzo, A. A., Borrelli, F., Capasso, R., Di Marzo, V., & Mechoulam, R. (2009). Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends in Pharmacological Sciences*, *30*(10), 515–527.

Kantarci, O. H., Pirko, I., & Rodriguez, M. (2014). Novel immunomodulatory approaches for the management of multiple sclerosis. *Clinical Pharmacology and Therapeutics*, *95*(1), 32–44.

Kapur, A., Zhao, P., Sharir, H., Bai, Y., Caron, M. G., Barak, L. S., & Abood, M. E. (2009). Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands. *Journal of Biological Chemistry*, *284*, 29817–19827.

Kathmann, M., Flau, K., Redmer, A., Tränkle, C., & Schlicker, E. (2006). Cannabidiol is an allosteric modulator at mu- and delta-opioid receptors. *Naunyn Schmiedebergs Archives of Pharmacology*, *372*(5), 354–361.

Kozela, E., Lev, N., Kaushanski, N., Eilam, R., Rimmerman, N., Levy, R., Ben-Nun, A., Juknat, A., & Vogel, Z. (2011). Cannabidiol inhibits pathogenic T cells, decreases spinal microglial activation and ameliorates multiple sclerosis-like disease in C57BL/6 mice. *British Journal of Pharmacology*, *163*(7), 1507–1519.

Krumbholz, M., Derfuss, T., Hohlfeld, R., & Meinl, E. (2012). B cells and antibodies in multiple sclerosis pathogenesis and therapy. *Nature Reviews Neurology*, *8*(11), 613–623.

Lassmann, H., Niedobitek, G., Aloisi, F., & Middeldorp, & The, J. M. NeuroproMISE EBV Working Group. (2011). Epstein–Barr virus in the multiple sclerosis brain: a controversial issue. *Brain*, *134*(Pt 9), 2772–2286.

Maresz, K., Pryce, G., Ponomarev, E. D., Marsicano, G., Croxford, J. L., Shriver, L. P., et al. (2007). Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB1 on neurons and CB2 on autoreactive T cells. *Nature Medicine*, *13*, 492–497.

Martino, G., Adorini, L., Rieckmann, P., Hillert, J., Kallmann, B., Comi, G., & Filippi, M. (2002). Inflammation in multiple sclerosis: the good, the bad, and the complex. *Lancet Neurology*, *1*, 499–509.

Matsuyama, S. S., & Fu, T. K. (1981). In vivo cytogenetic effects of cannabinoids. *Journal of Clinical Psychopharmacology*, *1*, 135–140.

Mecha, M., Carrillo-Salinas, F. J., Mestre, L., Feliu, A., & Guaza, C. (2013a). Viral models of multiple sclerosis: neurodegeneration and demyelination in mice infected with Theiler's virus. *Progress in Neurobiology*, *101*, 46–64.

Mecha, M., Feliu, A., Iñigo, P. M., Mestre, L., Carrillo-Salinas, F. J., & Guaza, C. (2013b). Cannabidiol provides long-lasting protection against the deleterious effects of inflammation in a viral model of multiple sclerosis: a role for A2A receptors. *Neurobiology of Disease*, *59*, 141–150.

Mechoulam, R., & Hanus, L. (2002). Cannabidiol: an overview of some chemical and pharmacological aspects. Part I: chemical aspects. *Chemistry and Physics of Lipids*, *121*(1–2), 35–43.

Mechoulam, R., Petersa, M., Murillo-Rodriguez, E., & Hanus, L. O. (2007). Cannabidiol – recent advances. *Chemistry and Biodiversity*, *4*(8), 1678–1692.

Mechoulam, R., Shani, A., Edery, H., & Grunfeld, Y. (1970). Chemical basis of hashish activity. *Science*, *169*, 611–612.

Mechoulam, R., & Shvo, Y. (1963). Hashish. I. The structure of cannabidiol. *Tetrahedron*, *19*, 2073–2078.

Mestre, L., Docagne, F., Correa, F., Loria, F., Hernangomez, M., Borrell, J., & Guaza, C. (2009). A cannabinoid agonist interferes with the progression of a chronic model of multiple sclerosis by downregulating adhesion molecules. *Molecular and Cellular Neuroscience*, *40*(2), 258–266.

Mestre, L., Iñigo, P. M., Mecha, M., Correa, F. G., Hernangomez-Herrero, M., Loria, F., Docagne, F., Borrell, J., & Guaza, C. (2011). Anandamide inhibits Theiler's virus induced VCAM-1 in brain endothelial cells and reduces leukocyte transmigration in a model of blood brain barrier by activation of CB(1) receptors. *Journal of Neuroinflammation*, *8*, 102.

Minagar, A. (2013). Current and future therapies for multiple sclerosis. *Scientifica*, *2013*, 249101.

Mishima, K., Hayakawa, K., Abe, K., Ikeda, T., Egashira, N., Iwasaki, K., & Fujiwara, M. (2005). Cannabidiol prevents cerebral infarction via a serotonergic 5-hydroxytryptamine1A receptor-dependent mechanism. *Stroke*, *36*(5), 1077–1082.

Nathan, C. (2002). Points of control in inflammation. *Nature*, *420*, 846–852.

Ni, X., Geller, E. B., Eppihimer, M. J., Eisenstein, T. K., Adler, M. W., & Tuma, R. F. (2004). Win 55212-2, a cannabinoid receptor agonist, attenuates leukocyte/endothelial interactions in an experimental autoimmune encephalomyelitis model. *Multiple Sclerosis*, *10*, 158–164.

Niedziela, N., Adamczyk-Sowa, M., & Pierzchala, K. (2014). Epidemiology and clinical record of multiple sclerosis in selected countries: a systematic review. *International Journal of Neuroscience*, *124*(5), 322–330.

Novotna, A., Mares, J., Ratcliffe, S., Novakova, I., Vachova, M., Zapletalova, O., Gasperini, C., Pozzilli, C., Cefaro, L., Comi, G., Rossi, P., Ambler, Z., Stelmasiak, Z., Erdmann, A., Montalban, X., Klimek, A., & Davies, P. Sativex Spasticity Study Group. (2011). A randomized double blind, placebo controlled, parallel-group, enriched-design study of Nabiximols (Sativex®) as an add therapy in subjects with refractory spasticity caused by multiple sclerosis. *European Journal of Neurology*, *18*, 1122–1131.

O'Sullivan, S. E., & Kendall, D. A. (2010). Cannabinoid activation of peroxisome proliferator/activated receptors: potential for modulation of inflammatory disease. *Immunobiology*, *215*, 611–616.

Perras, C. (2005). Sativex for the management of multiple sclerosis symptoms. *Issues in Emerging Health Technologies*, *72*, 1–4.

Pertwee, R. G. (2012). Targeting the endocannabinoid system with cannabinoid receptor agonists: pharmacological strategies and therapeutic possibilities. *Philosophical Transactions of the Royal Society B*, *367*(1607), 3353–3363.

Pertwee, R. G., Ross, R. A., Craib, S. J., & Thomas, A. (2002). (−)- cannabidiol antagonizes cannabinoid receptor agonists and noradrenaline in the mouse vas deferens. *European Journal of Pharmacology*, *456*, 99–106.

Ribeiro, A., Ferraz-de-Paula, V., Pinheiro, M. L., Vitoretti, L. B., Mariano-Souza, D. P., Quinteiro-Filho, W. M., Akamine, A. T., Almeida, V. I., Quevedo, J., Dal-Pizzol, F., Hallak, J. E., Zuardi, A. W., Crippa, J. A., & Palermo-Neto, J. (2012). Cannabidiol, a non-psychotropic plant-derived cannabinoid, decreases inflammation in a murine model of acute lung injury: role for the adenosine A(2A) receptor. *European Journal of Pharmacology*, *678*, 78–85.

Rog, D. J., Nurmikko, T. J., & Young, C. A. (2007). Oromucosal delta9-tetrahydrocannabinol/cannabidiol for neuropathic pain associated with multiple sclerosis: an uncontrolled, open-label, 2-year extension trial. *Clinical Therapeutics*, *29*, 2068–2079.

Rosenkrantz, H., Fleischman, R. W., & Grant, R. J. (1981). Toxicity of short-term administration of cannabinoids to rhesus monkeys. *Toxicology and Applied Pharmacology*, *58*, 118–131.

Russo, E. B., Burnett, A., Hall, B., & Parker, K. K. (2005). Agonistic properties of cannabidiol at 5-HT1A receptors. *Neurochemical Research*, *8*, 1037–1043.

Samara, E., Bialer, M., & Harvey, D. J. (1990a). Pharmacokinetics of urinary metabolites of cannabidiol in the dog. *Biopharmaceutics and Drug Dispositions*, *11*, 785–795.

Samara, E., Bialer, M., & Harvey, D. J. (1990b). Identification of glucose conjugates as major urinary metabolites of cannabidiol in the dog. *Xenobiotica*, *20*, 177–183.

Tait, A. R., & Straus, S. K. (2008). Phosphorylation of U24 from Human Herpes Virus type 6 (HHV-6) and its potential role in mimicking

myelin basic protein (MBP) in multiple sclerosis. *FEBS Letters*, *582*(18), 2685–2688.

Thomas, A., Baillie, G. L., Phillips, A. M., Razdan, R. K., Ross, R. A., & Pertwee, R. G. (2007). Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. *British Journal of Pharmacology*, *150*, 613–623.

Thomas, B. F., Gilliam, A. F., Burch, D. F., Roche, M. J., & Seltzman, H. H. (1998). Comparative receptor binding analyses of cannabinoid agonists and antagonists. *Journal of Pharmacology and Experimental Therapeutics*, *285*, 285–292.

Van der Mei, I. A., Ponsonby, A. L., Dwyer, T., Blizzard, L., Simmons, R., Taylor, B. V., Butzkueven, H., & Kilpatrick, T. (2003). Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study. *British Medical Journal*, *327*(7410), 316.

Wade, D. T., Collin, C., Stott, C., & Duncombe, P. (2010). Meta-analysis of the efficacy of Sativex (Nabiximols), on spasticity on people with multiple sclerosis. *Multiple Sclerosis Journal*, *16*, 707–714.

Whyte, L. S., Ryberg, E., Sims, N. A., Ridge, S. A., Mackie, K., Greasley, P. J., Ross, R. A., & Rogers, M. J. (2009). The putative cannabinoid receptor GPR55 affects osteoclast function in vitro and bone mass in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 16511–16516.

Yin, H., Chu, A., Li, W., Wang, B., Shelton, F., Otero, F., Nguyen, D. G., Caldwell, J. S., & Chen, Y. A. (2009). Lipid G protein-coupled receptor ligand identification using β-arrestin PathHunter assay. *Journal of Biological Chemistry*, *284*, 12328–12338.

Zajicek, J. P., Sanders, H. P., Wright, D. E., Vickery, P. J., Ingram, W. M., Reilly, S. M., Nunn, A. J., Teare, L. J., Fox, P. J., & Thompson, A. J. (2005). Cannabinoids in multiple sclerosis (CAMS) study: safety and efficacy data for 12 months follow up. *Journal of Neurology, Neurosurgery and Psychiatry*, *76*, 1664–1669.

Zhornitsky, S., & Potvin, S. (2012). Cannabidiol in humans: the quest for therapeutic targets. *Pharmaceuticals (Basel)*, *5*(5), 529–552.

CHAPTER

94

Cannabinoids and Their Effects on Painful Neuropathy

*D. Selvarajah**, *R. Gandhi***, *S. Tesfaye***

*Department of Human Metabolism, Medical School, University of Sheffield, Sheffield, United Kingdom

**Academic Department of Diabetes and Endocrinology, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, United Kingdom

SUMMARY POINTS

- Chronic neuropathic pain is a common and poorly managed condition.
- This chapter focuses on the effects of cannabinoids on neuropathic pain.
- There is considerable anecdotal and preclinical trial evidence supporting a possible beneficial role for cannabinoids.
- However, there have only been a limited range of short-term clinical trials with largely disappointing outcomes. The long term efficacy of cannabinoids have also not been established.
- By far the largest numbers of clinical trials conducted to date have examined the use of cannabinoids in central neuropathic pain caused by multiple sclerosis.
- There is a restricted role for cannabinoids in certain patient groups with severe painful neuropathy who are inadequately treated on currently available treatments
- Further adequately powered clinical trials are required to fully examine the long term safety and efficacy on pain and functional measures of these compounds.

KEY FACTS OF PAINFUL NEUROPATHY

- Neuropathic pain is a chronic painful condition that results from damage or dysfunction to the nervous system.
- Patients often report a number of typical painful symptoms which includes burning, sharp shooting pain, electric shocks, pins, and needles or tingling.
- Chronic neuropathic pain often results in considerable distress and disability leading to significant morbidity and reduction in quality of life.
- There is often accompanying mood disorders and sleep disturbance.
- Chronic neuropathic pain can be a challenge to manage. Unfortunately, pharmacological therapies are often ineffective and their use is limited by intolerable side effects.
- Patients are often managed by a multi-disciplinary team of specialists with a combination of pharmacotherapy, psychological treatment, physiotherapy, and neuromodulation.

LIST OF ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
CBME	Cannabis based medicinal extract
CNS	Central nervous system

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00109-5
Copyright © 2017 Elsevier Inc. All rights reserved.

DN	Diabetic neuropathy
EMEA	European Medicines Agency
FDA	Federal Drug Administration
HIV	Human immunodeficiency virus
HIV-PN	Human immunodeficiency virus painful neuropathy
MS	Multiple sclerosis
THC	Tetrahydrocannabinol

INTRODUCTION

Neuropathic pain is defined as "pain initiated or caused by a primary lesion or dysfunction in the peripheral or central nervous system" (Treede et al., 2008), and is estimated to affect up to 7–8% of the general population in Europe (Torrance, Smith, Bennett, & Lee, 2006). A number of different pathological processes that cause neuronal dysfunction can lead to neuropathic pain (Table 94.1). Common examples include diabetic polyneuropathies, postherpetic neuralgia, trigeminal neuralgia, spinal cord injury, and postsurgical/traumatic neuropathies. Regardless of etiology, chronic neuropathic pain often results in considerable distress and disability leading to significant comorbidity and an overall reduction in an individual's quality of life (Meyer-Rosberg et al., 2001). This leads to mood disorders, pathological sleep states (Gore et al., 2005), and social isolation (McCarberg & Billington, 2006). The management of patients with neuropathic pain is challenging, as current pharmacological treatments are often ineffective and use limited by intolerable side effects (Finnerup, Sindrup, & Jensen, 2010; Attal et al., 2010; Dworkin et al., 2007).

TABLE 94.1 Common Causes of Neuropathic Pain

Metabolic/nutritional	Diabetes mellitus, uremia, vitamin B12 and folate deficiencies, paraneoplastic, hypothyroidism
Toxic/drug related	Alcohol, antiretroviral drugs, antituberculosis drugs, chemotherapy (vincristine, cisplatinum, paclitaxel), fluoroquinolones
Viral	Human immunodeficiency virus, herpes zoster, and postherpetic neuralgia
Structural/pressure/trauma	Postsurgical/traumatic neuropathies, entrapment syndromes, neuropathic lower back pain, phantom limb pain, nerve root syndromes/radiculopathies, radiotherapy
Inflammatory	Vasculitis, Sjogren's syndrome, systemic lupus erythematosus
Others	Complex regional pain syndrome, ischemic neuropathies, neuromas, multiple-sclerosis vascular brain/spinal cord lesions (brainstem/thalamic)

The discovery of cannabinoid receptors and their endogenous ligands has resulted in a number of clinical trials involving cannabinoids in neuropathic pain. Presently, in the United Kingdom, the only approved indication for cannabinoids is for neuropathic pain and muscle spasticity in multiple sclerosis (MS) (Guindon & Hohmann, 2008). Uncertainties remain for its use in other neuropathic pain conditions. This chapter will examine the pathophysiology and mechanisms of neuropathic pain and review the existing literature on cannabinoid use in painful neuropathy.

NEUROPATHIC PAIN MECHANISMS

Despite the broad spectrum of etiologies, the clinical manifestations of neuropathic pain are similar across the different syndromes. This is because the symptoms and signs of neuropathic pain are caused by a combination of abnormal nerve activity (ectopic activity and central sensitization) and lesion-induced reduced sensations (Table 94.2) (Baron, Binder, & Wasner, 2010). Detailed history and clinical examination aimed at identifying these characteristic symptoms and sensory abnormalities are important for the accurate diagnosis of neuropathic pain and to distinguish this from other pain types.

PATHOPHYSIOLOGY OF NEUROPATHIC PAIN

Over the past decade, pain research has made considerable progress to uncover the pathophysiology of neuropathic pain (Fig. 94.1) (Baron et al., 2010). Although this has led to a better mechanistic understanding into human pain pathology, there is still a lack of effective mechanism-based treatment approaches and more efficient analgesia. According to data from basic research and human pain experiments a lesion to the afferent pathway is necessary for the development of neuropathic pain (Baron, 2006). This triggers a cascade of several different mechanisms leading to neuropathic pain. Different mechanisms can be involved in the same disease or in the same individual which could in turn lead to the same symptom (Baron et al., 2010). For example, on-going spontaneous pain and paroxysmal

TABLE 94.2 Symptoms and Signs of Neuropathic Pain

Positive symptoms and signs:
- Spontaneous: burning pain, paresthesia, sharp shooting pain, electric shocks
- Evoked pain: allodynia, hyperalgesia

Negative symptoms and signs:
- Hypoalgesia and hypoesthesia

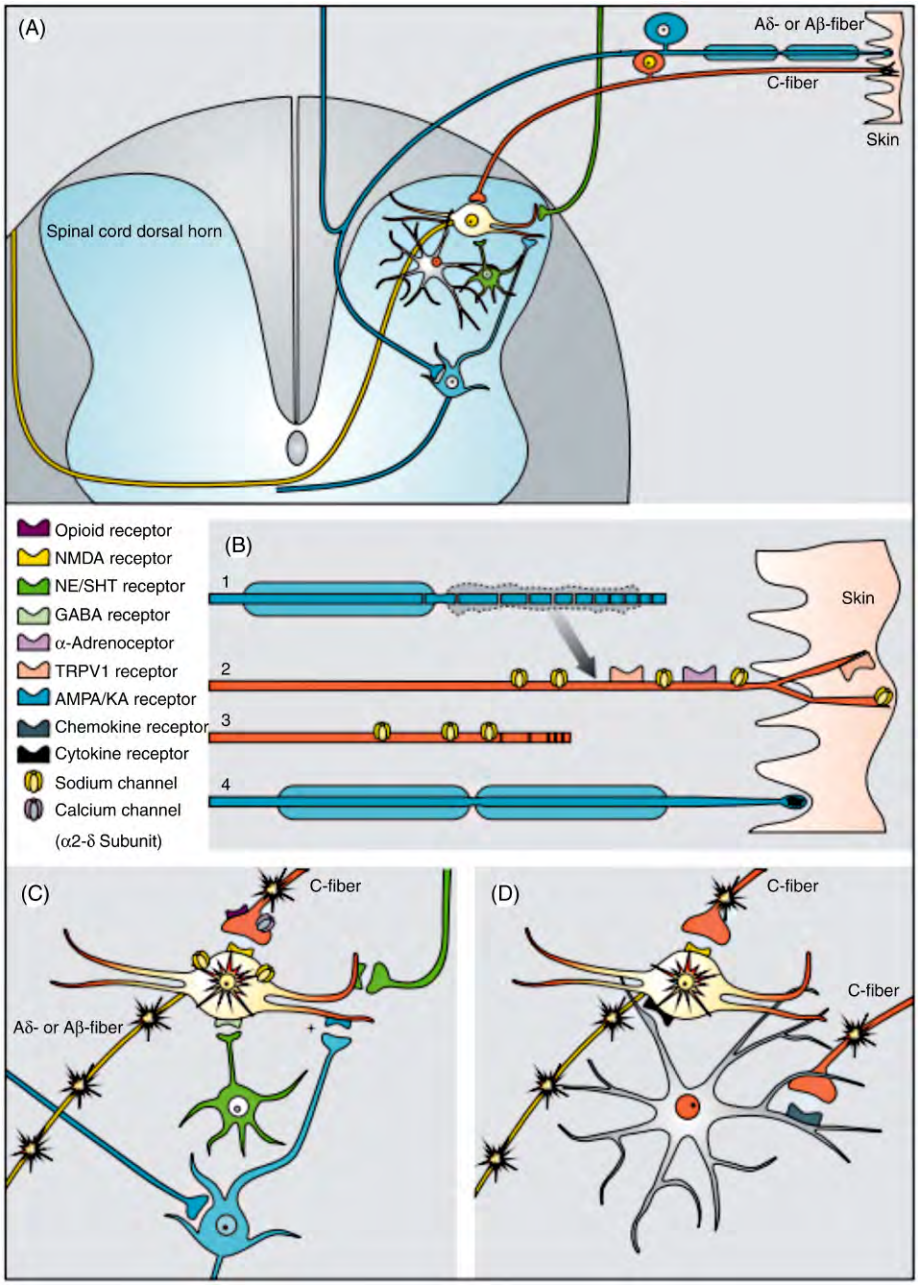


FIGURE 94.1 **Pathophysiological mechanisms of neuropathic pain.** (A) Primary afferent pathways and their connections in the spinal cord dorsal horn. Nociceptive C-fibers *(red)* terminate at spinothalamic projection neurons in the upper laminae (*yellow* neuron). Nonnociceptive myelinated A-fibers project to deeper laminae. The second-order projection neuron receives direct synaptic input from nociceptive terminals and also multisynaptic input from myelinated A-fibers (nonnoxious information, *blue* neuron system). Interaction with microglial (*gray* cell) facilitates synaptic transmission. GABAergic interneurons (*green* neuron) normally exert inhibitory synaptic input on second order neuronal projections. (B) Peripheral changes at primary afferent neurons after a partial nerve lesion, leading to peripheral sensitization. Note that some axons are damaged and degenerate (axons 1 and 3) and some are still intact and connect to the peripheral end organ (skin; axons 2 and 4). Expression of sodium channels is increased on damaged neurons (axon 3), triggered has a consequence of the lesion. Furthermore, products such as nerve growth factor, associated with Wallerian degeneration and release in the vicinity of spared fibers *(arrow)*, trigger expression of channels and receptors (eg, sodium channels, TRPV1 receptors, adrenoreceptors) on uninjured fibers. (C) Spontaneous activity in C-nociceptors induces secondary changes in central sensory processing, leading to spinal cord hyperexcitability (central sensitization of second-order nociceptive neurons, star in yellow neuron) that causes input from mechanoreceptors A-fibers (*blue* neuron system, light touching and punctuate stimuli) to be perceived as pain (dynamic and punctate mechanical allodynia, + indicates gating at synapse). Several presynaptic (opioid receptors, calcium channels) and postsynaptic molecular structures (glutamate receptors, AMPA/kainite receptors, sodium/5HT receptors, GABA receptors, sodium channels) are involved in central sensitization. Inhibitory interneurons and descending modulatory control systems (*green* neurons) are dysfunctional after nerve lesions, leading to disinhibition or facilitation of spinal cord dorsal horn neurons and to further central sensitization. (D) Peripheral nerve injury activates spinal cord glial cells (*gray* cell) via chemokines, such as CCL2 acting on chemokine receptors. Activated microglia further enhance excitability in second order neuronal projections by releasing chemokines and growth factors (eg, tumor necrosis factor, bone-derived nerve factor) and increasing glutamate concentrations. *Source: Adapted from Baron et al. (2010).*

shooting pain is the result of abnormal ectopic nociceptive nerve activity. Under normal physiologic conditions these unmyelinated c-fiber and thinly myelinated Aδ afferent nociceptive fibers usually have a high threshold for activation to indicate potential tissue damage. In neuropathic pain states, this threshold is lowered and results in spontaneous activity (Ørstavik et al., 2006; Ørstavik & Jørum, 2010; Nyström & Hagbarth, 1981). Changes in the voltage gated sodium channels, potassium channels, and upregulation of receptor proteins (eg, TRPV1) have all been shown to correlate with increased spontaneous nerve activity (Caterina & Julius, 2001; Fischer & Reeh, 2007; Ma, Zhang, Bantel, & Eisenach, 2005). Secondary allodynia and hyperalgesia on the other hand occurs as a result of involvement of the central nervous system (CNS) (Baron et al., 2010). In a process called central sensitization, lesions within first order afferent neurons induce postsynaptic changes within second-order nociceptive neurons that lowers the mechanosensitivity threshold of Aβ and Aδ afferent fibers (Hains, Saab, Klein, Craner, & Waxman, 2004; Ultenius, Linderoth, Meyerson, & Wallin, 2006). As a result, nonpainful tactile stimulation (light brushing) becomes painful. These mechanisms are thought to occur at both spinal and supraspinal levels of the CNS. These processes highlight clearly the complexity of neuropathic pain and the challenges posed to develop therapeutic regimens that are effective at treating and preventing this condition. Further research is needed to identify the mechanisms involved for an individual patient as this could lead to targeted treatment regimens for the different pain mechanisms.

One of the earliest documented uses of cannabis was in pain management. It was used for surgical anesthesia in ancient China and for pain relief in early Egyptian, Roman, and Indian civilizations (Peters & Nahas, 1999). More recent experimental studies in animal models of pain have demonstrated beneficial antinociceptive effects and provide substantial preclinical evidence (Guindon & Hohmann, 2009; Hohmann & Suplita, 2006). In a wide range of animal models of neuropathic pain, various studies have demonstrated that cannabinoid agonists reverse the common symptoms of neuropathic pain, including allodynia and hyperalgesia. In these studies, the analgesic properties of cannabinoids depend on a number of factors including administration route, assay conditions and the preparation of the drug (Harris, 1971). Of these factors, route of administration plays a critical role; when given intravenously, THS exhibits high degree of potency and efficacy comparable to morphine. In contract the efficacy of THC is considerably reduced when administered subcutaneously or intraperitoneally. Administration of cannabinoids to animals also elicits a number of significant adverse effects including sedation, motor, and cognitive impairments (Little, Compton, Johnson, Melvin, & Martin, 1988). Such side effects could potentially confound the interpretation of antinociceptive measurements in animal studies which generally rely on paw/tail withdrawal from a noxious stimulus. Thus, it remains to be determined if cannabinoids can produce pain relief at doses below the side effect threshold.

The analgesic properties of cannabinoids occur through a complex range of mechanisms at different levels of the nervous system. This is consistent with the presence of CB1 and CB2 receptors in three key areas involved in pain control (peripheral, spinal, and brain) (Agarwal et al., 2007; Herkenham, 1995; Van Sickle et al., 2005; Jhaveri, Sagar, Elmes, Kendall, & Chapman, 2007). These include the periaqueductal gray, spinal dorsal horn and dorsal root ganglion and are target sites of major endocannabinoids, such as *N*-arachidonoyl ethanolamide (anandamide) and 2-arachidonoyl glycerol (2-AG). They may also modulate analgesic responses via noncannabinoid receptor targets (eg, anandamide is also a ligand for the TRPV-1 receptor) (Cristino et al., 2006; Ahluwalia, Urban, Capogna, Bevan, & Nagy, 2000) and by working synergistically with the endogenous opioid system (Pertwee, 2001). Both endogenous cannabinoid and opioid systems display similarities in receptor structure and signal transduction (Fig. 94.2). Stimulation of these receptors blocks the conversion of noxious stimuli into electrochemical signals by inhibiting voltage-gated Ca^{2+} channels, stimulating K^+ inward channels, and subsequently inhibiting the Ca^{2+} dependent release of pronociceptive effectors such as substance P, calcitonin gene-related peptide, and bradykinin (Roques, Fournié-Zaluski, & Wurm, 2012).

PHARMACOLOGICAL TREATMENT OF NEUROPATHIC PAIN

Treatment of chronic neuropathic pain can be one of the most challenging areas of modern medical practice. In addition to suffering longstanding disabling pain, patients often have complex biological, psychological, and social problems. Their pain has often been diagnosed incorrectly and managed poorly. Pharmocotherapy remains the main pillar of pain management and can be divided into two broad classes, membrane stabilizing agents (antiepileptic drugs and anesthetics) and medications that enhance dorsal horn inhibitory mechanisms (Table 94.3) (Attal et al., 2010). Most agents, however, have multiple mechanisms of action and their effects often may overlap. Despite being the mainstay in the management of neuropathic pain, there are significant limitations to currently available pharmacological treatments. Lack of perceived efficacy,

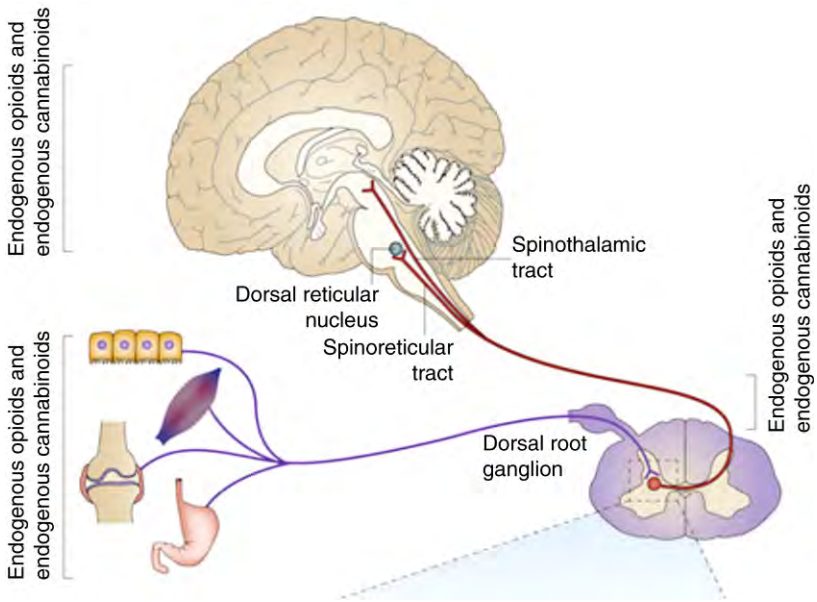


FIGURE 94.2 **Endogenous cannabinoids and opioids are present at all three levels of pain control.** Endogenous opioids and endogenous cannabinoids are distributed widely in the CNS, spinal cord, and peripheral organs. At the periphery, endogenous opioids, and endogenous cannabinoids are present in epithelial cells of the intestine and kidney, in the joints, lung, testis, and skin, as well as on various types of immune cells, including oligodendrocytes and Schwann cells surrounding nerve fibers. Cannabinoid receptors are found at the nociceptor level, and in immune cells, where inhibitors can block the noxious inputs. Noxiously stimulated cutaneous fibers converge to the dorsal horn, sometimes along with nonstimulated fibers from distant cutaneous, muscular or visceral areas. μ-opioid receptors (MORs) and δ-opioid receptors (DORs) are mainly located at the presynaptic end of afferent fibers in the spinal dorsal horn. The brain distribution of MORs and DORs are in structures involved in the control of pain and emotions, such as the periaqueductal gray, thalamus, cortex, and limbic system. Brain neurons enriched in cannabinoid receptor 1 (CB1R), includes structures involved in fear and emotions. *Source: Adapted from Roques et al. (2012).*

TABLE 94.3 Pharmacological Treatments for Neuropathic Pain

Anticonvulsants	Gabapentin, pregabalin, carbamazepine, oxcarbazepine, topiramate, lamotrigine
Antidepressants	
• Selective serotonin and noradrenaline reuptake inhibitor	Duloxetine, venlafaxine
• Tricyclic antidepressants	Amitriptyline, imipramine, nortriptyline
Opioids	Tramadol, morphine, oxycodone

unpleasant side effect profiles, drug interactions, and costs are some of the factors that limit the use of these agents. As a result patients with intractable neuropathic pain are often managed in a patient focused multidisciplinary approach which include attempts to treat the underlying disease (eg, diabetes), a rational titrated approach to medications, neuromodulation (eg, ablation therapy, or spinal cord stimulation), psychological, and physical therapies (Fig. 94.3) (Turk & Melzack, 2011).

CANNABINOIDS AND NEUROPATHIC PAIN

Cannabinoids have been evaluated in a number of different clinical pain studies for their effectiveness on acute postoperative and chronic neuropathic pain. These studies indicate that cannabinoids exhibit their greatest efficacy when employed in the management of neuropathic pain (Dyer, 2013). A number of different preparations and delivery methods (oral-mucosal spray and rectal suppositories—benefit of rapid action) have been examined in these clinical trials. This makes any direct comparisons difficult. For example, there are 460 known chemical constituents in cannabis and as a result the pharmacological properties of smoking cannabis are different from oral preparations of Δ^9-THC or different combinations of Δ^9-THC and cannabidiol (Huesis, 1999).

MULTIPLE SCLEROSIS CENTRAL PAINFUL NEUROPATHY

By far, the largest numbers of disease-related clinical studies conducted to date assessing the effects of cannabinoids have been in central neuropathic pain caused

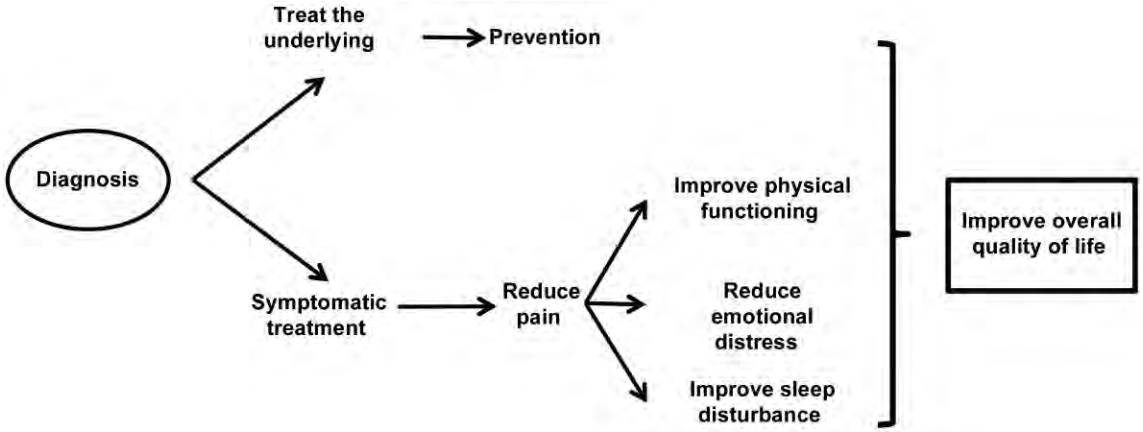


FIGURE 94.3 **Approach to the management of neuropathic pain.** The management of neuropathic pain should follow a two-pronged approach. Following diagnosis of chronic neuropathic pain, efforts should focus on treating (if possible) the underlying condition to prevent further progression of disease. At the same time, appropriate symptomatic treatment should be offered to reduce pain. The aim of symptomatic treatment should not only focus on pain relief but also improve an individual's physical functioning, reduce any emotional distress, and improve sleep duration and quality. This will ultimately result in an overall improvement in an individual's quality of life. *Source: Adapted from Turk and Melzack (2011).*

by multiple sclerosis. Most of these studies, however, focused on the treatment of spasticity. Altogether, there are three Class I, two Class II and four Class III studies of cannabinoids which also included treating central neuropathic pain (either as a secondary end-point or incorporated as part of a composite score) between 1970 and Sep. 2013 (Yadav et al., 2014).

These studies were included in a systematic review by the Guideline Development Subcommittee of the American Academy of Neurology which concluded that for patients with MS with central neuropathic pain or painful spasms, oral cannabinoid extract is effective for reducing central pain. Tetrahydrocannabinol and nabiximols were probably effective in treating MS-related pain or painful spasms. Standard medical therapy was continued in these studies, so no comment was made as to comparative effectiveness. This restricts the use of cannabinoids in multiple sclerosis (Level A) only if other treatments fail. It is difficult to tease out the clinical effectiveness of cannabinoids on the neuropathic pain components in many of these trials in the presence of both painful spasticity and dysesthesia. Some authors view spasm-related pain in multiple sclerosis as being neuropathic whereas others do not. Post-hoc attempts to examine the efficacy of cannabinoids on the different pain manifestation in multiple sclerosis have suggested that both components respond similarly to treatment (Rog, Nurmikko, Friede, & Young, 2005). Hence, from a patients' perspective such comparisons may be unnecessary. Two notable Class 1 studies have evaluated the efficacy of cannabinoids on central neuropathic pain in multiple sclerosis.

Rog et al. (2005) reported a single-center 4-week treatment randomized, double-blind, placebo-controlled trial in 66 patients with MS and central pain states (based on pain description and clinical examination, painless spasms excluded) of nabiximols (2.7 mg THC: 2.5 mg CBD, cannabis based medicinal extract, CBME) oromucosal spray as adjunctive analgesia. They demonstrated that treatment with nabiximols resulted in significant reduction in mean pain intensity [11-point numeric rating scale (NRS-11); nabiximols mean change –2.7, 95% CI: –3.4 to –2.0 vs placebo –1.4 (–2.0:–0.8); $p = 0.005$] and sleep disturbance [–2.5 (–3.4:–1.7) vs –0.8 (–1.5:–0.1); $p = 0.003$], when compared to placebo. Overall, nabiximol treatment was generally well tolerated, although more patients on active treatment reported dizziness, dry mouth, and somnolence compared with placebo. Patients in this study were taking on average, two other medications, with limited efficacy given baseline NRS-11 pain scores of 6.5. This would suggest as adjunctive treatment nabiximol treatment resulted in a significant treatment effect of –1.3 in the NRS-11 pain scores.

Svendsen, Jensen, and Bach (2004) reported a randomized, placebo-controlled crossover study to evaluate the effect of oral synthetic Δ^9-THC dronabinol on central neuropathic pain. In total, 24 patients with MS and central pain (based on clinical examination or quantitative sensory testing) received dronabinol (10 mg OD max) or placebo for 3-weeks separated by a 3-week wash-out period. Dronabinol treatment resulted in significantly lower spontaneous pain intensity compared to placebo treatment [4.0 (25th–75th CI: 2.3–6.0) vs 5.0 (4.0:6.4); $p = 0.02$]. The estimated difference between dronabinol and placebo treatments was –0.6 (95% CI: –1.9 to 0). Lower median radiating pain intensity and higher pain relief score was obtained during the last week of dronabinol treatment compared to placebo. Interestingly, there was a significantly higher pressure

pain threshold during dronabinol treatment (secondary outcome which was not subjected to correction for multiple comparisons) but no differences between treatments in cold sensibility, warm sensibility, tactile detection, tactile pain detection, vibration sense, temporal summation, or mechanical and cold allodynia. Adverse events were more common in the dronabinol treatment (96%) than during placebo treatment (46%). The most common adverse events during active treatment were dizziness, headaches, tiredness, myalgia, and muscle weakness. Overall, dronabinol has a modest analgesic effect on central pain in multiple sclerosis patients. Although the effect size is less than the nabiximol used by Rog et al. (2005) this should be interpreted with caution given the different study designs. Both treatments result in modest improvements in pain which arguably are a clinical relevant analgesic effect in MS patients with central pain. Although, it is suggested that a 30% or 2-point NRC score reduction in pain as being clinically significant, both studies included patients whose pain was controlled poorly on existing medications and even a relatively small reduction in pain intensity are often very appreciated by the patients. Both effect sizes are comparable to other treatment options available with numbers needed to treat to obtain one patient with more than 50% pain relief of 3.45 and 3.7 for dronabinol and CBME respectively (Sindrup & Jensen, 1999).

Systematic reviews of trials of cannabinoids in participants with other types of neuropathic pain have reported mixed results.

HIV PAINFUL NEUROPATHY

Distal symmetrical painful peripheral neuropathy is a frequent complication of HIV infection. Its prevalence is increasing despite (or because of) the introduction of otherwise successful antiretroviral therapy (Evans et al., 2011). The accompanying pain has a major impact on quality of life in otherwise largely healthy individuals. There are at least two etiologies that often coexist and are clinically indistinguishable (Pardo, McArthur, & Griffin, 2001). One associated with HIV disease itself and the other which complicates antiretroviral treatments. Both etiologies cause a distal symmetrical axonal sensory polyneuropathy that affects the feet and less frequently the hands. Often only a temporal association between the onset of symptoms and the starting of a particular antiretroviral agent will provide clues to the possible etiology. A recent systematic review and metaanalysis of randomized control trials was performed to evaluate the clinical effectiveness of analgesics for the treatment of painful HIV painful neuropathy (HIV-PN) (Phillips, Cherry, Cox, Marshall, & Rice, 2010). Of 44 studies identified only 14 met the inclusion criteria for metaanalysis. Authors reported evidence of efficacy only exists for smoked cannabis, capsaicin 8% topical cream, and recombinant human growth factor (clinically unavailable). The evidence for effectiveness of cannabis was based on two randomized clinical trials:

Ellis et al. (2008) reported on the double-blind, placebo-controlled, crossover trial of analgesia with smoked cannabis on neuropathic pain in HIV infection. Patients (n = 34) had intractable painful neuropathic symptoms refractory to at least two previous analgesic classes and consumed between 1% and 8% Δ^9-THC, 4 times daily for 5 consecutive days during a 2-week treatment phase. Active and placebo treatment was separated by a 2-week washout. Pain relief as assessed by the descriptor differential scale was greater in with cannabis than placebo (median difference DDS pain score change, 3.3 points, p = 0.02) among the completers [28 out of 34 subjects randomized (21.4% dropout)]. Proportion of subjects achieving at least 30% reduction in pain intensity with cannabis versus placebo were 0.46 (95% CI: 0.28–0.65) and 0.18 (0.03:0.32) respectively. Sensitivity analysis was performed to examine if blinding was preserved over the course of the study. When subjects were on placebo, accuracy of treatment guesses was no different from random guessing (50%), however, the majority of subjects (93%) were able to guess correctly during the cannabis treatment period. Hence, there was a high risk of performance and detection bias.

Abrams et al. (2007) compared smoked cannabis (3.56% Δ^9-THC tds) to placebo cigarettes in a parallel group RCT design. Only subjects with previous exposure to cannabis were included in this study (n = 27). Those who were current users were asked to discontinue prior to study. Again there was a significant reduction in overall pain intensity from baseline in the cannabis treated group at the end of Day 5 of treatment. A greater proportion of subjects in the cannabis treated group (13/27) reported at least a 30% reduction in VAS pain intensity compared to placebo (6/27). Sensitivity analysis was not performed to assess the robustness of placebo control. It is possible given subjects had a history of previous exposure to cannabis that unintentional unblinding may have occurred during the course of the study. Based on these two studies, the NNT for smoked cannabis was calculated as 3.38 (95% CI: 2.19–7.5). Smoking cannabis should not be clinically advocated. Further well designed clinical trials using alternative routes of administration should be assessed. Both these studies are also limited by small sample sizes, short duration, and restricted subject selection which limits the generalizability of reported findings. They do, however, suggest a "proof of concept" that cannabinoid therapy may be an effective option for pain relief in HIV-PN patients, which needs further investigation.

DIABETIC DISTAL SYMMETRICAL SENSORIMOTOR POLYNEUROPATHY

Diabetes affects approximately 415 million people worldwide (http://www.idf.org/worlddiabetesday/toolkit/gp/facts-figures). This is expected to rise considerably within the next 10 years. Complications of diabetes cause great distress, premature death, and disability to subjects, and is a major burden on national health services. Diabetic neuropathy is one of the commonest long-term complications of diabetes with a prevalence rate of up to 50% after 15–20 years of the disease (Dyck et al., 1993). Painful neuropathy (painful DN) will affect 10% of all subjects with diabetes and causes great distress and disability to sufferers and affects quality of life immensely (Galer, Gianas, & Jensen, 2000). Usually, if the pain persists for longer than 12 months it does not resolve and may thus last for many years. Apart from strict glycemic control there are no pharmacological therapies that have been proven to halt or reverse the neuropathic process. Unfortunately, additional drug treatments used to alleviate painful symptoms are often ineffective and complicated by side effects. A recent metaanalysis of randomized controlled trials for the treatment of painful DN has reported that at best less than a third of patients achieve at least a 30% reduction in pain intensity (Snedecor et al., 2014). Occasionally, in subjects who do not respond to drug treatment electrical spinal cord stimulation has been used. Two small studies have examined the use of cannabinoids in painful DN.

Selvarajah, Gandhi, Emery, and Tesfaye (2010) conducted a randomized, placebo-controlled, double-blind, controlled trial to assess the efficacy of Sativex, a cannabis based medicinal extract, as adjunctive treatment in painful DN. In this small study, 30 subjects were randomized to receive daily sativex or placebo. There was significant improvement in pain scores in both groups, but mean change between groups was not significant. In addition to a large placebo response, post-hoc analysis revealed that subjects with greater depressive symptoms reported higher pain scores at baseline and they improved regardless of intervention. In this first-ever trial assessing the efficacy of cannabis in painful DN showed it to be no more efficacious than placebo. The main limitations of this study were the small sample size and the use of concomitant medications which may have attenuated the analgesic response to sativex. In a subsequent study adopting a different trial design, Toth et al. (2012) reported evaluated add-on treatment of oral cannabinoid, nabilone, for refractory painful DN. They conducted a single center, enriched enrolment, placebo controlled, randomized withdrawal study. 37 subjects with pain scores greater than 4 (0–10 scale) received, single blinded adjuvant nabilone for 4 weeks. Subjects achieving greater than 30% pain relief (responders) were then randomized to either continue on going nabilone treatment or switch to placebo. Although, the study failed to randomize the number of subjects estimated by initial sample size calculation, it reported significant reduction in pain intensity with nabilone treatment compared to placebo in "responders." The number of subjects with more than 30% reduction in pain from baseline to endpoint was 11/13 (85%) in the nabilone group compared to 5/13 (38%) in the placebo group ($p < 0.05$). There were also improvements in anxiety scores, well-being, and sleep disturbance with nabilone treatment. Demonstration of improvement in these multiple measures adds veracity to study findings. However, the overall effect size was small (0.67) for nabilone compared to placebo despite the enriched study design, which also limits the generalizability of study findings. Both studies are limited by the relatively small sample sizes, short duration of follow-up, and risk of inadvertent unmasking with the enriched trial design. Once again, larger, well designed, adequately powered studies are required to truly examine the analgesic effects of cannabinoids in painful DN.

SPINAL CORD INJURY

A systematic review and metaanalysis of drugs for neuropathic pain associated with spinal cord injury examined nine placebo control trials which included one small crossover trial which evaluated dronabinol ($n = 7$) (Snedecor et al., 2013; Rintala, Fiess, Tan, Holmes, & Bruel, 2010). In this pilot study, the effects of dronabinol were comparable to an active placebo diphenhydramine (an antihistamine which does not possess analgesic properties but mimics some of the possible side effects of cannabinoids). On average, dronabinol was no more effective than diphenhydramine for relieving chronic neuropathic pain below the level of injury.

There have been very few head-to-head studies of cannabinoids with other analgesic treatments in neuropathic pain. One notable study compared dihydrocodeine and nabilone in a randomized, double blind, crossover trial of 14 weeks duration (Frank, Serpell, Hughes, Matthews, & Kapur, 2008). Ninety six subjects with neuropathic pain (confirmed on clinical examination) of mixed etiologies (majority 42/96 were nerve injury postsurgery) were randomized. There was a high drop-out rate which can be explained partially by the crossover design. The weak opioid dihydrocodeine was found to provide better pain relief than the cannabinoid in the treatment of chronic neuropathic pain [mean difference in 10.0 cm VAS between nabilone and dihydrocodeine was in favor of the latter: 0.6 cm (95% CI: 1.4–10.5), $p = 0.01$]. Dihydrocodeine use was also accompanied by fewer side effects, although no major adverse events occurred for either drug. Overall, there is a lack of clinical studies as well as a dearth

of comparative data with different pharmacologic therapies. As such, the effectiveness of cannabinoids in the treatment of other neuropathic pain remains unproven.

SIDE EFFECTS

Side effects were reported in all studies in a proportion of patients receiving cannabinoids. In the recent systematic review of cannabinoids in chronic noncancer pain, of the 18 trials examined there were no serious adverse events (Lynch & Campbell, 2011). Drug-related adverse events were generally described as well tolerated, transient or mild to moderate. Most frequently reported side effects were dizziness, loss of balance, feelings of intoxication, dry mouth, gastrointestinal effects, sedation, and hunger. In the longest extension study (up to three years) to date these side effects were reported by 90% of patients and no tolerance was observed (Wade, Makela, House, Bateman, & Robson, 2006). Most clinical studies however treated patients for less than 6 months and report a greater proportion of patients discontinuing medications because of adverse events in the active treatment group compared to placebo. Data on the symptoms that cause withdrawal of medications are often incomplete. Among patients treated with cannabinoids, this could either be a direct result of adverse events related to the drug or an indirect effect caused by interactions with other medications being taken, especially opiates for pain or a combination of both (Sutin & Nahas, 1999). Hence, adverse events are a significant concern with cannabinoid use especially in high risk populations with multiple medical comorbidities and polypharmacy. Hence, individual circumstances (medical and social) should be carefully considered when selecting potential patients for treatment. If medical use is likely to be long-term, patients should be warned that the adverse effects of long-term use are unclear, including the risk of dependence and cognitive impairment. It may be sensible to refer patients to specialist management of the condition, where trial of cannabinoids might be undertaken (Farrell, Buchbinder, & Hall, 2014).

FUTURE CLINICAL STUDIES

As highlighted earlier, there is a need for more robust, well-powered, randomized controlled clinical trials of cannabinoids (either vs placebo or head-to-head studies against established neuropathic pain treatments) in different models of neuropathic pain. Based on the currently available published literature, cannabinoids have at best only a modest effect on neuropathic pain, comparable to other established pharmacological therapies. One possible reason for this may be related to the fact that in most studies a summative pain score is used as the primary outcome. This does not sufficiently consider the heterogeneity of neuropathic pain syndromes. Hence, a comprehensive assessment of symptoms and signs of pain could be a more sensitive way than an overall assessment of pain in demonstrating and characterizing the effects of a given pharmacological treatment (Attal et al., 2011; Baron, Förster, & Binder, 2012). For example, studies have demonstrated that morphine and lidocaine act differently on different neuropathic pain symptoms and signs as captured using quantitative sensory testing and/or neuropathic pain questionnaires that better quantify the heterogeneity of neuropathic pain syndromes (Attal et al., 2002, 2000). A post-hoc analysis performed in the Combination versus monotherapy of pregabalin and duloxetine in diabetic neuropathy study (COMBO-DN) showed that adding pregabalin to existing duloxetine treatment seemed to have benefited those with pressing pain and evoked pain; whereas increasing the dose of duloxetine appeared more beneficial for parethesic/dysesthesic symptoms (Bouhassira et al., 2014). In the context of cannabinoids, this hypothesis is best supported by the recent demonstration that these agents show greater reduction in mechanical/thermal hyperalgesia and tactile allodynia than placebo. Future studies should explore these hypotheses as this might lead to more stratified treatment and potentially to personalized pain therapy where cannabinoids are most effective.

Finally, most studies in neuropathic pain conducted to date have assessed the efficacy of cannabinoids over a relatively short duration. The key question, therefore, is the long-term safety and efficacy of these agents. Large scale, long-term observational outcome studies or record linkage studies based on the use of cannabinoids for medically approved indications such as multiple sclerosis may quantify the risks and benefits of long term use (Farrell et al., 2014). In a recent opinion piece, Farrell et al. (2014) searched key clinical trials registers including US ClinicalTrials.gov, EU Clinical Trials Registry, and Australian and New Zealand Clinical trials Register and found 10 prospective studies of cannabinoids but was unable to identify key studies that would provide important information on the long-term safety and efficacy of these agents in the near future.

CONCLUSIONS

Chronic neuropathic pain is a common and poorly managed condition. Currently available treatments are often ineffective with side effect profiles that limit their use. This leads to significant morbidity and poor quality of life. In this context, patients with chronic neuropathic pain require access to better therapeutic care and additional treatment options. Although there is considerable anecdotal and preclinical trial evidence supporting

an antinociceptive role for cannabinoids, outcomes in the limited range of clinical trials that have been performed thus far have been largely disappointing. Most clinical studies have focused on narrow clinical indications with modest effect sizes not dissimilar to many of the currently used neuropathic pain treatments. As a result, the use of cannabinoids is restricted to specialist use for only a limited range of conditions. Adequately powered, placebo-controlled or head-to-head, multicenter, clinical studies in neuropathy pain models according to predetermined FDA/EMEA guidelines are required in order to fully examine the safety and efficacy on pain and functional measures of these compounds.

MINI-DICTIONARY

Adverse events These are predetermined recorded events that occur to subjects during participation in a clinical trial. Example of such events include hospital admission, injury or harm and death.
Allodynia This is a description of a type of painful symptom that can accompany nerve damage. It occurs when pain is evoked during nonpainful stimulus (eg, soft touch).
Electrical spinal cord stimulation This is a treatment of very severe neuropathic pain in the lower limbs. Low voltage current is delivered by electrodes are placed near the spinal cord. The duration and intensity of the current can be controlled using a portable, remote-control, radio-receiver unit.
Hyperalgesia This is a description of a type of painful symptom that can accompany nerve damage. It occurs when a normally painful stimulus (pressure pain) is report as very painful.
Myalgia Symptom of muscle aches and pains.
Neuromodulation Nonpharmacological treatment of neuropathic pain which can include nerve ablation or electrical spinal cord stimulation.
Neuropathic pain Chronic pain that results from nerve damage. This results in a particular description of either spontaneous or evoked painful symptoms, such as, burning, sharp shooting, or deep aching pain.
Paresthesia This is a description of a type of painful symptom that can accompany nerve damage. It is a description of symptoms of pins and needles or tingling.
Postherpetic neuralgia Nerve damage that follow herpes viral infection.
Post-hoc analysis Secondary or follow-up analysis performed on clinical trial data. This type of analysis of often not planned at the start of the study but informed by its outcome.
Randomized controlled trials These are clinical trials which are often conducted to investigate the effectiveness of a drug. Subjects are randomized to two (sometimes more) treatment arms to compare the effects of the drug under investigation against either placebo or current treatment.
Trigeminal neuralgia Neuropathic pain/symptoms that occurs as a result of damage to the trigeminal nerve.

References

Abrams, D. I., Jay, C. A., Shade, S. B., Vizoso, H., Reda, H., et al. (2007). Cannabis in painful HIV-associated sensory neuropathy: a randomized placebo-controlled trial. *Neurology*, *68*, 515–521.

Agarwal, N., Pacher, P., Tegeder, I., Amaya, F., Constantin, C. E., Brenner, G. J., … , Kuner, R. (2007). Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. *Nature Neuroscience*, *10*(7), 870–879.

Ahluwalia, J., Urban, L., Capogna, M., Bevan, S., & Nagy, I. (2000). Cannabinoid 1 receptors are expressed in nocceptive primary sensory neurons. *Neuroscience*, *100*, 685–688.

Attal, N., Bouhassira, D., Baron, R., Dostrovsky, J., Dworkin, R. H., & Finnerup, N. (2011). Assessing symptom profiles in neuropathic pain clinical trials: can it improve outcome? *European Journal of Pain*, *11*(15), 441–443.

Attal, N., Cruccu, G., Baron, R., Haanpää, M., Hansson, P., Jensen, T. S., & Nurmikko, T. (2010). EFNS guidelines on the pharmacological treatment of neuropathic pain: 2010 revision. *European Journal of Neurology*, *17*(9), 1113-e88.

Attal, N., Gaudé, V., Brasseur, L., Dupuy, M., Guirimand, F., & Parker, F. (2000). Intravenous lidocaine in central pain: a double-blind, placebo-controlled, psychophysical study. *Neurology*, *54*, 564–574.

Attal, N., Guirimand, F., Brasseur, L., Gaude, V., Chauvin, M., & Bouhassira, D. (2002). Effects of IV morphine in central pain: a randomized placebo-controlled study. *Neurology*, *26*, 554–563.

Baron, R. (2006). Mechanisms of disease: neuropathic pain—a clinical perspective. *Nature Clinical Practice Neurology*, *2*(2), 95–106.

Baron, R., Binder, A., & Wasner, G. (2010). Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. *The Lancet Neurology*, *9*(8), 807–819.

Baron, R., Förster, M., & Binder, A. (2012). Subgrouping of patients with neuropathic pain according to pain-related sensory abnormalities: a first step to a stratified treatment approach. *Lancet Neurology*, *11*, 999–1005.

Bouhassira, D., Wilhelm, S., Schacht, A., Perrot, S., Kosek, E., Cruccu, G., … , Tölle, T. (2014). Neuropathic pain phenotyping as a predictor of treatment response in painful diabetic neuropathy: data from the randomized, double-blind, COMBO-DN study. *Pain*, *155*(10), 2171–2179.

Caterina, M. J., & Julius, D. (2001). The vanilloid receptor: a molecular gateway to the pain pathway. *Annual Review of Neuroscience*, *24*(1), 487–517.

Cristino, L., De Petrocellis, L., Pryce, G., Baker, D., Guglielmotti, V., & Di Marzo, V. (2006). Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. *Neuroscience*, *139*, 1405–1415.

Dworkin, R. H., O'Connor, A. B., Backonja, M., Farrar, J. T., Finnerup, N. B., Jensen, T. S., … , Wallace, M. S. (2007). Pharmacologic management of neuropathic pain: evidence-based recommendations. *Pain*, *132*(3), 237–251.

Dyck, P. J., Kratz, K. M., Karnes, J. L., Litchy, W. J., Klein, R., Pach, J. M., …, Melton, L. (1993). The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort. The Rochester Diabetic Neuropathy Study. *Neurology*, *43*(4), 817.

Dyer, O. (2013). The growth of medical marijuana. *British Medical Journal*, *347*, f4755.

Ellis, R. J., Toperoff, W., Vaida, F., Van Den Brande, G., Gonzales, J., Gouaux, B., … , Atkinson, J. H. (2008). Smoked medicinal cannabis for neuropathic pain in HIV: a randomized, crossover clinical trial. *Neuropsychopharmacology*, *34*(3), 672–680.

Evans, S. R., Ellis, R. J., Chen, H., Yeh, T. M., Lee, A. J., Schifitto, G., … , Clifford, D. B. (2011). Peripheral neuropathy in HIV: prevalence and risk factors. *AIDS (London, England)*, *25*(7), 919.

Farrell, M., Buchbinder, R., & Hall, W. (2014). Should doctors prescribe cannabinoids? *British Medical Journal*, *348*, g2737.

Finnerup, N. B., Sindrup, S. H., & Jensen, T. S. (2010). The evidence for pharmacological treatment of neuropathic pain. *Pain*, *150*(3), 573–581.

Fischer, M. J., & Reeh, P. W. (2007). Sensitization to heat through G-protein-coupled receptor pathways in the isolated sciatic mouse nerve. *European Journal of Neuroscience*, *25*(12), 3570–3575.

Frank, B., Serpell, M. G., Hughes, J., Matthews, J. N. S., & Kapur, D. (2008). Comparison of analgesic effects and patient tolerability of nabilone and dihydrocodeine for chronic neuropathic pain: randomised, crossover, double blind study. *British Medical Journal*, *336*(7637), 199–201.

Galer, B. S., Gianas, A., & Jensen, M. P. (2000). Painful diabetic polyneuropathy: epidemiology, pain description, and quality of life. *Diabetes Research and Clinical Practice*, *47*(2), 123–128.

Gore, M., Brandenburg, N. A., Dukes, E., Hoffman, D. L., Tai, K. S., & Stacey, B. (2005). Pain severity in diabetic peripheral neuropathy is associated with patient functioning, symptom levels of anxiety and depression, and sleep. *Journal of Pain and Symptom Management*, *30*(4), 374–385.

Guindon, J., & Hohmann, A. G. (2008). Cannabinoid CB2 receptors: a therapeutic target for the treatment of inflammatory and neuropathic pain. *British Journal of Pharmacology*, *153*, 319–334.

Guindon, J., & Hohmann, A. G. (2009). The endocannabinoid system and pain. *CNS and Neurological Disorders Drug Targets*, *8*(6), 403.

Hains, B. C., Saab, C. Y., Klein, J. P., Craner, M. J., & Waxman, S. G. (2004). Altered sodium channel expression in second-order spinal sensory neurons contributes to pain after peripheral nerve injury. *The Journal of Neuroscience*, *24*(20), 4832–4839.

Harris, L. S. (1971). General and behavioral pharmacology. *Pharmacological Reviews*, *23*(4), 285–294.

Herkenham, M. (1995). Localization of cannabinoid receptors in brain and periphery. In R. G. Pertwee (Ed.), *Cannabinoid receptors* (pp. 145–166). London, UK: Academic Press.

Hohmann, A. G., & Suplita, R. L. (2006). Endocannabinoid mechanisms of pain modulation. *The AAPS Journal*, *8*(4), E693–E708.

Huesis, M. (1999). Pharmacokinetics of THC in inhaled and oral preparations. In G. G. Nahas, K. M. Sutin, D. J. Harvey, & S. Agurell (Eds.), *Marihuana and medicine* (pp. 3–7). Totowa, NJ: Humana Press Inc.

Jhaveri, M. D., Sagar, D. R., Elmes, S. J., Kendall, D. A., & Chapman, V. (2007). Cannabinoid CB2 receptor-mediated anti-nociception in models of acute and chronic pain. *Molecular Neurobiology*, *36*, 26–35.

Little, P. J., Compton, D. R., Johnson, M. R., Melvin, L. S., & Martin, B. R. (1988). Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *Journal of Pharmacology and Experimental Therapeutics*, *247*(3), 1046–1051.

Lynch, M. E., & Campbell, F. (2011). Cannabinoids for treatment of chronic non-cancer pain; a systematic review of randomized trials. *British Journal of Clinical Pharmacology*, *72*(5), 735–744.

Ma, W., Zhang, Y., Bantel, C., & Eisenach, J. C. (2005). Medium and large injured dorsal root ganglion cells increase TRPV-1, accompanied by increased α2C-adrenoceptor co-expression and functional inhibition by clonidine. *Pain*, *113*(3), 386–394.

McCarberg, B. H., & Billington, R. (2006). Consequences of neuropathic pain: quality-of-life issues and associated costs. *American Journal of Managed Care*, *12*(9 Suppl.), S263–S268.

Meyer-Rosberg, K., Kvarnström, A., Kinnman, E., Gordh, T., Nordfors, L. O., & Kristofferson, A. (2001). Peripheral neuropathic pain—a multidimensional burden for patients. *European Journal of Pain*, *5*(4), 379–389.

Nyström, B., & Hagbarth, K. E. (1981). Microelectrode recordings from transected nerves in amputees with phantom limb pain. *Neuroscience Letters*, *27*(2), 211–216.

Ørstavik, K., & Jørum, E. (2010). Microneurographic findings of relevance to pain in patients with erythromelalgia and patients with diabetic neuropathy. *Neuroscience Letters*, *470*(3), 180–184.

Ørstavik, K., Namer, B., Schmidt, R., Schmelz, M., Hilliges, M., Weidner, C., … , Torebjörk, H. E. (2006). Abnormal function of C-fibers in patients with diabetic neuropathy. *The Journal of Neuroscience*, *26*(44), 11287–11294.

Pardo, C. A., McArthur, J. C., & Griffin, J. W. (2001). HIV neuropathy: insights in the pathology of HIV peripheral nerve disease. *Journal of the Peripheral Nervous System*, *6*(1), 21–27.

Pertwee, R. G. (2001). Cannabinoid receptors and pain. *Progress in Neurobiology*, *63*(5), 569–611.

Peters, H., & Nahas, G. (1999). A brief history of four millennia (B.C. 2000–A.D. 1974). In G. G. Nahas, K. M. Sutin, D. J. Harvey, & S. Agurell (Eds.), *Marihuana and medicine* (pp. 3–7). Totowa, NJ: Humana Press Inc.

Phillips, T. J., Cherry, C. L., Cox, S., Marshall, S. J., & Rice, A. S. (2010). Pharmacological treatment of painful HIV-associated sensory neuropathy: a systematic review and meta-analysis of randomised controlled trials. *PLoS One*, *5*(12), e14433.

Rintala, D. H., Fiess, R. N., Tan, G., Holmes, S. A., & Bruel, B. M. (2010). Effect of dronabinol on central neuropathic pain after spinal cord injury: a pilot study. *American Journal of Physical Medicine Rehabilitation*, *89*, 840–848.

Rog, D. J., Nurmikko, T. J., Friede, T., & Young, C. A. (2005). Randomized, controlled trial of cannabis-based medicine in central pain in multiple sclerosis. *Neurology*, *65*, 812–819.

Roques, B. P., Fournié-Zaluski, M. C., & Wurm, M. (2012). Inhibiting the breakdown of endogenous opioids and cannabinoids to alleviate pain. *Nature Reviews Drug Discovery*, *11*(4), 292–310.

Selvarajah, D., Gandhi, R., Emery, C. J., & Tesfaye, S. (2010). Randomized placebo-controlled double-blind clinical trial of cannabis-based medicinal product (Sativex) in painful diabetic neuropathy depression is a major confounding factor. *Diabetes Care*, *33*(1), 128–130.

Sindrup, S. H., & Jensen, T. S. (1999). Efficacy of pharmacological treatments of neuropathic pain: an update and effect related to mechanism of drug action. *Pain*, *83*(3), 389–400.

Snedecor, S. J., Sudharshan, L., Cappelleri, J. C., Sadosky, A., Desai, P., & Jalundhwala, Y. J. (2013). Systematic review and comparison of pharmacologic therapies for neuropathic pain associated with spinal cord injury. *Journal of Pain Research*, *6*, 539–547.

Snedecor, S. J., Sudharshan, L., Cappelleri, J. C., Sadosky, A., Mehta, S., & Botteman, M. (2014). Systematic review and meta-analysis of pharmacological therapies for painful diabetic peripheral neuropathy. *Pain Practice*, *14*(2), 167–184.

Sutin, K. M., & Nahas, G. G. (1999). Physiological and pharmacological interactions of marihuana (THC) with drugs and anesthetics. In G. G. Nahas, K. M. Sutin, D. J. Harvey, & S. Agurell (Eds.), *Marihuana and medicine* (pp. 3–7). Totowa, NJ: Humana Press Inc..

Svendsen, K. B., Jensen, T. S., & Bach, F. W. (2004). Does the cannabinoid dronabinol reduce central pain in multiple sclerosis? Randomised double blind placebo controlled crossover trial. *British Medical Journal*, *329*, 253.

Torrance, N., Smith, B. H., Bennett, M. I., & Lee, A. J. (2006). The epidemiology of chronic pain of predominantly neuropathic origin. Results from a general population survey. *The Journal of Pain*, *7*(4), 281–289.

Toth, C., Mawani, S., Brady, S., Chan, C., Liu, C., Mehina, E., …, Korngut, L. (2012). An enriched-enrolment, randomized withdrawal, flexible-dose, double-blind, placebo-controlled, parallel assignment efficacy study of nabilone as adjuvant in the treatment of diabetic peripheral neuropathic pain. *Pain*, *153*(10), 2073–2082.

Treede, R. D., Jensen, T. S., Campbell, J. N., Cruccu, G., Dostrovsky, J. O., Griffin, J. W., …, Serra, J. (2008). Neuropathic pain redefinition and a grading system for clinical and research purposes. *Neurology*, *70*(18), 1630–1635.

Turk, D. C., & Melzack, R. (Eds.). (2011). *Handbook of pain assessment*. New York, NY: Guilford Press.

Ultenius, C., Linderoth, B., Meyerson, B. A., & Wallin, J. (2006). Spinal NMDA receptor phosphorylation correlates with the presence of neuropathic signs following peripheral nerve injury in the rat. *Neuroscience Letters*, *399*(1), 85–90.

Van Sickle, M. D., Duncan, M., Kingsley, P. J., Mouihate, A., Urbani, P., Mackie, K., Stella, N., Makriyannis, A., Piomelli, D., Davison, J. S., Marnett, L. J., Di Marzo, V., Pittman, Q. J., Patel, K. D., & Sharkey,

K. A. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science*, *310*, 329–332.

Wade, D. T., Makela, P. M., House, H., Bateman, C., & Robson, P. (2006). Long-term use of a cannabis-based medicine in the treatment of spasticity and other symptoms in multiple sclerosis. *Multiple Sclerosis*, *12*(5), 639–645.

Yadav, V., Bever, C., Bowen, J., Bowling, A., Weinstock-Guttman, B., Cameron, M., … , Narayanaswami, P. (2014). Summary of evidence-based guideline: complementary and alternative medicine in multiple sclerosis. Report of the Guideline Development Subcommittee of the American Academy of Neurology. *Neurology*, *82*(12), 1083–1092.

CHAPTER

95

Cannabis for Basal Ganglia Disorders (Parkinson Disease and Huntington Disease)

O.M.E. Abdel-Salam

Department of Toxicology and Narcotics, National Research Centre, Dokki, Greater Cairo, Egypt

SUMMARY POINTS

- This chapter summarizes the current clinical and experimental data related to the effect of cannabis and/or cannabinoids on motor dysfunction in basal ganglia movement disorders.
- In patients with Parkinson disease (PD), there are data to suggest some benefit for cannabis or cannabis-based medicines on motor symptoms and L-dopa-induced dyskinesia.
- Rodent models of PD suggest a modulatory effect for cannabinoid receptor agonists on L-dopa-induced dyskinesia
- Limited data in Huntington disease (HD) patients suggest a benefit from cannabidiol, herbal cannabis, nabilone, or Sativex.
- Cannabinoids showed mostly a neuroprotective effect in animal models of PD or HD.
- There is evidence for cannabinoid receptor 2 in decreasing activation of microglia cells and neuroinflammation.

KEY FACTS OF PARKINSON DISEASE

- The disease was first described by James Parkinson in 1817.
- It mainly occurs in old age but, in 5% of cases, young persons can be also affected.
- The patients have difficulty and slowness in initiating movement, muscle stiffness, and hand tremor.
- It is due to a gradual and progressive loss of brain cells that synthesize and release dopamine.
- Treatment thus is based on levodopa, a precursor of dopamine and other drugs that increase dopamine brain availability.
- With advancing disease, more cells are lost and the response to L-dopa frequently decreases.

KEY FACTS OF HUNTINGTON DISEASE

- It was described in 1872 by George Huntington.
- This is a rare disease which is genetically transmitted.
- It begins in midlife and progresses over 15–20 years.
- Patients may experience involuntary and unpredictable irregular jerky movements of the limbs and/or body.
- Patients deteriorate over time and emotional and personality changes occur along with the motor dysfunction.
- There is no cure but there are medications which help to lessen the manifestations of the disease.

LIST OF ABBREVIATIONS

2-AG	2-Arachidonoylglycerol
ACEA	Arachidonyl-2-chloroethylamide

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00110-1
Copyright © 2017 Elsevier Inc. All rights reserved.

AEA	*N*-arachidonoylethanolamine (anandamide)
AIMs	Abnormal involuntary movements
CB1	Cannabinoid receptor subtype 1
CB2	Cannabinoid receptor subtype 2
FAAH	Fatty acid amide hydrolase
GABA	Gamma aminobutyric acid
GPe	Globus pallidus external
GPi	Globus pallidus internal
HD	Huntington disease
iNOS	Inducible nitric oxide synthase
JNK	c-Jun-NH2-terminal kinase
L-dopa	Levodopa
MPP+	1-Methyl-4-phenylpyridinium
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
3NP	3-Nitropropionic acid
PD	Parkinson disease
PI3K	Phosphatidylinositol 3-kinase
PRARγ	Peroxisome proliferator activated receptor gamma
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
STN	Subthalamic nucleus
TH-ir	Tyrosine hydroxylase immunoreactive
Δ^9-THC	**Δ^9**-Tetrahydrocannabinol
THC	Tetrahydrocannabinol
Δ^9-THCA	Δ^9-Tetrahydrocannabinolic acid
Δ^9-THCV	Δ^9-Tetrahydrocannabivarin
TNF-α	Tumor necrosis factor-alpha
TRPV1	Transient receptor potential vanilloid type-1

INTRODUCTION

The basal ganglia are a group of related subcortical nuclei. These are the striatum (caudate nucleus and putamen), the internal and external segments of the globus pallidus (GPi and GPe), the substantia nigra pars reticulata (SNr), and pars compacta (SNc), and the subthalamic nucleus (STN). These structures are components of circuits involving portions of the cerebral cortex, thalamus, and brain stem. The basal ganglia have significant influence on motor function in that it controls and smoothens the execution of movements (Wichmann & DeLong, 2006). Parkinson disease (PD) is a progressive neurodegenerative disorder, the prevalence of which increases with age, with a peak age of onset around 60 years. There is a selective and progressive loss of dopamine-producing neurons of the SNc. The loss of these neurons produces a marked deficit of dopamine content in the striatum, and a consequent disruption of the basal ganglia circuits (Fig. 95.1). This leads to the characteristic motor abnormalities, namely rigidity, bradykinesia, tremor, and postural instability (Gunzler, Schoenberg, Riley, Walter, & Maciunas, 2011). Huntington disease (HD) is a relatively rare neurodegenerative disease with prevalence 10 per 100,000. It is genetically transmitted as an autosomal dominant trait. The disorder occurs typically in middle to late age, is characterized by insidious progression of motor dysfunction, cognitive, emotional, and behavioral deficits, and results in death within 15–20 years after the onset of symptoms. Motor dysfunction; initially include chorea and other hyperkinetic movements (myoclonic jerks and dystonia). Later bradykinesia, incoordination, and rigidity become more evident. The core pathology is the loss of medium-sized spiny GABAergic projection neurons in the striatum. Striatal degeneration is the first and most obvious neuropathology in the early stages of the disease. Later, there is profound loss of neurons in other brain regions, particularly the neocortex (Walker, 2007).

Cannabinoid CB1 receptors are expressed at high levels in the basal ganglia structures (striatum, GP, SNc). In these regions, CB1 receptors are primarily located on GABAergic and glutamaregic striatal nerve terminals, and their stimulation with Δ^9-THC or synthetic agonists inhibited synaptic release of GABA and glutamate in the striatum (Gerdeman & Fernández-Ruiz, 2008). Anandamide, and 2-arachidonoylglycerol (2-AG), the endogenous ligands for the CB1 receptor are present in the GP and SN, which suggests a role for endocannabinoids in control of motor activity (Di Marzo, Hill, Bisogno, Crossman, & Brotchie, 2000).

The present chapter aims to review the current available evidence pertaining to the effect of cannabis and/or cannabinoids in basal ganglia movement disorders namely PD and HD.

THE ENDOCANNABINOID SYSTEM IN PARKINSON DISEASE

There is evidence that the endocannabinoid system is altered in PD. In caudate nucleus and putamen from patients with PD postmortem and in MPTP-lesioned marmosets, CB1 receptor binding increased and this was amenable to L-dopa treatment (Lastres-Becker et al., 2001). Hurley, Mash, and Jenner (2003) found decreased CB1 receptor mRNA expression in the caudate nucleus, anterior dorsal putamen and GPe in PD brain post-mortem. Van Laere et al. (2012), however, using positron emission tomography (PET) with the CB1-selective radioligand [(18)F] MK-9470 and magnetic resonance imaging, found an absolute decrease in CB1 availability in SN, but a relative increase in nigrostriatal, mesolimbic, and mesocortical dopaminergic projection areas in PD patients.

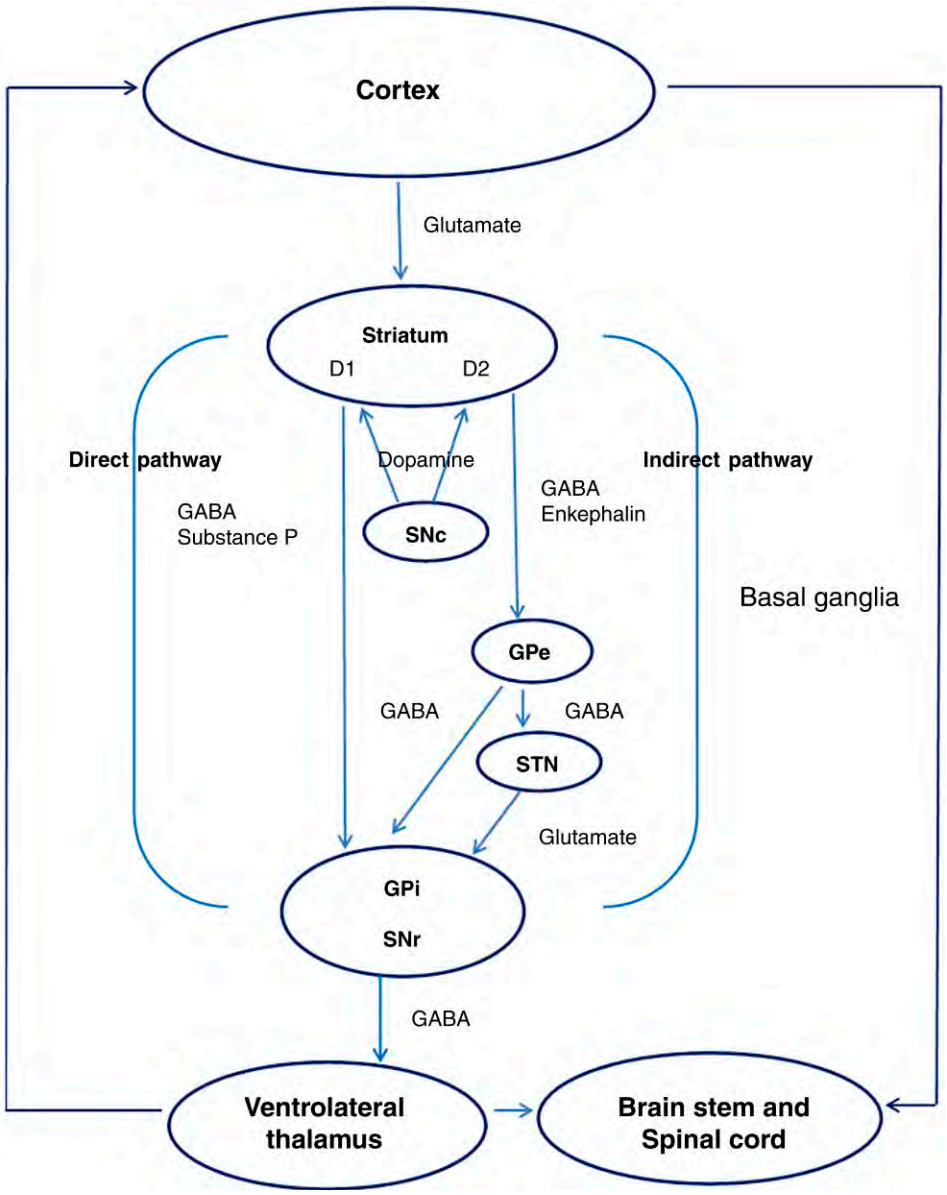


FIGURE 95.1 Schematic representation of the organization of basal ganglia–thalamocortical circuits. By means of direct and indirect pathways that run from the input structure (striatum) to the output nuclei (GPi, GPe, and SNr) the basal ganglia act to disinhibit or facilitate related thalamocortical neurons, and ultimately facilitate or inhibit movements initiated in the cortex. Neurotransmitters involved in each pathway are shown. *GABA*, Gamma aminobutyric acid; *GPe*, globus pallidus external; *GPi*, globus pallidus internal; *SNc*, substantia nigra pars compacta; *SNr*, substantia nigra pars reticulata; *STN*, subthalamic nucleus.

EFFECT OF CANNABINOIDS IN PARKINSON DISEASE

Human Studies

Venderová, Růzicka, Vorísek, and Visnovský (2004) found that PD subjects who used cannabis to alleviate symptoms reported mild or substantial alleviation of their symptoms in general (45%), improvement of resting tremor (30.6%), bradykinesia (44.7%), and of muscular rigidity (37.7%). Cannabis, however, worsened symptoms in 4–7% of patients. Most patients consumed a half teaspoon of fresh or dried leaves orally, usually with meals and mostly once a day (Venderová et al., 2004).

6-HYDROXYDOPAMINE MODEL OF PARKINSON DISEASE

In 6-hydroxydopamine-lesioned rats, the daily administration of Δ^9-THC or cannabidiol (a nonpsychotropic cannabinoid) for 2 weeks conferred significant protection against the toxin-induced decreases in dopamine, and TH-ir in the lesioned striatum. These effects were ascribed to an antioxidant properties or glial modulating effects (Lastres-Becker, Molina-Holgado, Ramos, Mechoulam, & Fernández-Ruiz, 2005). Other authors observed a small recovery in the dopamine depletion in the lesioned side after treatment with HU-308, a CB2 receptor agonist, but not after the CB1 receptor agonist ACEA or the nonselective agonist WIN55,212-2 (García-Arencibia et al., 2007). *N*-(4-hydroxyphenyl)-arachidonamide (AM404), an inhibitor of endocannabinoid uptake (which enhances anandamide availability) was found to have antiparkinsonian effects, possibly through stimulation of striatal 5-HT(1B) serotonin receptor function (Fernandez-Espejo et al., 2004) or possibly due to an antioxidant properties being derived from the phenolic group in its structure (García-Arencibia et al., 2007). In rats, adrenal phaeochromocytoma PC12 cells, the endocannabinoid anandamide inhibited 6-hydroxydopamine-induced apoptosis (caspase-3 activity), independently of cannabinoid receptors or TRPV1. This antiapoptotic effect of anandamide involved activation of phosphatidylinositol 3-kinase and inhibition of JNK signaling (Mnich, Finn, Dowd, & Gorman, 2010).

Motor inhibition due to 6-hydroxydopamine in rats was also alleviated following the acute administration of Δ^9-THCV, a phytocannabinoid with very weak or no psychotropic effects. Moreover, the loss of TH-ir neurons decreased by its chronic administration. These effects of Δ^9-THCV involved changes in glutamatergic transmission and an antioxidant effect (García et al., 2011).

Rimonabant, a selective antagonist of CB1 receptors, displayed antihypokinetic activity at dose of 0.1 mg/kg in 6-hydroxydopamine-lesioned rats. Rimonabant, however, had no effect on the dopamine deficit or on GABA or glutamate transmission in the caudate-putamen (González et al., 2006). In the same model, rimonabant (0.1 and 1 mg/kg) caused marked striatal glutamate release. Rimonabant (1 mg/kg) caused a slight increase in striatal glutamate release in control rats (García-Arencibia, Ferraro, Tanganelli, & Fernández-Ruiz, 2008). Rimonabant was also found to augment the therapeutic effect of a moderate, but not a high, dyskinetic dose of L-dopa (Kelsey, Harris, & Cassin, 2009).

In cultures of mouse cerebellar granule cells exposed to 6-hydroxydopamine, treatment with the nonselective cannabinoid agonist HU-210 increased cell survival by regulating glial influence to neurons (Lastres-Becker et al., 2005). Interestingly, the cannabinoid HU 210 and Δ^9-THC increased TH expression and dopamine content in the striatum of normal rats (Bosier et al., 2012).

1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE MODEL OF PARKINSON DISEASE

It was found that lack of both CB1 and CB2 receptors did not alter the susceptibility to the neurotoxic effect of MPTP in mice (Simkins et al., 2012). Genetic ablation of CB2 receptors, however, exacerbated MPTP toxicity (Price et al., 2009). The cannabinoid agonist WIN55,212-2 protected against nigrostriatal cell loss induced by MPTP. This effect was independent of CB1 cannabinoid receptor activation, but involved reduced MPTP-induced microglial activation (via CB2 cannabinoid receptor activation) (Price et al., 2009). In CB1 knockout mice, WIN55,212-2 was also neuroprotective against MPTP toxicity (Chung et al., 2011). WIN55,212-2 and HU210 increased survival of dopaminergic SN neurons and improved motor function. This effect appears to involve CB1 receptor-mediated inhibition of NADPH oxidase reactive oxygen species production and reduced expression of proinflammatory cytokines from activated microglia (Chung et al., 2011). Suppression of microglia-mediated inflammation might therefore underlie some of the neuroprotective effects ascribed for cannabinoids.

In SH-SY5Y neuroblastoma cells exposed to MPP+ (a complex I inhibitor), lactacystin and paraquat, Δ^9-THC and also WIN55,212-2 protected against toxin-induced cell death (though not α-tocopherol and butylhydroxytoluene, nabilone, and cannabidiol). The effect of Δ^9-THC depended on CB1 receptors and involved increased activation of PRARγ (Carroll, Zeissler, Hanemann, & Zajicek, 2012). In murine mesencephalic culture exposed to MPP+, Δ^9-THCA, Δ^9-THC and cannabidiol showed antioxidant effect and protected dopaminergic neurons in culture against MPP(+)-induced cell death. THC increased dopaminergic cell count. Cannabidiol, however, decreased cell count viability, and decreased TH-ir neurons (Moldzio et al., 2012).

ROTENONE MODEL OF PARKINSON DISEASE

In mouse, mesencephalic cultures and neuroblastoma cells, Δ^9-THC or cannabidiol decreased the rotenone-induced degeneration of dopaminergic cells along with significant restoration of glutathione levels. Either cannabinoid failed to scavenge superoxide radicals in neuroblastoma cells exposed to rotenone (Pöhn et al., 2012). In vivo, cannabis extract (20 mg/kg, expressed as Δ^9-THC, subcutaneously daily) given for 2 weeks in mice,

TABLE 95.1 Effect of Cannabinoids or Modulators of Endogenous Cannabinoids on Nigrostriatal Damage Induced by 6-Hydroxydopamine

Model	Agent	Neuroprotection	Suggested mechanism/CB receptor involvement	Study
Unilateral injection of 6-hydroxydopamine into the rat medial forebrain bundle	Δ^9-THC, cannabidiol	√√√	Possible antioxidant (cannabinoid receptor-independent) or antiinflammatory effect	Lastres-Becker et al. (2005)
Unilateral injection of 6-hydroxydopamine into rat striatum	HU-308 (CB2 receptor agonist)	√	Possible antioxidant effect	García-Arencibia et al. (2007)
Unilateral injection of 6-hydroxydopamine into rat striatum	Arachidonyl-2-chloroethylamide (ACEA)(CB1 receptor agonist)	—	—	García-Arencibia et al. (2007)
Unilateral injection of 6-hydroxydopamine into rat striatum	WIN55,212-2 (nonselective cannabinoid agonist)	—	—	García-Arencibia et al. (2007)
Unilateral injection of 6-hydroxydopamine into rat striatum	AM404 (inhibitor of endocannabinoid inactivation)	√√√	Possible antioxidant effect	García-Arencibia et al. (2007)
Unilateral injection of 6-hydroxydopamine into rat striatum	UCM707 (inhibitor of endocannabinoid inactivation)	—	—	García-Arencibia et al. (2007)
Unilateral injection of 6-hydroxydopamine into rat striatum	Cannabidiol	√√√	Antioxidant effect: upregulation of mRNA levels for Cu,Zn-superoxide dismutase	García-Arencibia et al. (2007)
Unilateral injection of 6-hydroxydopamine into rat striatum	*N*-(4-hydroxyphenyl)-arachidonamide (AM404), an inhibitor of endocannabinoid uptake	√√√	Stimulation of striatal 5-HT(1B) serotonin receptor function	Fernandez-Espejo et al. (2004)
Mouse cerebellar granule cells exposed to 6-hydroxydopamine	HU-210 (nonselective cannabinoid agonist)	√√√	Modulation of glial function	Lastres-Becker et al. (2005)
6-Hydroxydopamine-treated pheochromocytoma PC12 cells	Anandamide	√√√	PI3K activation and inhibition of JNK signaling	Mnich et al. (2010)
Unilateral injection of 6-hydroxydopamine into rat striatum	Δ^9-THCV	√√√	Modulation of glutamatergic neurotransmission and antioxidant properties	García et al. (2011)
	Cannabidiol-enriched botanical extract	√√√	Antioxidant effect	García et al. (2011)
Unilateral injection of lipopolysaccharide into substantia nigra of mice	Δ^9-THCV	√√√	CB2 receptor-mediated mechanism	García et al. (2011)
	HU-308 (CB2 receptor agonist)	√√√	CB2 receptor-mediated mechanism	García et al. (2011)

√√√, Neuroprotective.

failed to reduce nigrostriatal damage due to rotenone, lipopolysaccharide or both (Abdel-Salam, Omara, El-Shamarka, & Hussein, 2013) (Tables 95.1 and 95.2).

PRIMATE MODELS OF PARKINSON DISEASE

In cynomolgus monkeys, the cannabinoid agonist levonantradol decreased general and locomotor activity and increased bradykinesia, while tetrahydrocannabinol increased the bradykinesia only. Rimonabant, however, had no significant effects compared with vehicle. Moreover, the authors found that the rimonabant failed to alleviate the motor deficits in an early and advanced stage of MPTP-induced parkinsonism (Meschler, Howlett, & Madras, 2001). In the MPTP-marmoset model, Δ^9-THC (4 mg/kg) improved locomotor activity and hand-eye coordination performance (van Vliet, Vanwersch, Jongsma, Olivier, & Philippens, 2008). In L-DOPA-primed MPTP-treated rhesus monkeys with moderate and severe parkinsonism, selective CB1 antagonism increased the duration responses to L-dopa by 30% (Cao et al., 2007). In the same model, systemic administration

TABLE 95.2 Effect of Cannabinoids on Nigrostriatal Damage Induced by 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) or 1-Methyl-4-Phenyl Pyridinium [MPP(+)]

Model	Agent	Neuroprotection	Suggested mechanism/CB receptor involvement	Study
Mice injected with MPTP	WIN55212-2 (nonselective cannabinoid agonist)	√√√	↓ Microglial activation/ infiltration (CB2 receptor-dependent)	Price et al. (2009)
Mice injected with MPTP	WIN55212-2 HU210 (nonselective cannabinoid agonist)	√√√ √√√	↓ Microglial activation (CB1 receptor-dependent) resulting in suppression of NADPH oxidase reactive oxygen species production and reduced expression of proinflammatory cytokines from activated microglia.	Chung et al. (2011)
Mice mesencephalic culture exposed to MPP+	Δ^9-THCA Δ^9-THC Cannabidiol	√√√ √√√ √√√	Antioxidant effect	Moldzio et al. (2012)
SH-SY5Y neuroblastoma cells exposed to MPP+, lactacystin and paraquat	Δ^9-THC WIN55,212-2	√√√ √√√	↑ PRARγ activation (CB1 receptor-dependent)	Carroll et al. (2012)
SH-SY5Y neuroblastoma cells exposed to MPP+, lactacystin and paraquat	Cannabidiol	—	—	Carroll et al. (2012)
SH-SY5Y neuroblastoma cells exposed to MPP+, lactacystin and paraquat	Nabilone (synthetic THC)	—	—	Carroll et al. (2012)
SH-SY5Y neuroblastoma cells exposed to MPP+, lactacystin and paraquat	α-Tocopherol	—	—	Carroll et al. (2012)
SH-SY5Y neuroblastoma cells exposed to MPP+, lactacystin and paraquat	Butylhydroxytoluene (antioxidant)	—	—	Carroll et al. (2012)

√√√, Neuroprotective; ↑, increase; ↓, decrease.

of rimonabant displayed antiparkinsonian actions (71% increase in motor activity) (van der Stelt et al., 2005).

CANNABINOIDS AND L-DOPA-INDUCED DYSKINESIA

The mainstay of PD treatment is L-dopa (the biosynthetic precursor of dopamine). Several years after L-dopa, however, a significant proportion of patients experience motor complications, particularly end-of-dose motor fluctuations and dyskinesias or uncontrolled involuntary hyperkinetic movements which most commonly represent a peak-dose effect (Table 95.3). In L-dopa-induced dyskinesia, there is overactivity of the direct striatal output pathway (Fabbrini, Brotchie, Grandas, Nomoto, & Goetz, 2007).

Human Studies

Improvement of L-dopa-induced dyskinesia has been reported in 14.1% of PD subjects taking fresh or dried cannabis leaves orally (Venderová et al., 2004). In another study, oral cannabis extract given for 4 weeks had no effect on L-dopa induced dyskinesia in PD patients (Carroll et al., 2004). In a randomized, double-blind, placebo-controlled, crossover trial in seven PD patients, L-dopa-induced dyskinesia was significantly reduced by the cannabinoid receptor agonist nabilone (Sieradzan et al., 2001).

Studies in Nonhuman Primates

In the MPTP-lesioned marmoset model of PD, nabilone (0.1 mg/kg) decreased levodopa-induced dyskinesia. Nabilone (0.03 mg/kg) also increased the duration of antiparkinsonian action of levodopa by 76% (Fox et al., 2002). L-dopa-primed MPTP-treated marmosets, coadministration of L-dopa and CB1 receptor antagonist rimonabant resulted in significantly less dyskinesia compared with L-dopa alone (van der Stelt et al., 2005). In the same model, a selective cannabinoid CB1 antagonist (CE) had no effect on the L-dopa-induced dyskinesia (Cao et al., 2007).

EXPERIMENTAL DATA

Studies in rodent models of PD suggested that stimulation of CB1 receptors exerts antidyskinetic effects. Thus, the cannabinoid agonist WIN55,212-2 decreased the AIMs in the 6-hydroxydopamine-lesioned rat treated

TABLE 95.3 Effect of Cannabinoids or Modulators of Endogenous Cannabinoids on L-Dopa-Induced Dyskinesia

Model	Agent	Effect	Suggested mechanism/CB receptor involvement	Study
Rats with 6-OHDA lesions chronically treated with L-dopa	WIN 55,212-2 (nonselective cannabinoid agonist)	↓ L-dopa-induced axial, limb and oral abnormal involuntary movements ↓↑ Locomotive abnormal involuntary movements	CB1 mediated	Morgese, Cassano, Cuomo, and Giuffrida (2007)
Rats with 6-OHDA lesions chronically treated with L-dopa	URB597 (FAAH inhibitor: increase anandamide concentration throughout the basal ganglia)	↓↑ Abnormal involuntary movements	—	Morgese et al. (2007)
Rats with 6-OHDA lesions chronically treated with L-dopa	URB597 + capsazepine (TRPV1 antagonist)	↓ Abnormal involuntary movements	CB1 and TRPV1 receptor mediated	Morgese et al. (2007)
Rats with 6-OHDA lesions chronically treated with L-dopa	HU210 (nonselective cannabinoid agonist)	↓ Abnormal involuntary movements ↓ Normal motor functioning	—	Walsh, Gorman, Finn, and Dowd (2010)
Rats with 6-OHDA lesions chronically treated with L-dopa	AM251 (CB1 receptor antagonist/inverse agonist)	↓↑ Abnormal involuntary movements	—	Walsh et al. (2010)
MPTP-lesioned marmoset model of Parkinson disease	Nabilone (synthetic THC: cannabinoid receptor agonist)	↓ Total dyskinesia score (nabilone 0.1 mg/kg) ↑ Duration of antiparkinsonian action of L-dopa by 76% (nabilone 0.03 mg/kg)	—	Fox, Henry, Hill, Crossman, and Brotchie (2002)
Reserpine-treated rat administered a high dose of L-dopa	SR141716 (Rimonabant : CB1 receptor antagonist)	↓ Dyskinesia (↓ increase in vertical activity)	CB1 mediated	Segovia, Mora, Crossman, and Brotchie (2003)
Reserpine-treated rat administered a high dose of L-dopa	WIN 55,212-2 (nonselective cannabinoid agonist)	↓ Dyskinesia	CB1 mediated	Segovia et al. (2003)
Reserpine-treated rat administered a high dose of L-dopa	AM404 (inhibitor of endocannabinoid transport)	↓↑	—	Segovia et al. (2003)
L-dopa-primed MPTP-treated rhesus monkeys with moderate and severe parkinsonism.	Selective CB1 antagonist (CE)	↓↑ L-dopa-induced dyskinesias ↑ Responses to L-dopa ↓↑ L-dopa pharmacokinetics	CB1 mediated (↓ CB1 signaling)	Cao et al. (2007)
L-dopa-primed MPTP-treated marmosets	Rimonabant (selective CB1 antagonist)	↓ L-dopa-induced dyskinesia	CB1 mediated (↓ CB1 signaling)	van der Stelt et al. (2005)
Posttransplantation amphetamine-induced dyskinesia in the 6-OHDA lesioned rat model of PD	WIN 55,212-2 (nonselective cannabinoid agonist)	↓↑ Abnormal involuntary movements	—	Smith, Breger, Lane, and Dunnett (2012)

↑, Increase; ↓, decrease; ↑↓, no effect.

with L-dopa (reversed by the CB1 antagonist rimonabanat (SR141716A) (Ferrer, Asbrock, Kathuria, Piomelli, & Giuffrida, 2003, Morgese et al., 2007). The antidyskinetic properties of WIN55,212-2 involved changes of dopamine and glutamate outputs across brain hemispheres (Morgese et al., 2009). In the same model, WIN55212-2 ameliorated L-dopa-induced AIMs and reversed the associated elevation in striatal protein kinase A activity via CB1-mediated mechanism (Martinez, Macheda, Morgese, Luigia, & Giuffrida, 2012). WIN55,212-2, however, failed to alter fetal cell graft-induced dyskinesia in response to an amphetamine challenge in the 6-hydroxydopamine lesioned rat (Smith et al., 2012). In 6-hydroxydopamine-lesioned rats made dyskinetic by daily L-dopa injections, the cannabinoid receptor agonist, HU210 reduced AIMs. This however was achieved at a dose which impaired normal motor functioning (Walsh et al., 2010). Morgese et al. (2007) suggested that CB1 and TRPV1 receptors play opposite roles in L-dopa-induced dyskinesias- and that the antidyskinetic effect of FAAH

inhibitors (which increase anandamide concentration in basal ganglia) is masked by the action of anandamide on TRPV1 receptors. In the reserpine-treated rat, the dyskinetic activity of L-dopa decreased following treatment with rimonabant or the CB1 agonist WIN55,212-2 which also exerted antiparkinsonian effect at a higher dose. This suggested that both cannabinoid agonists and antagonists are useful in L-dopa-induced dyskinesia (Segovia et al., 2003). Other researchers, however, found that in the nondyskinetic 6-hydroxydopamine lesioned rats treated with L-dopa injections, rimonabant precipitated certain subtypes of AIMs (Walsh et al., 2010).

CANNABINOID SIGNALING IN HUNTINGTON DISEASE

Studies revealed alterations in cannabinoid receptor density in the basal ganglia in brains from HD patients, and in transgenic and toxic rodent models (Table 95.4). The loss of cannabinoid receptor occurs early in the course of the disease and is largely thought to be involved in the pathogenesis of HD (Lastres-Becker, Gómez, De Miguel, Ramos, & Fernández-Ruiz, 2002a; Dowie et al., 2009). In brains of HD patients there is severe loss (97.5%) of cannabinoid receptor binding on striatonigral terminals in SNr (Glass, Faull, & Dragunow, 1993). Early and widespread profound reduction of CB1 availability throughout the gray matter of the cerebrum, cerebellum, and brain stem in HD patients was also demonstrated in vivo using PET studies with CB1 ligand. Moreover, CB1 availability in the prefrontal and premotor cortex inversely correlated with the number of CAG repeats in the huntingtin gene (Van Laere et al., 2010).

Fatty acid amide hydrolase is the enzyme that degrades the endocannabinoid anandamide. In peripheral lymphocytes from HD patients the activity of this enzyme was found to be decreased by >90% compared to healthy subjects. Accordingly, anandamide levels were elevated (by ~sixfold). Moreover, defective enzyme activity was observed in pre-HD patients and in brains of HD patients (Battista et al., 2007). Studies also indicated alterations in the brain levels of endocannabinoids in genetic mouse models of HD (Dowie et al., 2009; Blázquez et al., 2011).

EFFECT OF CANNABINOIDS IN HUNTINGTON DISEASE

Clinical Data

One study involving three patients with HD reported improvement in choreic movements by orally administered cannabidiol. The cannabinoid was administered initially at 300 mg/day and increased 1 week later to 600 mg/day for the next 3 weeks. Choreic movements improved by 5–15% after the first week and by 20–40% after the second week of cannabidiol, and this remained stable for 2 more weeks (Sandyk, Consroe, Stern, & Snider, 1986). In a double-blind, randomized crossover study in 15 neuroleptic-free patients with HD, cannabidiol given daily for 6 weeks was without effect relative to placebo (sesame oil). Cannabidiol was administered orally at an average daily dose of about 700 mg/day (10 mg/kg per day) (Consroe et al., 1991). In one patient with HD disease patient, improvement in behavior (irritability), and reduction of chorea coincided with smoking cannabis. This was maintained by daily administration of 1 mg/kg of nabilone (an orally active synthetic THC) (Curtis & Rickards, 2006). Nabilone was evaluated in a double-blind, placebo-controlled, crossover study where 44 randomized patients received either nabilone (1 or 2 mg) followed by placebo ($n = 22$), or placebo followed by nabilone ($n = 22$). Nabilone was found of benefit in improving chorea and cognition (Curtis, Mitchell, Patel, Ives, & Rickards, 2009). A double blind, crossover, placebo-controlled, phase II trial of Sativex conducted on 24 patients reported improvement in symptoms (Caldentey et al., 2012).

3-NITROPROPIONIC ACID-INDUCED STRIATAL DAMAGE

Increasing CB1 receptor activity might improve the hyperkinetic manifestations early in the disease. One approach involved augmenting the residual CB1 receptor activity by inhibiting endocannabinoid reuptake or hydrolysis (Lastres-Becker et al., 2002a,b, 2003b). In this respect, AM404, an inhibitor of endocannabinoid uptake, was found to reduce motor disturbances, and improve the deficits in GABA and dopamine observed in basal ganglia in the early phase of hyperactivity in 3-nitropropionic acid (3NP)-injected rats (Lastres-Becker et al., 2002b). These effects of AM404 were reversed by the TRPV1 antagonist capsazepine, while the improvements in neurochemical deficits in the caudate-putamen caused by AM404, were abolished by rimonabant. In contrast, VDM11 or AM374, two selective inhibitors or the endocannabinoid reuptake or hydrolysis, respectively, failed to reduce hyperkinesia in 3NP-lesioned rats, while CB1 receptor stimulation (with CP55,940) was antihyperkinestic. Interestingly, capsaicin, a TRPV1 receptor agonist exerted a strong antihyperkinetic activity and attenuated the reductions in dopamine and GABA transmission provoked by 3NP (Lastres-Becker et al., 2003b). Thus compounds with VR1 stimulant activity would be of more value in treating hyperkinesia in this case (Lastres-Becker et al., 2003b). Consistent with this notion, arvanil

TABLE 95.4 Effect of Cannabinoids or Modulators of Endogenous Cannabinoids in Toxic Models of Huntington Disease

Model	Agent	Neuroprotection	Suggested mechanism/CB receptor involvement	Study
3-Nitropropionic acid (3NP)-injected into rat striata	AM404 (inhibitor of endocannabinoid uptake)	√√√	↑Endocannabinoid activity and resultant activation of the remaining population of CB1 receptors (VR1 and CB1 receptor-dependent)	Lastres-Becker et al. (2003b, 2002b)
3NP-injected into rat striata	VDM11 (selective inhibitor of endocannabinoid reuptake)	—	—	Lastres-Becker et al. (2003b)
3NP-injected into rat striata	AM374 (selective inhibitor of endocannabinoid hydrolysis)	—	—	Lastres-Becker et al. (2003b)
Malonate injection into the rat striatum	O-3841 (inhibitor of diacylglycerol lipases; catalyze 2-AG biosynthesis)	√√√	↓ Formation of proneuroinflammatory prostaglandin E2 glyceryl ester (formed from COX-2-mediated oxygenation of the endocannabinoid 2- arachidonoyl-glycerol)	Valdeolivas, Satta, Pertwee, Fernández-Ruiz, and Sagredo (2012)
Malonate injection into the rat striatum	JZL184 (inhibitor of 2-AG inactivation by monoacylglycerol lipase)	↑ Striatal damage	—	Valdeolivas et al. (2013)
M-213 cells exposed to malonate	JZL184	↑ Neurotoxicity	—	Valdeolivas et al. (2013)
Malonate injection into the rat striatum	Δ^9-THC	↑ Striatal damage	—	Lastres-Becker et al. (2003a)
Malonate injection into the rat striatum	SR141716 (selective CB1 receptor antagonist)	↑ Striatal damage	—	Lastres-Becker et al. (2002a)
3NP-injected into rat striata	Cannabidiol	√√√	Possible antioxidant effects (CB1 receptor independent)	Sagredo, Ramos, Decio, Mechoulam, and Fernández-Ruiz (2007)
3NP-injected into rat striata	Arachidonyl-2-chloroethylamide (ACEA) (CB1 agonist)	—	—	Sagredo et al. (2007)
3NP-injected into rat striata	HU-308 (CB2 agonist)	—	—	Sagredo et al. (2007)
3NP-injected into rat striata	Δ^9-THC- and CBD-enriched botanical extracts	√√√	Possible antioxidant and/or antiinflammatory properties (CB1 and CB2 receptor-independent)	Sagredo et al. (2011)
Malonate injection into the rat striatum	HU-308 (CB2 receptor agonist)	√√√	↓ TNF-α from activated microglial cells (CB2 receptor-dependent)	Sagredo et al. (2009)
Malonate injection into the rat striatum	Sativex-like preparation (1:1 combination of botanical extracts enriched in either Δ^9-THC or cannabidiol)	√√√	↓ Glial activation, ↓ Expression of inducible nitric oxide synthase ↓ Expression of neurotrophin IGF-1 (cannabinoid receptor-dependent actions)	Valdeolivas et al. (2012)
Quinolinic acid injection into the rat striatum	WIN 55,212-2 (nonselective cannabinoid agonist)	√√√	Stimulation of CB1 receptors	Pintor et al. (2006)
Quinolinic acid injection into the rat striatum	CB2 receptor-selective agonists	√√√	Microglial activation (CB2-mediated)	Palazuelos et al. (2009)

√√√, Neuroprotective; ↑, increase; ↓, decrease.

(*N*-arachidonoyl-vanillyl-amide), an endocannabinoid/vanilloid structural "hybrid," reduced the hyperkinesia (increased ambulation), and also increased the inactivity in 3NP-lesioned rats. Arvanil increased glutamate content in globus pallidus and thus enhanced excitatory transmission at this region (though in control rats, it enhanced pallidal GABA-ergic transmission (de Lago, Urbani, Ramos, Di Marzo, & Fernández-Ruiz, 2005).

On the other hand, it has been observed that neurodegeneration caused by injection of malonate (complex II inhibitor) into the rat striatum decreased after inhibition of striatal 2-AG biosynthesis by an inhibitor of diacylglycerol lipase (the enzyme that catalyze 2-AG biosynthesis). This effect is likely to be mediated by the proneuroinflammatory prostaglandin E2 glyceryl ester formed from COX-2-mediated oxygenation of 2-AG. In contrast, inhibition of 2-AG inactivation by monoacylglycerol lipase exacerbated the malonate-induced striatal damage (Valdeolivas et al., 2013). In the late akinetic phase of 3-NP-treated rats, administering rimonabant had no effect on akinesia (Lastres-Becker et al., 2002a). In 3-NP-treated rats, Δ^9-THC- and cannabidiol-enriched botanical extracts attenuated neuronal loss, GABA deficiency, the neurotrophin IGF-1 expression, and the upregulation of iNOS, while reversing the reduction in superoxide dismutase-1 expression (Sagredo et al., 2011). It was also found that, cannabidiol (though not the CB1 agonist ACEA or the CB2 agonist HU-308) reversed 3NP-induced reductions in mRNA levels substance P, neuronal-specific enolase, and superoxide dismutase-2 (which are markers of striatal GABAergic projection neurons). Cannabidiol effects were independent of CB1, TRPV1 or adenosine A2A receptors (Sagredo et al., 2007). Thus it is possible to assume that the beneficial effects observed with Sativex are due to its cannabidiol content.

MALONATE-INDUCED STRIATAL DAMAGE

In rats, treatment with Δ^9-THC or with SR141716 (rimonabant) increased striatal damage due to malonate (Lastres-Becker et al., 2002a). In mice, CB2 receptor upregulation occurred in response to malonate injection. Mice deficient in CB2 receptors exhibited increased striatal degeneration caused by malonate. Neither the CB1 agonist arachidonyl-2-chloroethylamide nor cannabidiol decreased striatal neurochemical deficits induced by malonate. Striatal damage, however, decreased upon treatment with HU-308 (CB2 receptor agonist), which also lessened the decreases in GABA, dopamine and neuron-specific enolase mRNA. The protective effect of HU-308 appeared to involve decreased TNF-α level in the striatum (Sagredo et al., 2009). In malonate-lesioned rats, treatment with a combination of phytocannabinoids (Δ^9-THC:cannabidiol = 1:1) reduced oedema and neurodegeneration, attenuated glial activation and both iNOS and IGF-1 expressions. These effects of the Sativex-like preparation were CB1 and CB2 receptor-dependent (Valdeolivas et al., 2012).

QUINOLINIC ACID-INDUCED STRIATAL DAMAGE

In this model, intrastriatal WIN 55,212-2 attenuated the striatal damage (Pintor et al., 2006). WIN 55,212-2 increased the expression of neuronal cytoskeletal proteins, synuclein expression, number of synaptic vesicles, and thickness of the postsynaptic densities in rat brain. This sprouting phenomenon observed after 2 weeks therapy of WIN 55,212-2 is likely to be involved in the neuroprotective effects of cannabinoids (Tagliaferro et al., 2006). Quniolinic acid-induced striatal neuroinflammation, loss of neurons and motor symptoms also decreased following treatment with selective CB2 receptor agonists (Palazuelos et al., 2009).

In Vitro Studies

In cell culture model of striatal neurons, treatment with ACEA or anandamide increased CB1 promoter activity, mRNA, and protein expression. Moreover, in cells expressing the mutant huntingtin protein, cannabinoid treatment increased CB1 mRNA levels, improved ATP production, increased the expression of brain derived neurotrophic factor-2, and the mitochondrial regulator PGC1α, and reduced spontaneous GABA release (Laprairie, Kelly, & Denovan-Wright, 2013).

GENETIC MODELS OF HUNTINGTON DISEASE

The administration of Δ^9-THC to mice expressing human mutant huntingtin exon 1 exerted a therapeutic effect (Blázquez et al., 2011). In R6/1 transgenic mice, however, eight weeks of daily treatment with the cannabinoid agonists Δ^9-THC and HU210, or the inhibitor of endocannabinoid metabolism URB597 did not alter the progressive deterioration in motor performance. The cannabinoid agonists did not alter the ligand binding of cannabinoid CB1, dopamine D1, D2, serotonin 5HT2A or GABA (A) receptors, nor CB1 or fatty acid amide hydrolase mRNA expression (Table 95.5). URB597, however, preserved striatal CB1 receptors. Surprisingly, HU210 increased ubiquitinated aggregates in the striatum, suggesting a different effect CB1 receptor agonists and endocannabinoids on disease process (Dowie et al., 2010). In pheochromocytoma cells expressing exon 1 of mutant

TABLE 95.5 Effect of Cannabinoids in Genetic Models of Huntington Disease

Model	Agent	Neuroprotection	Suggested mechanism/CB receptor involvement	Study
R6/2 genetic HD mouse model mice	$CB2^{-/-}$	√√√ Accelerates the onset of motor deficits ↑ Severity of motor deficits	CB2 receptors are neuroprotective	Bouchard et al. (2012)
R6/2 transgenic HD mouse model	$CB2^{-/-}$	↑ Motor deficits ↓ Lifespan	↑ Microglial activation (CB2 receptors ↓ microglial activation)	Palazuelos et al. (2009)
R6/2 transgenic HD mouse model	CB2 receptor agonist	√√√ ↓Motor deficits ↓Synapse loss ↑ Lifespan	↓ Interleukin-6 (IL-6) blood levels and neuroinflammation (CB2 receptor mediated)	Bouchard et al. (2012)
R6/2 transgenic HD mouse model	Δ^9-THC	√√√ ↓Motor deficits ↓Synapse loss	↓ Excitotoxic damage (CB2 receptor mediated)	Blázquez et al. (2011)
R6/1 transgenic HD mouse model	HU210 (nonselective cannabinoid agonist)	↑ Seizure events ↑ Ubiquitinated aggregates in striatum	—	Dowie et al, (2010)
R6/1 transgenic HD mouse model	Δ^9-THC	↑↓ Motor deficits	—	Dowie et al. (2010)
R6/1 transgenic HD mouse model	URB597 (inhibitor of endocannabinoid metabolism)	↑↓ Motor deficits Preserved striatal CB1 receptors	—	Dowie et al. (2010)
R6/2 transgenic HD mouse model	$CB2^{-/-}$	↑ Motor deficits ↓ Lifespan	↑ Microglial activation	Palazuelos et al. (2009)
Striatal neurons in culture expressing the mutant huntingtin protein	ACEA (selective CB1 receptor agonist) Anandamide	√√√	↑ ATP production ↑ BDNF-2 expression ↑ Mitochondrial regulator PGC1α expression ↓ Spontaneous GABA release (CB1 receptor-mediated effects)	Laprairie et al. (2013)
Pheochromocytoma cells expressing exon 1 of mutant huntingtin	HU210 (nonselective cannabinoid agonist)	√ Small yet significant ↓ in cell death ↑ Mutant huntingtin aggregates	CB1 receptor mediated	Scotter et al. (2010)

√√√, Neuroprotective; ↑, increase; ↓, decrease; ↑↓, no effect.

huntingtin, HU210 also increased mutant huntingtin aggregates. This occurred despite a small, yet significant, protective effect for HU210 on mutant huntingtin-induced cell death. It was suggested that the protective potential of CB1 receptor stimulation is limited by coupling of CB1 to G-protein alpha subtype s (Gs) G-protein, the stimulation of cAMP formation and increased aggregate formation and that therapies selective for the G(i/o), ERK pathway are likely to be of benefit (Scotter, Goodfellow, Graham, Dragunow, & Glass, 2010).

Studies suggested a role for CB2 receptors in preventing neurodegeneration (Palazuelos et al., 2009; Bouchard et al., 2012). In R6/2 mice, genetic deletion of CB2 receptors, enhanced microglial activation, aggravated disease symptomatology and reduced mice lifespan. Moreover, in $CB2^{-/-}$ mice, quinolinic acid induced more striatal neurodegeneration and inflammation. CB2 receptor stimulation leads to decreased activation of microglia cells, the resident immune cells of the CNS (Palazuelos et al., 2009). Other researchers found increased IL-6 blood levels (though not TNF-α) in $CB2^{-/-}$ R6/2 mice. IL-6 levels decreased following treatment with a selective CB2 receptor agonist which also improved motor deficits, synapse loss, neuroinflammation, and extended mice life span, effects that were prevented by the selective CB2 receptor antagonist SR144528. These data suggested a role for peripherally released IL-6 in the neuronal damage and symptomatology in HD and which could be modulated by CB2 receptor signaling in peripheral immune cells (Bouchard et al., 2012).

MINI-DICTIONARY

Akinesia Absence of movement which occur in advancing PD.
Bradykinesia This describes difficulty in starting and slowness of voluntary movements.
Chorea Involuntary, spontaneous, irregular, and jerky movements.
Dystonia Excessive involuntary motor contractions resulting in unusual fixed postures lasting seconds to minutes.
Hyperkinesia Excessive, spontaneous, involuntary, irregular movements. It might be local involving the hands or more generalized involving the limbs and trunk.
L-dopa-induced dyskinesia Involuntary hyperkinetic movements that develop after years of L-dopa therapy. These are caused by excessive levels of dopamine in brain.
Rigidity Muscle stiffness or increased muscle tone resulting in increased resistance to movement.

References

Abdel-Salam, O. M. E., Omara, E. A., El-Shamarka, M. E. -S., & Hussein, J. S. (2013). Nigrostriatal damage after systemic rotenone and/or lipopolysaccharide and the effect of cannabis. *Comparative Clinical Pathology*, *2014*(23), 1343–1358.

Battista, N., Bari, M., Tarditi, A., Mariotti, C., Bachoud-Lévi, A. C., Zuccato, C., Finazzi-Agrò, A., Genitrini, S., Peschanski, M., Di Donato, S., Cattaneo, E., & Maccarrone, M. (2007). Severe deficiency of the fatty acid amide hydrolase (FAAH) activity segregates with the Huntington's disease mutation in peripheral lymphocytes. *Neurobiology of Disease*, *27*, 108–116.

Blázquez, C., Chiarlone, A., Sagredo, O., Aguado, T., Pazos, M. R., Resel, E., Palazuelos, J., Julien, B., Salazar, M., Börner, C., Benito, C., Carrasco, C., Diez-Zaera, M., Paoletti, P., Díaz-Hernández, M., Ruiz, C., Sendtner, M., Lucas, J. J., de Yébenes, J. G., Marsicano, G., Monory, K., Lutz, B., Romero, J., Alberch, J., Ginés, S., Kraus, J., Fernández-Ruiz, J., Galve-Roperh, I., & Guzmán, M. (2011). Loss of striatal type 1 cannabinoid receptors is a key pathogenic factor in Huntington's disease. *Brain*, *134*, 119–136.

Bosier, B., Muccioli, G. G., Mertens, B., Sarre, S., Michotte, Y., Lambert, D. M., & Hermans, E. (2012). Differential modulations of striatal tyrosine hydroxylase and dopamine metabolism by cannabinoid agonists as evidence for functional selectivity in vivo. *Neuropharmacology*, *62*, 2328–2336.

Bouchard, J., Truong, J., Bouchard, K., Dunkelberger, D., Desrayaud, S., Moussaoui, S., Tabrizi, S. J., Stella, N., & Muchowski, P. J. (2012). Cannabinoid receptor 2 signaling in peripheral immune cells modulates disease onset and severity in mouse models of Huntington's disease. *Journal of Neuroscience*, *32*(50), 18259–18268.

Caldentey, J. G., Lopéz-Sendón, J. L., Trigo, P., Ruiz, C., Ribas, G. G., Aguilar, M., Galvez, M. A., Pablo, I., Galve-Roth, I., Sagredo, O., Fernandez-Ruiz, J. J., Guzman, M., & García, J. (2012). A double blind, cross over, placebo-controlled, phase II trial of Sativex in Huntington's disease. *Journal of Neurology, Neurosurgery and Psychiatry*, *83*, A62.

Cao, X., Liang, L., Hadcock, J. R., Iredale, P. A., Griffith, D. A., Menniti, F. S., Factor, S., Greenamyre, J. T., & Papa, S. M. (2007). Blockade of cannabinoid type 1 receptors augments the antiparkinsonian action of levodopa without affecting dyskinesias in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics*, *323*, 318–326.

Carroll, C. B., Bain, P. G., Teare, L., Liu, X., Joint, C., Wroath, C., Parkin, S. G., Fox, P., Wright, D., Hobart, J., & Zajicek, J. P. (2004). Cannabis for dyskinesia in Parkinson disease: a randomized double-blind crossover study. *Neurology*, *63*, 1245–1250.

Carroll, C. B., Zeissler, M. L., Hanemann, C. O., & Zajicek, J. P. (2012). Δ^9-Tetrahydrocannabinol (Δ^9-THC) exerts a direct neuroprotective effect in a human cell culture model of Parkinson's disease. *Neuropathology and Applied Neurobiology*, *38*, 535–547.

Chung, Y. C., Bok, E., Huh, S. H., Park, J. Y., Yoon, S. H., Kim, S. R., Kim, Y. S., Maeng, S., Park, S. H., & Jin, B. K. (2011). Cannabinoid receptor type 1 protects nigrostriatal dopaminergic neurons against MPTP neurotoxicity by inhibiting microglial activation. *Journal of Immunology*, *87*, 6508–6517.

Consroe, P., Laguna, J., Allender, J., Snider, S., Stern, L., Sandyk, R., Kennedy, K., & Schram, K. (1991). Controlled clinical trial of cannabidiol in Huntington's disease. *Pharmacology Biochemistry and Behavior*, *40*, 701–708.

Curtis, A., Mitchell, I., Patel, S., Ives, N., & Rickards, H. (2009). A pilot study using nabilone for symptomatic treatment in Huntington's disease. *Movement Disorders*, *24*, 2254–2259.

Curtis, A., & Rickards, H. (2006). Nabilone could treat chorea and irritability in Huntington's disease. *Journal of Neuropsychiatry and Clinical Neurosciences*, *18*, 553–554.

de Lago, E., Urbani, P., Ramos, J. A., Di Marzo, V., & Fernández-Ruiz, J. (2005). Arvanil, a hybrid endocannabinoid and vanilloid compound, behaves as an antihyperkinetic agent in a rat model of Huntington's disease. *Brain Research*, *1050*, 210–216.

Di Marzo, V., Hill, M. P., Bisogno, T., Crossman, A. R., & Brotchie, J. M. (2000). Enhanced levels of endogenous cannabinoids in the globus pallidus are associated with a reduction in movement in an animal model of Parkinson's disease. *FASEB Journal*, *14*, 1432–1438.

Dowie, M. J., Bradshaw, H. B., Howard, M. L., Nicholson, L. F., Faull, R. L., Hannan, A. J., & Glass, M. (2009). Altered CB1 receptor and endocannabinoid levels precede motor symptom onset in a transgenic mouse model of Huntington's disease. *Neuroscience*, *163*, 456–465.

Dowie, M. J., Howard, M. L., Nicholson, L. F., Faull, R. L., Hannan, A. J., & Glass, M. (2010). Behavioural and molecular consequences of chronic cannabinoid treatment in Huntington's disease transgenic mice. *Neuroscience*, *170*, 324–336.

Fabbrini, G., Brotchie, J. M., Grandas, F., Nomoto, M., & Goetz, C. G. (2007). Levodopa-induced dyskinesias. *Movement Disorders*, *22*, 1379–1389.

Fernandez-Espejo, E., Caraballo, I., Rodriguez de Fonseca, F., Ferrer, B., El Banoua, F., Flores, J. A., & Galan-Rodriguez, B. (2004). Experimental parkinsonism alters anandamide precursor synthesis, and functional deficits are improved by AM404: a modulator of endocannabinoid function. *Neuropsychopharmacology*, *29*, 1134–1142.

Ferrer, B., Asbrock, N., Kathuria, S., Piomelli, D., & Giuffrida, A. (2003). Effects of levodopa on endocannabinoid levels in rat basal ganglia: implications for the treatment of levodopa-induced dyskinesias. *European Journal of Neuroscience*, *18*, 1607–1614.

Fox, S. H., Henry, B., Hill, M., Crossman, A., & Brotchie, J. (2002). Stimulation of cannabinoid receptors reduces levodopa-induced dyskinesia in the MPTP-lesioned nonhuman primate model of Parkinson's disease. *Movement Disorders*, *17*, 1180–1187.

García, C., Palomo-Garo, C., García-Arencibia, M., Ramos, J., Pertwee, R., & Fernández-Ruiz, J. (2011). Symptom-relieving and neuroprotective effects of the phytocannabinoid Δ^9-THCV in animal models of Parkinson's disease. *British Journal of Pharmacology*, *163*, 1495–1506.

García-Arencibia, M., Ferraro, L., Tanganelli, S., & Fernández-Ruiz, J. (2008). Enhanced striatal glutamate release after the administration of rimonabant to 6-hydroxydopamine-lesioned rats. *Neuroscience Letters*, *438*, 10–13.

García-Arencibia, M., González, S., de Lago, E., Ramos, J. A., Mechoulam, R., & Fernández-Ruiz, J. (2007). Evaluation of the neuroprotective effect of cannabinoids in a rat model of Parkinson's disease: importance of antioxidant and cannabinoid receptor-independent properties. *Brain Research*, *1134*, 162–170.

Gerdeman, G. L., & Fernández-Ruiz, J. (2008). The Endocannabinoid system in the physiology and pathology of the basal ganglia. In F. A. Kofalvi (Ed.), *Cannabinoids and the brain*. New York, NY: Springer (chapter 21).

Glass, M., Faull, R. L., & Dragunow, M. (1993). Loss of cannabinoid receptors in the substantia nigra in Huntington's disease. *Neuroscience*, *56*, 523–527.

González, S., Scorticati, C., García-Arencibia, M., de Miguel, R., Ramos, J. A., & Fernández-Ruiz, J. (2006). Effects of rimonabant, a selective cannabinoid CB1 receptor antagonist, in a rat model of Parkinson's disease. *Brain Research*, *16*, 1073–1074.

Gunzler, S. A., Schoenberg, M. R., Riley, D. E., Walter, B., & Maciunas, R. J. (2011). Parkinson's disease and other movement disorders. In M. R. Schoenberg, & J. G. Scott (Eds.), *The little black book of neuropsychology: A syndrome-based approach* (pp. 567–646). New York, Dordrecht, Heidelberg, London: Springer.

Hurley, M. J., Mash, D. C., & Jenner, P. (2003). Expression of cannabinoid CB1 receptor mRNA in basal ganglia of normal and parkinsonian human brain. *Journal of Neural Transmission*, *110*, 1279–1288.

Kelsey, J. E., Harris, O., & Cassin, J. (2009). The CB(1) antagonist rimonabant is adjunctively therapeutic as well as monotherapeutic in an animal model of Parkinson's disease. *Behavioural Brain Research*, *203*, 304–307.

Laprairie, R. B., Kelly, M. E., & Denovan-Wright, E. M. (2013). Cannabinoids increase type 1 cannabinoid receptor expression in a cell culture model of striatal neurons: implications for Huntington's disease. *Neuropharmacology*, *72*, 47–57.

Lastres-Becker, I., Cebeira, M., de Ceballos, M., Zeng, B. -Y., Jenner, P., Ramos, J. A., & Fernandez-Ruiz, J. (2001). Increased cannabinoid CB1 receptor binding and activation of GTP-binding proteins in the basal ganglia of patients with Parkinson's syndrome and of MPTP-treated marmosets. *European Journal of Neuroscience*, *14*, 1827–1832.

Lastres-Becker, I., Bizat, N., Boyer, F., Hantraye, P., Brouillet, E., & Fernández-Ruiz, J. (2003a). Effects of cannabinoids in the rat model of Huntington's disease generated by an intrastriatal injection of malonate. *Neuroreport*, *14*(6), 813–816.

Lastres-Becker, I., de Miguel, R., De Petrocellis, L., Makriyannis, A., Di Marzo, V., & Fernández-Ruiz, J. (2003b). Compounds acting at the endocannabinoid and/or endovanilloid systems reduce hyperkinesia in a rat model of Huntington's disease. *Journal of Neurochemistry*, *84*, 1097–1109.

Lastres-Becker, I., Gómez, M., De Miguel, R., Ramos, J. A., & Fernández-Ruiz, J. (2002a). Loss of cannabinoid CB(1) receptors in the basal ganglia in the late akinetic phase of rats with experimental Huntington's disease. *Neurotoxicity Research*, *4*, 601–608.

Lastres-Becker, I., Hansen, H. H., Berrendero, F., De Miguel, R., Pérez-Rosado, A., Manzanares, J., Ramos, J. A., & Fernández-Ruiz, J. (2002b). Alleviation of motor hyperactivity and neurochemical deficits by endocannabinoid uptake inhibition in a rat model of Huntington's disease. *Synapse*, *44*, 23–35.

Lastres-Becker, I., Molina-Holgado, F., Ramos, J. A., Mechoulam, R., & Fernández-Ruiz, J. (2005). Cannabinoids provide neuroprotection against 6-hydroxydopamine toxicity in vivo and in vitro: relevance to Parkinson's disease. *Neurobiology of Diseases*, *19*, 96–107.

Martinez, A., Macheda, T., Morgese, M. G., Luigia, T., & Giuffrida, A. (2012). The cannabinoid agonist WIN55212-2 decreases L-DOPA induced PKA activation and dyskinetic behavior in 6-OHDA treated rats. *Neuroscience Research*, *72*, 236–242.

Meschler, J. P., Howlett, A. C., & Madras, B. K. (2001). Cannabinoid receptor agonist and antagonist effects on motor function in normal and 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP)-treated non-human primates. *Psychopharmacology (Berlin)*, *156*, 79–85.

Mnich, K., Finn, D. P., Dowd, E., & Gorman, A. M. (2010). Inhibition by anandamide of 6-hydroxydopamine-induced cell death in PC12 cells. *International Journal of Cell Biology*, *2010*, 818497.

Moldzio, R., Pacher, T., Krewenka, C., Kranner, B., Novak, J., Duvigneau, J. C., & Rausch, W. D. (2012). Effects of cannabinoids Δ(9)-tetrahydrocannabinol, Δ(9)-tetrahydrocannabinolic acid and cannabidiol in MPP+ affected murine mesencephalic cultures. *Phytomedicine*, *19*, 819–824.

Morgese, M. G., Cassano, T., Cuomo, V., & Giuffrida, A. (2007). Antidyskinetic effects of cannabinoids in a rat model of Parkinson's disease: role of CB(1) and TRPV1 receptors. *Experimental Neurology*, *208*, 110–119.

Morgese, M. G., Cassano, T., Gaetani, S., Macheda, T., Laconca, L., Dipasquale, P., Ferraro, L., Antonelli, T., Cuomo, V., & Giuffrida, A. (2009). Neurochemical changes in the striatum of dyskinetic rats after administration of the cannabinoid agonist WIN55,212-2. *Neurochemistry International*, *54*, 56–64.

Palazuelos, J., Aguado, T., Pazos, M. R., Julien, B., Carrasco, C., Resel, E., Sagredo, O., Benito, C., Romero, J., Azcoitia, I., Fernández-Ruiz, J., Guzmán, M., & Galve-Roperh, I. (2009). Microglial CB2 cannabinoid receptors are neuroprotective in Huntington's disease excitotoxicity. *Brain*, *132*, 3152–3164.

Pintor, A., Tebano, M. T., Martire, A., Grieco, R., Galluzzo, M., Scattoni, M. L., Pèzzola, A., Coccurello, R., Felici, F., Cuomo, V., Piomelli, D., Calamandrei, G., & Popoli, P. (2006). The cannabinoid receptor agonist WIN 55,212-2 attenuates the effects induced by quinolinic acid in the rat striatum. *Neuropharmacology*, *51*, 1004–1012.

Pöhn, B., Krewenka, C., Kranner, B., Duvigneau, J. C., Rausch, W. D., & Moldzio, R. (2012). Phytocannabinoids tetrahydrocannabinol and cannabidiol act against rotenone induced damages in murine cell cultures. *Planta Medica*, *78*, PD171.

Price, D. A., Martinez, A. A., Seillier, A., Koek, W., Acosta, Y., Fernandez, E., Strong, R., Lutz, B., Marsicano, G., Roberts, J. L., & Giuffrida, A. (2009). WIN55,212-2, a cannabinoid receptor agonist, protects against nigrostriatal cell loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *European Journal of Neuroscience*, *29*, 2177–2186.

Sagredo, O., González, S., Aroyo, I., Pazos, M. R., Benito, C., Lastres-Becker, I., Romero, J. P., Tolón, R. M., Mechoulam, R., Brouillet, E., Romero, J., & Fernández-Ruiz, J. (2009). Cannabinoid CB2 receptor agonists protect the striatum against malonate toxicity: relevance for Huntington's disease. *Glia*, *57*, 1154–1167.

Sagredo, O., Pazos, M. R., Satta, V., Ramos, J. A., Pertwee, R. G., & Fernández-Ruiz, J. (2011). Neuroprotective effects of phytocannabinoid-based medicines in experimental models of Huntington's disease. *Journal of Neuroscience Research*, *89*, 1509–1518.

Sagredo, O., Ramos, J. A., Decio, A., Mechoulam, R., & Fernández-Ruiz, J. (2007). Cannabidiol reduced the striatal atrophy caused 3-nitropropionic acid in vivo by mechanisms independent of the activation of cannabinoid, vanilloid TRPV1 and adenosine A2A receptors. *European Journal of Neuroscience*, *26*, 843–851.

Sandyk, R., Consroe, P., Stern, L. Z., & Snider, S. R. (1986). Effects of cannabidiol in Huntington's disease. *Neurology*, *36*(Suppl. 1), 342.

Scotter, E. L., Goodfellow, C. E., Graham, E. S., Dragunow, M., & Glass, M. (2010). Neuroprotective potential of CB1 receptor agonists in an in vitro model of Huntington's disease. *British Journal of Pharmacology*, *160*, 747–761.

Segovia, G., Mora, F., Crossman, A. R., & Brotchie, J. M. (2003). Effects of CB1 cannabinoid receptor modulating compounds on the hyperkinesia induced by high-dose levodopa in the reserpine-treated rat model of Parkinson's disease. *Movement Disorders*, *18*, 138–149.

Sieradzan, K. A., Fox, S. H., Hill, M., Dick, J. P., Crossman, A. R., & Brotchie, J. M. (2001). Cannabinoids reduce levodopa-induced dyskinesia in Parkinson's disease: a pilot study. *Neurology*, *57*, 2108–2111.

Simkins, T. J., Janis, K. L., McClure, A. K., Behrouz, B., Pappas, S. S., Lehner, A., Kaminski, N. E., Goudreau, J. L., Lookingland, K. J., & Kaplan, B. L. (2012). Comparison of the D2 receptor regulation and

neurotoxicant susceptibility of nigrostriatal dopamine neurons in wild-type and CB1/CB2 receptor knockout mice. *Journal of Neuroimmune Pharmacology*, *7*, 533–538.

Smith, G. A., Breger, L. S., Lane, E. L., & Dunnett, S. B. (2012). Pharmacological modulation of amphetamine-induced dyskinesia in transplanted hemi-parkinsonian rats. *Neuropharmacology*, *63*, 818–828.

Tagliaferro, P., Javier Ramos, A., Onaivi, E. S., Evrard, S. G., Lujilde, J., & Brusco, A. (2006). Neuronal cytoskeleton and synaptic densities are altered after a chronic treatment with the cannabinoid receptor agonist WIN 55,212-2. *Brain Research*, *1085*, 163–176.

Valdeolivas, S., Satta, V., Pertwee, R. G., Fernández-Ruiz, J., & Sagredo, O. (2012). Sativex-like combination of phytocannabinoids is neuroprotective in malonate-lesioned rats, an inflammatory model of Huntington's disease: role of CB1 and CB2 receptors. *ACS Chemical Neuroscience*, *3*, 400–406.

Valdeolivas, S., Pazos, M. R., Bisogno, T., Piscitelli, F., Iannotti, F. A., Allarà, M., Sagredo, O., Di Marzo, V., & Fernández-Ruiz, J. (2013). The inhibition of 2-arachidonoyl-glycerol (2-AG) biosynthesis, rather than enhancing striatal damage, protects striatal neurons from malonate-induced death: a potential role of cyclooxygenase-2-dependent metabolism of 2-AG. *Cell Death Diseases*, *4*, e862.

van der Stelt, M., Fox, S. H., Hill, M., Crossman, A. R., Petrosino, S., Di Marzo, V., & Brotchie, J. M. (2005). A role for endocannabinoids in the generation of parkinsonism and levodopa-induced dyskinesia in MPTP-lesioned non-human primate models of Parkinson's disease. *FASEB Journal*, *19*, 1140–1142.

Van Laere, K., Casteels, C., Dhollander, I., Goffin, K., Grachev, I., Bormans, G., & Vandenberghe, W. (2010). Widespread decrease of type 1 cannabinoid receptor availability in Huntington disease in vivo. *Journal of Nuclear Medicine*, *51*, 1413–1417.

Van Laere, K., Casteels, C., Lunskens, S., Goffin, K., Grachev, I. D., Bormans, G., & Vandenberghe, W. (2012). Regional changes in type 1 cannabinoid receptor availability in Parkinson's disease in vivo. *Neurobiology of Aging*, *33*, 620.e1–620.e8.

van Vliet, S. A., Vanwersch, R. A., Jongsma, M. J., Olivier, B., & Philippens, I. H. (2008). Therapeutic effects of Delta9-THC and modafinil in a marmoset Parkinson model. *European Neuropsychopharmacology*, *18*, 383–389.

Venderová, K., Růzicka, E., Vorísek, V., & Visnovský, P. (2004). Survey on cannabis use in Parkinson's disease: subjective improvement of motor symptoms. *Movement Disorders*, *19*, 1102–1106.

Walker, F. O. (2007). Huntington's disease. *Seminars in Neurology*, *27*, 143–150.

Walsh, S., Gorman, A. M., Finn, D. P., & Dowd, E. (2010). The effects of cannabinoid drugs on abnormal involuntary movements in dyskinetic and non-dyskinetic 6-hydroxydopamine lesioned rats. *Brain Research*, *1363*, 40–84.

Wichmann, T., & DeLong, M. R. (2006). Neurotransmitters and disorders of the basal ganglia. In S. Brady, G. Siegel, R. Wayne Albers, & D. Price (Eds.), *Basic neurochemistry: molecular, cellular and medical aspects* (7th ed., pp. 761–779). San Diego, CA: American Society for Neurochemistry-Elsevier Academic Press, Part VI: Inherited and neurodegenerative diseases (chapter 46).

CHAPTER

96

Medical Cannabis for the Treatment of Inflammatory Bowel Disease

A. Lahat

Department of Gastroenterology, Chaim Sheba Medical Center, Tel-Hashomer, Israel

SUMMARY POINTS

- Crohn disease (CD) and ulcerative colitis (UC) are chronic inflammatory bowel diseases (IBD).
- While CD can affect the whole digestive system from mouth to anus, UC affects mainly the colon.
- Both diseases cause diminished life quality.
- The main symptoms in IBD include abdominal pain, diarrhea, and loss of appetite.
- Cannabis was shown in animal models to reduce intestinal motility and inhibit inflammatory processes.
- Cannabis was also shown to stimulate appetite and cause weight gain.
- Cannabis is a known potent analgesic.
- Clinical data assessing the utility of cannabis in treating IBD patients is only available from recent years.
- In small clinical studies, cannabis was shown to cause significant improvement in patients' abdominal pain and diarrhea.
- Cannabis was also shown to increase appetite, cause weight gain, and improved quality of life in IBD patients.
- More studies are warranted in order to assess the utility, indications, and contraindications to medical use of cannabis in IBD patients.

KEY FACTS OF IBD

- Crohn disease (CD) and ulcerative colitis (UC) are chronic inflammatory bowel diseases (IBD).
- The incidence and prevalence of IBD is rising. The diseases are more prevalent in Europe and in the US.
- The highest annual incidence of UC is 24.3 per 100,000 person-years in Europe, and the highest annual incidence of CD is 12.7 per 100,000 person-years in Europe.
- The highest reported prevalence values for IBD were in Europe (UC: 505 per 100,000 persons; CD: 322 per 100,000 persons) and North America (UC: 249 per 100,000 persons; CD: 319 per 100,000 persons).
- CD and UC are autoimmune diseases. Theirs cause is believed to be multifactorial—the result of the interaction of environmental and genetic factors.
- The main symptoms include abdominal pain, vomiting, diarrhea, rectal bleeding, and weight loss.
- Both diseases can cause major morbidity and diminished life quality.
- Treatment is individualized according to disease characters and severity. It mainly includes antiinflammatory drugs as 5-aminosalicylic acid (5 ASA), and immunosuppressive drugs as as prednisone, TNF inhibition, azathioprine methotrexate, or 6-mercaptopurine, etc.

LIST OF ABBREVIATIONS

5-ASA	5-Aminosalicylic acid
AER	Ascending enteric reflex

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00111-3
Copyright © 2017 Elsevier Inc. All rights reserved.

anti-TNFα	Antitumor necrosis factor alpha
CB1, CB2	Cannabinoid receptors type 1 and 2
CD	Crohn disease
CDAI	Crohn disease activity index
IBD	Inflammatory bowel disease
LES	Lower esophageal sphincter
LPS	Lipopolysaccharides
SIBDQ	Short inflammatory bowel disease questionnaire
UC	Ulcerative colitis

INTRODUCTION

Crohn disease (CD) is a chronic inflammatory disorder that may affect the gastrointestinal (GI) tract from the mouth to the anus. The inflammation is transmural, and might therefore be complicated by fistula and abscess formation, perforations, and fibrotic strictures. Ulcerative colitis (UC) is a form of inflammatory bowel disease (IBD) which affects mainly the colon, thus causing abdominal pain, bloody diarrhea, and weight loss. Both diseases may cause significant morbidity and diminished life quality (Love, Irvine, & Fedorak, 1992; Casellas, López-Vivancos, Badia, Vilaseca, & Malagelada, 2001; Rubin, Hungin, Chinn, & Dwarakanath, 2004; Canavan, Abrams, Hawthorne, Drossman, & Mayberry, 2006; Hjortswang et al., 2003).

Treatment options include a variety of antiinflammatory and immunosuppressant agents. Most commonly used are the 5-aminosalicylic acid (5- ASA) antiinflammatory drugs, corticosteroids, thiopurine drugs (azathioprine and 6-mercaptopurine), methotrexate and antitumor necrosis factor alpha (anti-TNFα) agents. Most of these treatments, however, carry a considerable risk for severe side effects. (Gearry, Barclay, Burt, Collett, & Chapman, 2004; Fraser, Orchard, & Jewell, 2002; Colombel et al., 2004; Schnitzler et al., 2009; Sandborn & Loftus, 2004; Lichtenstein et al., 2004).

Cumulative data suggest that endocannabinoids have a role in various important physiological and pathophysiological functions in the GI tract. The relevance of these effects to IBD patients could be speculated to stem from its effect on GI motility, on the immune system and its potent action as analgesics.

The plant *Cannabis sativa* is the source of more than 60 aromatic hydrocarbon compounds called cannabinoids, of which Δ^9-tetrahydrocannabinol is the main psychotropic constituent. This agent acts via at least two types of cannabinoid receptors, named CB1 and CB2 receptors (Pertwee, 1997). CB1 receptors are located mainly on central and peripheral neurons, and in the GI tract in the enteric nervous system. Through these receptors, the cannabinoids cause inhibition of the gastrointestinal motility, mainly by inhibiting ongoing contractile transmitter release) (Shook & Burks, 1989; Izzo, Mascolo, Pinto, Capasso, & Capasso, 1999; Pertwee, 2001).

The CB2 receptors are located mainly on immune cells (Hornby & Prouty, 2004), but also on some afferent (sensory) nerve terminals (Stander, Schmelz, Metze, Luger, & Rukwied, 2005).

Cannabinoid receptor agonists were shown to inhibit small bowel peristalsis both in vitro (Izzo, Mascolo, Tonini, & Capasso, 2000; Heinemann, Shahbazian, & Holzer, 1999) and in vivo (Shook & Burks, 1989) in animal models. This effect was produced by activating CB1 receptor of rodents. When activated, CB1 receptor suppresses propulsive peristalsis and ascending enteric reflex (AER) contractions by inhibiting cholinergic and noncholinergic transmission. Cannabinoids were also shown to delay gastric empting in rodents (Shook & Burks, 1989), and to reduce both contractile activity of stomach and duodenum, and intragastric pressure (Shook & Burks, 1989; Krowicki, Moerschbaecher, Winsauer, Digavalli, & Hornby, 1999).

This inhibitory effect is probably also mediated through the CB1 receptor. Another action of the cannabinoids is the inhibition of gastric acid secretion (Coruzzi, Adami, Coppelli, Frati, & Soldani, 1999).

This effect was shown also in 90 heavy cannabis smokers (Nalin et al., 1978). These inhibitory effects on GI tract motility and secretion may be beneficial in patients with diarrhea. Indeed, some anecdotal reports suggest the effective use of cannabis against dysentery and cholera (Mechoulam, 1986)

Cannabinoid receptor agonists were shown to inhibit (through CB1 receptor activation) LES (lower esophageal sphincter) relaxation in dogs (Lehmann et al., 2002) and ferrets (Partosoedarso, Abrahams, Scullion, Moerschbaecher, & Hornby, 2003).

This effect was shown to decrease gastroesophageal reflux (Lehmann et al., 2002), and was caused by central and peripheral vagal mechanisms. In the inflamed gut, both CB1 and CB2 receptor activation may reduce inflammation-associated hypermotility. In a mouse model of ileitis induced by irritant croton oil, intestinal CB1 receptors are hyperexpressed, and cannabinoid agonists were proven effective in reducing intestinal motility compared to control sham-treated mice (Izzo et al., 2001).

In CB1 receptor-knockout mice, experimental colitis is significantly more severe than in wild-type mice, indicating that CB1 receptors have a role in protecting the bowel from inflammation (Massa et al., 2004)

Activation of CB2 receptors also inhibits both the accelerated intestinal motility (Mathison, Ho, Pittman, Davison, & Sharkey, 2004) and the extent of inflammation (Kimball, Schneider, Wallace, & Hornby, 2006) in experimental bowel inflammation.

The effect of cannabinoids on the immune system is complex, and mediated through CB1 and CB2 receptors

(Klein, 2005). Both receptors are expressed on B cells, NK cells, and mast cells (Klein et al., 2003; Samson et al., 2003). Cannabinoid receptors are also found on gut-associated lymphoid tissue (Lynn & Herkenham, 1994; Kulkarni-Narla & Brown, 2000).

Cannabinoids suppress cell mediated immunity and Th1 cytokine production, and enhance adaptive immunity and Th2 cytokine production, primary via CB2 receptors (Roth, Baldwin, & Tashkin, 2002). Secretion of TNF-α is directly inhibited by cannabinoids (Samson et al., 2003; Facchinetti, Del Giudice, Furegato, Passarotto, & Leon, 2003; Chang, Lee, & Lin, 2001).

Cannabinoids inhibit LPS-evoked macrophage activation (Chang et al., 2001), suppress activated macrophages, and mast cells (Small-Howard, Shimoda, Adra, & Turner, 2005; Vannacci et al., 2003).

The use of cannabis is also known to stimulate appetite and cause weight gain (Abel, 1975), (Berry & Mechoulam, 2002). Recent studies show involvement of the plant in the regulation of appetite, food intake, and energy metabolism (Di Marzo & Matias, 2005; Sharkey & Pittman, 2005; Pagotto, 2006).

Cannabinoids are also potent analgesics, mainly affecting chronic neuropathic and inflammatory pain (Richardson, Aanonsen, & Hargreaves, 1998; Siegling, Hofmann, Denzer, Mauler, & De Vry, 2001).

Visceral sensory nerves of the gut are inhibited by CB1 and CB2 receptor selective agonists (Sanson, Bueno, & Fioramonti, 2006; Hillsley et al., 2007).

CB2 receptor agonists normalize sensory hypersensitivity caused by colon distension in experimental colitis (Sanson et al., 2006).

Thus, treatment with cannabis may have a beneficial effect in patients with IBD who suffer from alerted bowel movements, abdominal pain, and loss of appetite. However, despite the fact that sporadic patients self-administer cannabis, investigational data in the literature are scarce.

CANNABIS TREATMENT IN IBD

The beneficial effect of cannabinoids on the GI tract has been recognized for centuries, and, apparently, many IBD patients self-administrated the plant in order to control their symptoms. However, until recently, little attention was attributed toward the medical usage of cannabis in patients suffering from IBD. Searching the literature reveals only seven papers addressing this topic, all of them published during the past years. Of these papers, only four are clinical trials in IBD patients, one is a trial performed in a murine model of colitis, and the rest are review articles.

This chapter will focus on clinical data from clinical trials in IBD patients. The first paper published in 2011 is a survey conducted on IBD patients in Mount Sinai Hospital, Toronto, Canada (Lal et al., 2011). The aim of this study was to evaluate cannabis use in IBD patients attending the outpatient clinic. Patients were asked to complete an anonymous questionnaire regarding their disease, treatment, demographic, and psychological data. Approximately 50% of 291 patients included reported lifetime cannabis use. Fifty percent of CD patients and 33% of UC patients that reported lifetime cannabis use consumed the cannabis in order to relief IBD symptoms, including pain relief, loss of appetite, and diarrhea. Patients were more likely to use cannabis for symptom relief if they had a history of abdominal surgery (60% vs 32%), chronic analgesic use (71% vs 31%), used complimentary alternative medicine (55% vs 32%) or had a lower short inflammatory bowel disease questionnaire (SIBDQ) score (45.1 ± 2.1 vs 50.3 ± 1.5) Notably, this survey did not asses the clinical efficacy of cannabis in IBD patients, only its consumption.

The first study that directly measured the impact of cannabis treatment on IBD patients' quality of life, weight and disease activity was published in 2012 (Lahat, Lang, & Ben-Horin, 2012).

In this prospective study, a small group of long standing severe IBD patients initiated treatment with inhaled cannabis. Disease activity, weight, and quality of life were assessed before and after 3 months treatment. After 3 months treatment patients reported improvement in general health perception, social functioning, ability to work, physical pain, and depression (Tables 96.1 and 96.2).

Patients had a weight gain of 4.3 ± 2 kg during treatment, and an average rise in BMI of 1.4 ± 0.61 (Fig. 96.1). Disease activity Harvey–Bradshaw index was reduced from 11.36 ± 3.17 to 5.72 ± 2.68 ($p = 0.001$) (Table 96.3). No adverse events were reported.

A small prospective placebo controlled study was published in 2013 (Naftali et al., 2013).

In this study, 21 patients with severe active CD were randomized to receive either inhaled cannabis or placebo containing cannabis flowers from which THC had been extracted. Patients were treated with inhaled cannabis for 8 weeks. CD activity index (CDAI) was calculated before treatment, after 2 and after 8 weeks treatment. After 8 weeks treatment, 45% of the study group achieved complete remission, as compared to 10% in the placebo group. However, these results did not reach statistical significance—probably due to the small sample size (11 patients in the study group, 10 in the placebo). The response rate (decrease of more than 100 points in the CDAI) was 90% in the study group, as compared to 30% in the placebo group ($p = 0.05$).

Patients in the study group reported a significant improvement in quality of life as measured by a rise in the SF-36 health survey results (from 68 at week 0–86

TABLE 96.1 Results of the SF-36v2 Health Survey

	Before treatment	After treatment	*p* Value
General health	4.38 ± 0.65	2.92 ± 0.76	$p = 0.0005$
General health today compared to 1 year ago	3.77 ± 0.92	2.07 ± 1.18	$p = 0.001$
Restrictions in daily activities	2.01 ± 0.73	2.61 ± 0.59	$p < 0.0001$
Limitations in daily activities due to physical condition	1.86 ± 0.71	3.48 ± 0.78	$p < 0.0001$
Limitations in daily activities due to emotional problems	2.52 ± 0.97	3.86 ± 0.86	$p < 0.0001$
Restrictions in social life	3.92 ± 0.86	2.3 ± 0.94	$p = 0.0002$
Physical pain	4.84 ± 0.55	3 ± 1.03	$p = 0.0002$
Limitations in working ability following pain	4.15 ± 0.55	2.23 ± 0.92	$p = 0.0002$
General well being	3.05 ± 0.99	3.17 ± 0.93	$p = 0.4758$
Frequency of restrictions in social life	2.07 ± 0.76	3.69 ± 0.75	$p = 0.002$
Self-perception of health condition	3.51 ± 1.39	3.26 ± 1.19	$p = 0.0797$

From Lahat A., Lang A., Ben-Horin S (2012). Impact of cannabis treatment on the quality of life, weight and clinical disease activity in inflammatory bowel disease patients: a pilot prospective study. Digestion, *85(1), 1–8. With permission from Karger publishers.*

TABLE 96.2 Results of the EQ-5D Health Survey

	Before treatment	After treatment	*p* Value
Mobility	1.53 ±0.66	1.3 ± 0.48	$p = 0.9$
Self-care ability	1.15 ± 0.55	1.05 ± 0.277	$p = 0.9$
Daily activities	1.92 ± 0.64	1.53 ± 0.51	$p = 0.06$
Pain	2.46 ± 0.66	1.76 ± 0.43	$p = 0.004$
Depression	1.84 ± 0.68	1.23 ± 0.43	$p = 0.007$
Schematic scale	4.1 ± 1.43	7 ± 1.42	$p = 0.0002$

From Lahat A., Lang A., Ben-Horin S (2012). Impact of cannabis treatment on the quality of life, weight and clinical disease activity in inflammatory bowel disease patients: a pilot prospective study. Digestion, *85(1), 1–8. With permission from Karger publishers.*

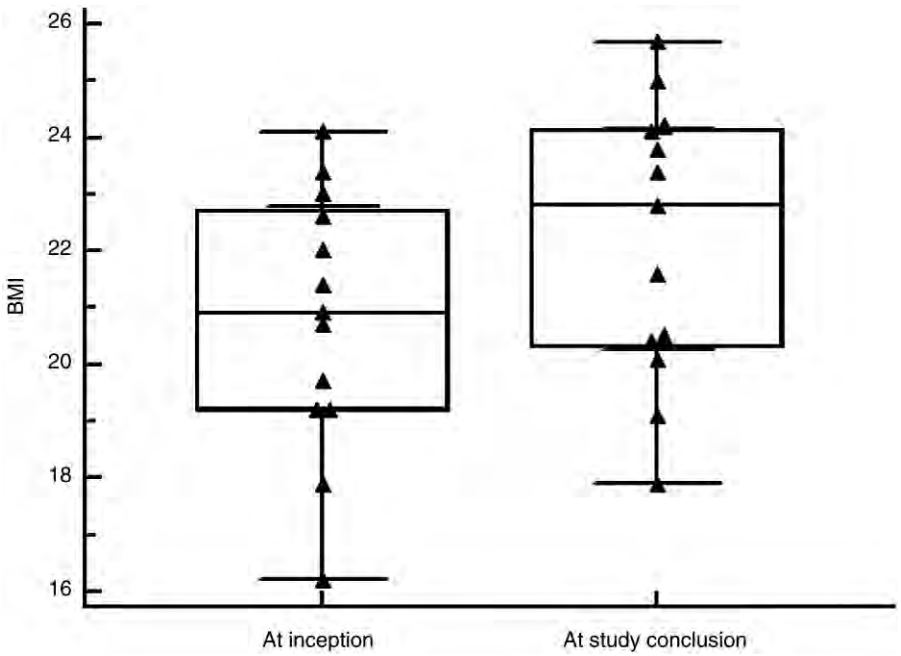


FIGURE 96.1 **BMI at study inception and after 3 months of treatment.** *Source: Figure from Lahat A., Lang A., Ben-Horin S. (2012). Impact of cannabis treatment on the quality of life, weight and clinical disease activity in inflammatory bowel disease patients: a pilot prospective study.* Digestion, *85(1), 1–8. With permission from Karger publishers.*

TABLE 96.3 Results of the Harvey–Bradshaw Index

	Before treatment	After treatment	*p* Value
General well being	2.63 ± 0.67	0.81 ± 0.87	$p = 0.0010$
Abdominal pain	2.36 ± 0.92	1.09 ± 0.7	$p = 0.0039$
Number of liquid stools/day	5.27 ± 2.93	3 ± 1.73	$p = 0.0039$
Abdominal mass	0.27 ± 0.9	0.18 ± 0.6	$p = 0.7$
complications	0.63 ± 0.5	0.63 ± 0.5	$p = 1$

From Lahat A., Lang A., Ben-Horin S (2012). Impact of cannabis treatment on the quality of life, weight and clinical disease activity in inflammatory bowel disease patients: a pilot prospective study. Digestion, 85*(1), 1–8. With permission from Karger publishers.*

TABLE 96.4 Beneficial Effects of Cannabis Use in Patients With IBD

Symptoms where cannabis helps	
Abdominal pain	47 (83.9)
Abdominal cramps	43 (76.8)
Joint pain	27 (48.2)
Diarrhea	16 (28.6)
Fever	5 (8.9)
Fistula problems	5 (8.9)
CANNABIS USE	*YES*
Allowed me to decrease the dose of my conventional IBD medications	11 (19.6)
Allowed me to stop my conventional IBD medications	7 (12.5)
Made me need to increase my conventional IBD medications	2 (3.6)
Allowed me to avoid surgery that was recommended to me	6 (10.7)
Was a waste of my money	1 (1.8)
Cured my inflammatory bowel disease	4 (7.1)
Caused only side effects	3 (5.4)

Data shown as *n* (%).
From Storr M., Devlin S., Kaplan G. G., Panaccione R., Andrews C. N. (2014). Cannabis use provides symptom relief in patients with inflammatory bowel disease but is associated with worse disease prognosis in patients with Crohn disease. Inflammatory Bowel Disease, 20*(3), 472–480. With permission from Wolters Kluwer Health publishers.*

after 8 weeks treatment, $p = 0.05$). Patients in the control group showed no statistically significant improvement (71 vs 79, $p = 0.5$).

There were no significant changes laboratory results during the study period.

Side effects did not differ between the study groups. Patients from the study group reported significantly less pain, a rise in appetite, and higher satisfaction from the treatment.

The most recent study assessing cannabis' impact on IBD patients was published in 2014 (Storr, Devlin, Kaplan, Panaccione, & Andrews, 2014).

In this study, patients from the University of Calgary Gastroenterology outpatient clinic were asked to complete an anonymous questionnaire regarding self-administration of cannabis for the treatment of IBD and IBD related symptoms. Patients' demographic and clinical characteristics, as well as overall effectiveness and side effects were evaluated. Approximately 18% of patients used cannabis specifically to relieve IBD related symptoms, 96% of them used inhaled cannabis. Eighty four percent of patients reported improved abdominal pain and 77% reported improvement in abdominal cramps with cannabis use. Other beneficial effects were reduced joint pain (48%) and diarrhea (29%). (Table 96.4)

Patients that used cannabis for IBD tented more to suffer from sever disease (32% vs 22%, $p < 0.001$), to have more abdominal pain (59% vs 31%, $p < 0.001$) and to had more IBD relates hospitalizations (85% vs 31%, $p < 0.001$) than patients who did not use cannabis (Table 96.5)

Patient that used cannabis were at higher risk for surgery (OR 5) and for hospitalization (OR 2.855) than patients that did not use cannabis. The study could not assess causality. However, a reasonable option is that patients that used cannabis suffered from a more severe disease, and therefore underwent more operations and more IBD related hospitalizations.

TABLE 96.5 Activity, Clinical Characteristics, and Current Treatment of IBD Patients: Users Versus Nonusers of Cannabis

Variable	Total (*n*=319)	Nonusers (*n*=263)	Users (*n*=56)	*p* Value
ACTIVITY OF IBD				
Inactive	6 (1.9%)	4 (1.5%)	2 (3.6%)	0.515
Moderate	270 (84.7%)	235 (89.4%)	35 (62.5%)	<0.001
Severe	76 (23.8%)	58 (22.1%)	18 (32.1%)	<0.001
Extremely sever	2 (0.6%)	1 (0.4%)	1 (1.8%)	1
MOST BOTHERSOME SYMPTOMS				
Abdominal pain	114 (35.8%)	81 (30.8%)	33 (58.9%)	<0.001
Abdominal cramping	84 (26.3%)	62 (23.6%)	22 (39.3%)	0.015
Diarrhea	166 (52.0%)	140 (53.2%)	26 (46.4%)	0.355
Joint pains	52 (16.3%)	38 (14.4%)	14 (25.0%)	0.052
Problem with fistula	32 (10.0%)	24 (9.1%)	8 (14.3%)	0.02
Fever	4 (1.3%)	0 (0%)	4 (7.1%)	0.555
Hospitalized because of IBD	214 (67.1%)	166 (63.1%)	48 (85.7%)	0.001
Surgery of IBD	145 (45.5%)	111 (42.2%)	34 (60.7%)	0.012
Flares or constant trouble within the last 12 months	214 (67.1%)	171 (65.0%)	43 (76.8%)	<0.001
No flares within the last 12 months	63 (19.7%)	54 (20.5%)	9 (16.1%)	0.005
MEDICATION USE (LAST 12 MONTHS)				
Aminosalicylates	114 (35.7%)	100 (38.0%)	14 (25.0%)	0.065
Steroids	136 (42.6%)	107 (40.7%)	29 (51.8%)	0.127
Immunomodulators	132 (41.4%)	110 (41.8%)	22 (39.3%)	0.726
Analgesics	121 (37.9%)	93 (35.4%)	28 (50.0%)	0.04
Narcotics	79 (24.8%)	53 (20.2%)	26 (46.4%)	<0.001
Loperamide	55 (17.2%)	42 (16.0%)	13 (23.2%)	0.193
Biologics	102 (32.0%)	81 (30.8%)	21 (37.5%)	0.329
IV medication	31 (9.7%)	24 (9.1%)	7 (12.5%)	0.439
CAM	102 (32.0%)	73 (27.8%)	29 (51.8%)	<0.001

CAM, Complementary and alternative medicine; IV, intravenous.
From Storr M., Devlin S., Kaplan G. G., Panaccione R., Andrews C. N. (2014). Cannabis use provides symptom relief in patients with inflammatory bowel disease but is associated with worse disease prognosis in patients with Crohn disease. Inflammatory Bowel Disease, 20*(3), 472–480. With permission from Wolters Kluwer Health publishers.*

Patients in this study did report side effects—more than 75% reported dry mouth, drowsiness, increased appetite, and sensation of "high." However, in most cases, these symptoms were described as mild.

In conclusion, cannabis was shown to be beneficial for IBD symptoms relief. Patients reported significant improvement in abdominal pain, diarrhea, increased appetite, and improved quality of life. Side effects, if any, were mild. However, these small studies are insufficient to establish the role of cannabis in IBD treatment. More double-blind placebo-controlled large volume studies are warranted in order to assess the utility, indications, and contraindications to cannabis use as therapeutic tool in IBD patients.

MINI-DICTIONARY

5-ASA 5-Aminosalicylic acid (mesalamine), a group of antiinflammatory drugs for IBD treatment.
anti-TNFα Antitumor necrosis factor alpha, a biological medication composed of IgG1 monoclonal antibody against tumor necrosis factor alpha.
CDAI Crohn disease activity index is a research tool used to quantify the symptoms of patients with Crohn disease. The index is based on the number of liquid or soft stools , abdominal pain, general wellbeing, presence of complications, treatment with antidiarrhea drugs, and presence of an abdominal mass.
EQ 5D A standardized instrument for use as a measure of health outcome.
Harvey–Bradshaw index A simpler version of the Crohn disease activity index (CDAI) for data collection purposes. It consists of

only clinical parameters: the first three items are scored for the previous day. Harvey–Bradshaw index is far simpler to use than the CDAI and does not require biochemical tests.

SF 36 The Short Form (36) Health Survey is a patient-reported survey of patient health.

SIBDQ Short inflammatory bowel disease questionnaire (SIBDQ), a health-related quality of life (HRQoL) tool measuring physical, social, and emotional status (score 10–70, poor to good HRQoL).

References

Abel, E. L. (1975). Cannabis: effects on hunger and thirst. *Behavioral Biology*, *15*, 255–281.

Berry, E. M., & Mechoulam, R. (2002). Tetrahydrocannabinol and endocannabinoids in feeding and appetite. *Pharmacology and Therapeutics*, *95*, 185–190.

Canavan, C., Abrams, K. R., Hawthorne, B., Drossman, D., & Mayberry, J. F. (2006). Long-term prognosis in Crohn's disease: factors that affect quality of life. *Alimentary Pharmacology and Therapeutics*, *23*, 377–385.

Casellas, F., Arenas, J. I., Baudet, J. S., Fábregas, S., García, N., Gelabert, J., Medina, C., Ochotorena, I., Papo, M., Rodrigo, L., & Malagelada, J. R. (2005). Impairment of health-related quality of life in patients with inflammatory bowel disease: a Spanish multicenter study. *Inflammatory Bowel Disease*, *11*, 488–496.

Casellas, F., López-Vivancos, J., Badia, X., Vilaseca, J., & Malagelada, J. R. (2001). Influence of inflammatory bowel disease on different dimensions of quality of life. *European Journal of Gastroenterology and Hepatology*, *13*, 567–572.

Chang, Y. H., Lee, S. T., & Lin, W. W. (2001). Effects of cannabinoids on LPS-stimulated inflammatory mediator release from macrophages: involvement of eicosanoids. *Journal of Cellular Biochemistry*, *81*, 715–723.

Colombel, J. F., Loftus, E. V., Jr., Tremaine, W. J., Egan, L. J., Harmsen, W. S., Schleck, C. D., Zinsmeister, A. R., & Sandborn, W. J. (2004). The safety profile of infliximab in patients with Crohn's disease: the Mayo clinic experience in 500 patients. *Gastroenterology*, *126*, 19–31.

Coruzzi, G., Adami, M., Coppelli, G., Frati, P., & Soldani, G. (1999). Inhibitory effect of the cannabinoid receptor agonist WIN 55,212-2 on pentagastrin-induced gastric acid secretion in the anaesthetized rat. *Naunyn Schmiedebergs Archives of Pharmacology*, *360*, 715–718.

Di Marzo, V., & Matias, I. (2005). Endocannabinoid control of food intake and energy balance. *Nature Neuroscience*, *8*, 585–589.

Facchinetti, F., Del Giudice, E., Furegato, S., Passarotto, M., & Leon, A. (2003). Cannabinoids ablate release of TNFalpha in rat microglial cells stimulated with lypopolysaccharide. *Glia*, *41*, 161–168.

Fraser, A. G., Orchard, T. R., & Jewell, D. P. (2002). The efficacy of azathioprine for the treatment of inflammatory bowel disease: a 30 year review. *Gut*, *50*, 485–489.

Gearry, R. B., Barclay, M. L., Burt, M. J., Collett, J. A., & Chapman, B. A. (2004). Thiopurine drug adverse effects in a population of New Zealand patients with inflammatory bowel disease. *Pharmacoepidemiology and Drug Safety*, *13*, 563–567.

Heinemann, A., Shahbazian, A., & Holzer, P. (1999). Cannabinoid inhibition of guinea pig intestinal peristalsis via inhibition of excitatory and activation of inhibitory neural pathways. *Neuropharmacology*, *38*, 1289–1297.

Hillsley, K., McCaul, C., Aerssens, J., Peeters, P. J., Gijsen, H., Moechars, D., Coulie, B., Grundy, D., & Stead, R. H. (2007). Activation of the cannabinoid 2 (CB2) receptor inhibits murine mesenteric afferent nerve activity. *Neurogastroenterology and Motility*, *19*, 769–777.

Hjortswang, H., Järnerot, G., Curman, B., Sandberg-Gertzén, H., Tysk, C., Blomberg, B., Almer, S., & Ström, M. (2003). The influence of demographic and disease-related factors on health-related quality of life in patients with ulcerative colitis. *European Journal of Gastroenterology and Hepatology*, *15*, 1011–1020.

Hornby, P. J., & Prouty, S. M. (2004). Involvement of cannabinoid receptors in gut motility and visceral perception. *British Journal of Pharmacology*, *141*, 1335–1345.

Izzo, A. A., Fezza, F., Capasso, R., Bisogno, T., Pinto, L., Iuvone, T., Esposito, G., Mascolo, N., Di Marzo, V., & Capasso, F. (2001). Cannabinoid CB1-receptor mediated regulation of gastrointestinal motility in mice in a model of intestinal inflammation. *British Journal of Pharmacology*, *134*, 563–570.

Izzo, A. A., Mascolo, N., Pinto, L., Capasso, R., & Capasso, F. (1999). The role of cannabinoid receptors in intestinal motility, defaecation and diarrhoea in rats. *European Journal of Pharmacology*, *384*, 37–42.

Izzo, A. A., Mascolo, N., Tonini, M., & Capasso, F. (2000). Modulation of peristalsis by cannabinoid CB_1 ligands in the isolated guinea pig ileum. *British Journal of Pharmacology*, *129*, 984–990.

Kimball, E. S., Schneider, C. R., Wallace, N. H., & Hornby, P. J. (2006). Agonists of cannabinoid receptor 1 and 2 inhibit experimental colitis induced by oil of mustard and by dextran sulfate sodium. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *291*, G364–G371.

Kirkham, T. C. (2005). Endocannabinoids in the regulation of appetite and body weight. *Behavioural Pharmacology*, *16*, 297–313.

Klein, T. W. (2005). Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nature Reviews Immunology*, *5*, 400–411.

Klein, T. W., Newton, C., Larsen, K., Chou, J., Perkins, I., Lu, L., Nong, L., & Friedman, H. (2004). Cannabinoid receptors and T helper cells. *Journal of Neuroimmunology*, *147*, 91–94.

Klein, T. W., Newton, C., Larsen, K., Lu, L., Perkins, I., Nong, L., & Friedman, H. (2003). The cannabinoid system and immune modulation. *Journal of Leukocyte Biology*, *74*, 486–496.

Krowicki, Z. K., Moerschbaecher, J. M., Winsauer, P. J., Digavalli, S. V., & Hornby, P. J. (1999). Δ^9-tetrahydrocannabinol inhibits gastric motility in the rat through cannabinoid CB_1 receptors. *European Journal of Pharmacology*, *371*, 187–196.

Kulkarni-Narla, A., & Brown, D. R. (2000). Localization of CB1-cannabinoid receptor immunoreactivity in the porcine enteric nervous system. *Cell and Tissue Research*, *302*, 73–80.

Lahat, A., Lang, A., & Ben-Horin, S. (2012). Impact of cannabis treatment on the quality of life, weight and clinical disease activity in inflammatory bowel disease patients: a pilot prospective study. *Digestion*, *85*, 1–8.

Lal, S., Prasad, N., Ryan, M., Tangri, S., Silverberg, M. S., Gordon, A., & Steinhart, H. (2011). Cannabis use amongst patients with inflammatory bowel disease. *European Journal of Gastroenterology and Hepatology*, *23*, 891–896.

Lehmann, A., Blackshaw, L. A., Branden, L., Carlsson, A., Jensen, J., Nygren, E., & Smid, S. D. (2002). Cannabinoid receptor agonism inhibits transient lower esophageal sphincter relaxation and reflux in dogs. *Gastroenterology*, *123*, 1129–1134.

Lichtenstein, G. R., Feagan, B. G., Cohen, R. D., Salzberg, B. A., Diamond, R. H., Chen, D. M., Pritchard, M. L., & Sandborn, W. J. (2004). Serious infections and mortality in association with therapies for Crohn's disease: TREAT registry. *Clinical Gastroenterology and Hepatology*, *4*, 621–630.

Love, J. R., Irvine, E. J., & Fedorak, R. N. (1992). Quality of life in IBD. *Journal of Clinical Gastroenterology*, *14*, 15–19.

Lynn, A. B., & Herkenham, M. (1994). Localization of cannabinoid receptors and nonsaturable high-density cannabinoid binding sites in peripheral tissues of the rat: Implications for receptor-mediated immune modulation by cannabinoids. *Journal of Pharmacology and Experimental Therapeutics*, *268*, 1612–1623.

Massa, F., Marsicano, G., Hermann, H., Cannich, A., Monory, K., Cravatt, B. F., Ferri, G. L., Sibaev, A., Storr, M., & Lutz, B. (2004). The endogenous cannabinoid system protects against colonic inflammation. *Journal of Clinical Investigations*, *113*, 1202–1209.

Mathison, R., Ho, W., Pittman, Q. J., Davison, J. S., & Sharkey, K. A. (2004). Effects of cannabinoid receptor-2 activation on accelerated gastrointestinal transit in lipopolysaccharide-treated rats. *British Journal of Pharmacology*, *142*, 1247–1254.

Mechoulam, R. (1986). The pharmacohistory of *Cannabis sativa*. In R. Mechoulam (Ed.), *Cannabinoids as therapeutic agents* (pp. 1–19). Boca Raton, FL: CRC Press.

Naftali, T., Bar-Lev Schleider, L., Dotan, I., Lansky, E. P., Sklerovsky Benjaminov, F., & Konikoff, F. M. (2013). Cannabis induces a clinical response in patients with Crohn's disease: a prospective placebo-controlled study. *Clinical Gastroenterology and Hepatology*, *11*, 1276–1280.

Nalin, D. R., Rhead, J., Rennels, M., Bergquist, E., Rennels, M., Hughes, T., O'Donnel, S., & Hornick, R. B. (1978). Cannabis, hypochlorhydria and cholera. *Lancet*, *2*, 859–862.

Pagotto, U. (2006). The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocrine Review*, *27*, 73–100.

Partosoedarso, E. R., Abrahams, T. P., Scullion, R. T., Moerschbaecher, J. M., & Hornby, P. J. (2003). Cannabinoid1 receptor in the dorsal vagal complex modulates lower oesophageal sphincter relaxation in ferrets. *Journal of Physiology*, *550*, 149–158.

Pertwee, R. G. (1997). Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacology and Therapeutics*, *74*, 129–180.

Pertwee, R. G. (2001). Cannabinoids and the gastrointestinal tract. *Gut*, *48*, 859–867.

Richardson, J. D., Aanonsen, L., & Hargreaves, K. M. (1998). Antihyperalgesic effects of spinal cannabinoids. *European Journal of Pharmacology*, *345*, 145–153.

Roth, M. D., Baldwin, G. C., & Tashkin, D. P. (2002). Effects of delta-9-tetrahydrocannabinol on human immune function and host defense. *Chemistry and Physics of Lipids*, *121*, 229–239.

Rubin, G. P., Hungin, A. P., Chinn, D. J., & Dwarakanath, D. (2004). Quality of life in patients with established inflammatory bowel disease: a UK general practice survey. *Alimentary Pharmacology and Therapeutics*, *19*, 529–535.

Samson, M. T., Small-Howard, A., Shimoda, L. M., Koblan-Huberson, M., Stokes, A. J., & Turner, H. (2003). Differential roles of CB1 and CB2 cannabinoid receptors in mast cells. *Journal of Immunology*, *170*, 4953–4962.

Sandborn, W. J., & Loftus, E. V. (2004). Balancing the risks and benefits of infliximab in the treatment of inflammatory bowel disease. *Gut*, *53*, 780–782.

Sanson, M., Bueno, L., & Fioramonti, J. (2006). Involvement of cannabinoid receptors in inflammatory hypersensitivity to colonic distension in rats. *Neurogastroenterology and Motility*, *18*, 949–956.

Schnitzler, F., Fidder, H., Ferrante, M., Noman, M., Arijs, I., Van Assche, G., Hoffman, I., Van Steen, K., Vermeire, S., & Rutgeerts, P. (2009). Long-term outcome of treatment with infliximab in 614 patients with Crohn's disease: results from a single-centre cohort. *Gut*, *58*, 492–500.

Sharkey, K. A., & Pittman, Q. J. (2005). Central and peripheral signaling mechanisms involved in endocannabinoid regulation of feeding: a perspective on the munchies. *Science's STKE*, *2005*(277), pe15.

Shook, J. E., & Burks, T. F. (1989). Psychoactive cannabinoids reduce gastrointestinal propulsion and motility in rodents. *Journal of Pharmacology and Experimental Therapeutics*, *249*, 444–449.

Siegling, A., Hofmann, H. A., Denzer, D., Mauler, F., & De Vry, J. (2001). Cannabinoid CB(1) receptor upregulation in a rat model of chronic neuropathic pain. *European Journal of Pharmacology*, *415*, R5–R7.

Small-Howard, A. L., Shimoda, L. M., Adra, C. N., & Turner, H. (2005). Anti-inflammatory potential of CB1-mediated cAMP elevation in mast cells. *Biochemical Journal*, *388*, 465–473.

Stander, S., Schmelz, M., Metze, D., Luger, T., & Rukwied, R. (2005). Distribution of cannabinoid receptor 1 (CB1) and 2 (CB2) on sensory nerve fibers and adnexal structures in human skin. *Journal of Dermatological Science*, *38*, 177–188.

Storr, M., Devlin, S., Kaplan, G. G., Panaccione, R., & Andrews, C. N. (2014). Cannabis use provides symptom relief in patients with inflammatory bowel disease but is associated with worse disease prognosis in patients with Crohn's disease. *Inflammatory Bowel Disease*, *20*, 472–480.

Vannacci, A., Passani, M. B., Pierpaoli, S., Giannini, L., Mannaioni, P. F., & Masini, E. (2003). Nitric oxide modulates the inhibitory effect of cannabinoids on the immunological activation of guinea pig mast cells. *Inflammation Research*, *52*(Suppl. 1), S7–S8.

Yuan, M., Kiertscher, S. M., Cheng, Q., Zoumalan, R., Tashkin, D. P., & Roth, M. D. (2002). Delta 9-tetrahydrocannabinol regulates Th1/Th2 cytokine balance in activated human T cells. *Journal of Neuroimmunology*, *133*, 124–131.

CHAPTER

97

Cannabidiol for the Treatment of Drug Use Disorders

R.G. dos Santos, J.E.C. Hallak, A.W. Zuardi, J.A. de Souza Crippa

Department of Neuroscience and Behavior, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil

SUMMARY POINTS

- This chapter focuses on cannabidiol (CBD), which is a naturally occurring plant-derived compound.
- CBD is produced by the cannabis plant, which produces several other chemically related compounds collectively called phytocannabinoids or simply cannabinoids.
- CBD does not produce the psychoactive effects typically produced by Δ^9-tetrahydrocannabinol (Δ^9-THC), which is the main active compound in cannabis.
- Preclinical evidence suggest that CBD has antiaddictive effects in animal models using opiates/opioids and stimulants.
- Preliminary studies in humans suggest that CBD reduces cigarette consumption and reduces symptoms of cannabis dependence/withdrawal.
- Given the good safety profile of CBD and its promising antiaddictive effects, there is a clear need to perform randomized, controlled clinical trials with this phytocannabinoid.

KEY FACTS OF CANNABIDIOL

- Cannabis is a plant original from Central Asia that has been used for ritual and medicinal purposes for thousands of years.
- The cannabis plant is rich in unique chemical compounds called cannabinoids.
- The main cannabinoids are Δ^9-tetradydrocannabinol (THC), responsible for producing most of the subjective effects of cannabis, and cannabidiol (CBD), which lacks the psychoactivity of THC.
- The chemical structure of CBD was elucidated in 1963, while THC, the main active component in cannabis, was isolated in a pure form and its structure determined in 1964.
- Preclinical evidence suggest that CBD has antiaddictive effects in animal models using opiates/opioids and stimulants.
- Preliminary studies in humans suggest that CBD reduces cigarette consumption and reduces symptoms of cannabis dependence/withdrawal.

LIST OF ABBREVIATIONS

CB_1	Cannabinoid receptor 1
CB_2	Cannabinoid receptor 2
CBD	Cannabidiol
CNS	Central nervous system
COMT	Catechol-O-methyl transferase
COX	Cyclooxygenase
D_2	Dopaminergic receptor 2
FAAH	Fatty acid amide hydrolase
fMRI	Functional magnetic resonance imaging
GABA	γ-Aminobutyric acid
$GABA_A$	GABAergic receptor A
$GABA_B$	GABAergic receptor B
GluR1	Glutamatergic AMPA receptor 1

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00113-7
Copyright © 2017 Elsevier Inc. All rights reserved.

11-HO-delta-8-THC	11-Hydroxy-delta-8-tetrahydrocannabinol
5-HT$_{1A}$	Serotonergic receptor 1A
IFN-γ	Interferon-γ
ICSS	Intracranial self-stimulation
NAc	Nucleus accumbens
NO	Nitric oxide
PGE2	Prostaglandin E2
rCBF	Regional cerebral blood flow
SAD	Social anxiety disorder
SPECT	Single photon emission computed tomography
Δ^8-THC	Δ^8-Tetrahydrocannabinol
Δ^9-THC	Δ^9-Tetrahydrocannabinol
TNF	Tumor necrosis factor
TRPV$_1$	Transient receptor potential vanilloid 1 (vanilloid receptor 1)
TRPV$_2$	Transient receptor potential vanilloid 2 (vanilloid receptor 2)
VTA	Ventral tegmental area

INTRODUCTION

Although cannabis and stimulants (cocaine, amphetamine, methamphetamine) are among the most consumed illegal recreational drugs worldwide (Nutt, King, & Phillips, 2010; Greydanus, Hawver, Greydanus, & Merrick, 2013; Morgan, Noronha, Muetzelfeldt, Fielding, & Curran, 2013a; van Amsterdam, Pennings, Brunt, & van den Brink, 2013; Hall & Degenhardt, 2014; United Nations Office on Drugs & Crime, 2014), there is no established medication for use in the treatment of cannabis or stimulant use disorders to date (Haile, Mahoney, Newton, & De La Garza, 2012; Stoops & Rush, 2013). Moreover, there has been a noticeable increase in the number of persons seeking treatment for cannabis use disorders over the past decade, particularly in the Americas, Oceania and Europe (Hall & Degenhardt, 2009; United Nations Office on Drugs & Crime, 2014). In the case of the opiates, although treatment strategies including methadone have improved substance abuse outcome in opiate-dependent subjects, these treatment strategies do not effectively block opiate craving in all patients. Considering this context, new molecules should be explored for the treatment of drug use disorders that lack an effective treatment.

Preliminary evidence suggests that cannabidiol (CBD), a phytocannabinoid devoid of the subjective and harmful effects associated with Δ^9-tetrahydrocannabinol (Δ^9-THC), may have therapeutic effects for treating drug use disorders (Hine, Torrelio, & Gershon, 1975; Bhargava, 1976; Chesher & Jackson, 1985; Parker, Burton, Sorge, Yakiwchuk, & Mechoulam, 2004; Zuardi, 2008; Ren, Whittard, Higuera-Matas, Morris, & Hurd, 2009; Crippa, Zuardi, & Hallak, 2010; Crippa et al., 2013; Katsidoni, Anagnostou, & Panagis, 2013). CBD is not associated with adverse cognitive effects, presents good safety and tolerability profiles in humans, and has a broad pharmacological spectrum of action (Zuardi, 2008; Crippa et al., 2010; Bergamaschi, Queiroz, Zuardi, & Crippa, 2011; Zhornitsky & Potvin, 2012; Niesink & van Laar, 2013).

The objective of this chapter is to review data from preclinical and human studies suggesting that CBD has potential therapeutic effects for the treatment of drug use disorders.

CANNABIDIOL

Cannabis contains over 100 compounds called cannabinoids, which are unique to the plant (Mechoulam & Carlini, 1978; Mechoulam & Hanus, 2012). THC is the cannabinoid most closely associated with euphoria, dependence, and mental health side effects associated with cannabis consumption (Bhattacharyya et al., 2010; Morgan, Freeman, Schafer, & Curran, 2010; Maldonado, Berrendero, Ozaita, & Robledo, 2011; Carbuto et al., 2012; Niesink & van Laar, 2013). The other main cannabinoid in the *Cannabis* plant, CBD, lacks the psychoactivity of THC (Zuardi, 2008; Bhattacharyya et al., 2010; Morgan et al., 2010; Niesink & van Laar, 2013).

Human studies reported that CBD has sedative, anxiolytic, antidepressant, mood stabilizer, and anticraving properties (Mechoulam & Carlini, 1978; Zuardi, 2008; Crippa et al., 2010; Bergamaschi et al., 2011; Crippa et al., 2011; Schier et al., 2012; Zhornitsky & Potvin, 2012; Niesink & van Laar, 2013; Robson, 2014).

CBD has multiple mechanisms of action, and its pharmacology is not completely understood. CBD has little affinity to cannabinoids receptors CB$_{1/2}$, antagonizes CB$_{1/2}$ receptor agonists, inhibits the reuptake of the endocannabinoid anandamide and the activity of fatty acid amide hydrolase (FAAH), the enzyme that catalyzes anandamide, and also activates 5-HT$_{1A}$ serotonergic receptors (Zuardi, 2008; Crippa et al., 2010; Bergamaschi et al., 2011; Zhornitsky & Potvin, 2012).

Single photon emission computed tomography (SPECT) and functional magnetic resonance imaging (fMRI) studies reported that CBD effects are associated with the modulation of the functional activity of tempo-ro-limbic structures (amygdala-hippocampus complex and parahippocampal gyrus) and paralimbic regions, including the cingulate cortex (Crippa et al., 2010, 2011; Schier et al., 2012). The central effects of CBD on regional cerebral blood flow (rCBF) were investigated using SPECT in patients with social anxiety disorder (SAD) and in healthy volunteers (Crippa et al., 2010, 2011; Schier et al., 2012). Relative to placebo, CBD (400 mg) produced significant decreases in subjective anxiety

induced by the SPECT procedure in patients with SAD, and these effects were associated with an action on limbic and paralimbic brain areas (Crippa et al., 2010, 2011; Schier et al., 2012). The same brain areas were modulated by CBD in healthy volunteers (Crippa et al., 2010, 2011; Schier et al., 2012). Other neuroimaging studies reported that CBD (600 mg) attenuated fMRI responses during the recognition of fearful facial expressions in the amygdala and the anterior cingulate (Crippa et al., 2010, 2011; Schier et al., 2012).

CBD VERSUS OPIATES/OPIOIDS

Although opiates/opioids top the list of problem drugs that cause the most burden of disease and drug-related deaths worldwide, only a minority of dependent subjects receives treatment, which has traditionally targeted μ-opioid receptors and do not effectively block opiate craving in all patients (Nutt et al., 2010; Morgan et al., 2013a; van Amsterdam et al., 2013; United Nations Office on Drugs & Crime, 2014).

Some studies have investigated the effects of CBD and other cannabinoids in inhibiting withdrawal in morphine-dependent rodents. A study in rats reported that CBD (10 mg/kg) had little effect on abstinence scores in morphine-dependent rats, but significantly increased the abstinence attenuating properties of THC (2 mg/kg) (Hine et al., 1975). A study in mice reported that THC (2.5–20 mg/kg), Δ^8-tetrahydrocannabinol (Δ^8-THC) (5–10 mg/kg), 11-hydroxy-delta-8-tetrahydrocannabinol (11-HO-delta-8-THC) (5–10 mg/kg), cannabinol (CBN) (5–20 mg/kg), and CBD (5–20 mg/kg), significantly inhibited the naloxone-precipitated withdrawal in morphine-dependent mice (Bhargava, 1976). The relative effectiveness of the cannabinoids in inhibiting morphine abstinence appeared to be in the following order: THC > Δ^8-THC > 11-HO-delta-8-THC > CBD > CBN (Bhargava, 1976). Another study in rats reported that THC (5–10 mg/kg) and CBN (5–80 mg/kg) produced a significant reduction in withdrawal symptoms, while CBD (5–80 mg/kg) did not produce any effect (Chesher & Jackson, 1985).

The effect of CBD on heroin self-administration and drug-seeking behavior was investigated using an experimental rat model (Ren et al., 2009). CBD (5–20 mg/kg) did not alter stable intake of heroin self-administration, extinction behavior, or drug-seeking induced by a heroin prime injection. However, CBD specifically attenuated heroin-seeking behavior reinstated by exposure to a conditioned stimulus cue, both 24 h and 2 weeks after treatment with the cannabinoid. The expression of cannabinoid CB_1 and glutamatergic AMPA GluR1 receptors mRNA and protein levels were significantly increased in the nucleus accumbens (NAc) in heroin-treated rats that received vehicle treatment, and CBD reversed these effects both 24 h and 2 weeks after treatment. These results suggest that CBD has neurobiological effects to counter long-lasting neuroadaptations that specifically govern conditioned cue-induced drug-seeking behavior and relapse.

A recent study in rats investigated the effects of CBD on brain reward function and on the reward-facilitating effect of morphine using the intracranial self-stimulation (ICSS) paradigm (Katsidoni et al., 2013). CBD (5 mg/kg) inhibited the reward-facilitating effect of morphine, and this effect was reversed by pretreatment with an intradorsal raphe injection of a selective 5-HT_{1A} receptor antagonist. Moreover, CBD (5 mg/kg) did not affect the reinforcing efficacy of brain stimulation, whereas higher doses (10–20 mg/kg) increased ICSS thresholds, suggesting that acute administration of CBD reduces brain reward function. Morphine lowered ICSS thresholds while CBD increased it, suggesting that CBD is not likely to have abuse potential (Katsidoni et al., 2013).

Table 97.1 shows the effects of CBD in animals exposed to opiates/opioids.

Taken together, these results suggest that CBD may be clinically useful in attenuating the rewarding effects of opiates/opioids. Moreover, these data reinforce the strong neurobiological interactions between the cannabinoid and opioid systems (Ren et al., 2009; Maldonado et al., 2011).

CBD VERSUS STIMULANTS

The development of pharmacotherapies for the treatment of stimulant dependence has been a high priority in addiction research for more than two decades, yet no medication has been approved by the Food and Drug Administration (FDA) in the United Sates or by similar agencies in other countries for this disease (Haile et al., 2012; Stoops & Rush, 2013). The main mechanism of action associated with the euphoria and abuse potential of stimulants is the increase in dopamine levels in the brain, and a growing preclinical and clinical literature suggest that dopamine uptake blockers and releasers (*d*-amphetamine, methamphetamine, methylphenidate, bupropion, and modafinil) may be efficacious for treating stimulant abuse and dependence (Haile et al., 2012; Stoops & Rush, 2013). However, methylphenidate and *d*-amphetamine have significant abuse and diversion potential, and bupropion and modafinil appear to have limited clinical efficacy (Haile et al., 2012; Stoops & Rush, 2013). Thus novel approaches are necessary to help those with problematic stimulant use who are seeking treatment.

CBD inhibits the uptake of dopamine, but the pharmacological relevance of this effect in humans is unknown (Zuardi, 2008; Crippa et al., 2010; Bergamaschi et al., 2011; Zhornitsky & Potvin, 2012). A study

TABLE 97.1 Effects of CBD in Animals Exposed to Opiate/Opioid

Model	Animals	Results (symbol)[a]		Dose with significant results	References
Morphine dependence	Rats	Abstinence scores	(–)[b]	10 mg/kg	Hine et al. (1975)
Naloxone-precipitated withdrawal	Mice	Withdrawal symptoms	(+)	5–20 mg/kg	Bhargava (1976)
Morphine withdrawal syndrome	Rats	Withdrawal symptoms	(–)	5–80 mg/kg	Chesher and Jackson (1985)
Heroin self-administration and drug-seeking	Rats	Self-administration, extinction behavior, drug-seeking induced by prime injection	(–)	5–20 mg/kg	Ren et al. (2009)
Heroin self-administration and drug-seeking	Rats	Drug-seeking behavior reinstated by exposure to a conditioned stimulus cue	(+)	5–20 mg/kg	Ren et al. (2009)
Heroin self-administration and drug-seeking	Rats	Expression of cannabinoid and glutamatergic receptors mRNA/protein levels in the nucleus accumbens	(+)	5–20 mg/kg	Ren et al. (2009)
Rewarding effects of morphine/intracranial self-stimulation	Rats	Reward-facilitating effect of morphine	(+)	5 mg/kg	Katsidoni et al. (2013)

[a] *(+)Therapeutic action, (–) no therapeutic action.*
[b] *CBD (10 mg/kg) significantly increased the abstinence attenuating properties of THC (2 mg/kg).*

TABLE 97.2 Effects of CBD in Animals Exposed to Stimulants

Model	Animals	Results (symbol)[a]		Dose with significant results	References
Cocaine- and amphetamine-induced conditioned place-preference	Rats	Extinction of conditioned place-preference	(+)	5 mg/kg	Parker et al. (2004)
Rewarding effects of cocaine/intracranial self-stimulation	Rats	Reward-facilitating effect of cocaine	(–)	5–20 mg/kg	Katsidoni et al. (2013)

[a] *(+)therapeutic action, (–) no therapeutic action.*

evaluated if THC and CBD could potentiate extinction of a cocaine-induced and amphetamine-induced conditioned place-preference in rats (Parker et al., 2004). THC (0.5 mg/kg) and CBD (5 mg/kg) potentiated the extinction of both cocaine-induced and amphetamine-induced conditioned place-preference learning, and this effect was not reversed by a CB_1 receptor antagonist, suggesting that other neurochemical mechanisms may be involved. Moreover, CBD was not hedonic on its own (Parker et al., 2004). On the other hand, a recent study in rats investigating the effects of CBD on brain reward function and on the reward-facilitating effect of cocaine using a self-stimulation paradigm reported that CBD (5–20 mg/kg) did not inhibit the reward-facilitating effect of cocaine (Katsidoni et al., 2013).

Table 97.2 shows studies of the effects of CBD in animals exposed to stimulants.

CBD VERSUS NICOTINE

A recent pilot study assessed the impact of the ad-hoc use of low dose CBD in smokers who wished to stop smoking (Morgan, Das, Joye, Curran, & Kamboj, 2013b). In a double-blind, placebo-controlled study, 24 smokers were recruited from the community and were randomized to receive an inhaler of CBD (n = 12; 6 females) or placebo (n = 12; 6 females) for 1 week, and they were instructed to use the inhaler when they felt the urge to smoke. Each depress of the solution aerosol in the inhaler administered a dose of 400 μg CBD. CBD significantly reduced the number of cigarettes smoked by ~40% over the treatment week, while the placebo group showed no differences in number of cigarettes smoked. Moreover, results also indicated some maintenance of this effect at follow-up. The reduction in the number of cigarettes smoked occurred in the absence of a change in cigarette craving reported daily across the week, suggesting that participants using the CBD inhaler reduced the number of cigarettes they smoked, without increased craving for nicotine.

The authors suggested that the decrease in smoking could be related to three putative mechanisms produced by CBD: (1) action at the CB_1 receptor, given previous literature on similar reductions following treatment with rimonabant, a CB_1 receptor antagonist; (2) FAAH inhibition, as recent research has shown that the reinforcing and neurochemical effects of nicotine in rats are reduced

TABLE 97.3 Effects of CBD in Smokers

Study design	Volunteers/methods	Results (symbol)[a]		Dose with significant results	Reference
Pilot double-blind, placebo-controlled study	24 smokers randomized to receive an inhaler of CBD (n = 12; 6 females) or placebo (n = 12; 6 females) for 1 week	Number of cigarettes smoked (~40% reduction)	(+)	CBD inhaler used when volunteers felt the urge to smoke (each depress = 400 μg CBD)	Morgan, Das, Joye, Curran, and Kamboj (2013b)
Pilot double-blind, placebo-controlled study	24 smokers randomized to receive an inhaler of CBD (n = 12; 6 females) or placebo (n = 12; 6 females) for 1 week	Daily cigarette craving	(–)	CBD inhaler used when volunteers felt the urge to smoke (each depress = 400 μg CBD)	Morgan et al. (2013b)

[a] *(+) Therapeutic action, (–) no therapeutic action.*

by FAAH-inhibitors; and (3) modulation of the salience of smoking cues and subsequent reduction of the attentional bias of smokers to smoking stimuli, consistent with preclinical studies (Ren et al., 2009) and a naturalistic study that found CBD to reduce the attentional bias of dependent cannabis users to cannabis stimuli (Morgan et al., 2010).

These preliminary data suggest that CBD may be a potential treatment for nicotine addiction, and warrants further exploration. The therapeutic effects of CBD described by Morgan et al. (2013b) are summarized in Table 97.3.

CBD VERSUS CANNABIS

In 2010, there were an estimated 13.1 million cannabis dependent people globally, and in 2012, of the approximately 31.7 million Americans who reported using cannabis at least once, over 13% (4.3 million people) reported cannabis dependence or abuse (Degenhardt et al., 2013; Balter, Cooper, & Haney, 2014). Nevertheless, no currently available pharmacological therapy is considered adequate to treat cannabis abuse, dependence, and withdrawal (Budney, Vandrey, & Stanger, 2010; Crippa et al., 2010; Danovitch & Gorelick, 2012; Crippa et al., 2013; Balter et al., 2014).

An approach that has been investigated for treating cannabis withdrawal is the use of agonist replacement therapies. This approach can be implemented using THC, synthetic THC (dronabinol), a synthetic THC analogue (nabilone), or Sativex (GW Pharmaceuticals, United Kingdom), a buccal spray with an approximately 1:1 ratio of THC and CBD (Budney et al., 2010; Danovitch & Gorelick, 2012; Balter et al., 2014). Other substances also produce some improvement in symptoms of cannabis withdrawal, like the antidepressants mirtazapine (norepinephrine/serotonin modulator) and nefazodone (norepinephrine/serotonin reuptake inhibitor, 5-HT_2 antagonist), the mood stabilizer lithium, the α_2 adrenergic receptor agonist lofexidine, the $GABA_A$ receptor agonist zolpidem, the glutamatergic drug *N*-acetylcysteine, and the antiepileptic and analgesic gabapentin (Budney et al., 2010; Danovitch & Gorelick, 2012; Balter et al., 2014). On the other hand, the antidepressants buproprion (norepinephrine/dopamine reuptake inhibitor), venlafaxine (serotonin/norepinephrine reuptake inhibitor), fluoxetine and escitalopram (selective serotonin reuptake inhibitors), the $GABA_B$ receptor agonist baclofen, the atypical antipsychotic quetiapine (dopamine/serotonin/norepinephrine receptors antagonist), as well as the anticonvulsant and mood stabilizer divalproex, failed to produce positive results, or even worsened some symptoms (Budney et al., 2010; Danovitch & Gorelick, 2012; Balter et al., 2014).

Regarding cannabis dependence, a treatment option with positive results is agonist replacement with THC, dronabinol, or nabilone, but other drugs also showed promising results, like the catechol-O-methyl transferase (COMT) inhibitor entacapone, the selective norepinephrine reuptake inhibitor atomoxetine, the anxiolytic buspirone (5-HT_{1A} agonist, D_2 antagonist), gabapentin, and *N*-acetylcysteine (Budney et al., 2010; Danovitch & Gorelick, 2012; Balter et al., 2014). Many of the drugs that failed to reduce cannabis withdrawal symptoms (mirtazapine, escitalopram, buspirona, fluoxetine, venlafaxine, baclofen, and quetiapine) also failed to produce positive results on cannabis dependence symptoms (Budney et al., 2010; Danovitch & Gorelick, 2012; Balter et al., 2014).

An alternative to using exogenous cannabinoid agonists (THC, dronabinol, or nabilone) as a potential treatment for cannabis use disorders is to increase cannabinoid signaling by modulating the endogenous endocannabinoids (Budney et al., 2010; Danovitch & Gorelick, 2012; Balter et al., 2014). CBD inhibits the reuptake and hydrolysis of anandamide, and has little affinity to cannabinoids receptors $CB_{1/2}$, although it is capable of antagonizing $CB_{1/2}$ receptor agonists (Zuardi, 2008; Crippa et al., 2010; Bergamaschi et al., 2011; Zhornitsky & Potvin, 2012). Moreover, CBD has a broad spectrum of pharmacological effects, including sedative, anxiolytic, antidepressive, mood stabilizer, and anticraving properties (Mechoulam & Carlini, 1978; Zuardi, 2008;

TABLE 97.4 Effects of CBD on Cannabis Withdrawal

Study design	Patient	Results	Dose with significant results	References
Case report	19-year-old female (4–8 cigarettes/day) since 13 years	Reduction in withdrawal, dissociative, depression and anxiety symptoms[a]	Day 1: 300 mg Day 2–10: 600 mg/day divided into two doses Day 11: 300 mg	Crippa et al. (2013)

[a] *Withdrawal discomfort score, marijuana withdrawal symptom checklist, Beck anxiety inventory, and Beck depression inventory.*

Crippa et al., 2010, 2011; Bergamaschi et al., 2011; Schier et al., 2012; Zhornitsky & Potvin, 2012; Niesink & van Laar, 2013; Robson, 2014).

Among the various symptoms present in cannabis withdrawal, anxiety and insomnia are the principal manifestations and are considered disruptive to daily living (Budney et al., 2010; Crippa et al., 2010, 2013; Danovitch & Gorelick, 2012; Greydanus et al., 2013; Balter et al., 2014). Moreover, across laboratory and survey studies, sleep difficulty is one of the most common and highest magnitude cannabis withdrawal effects, and was the withdrawal symptom most associated with relapse to cannabis use across multiple studies (Budney et al., 2010; Crippa et al., 2010, 2013).

Based on previous evidence suggesting anxiolytic and sedative/hypnotic properties of CBD, our group recently investigated the effects of this cannabinoid in a subject with cannabis dependence and a history of cannabis withdrawal syndrome (Crippa et al., 2013). A 19-year-old female had a history of heavy and continued use of cannabis (four to eight cigarettes/day) since 13 years of age. She denied co-use or dependence on any other substance, and used almost all her salary to buy cannabis, dedicating much time and effort to obtain the substance. The patient complained of memory, concentration, and attention problems, which interfered with her studies and performance at work. She had tried to stop using cannabis at least 4 times, always presenting, among others, with insomnia, anxiety, total loss of appetite, restlessness, migraines, irritability, nightmares, and sudoresis around the 4th to 6th day after cessation of use. Resuming cannabis use resulted in immediate relief of symptoms. The subject was hospitalized and received oral CBD (300 mg) on the 1st day, CBD 600 mg/day, divided into two doses, from the 2nd to the 10th day, and CBD 300 mg on the 11th day. Serum levels of hepatic enzymes and plasma levels of CBD and THC were monitored daily. Under CBD, the patient did not report any cannabis withdrawal symptoms (Crippa et al., 2013). The therapeutic effects of CBD described by Crippa et al. (2013) are summarized in Table 97.4.

CBD can antagonize or inhibit some behavioral effects of THC both in animals and in humans (Zuardi, 2008; Bhattacharyya et al., 2010; Crippa et al., 2010; Morgan et al., 2010; Bergamaschi et al., 2011; Niesink & van Laar, 2013). Smokers of high CBD:THC strains showed reduced attentional bias to drug and food stimuli, compared with smokers of low CBD:THC, and also showed lower self-rated liking of cannabis stimuli (Morgan et al., 2010). An fMRI study in healthy volunteers reported that THC (10 mg) and CBD (600 mg) had opposite effects on brain activation in the striatum, hippocampus, amygdala, superior temporal cortex, and occipital cortex (Bhattacharyya et al., 2010).

Considering that CBD antagonizes/inhibits some behavioral effects of THC, inhibits the reuptake and hydrolysis of anandamide, thus increasing cannabinoid signaling, and has anxiolytic and hypnotic properties, the anticraving and antiaddictive properties of this cannabinoid should be further investigated in randomized, controlled clinical trials.

CONCLUSIONS

The problematic use of psychoactive substances like cannabis, stimulants, opiates/opioids, and nicotine, carry a series of health, economic, and social costs to individuals and society. Although pharmacological treatments for opiates/opioids (methadone or buprenorphine) and nicotine (nicotine patch, etc.) abuse or dependence are available, there is no approved medication for the treatment of cannabis and stimulant disorders.

CBD antagonizes behavioral effects of THC, inhibits the reuptake and hydrolysis of anandamide, increases cannabinoid signaling, and has anxiolytic and hypnotic properties. CBD has showed therapeutic effects in animal models of opiate/opioid and stimulant dependence and withdrawal, and preliminary studies in humans suggest that CBD may reduce cigarette consumption, and the symptoms of cannabis dependence/withdrawal.

The anticraving and antiaddictive effects of CBD should be investigated in controlled clinical trials.

MINI-DICTIONARY

Anandamide *N*-arachidonoylethanolamine. From the Sanskrit word "ananda"—bliss, delight. Endogenous cannabinoid neurotransmitter.

Cannabidiol (CBD) One of the main phytocannabinoids. It lacks the psychoactive characteristics of Δ^9-Tetradydrocannbinol (THC), and has several therapeutic potentials.

Cannabinoid receptors G-protein-coupled receptors present in the endocannabinoid system.

Cannabinoids Chemical compounds present exclusively in the *Cannabis* plant. More than 100 cannabinoids have been identified. Cannabinoids are also called phytocannabinoids, in opposition to endocannabinoids.

Cannabis A plant of the Cannabaceae botanical family, with three different species: *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis*.

CB_1 cannabinoid receptor Cannabinoid receptors mainly distributed in the central nervous system. CB_1 receptors are densely distributed in the frontal cerebral cortex, basal ganglia, cerebellum, hippocampus, hypothalamus, and anterior cingulate cortex, but rarely in the brainstem nuclei.

CB_2 cannabinoid receptor Cannabinoid receptors mainly distributed in immune cells. CB_2 receptors are also expressed in microglia, spinal cord, and sparsely in several brain regions, such as, the cerebellum, cortex, and brainstem.

Drug dependence A psychiatric disorder characterized by tolerance, impaired control over drug use, difficulty in ceasing drug use despite harms caused by it, and by personal, family, and professional problems.

Endocannabinoids Endogenous cannabinoid neurotransmitters. Endocannabinoids are chemically and pharmacologically related to phytocannabinoids, both groups of substances acting at the endocannabinoid system, which is basically composed by endocannabinoids, cannabinoid receptors, and the enzymes responsible for endocannabinoid synthesis and degradation.

Δ^9-Tetrahydrocannabinol (Δ^9-THC) The main chemical in the cannabis plant responsible for producing the typical subjective effects reported for cannabis consumption.

References

Balter, R. E., Cooper, Z. D., & Haney, M. (2014). Novel pharmacologic approaches to treating cannabis use disorder. *Current Addiction Reports*, *1*, 137–143.

Bergamaschi, M. M., Queiroz, R. H., Zuardi, A. W., & Crippa, J. A. (2011). Safety and side effects of cannabidiol, a *Cannabis sativa* constituent. *Current Drug Safety*, *6*, 237–249.

Bhargava, H. N. (1976). Effect of some cannabinoids on naloxone-precipitated abstinence in morphine-dependent mice. *Psychopharmacology*, *49*, 267–270.

Bhattacharyya, S., Morrison, P. D., Fusar-Poli, P., Martin-Santos, R., Borgwardt, S., Winton-Brown, T., Nosarti, C., O'Carroll, C. M., Seal, M., Allen, P., Mehta, M. A., Stone, J. M., Tunstall, N., Giampietro, V., Kapur, S., Murray, R. M., Zuardi, A. W., Crippa, J. A., Atakan, Z., & McGuire, P. K. (2010). Opposite effects of Δ-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology*, *35*, 764–774.

Budney, A. J., Vandrey, R. G., & Stanger, C. (2010). Pharmacological and psychosocial interventions for cannabis use disorders. *Revista Brasileira de Psiquiatria*, *32*, S46–55.

Carbuto, M., Sewell, R. A., Williams, A., Forselius-Bielen, K., Braley, G., Elander, J., Pittman, B., Schnakenberg, A., Bhakta, S., Perry, E., Ranganathan, M., & D'Souza, D. C. Yale THC Study Group. (2012). The safety of studies with intravenous Δ^9-tetrahydrocannabinol in humans, with case histories. *Psychopharmacology*, *219*, 885–896.

Chesher, G. B., & Jackson, D. M. (1985). The quasi-morphine withdrawal syndrome: effect of cannabinol, cannabidiol and tetrahydrocannabinol. *Pharmacology, Biochemistry and Behavior*, *23*, 13–15.

Crippa, J. A., Zuardi, A. W., & Hallak, J. E. (2010). Therapeutical use of the cannabinoids in psychiatry. *Revista Brasileira de Psiquiatria*, *32*, S56–66.

Crippa, J. A., Derenusson, G. N., Ferrari, T. B., Wichert-Ana, L., Duran, F. L., Martin-Santos, R., Simões, M. V., Bhattacharyya, S., Fusar-Poli, P., Atakan, Z., Santos Filho, A., Freitas-Ferrari, M. C., McGuire, P. K., Zuardi, A. W., Busatto, G. F., & Hallak, J. E. (2011). Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. *Journal of Psychopharmacology*, *25*, 121–130.

Crippa, J. A., Hallak, J. E., Machado-de-Sousa, J. P., Queiroz, R. H., Bergamaschi, M., Chagas, M. H., & Zuardi, A. W. (2013). Cannabidiol for the treatment of cannabis withdrawal syndrome: a case report. *Journal of Clinical Pharmacy and Therapeutics*, *38*, 162–164.

Danovitch, I., & Gorelick, D. A. (2012). State of the art treatments for cannabis dependence. *Psychiatric Clinics of North America*, *35*, 309–326.

Degenhardt, L., Ferrari, A. J., Calabria, B., Hall, W. D., Norman, R. E., McGrath, J., Flaxman, A. D., Engell, R. E., Freedman, G. D., Whiteford, H. A., & Vos, T. (2013). The global epidemiology and contribution of cannabis use and dependence to the global burden of disease: results from the GBD 2010 study. *PLoS One*, *8*, e76635.

Greydanus, D. E., Hawver, E. K., Greydanus, M. M., & Merrick, J. (2013). Marijuana: current concepts. *Front Public Health*, *1*, 42.

Haile, C. N., Mahoney, J. J., III., Newton, T. F., & De La Garza, R., II. (2012). Pharmacotherapeutics directed at deficiencies associated with cocaine dependence: focus on dopamine, norepinephrine and glutamate. *Pharmacology and Therapeutics*, *134*, 260–277.

Hall, W., & Degenhardt, L. (2009). Adverse health effects of non-medical cannabis use. *Lancet*, *374*, 1383–1391.

Hall, W., & Degenhardt, L. (2014). The adverse health effects of chronic cannabis use. *Drug Testing and Analysis*, *6*, 39–45.

Hine, B., Torrelio, M., & Gershon, S. (1975). Interactions between cannabidiol and Δ^9-THC during abstinence in morphine-dependent rats. *Life Sciences*, *17*, 851–857.

Katsidoni, V., Anagnostou, I., & Panagis, G. (2013). Cannabidiol inhibits the reward-facilitating effect of morphine: involvement of 5-HT_{1A} receptors in the dorsal raphe nucleus. *Addiction Biology*, *18*, 286–296.

Maldonado, R., Berrendero, F., Ozaita, A., & Robledo, P. (2011). Neurochemical basis of cannabis addiction. *Neuroscience*, *181*, 1–17.

Mechoulam, R., & Carlini, E. A. (1978). Toward drugs derived from cannabis. *Naturwissenschaften*, *65*, 174–179.

Mechoulam, R., & Hanus, L. (2012). Other cannabinoids. In D. Castle, R. M. Murray, & D. C. D'Souza (Eds.), *Marijuana and madness* (2nd ed., pp. 17–22). Cambridge, UK: Cambridge University Press.

Morgan, C. J., Freeman, T. P., Schafer, G. L., & Curran, H. V. (2010). Cannabidiol attenuates the appetitive effects of Δ^9-tetrahydrocannabinol in humans smoking their chosen cannabis. *Neuropsychopharmacology*, *35*, 1879–1885.

Morgan, C. J., Noronha, L. A., Muetzelfeldt, M., Fielding, A., & Curran, H. V. (2013a). Harms and benefits associated with psychoactive drugs: findings of an international survey of active drug users. *Journal of Psychopharmacology*, *27*, 497–506.

Morgan, C. J., Das, R. K., Joye, A., Curran, H. V., & Kamboj, S. K. (2013b). Cannabidiol reduces cigarette consumption in tobacco smokers: preliminary findings. *Addictive Behaviors*, *38*, 2433–2436.

Niesink, R. J., & van Laar, M. W. (2013). Does cannabidiol protect against adverse psychological effects of THC? *Frontiers in Psychiatry*, *4*, 130.

Nutt, D. J., King, L. A., & Phillips, L. D. Independent Scientific Committee on Drugs. (2010). Drug harms in the UK: a multicriteria decision analysis. *Lancet*, *376*, 1558–1565.

Parker, L. A., Burton, P., Sorge, R. E., Yakiwchuk, C., & Mechoulam, R. (2004). Effect of low doses of Δ^9-tetrahydrocannabinol and cannabidiol on the extinction of cocaine-induced and amphetamine-induced conditioned place preference learning in rats. *Psychopharmacology*, *175*, 360–366.

Ren, Y., Whittard, J., Higuera-Matas, A., Morris, C. V., & Hurd, Y. L. (2009). Cannabidiol, a nonpsychotropic component of cannabis, inhibits cue-induced heroin seeking and normalizes discrete mesolimbic neuronal disturbances. *Journal of Neuroscience*, *29*, 14764–14769.

Robson, P. J. (2014). Therapeutic potential of cannabinoid medicines. *Drug Testing and Analysis*, *6*, 24–30.

Schier, A. R., Ribeiro, N. P., Silva, A. C., Hallak, J. E., Crippa, J. A., Nardi, A. E., & Zuardi, A. W. (2012). Cannabidiol, a Cannabis sativa constituent, as an anxiolytic drug. *Revista Brasileira de Psiquiatria*, *34*, S104–S110.

Stoops, W. W., & Rush, C. R. (2013). Agonist replacement for stimulant dependence: a review of clinical research. *Current Pharmaceutical Design*, *19*, 7026–7035.

United Nations Office on Drugs and Crime. (2014). *World drug report (2014)*. Vienna, Austria: United Nations.

van Amsterdam, J., Pennings, E., Brunt, T., & van den Brink, W. (2013). Physical harm due to chronic substance use. *Regulatory Toxicology and Pharmacology*, *66*, 83–87.

Zhornitsky, S., & Potvin, S. (2012). Cannabidiol in humans – the quest for therapeutic targets. *Pharmaceuticals*, *5*, 529–552.

Zuardi, A. W. (2008). Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. *Revista Brasileira de Psiquiatria*, *30*, 271–280.

CHAPTER

98

Cannabinoids and Effects on the Gastrointestinal Tract: A Focus on Motility

G. Vera[*,**], *J. Fichna*[†], *R. Abalo*[*,**]

*Area of Pharmacology and Nutrition, Faculty of Health Sciences, University Rey Juan Carlos, Alcorcón, Madrid, Spain

**Associated Unit I+D+i of the Institute of Medicinal Chemistry (IQM) and of the Institute of Research in Food Sciences (CIAL), Spanish National Research Council (CSIC), Madrid, Spain

†Department of Biochemistry, Faculty of Medicine, Medical University of Lodz, Lodz, Poland

SUMMARY POINTS

- This chapter focuses on the role of cannabinoids in gut motility.
- It describes the location of the ECS in the myenteric plexus and other structures innervating the gut.
- Cannabis receptor type 1 (CB1R) in the excitatory myenteric neurons reduce acetylcholine release, leading to a decrease in smooth muscle contractility and delayed gastrointestinal transit.
- Stimulation of cannabinoid receptors may alleviate chemotherapy-induced nausea and vomiting (CINV), gastroesophageal reflux disease (GERD), ulcerative colitis (UC), and Crohn disease (CD).
- Conversely, inactivation of CB1R receptors could be useful in the treatment of paralytic ileus.
- The major limitations of cannabinoids for the treatment of gastrointestinal disorders are their central side effects.
- Chronic cannabinoid use may induce cannabinoid hyperemesis syndrome.

KEY FACTS OF CINV

- CINV is the most feared cancer treatment-related side effect.
- Usually classified into three categories: acute onset, occurring within 24 h of the initial administration of chemotherapy; delayed onset, occurring 24 h to several days after initial treatment; and anticipatory nausea and vomiting, triggered by taste, odor, sight, thoughts, or anxiety secondary to a history of poor response to antiemetic agents.
- The introduction of 5-HT3 antagonists has controlled CINV in many patients.
- Conventional antiemetics are not as successful in treating nausea as they are in treating emesis.
- Cannabinoids are frequently used to prevent CINV.

KEY FACTS OF CANNABINOID HYPEREMESIS SYNDROME

- First reported in the Adelaide Hills of South Australia, by (Allen, de Moore, Heddle, & Twartz, 2004).
- Cyclic nausea and vomiting can be induced by the long-term and intense marijuana use.
- Synthetic cannabinoids may also induce it.
- Relief can be obtained with hot water bath, or cannabis cessation.
- Dysregulation of peripheral enteric nerves causing delayed gastric emptying could be implicated.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00114-9
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

CB1R	Cannabinoid receptor type 1
CB2R	Cannabinoid receptor type 2
CD	Crohn disease
CINV	Chemotherapy-induced nausea and vomiting
CNS	Central nervous system
DMV	Dorsal motor nucleus of the vagus
ECS	Endogenous cannabinoid (or endocannabinoid) system
ENS	Enteric nervous system
FAAH	Fatty acid amide hydrolase
GERD	Gastroesophageal reflux disease
GI	Gastrointestinal
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
LES	Lower esophageal sphincter
MAGL	Monoacylglycerol lipase
PPAR	Peroxisome proliferator-activated receptor
TLESRs	Transient lower esophageal sphincter relaxations
Δ^9-THC	Δ^9-Tetrahydrocannabinol
TRPV1	Transient receptor potential vanilloid type 1
UC	Ulcerative colitis

INTRODUCTION

Cannabis has been used empirically and traditionally to treat different disorders including those of the gut, ranging from enteric infections and inflammatory conditions to motility alterations, emesis, and abdominal pain (Izzo & Coutts, 2005). Cannabis is also the most widely used recreational drug in the Western world for its psychotropic properties, mainly exerted by its major component, Δ^9-Tetrahydrocannabinol (Δ^9-THC) (Di Carlo & Izzo, 2003). Other cannabinoids contained in cannabis, with fewer psychotropic actions, include cannabidiol, Δ^9-tetrahydocannabivarin, cannabichromene, cannabigerol, cannabidivarin, and acidic cannabinoids, such as, Δ^9-tetrahydrocannabinolic acid and cannabidiolic acid (Kumar, Chambers, & Pertwee, 2001).

All the components of the endogenous cannabinoid system (ECS), including the CB1 and CB2 receptors (CB1R and CB2R), "nonclassical" receptors, such as GPR55, peroxisome proliferator-activated receptors (PPAR) α and γ, and transient receptor potential vanilloid-1 (TRPV1) channels, endogenous ligands (anandamide, 2-arachidonoylglycerol, noladin ether), their biosynthetic and degradative enzymes [fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MAGL)], and a hypothetical membrane transporter have been investigated in the gut. Another Chapter 56 in this book (*Cannabis and Cannabinoids, and the Effects on Gastrointestinal Function: an Overview*) offers an overview of the different actions exerted by cannabinoids within the gastrointestinal (GI) tract. Here we will focus on their involvement exclusively in GI motor function.

THE ECS AND GUT INNERVATION CONTROLLING MOTILITY

The enteric nervous system (ENS) is the major site of action of cannabinoids in the gut. The ENS is composed of two major plexuses found within the gut wall: the myenteric plexus that lies between the external muscle layers (namely, the inner circular and the outer longitudinal layers), and the submucosal plexus that is located in the submucosa. The myenteric plexus is the main system responsible for control of GI motility. Coordinated GI motor patterns, such as peristalsis, do not need any inputs from the central nervous system (CNS), although these may additionally modify gut motility (Brookes & Costa, 2002).

The main myenteric neuronal populations are represented in Fig. 98.1. Immunohistochemical methods have allowed the identification of the myenteric neuronal populations immunoreactive for different components of the ECS within the ENS, as shown also in Fig. 98.1 for CB1R. Table 98.1 offers a summary of the immunohistochemical findings for CB1R, CB2R, TRPV1, and FAAH in the main myenteric neuronal populations. These data suggest that the excitatory component of peristalsis may be modified by cannabinoids, whereas the inhibitory component of the peristaltic reflex seems to lack the necessary receptors and enzymes (Kulkarni-Narla & Brown, 2000; Izzo & Sharkey, 2010).

The extrinsic innervation of the gut controlling motility is represented in Fig. 98.2. The GI tract from the esophagus to the proximal parts of the colon is innervated by parasympathetic vagal efferent fibers from the dorsal motor nucleus of the vagus (DMV) in the medulla oblongata. CB1R have been detected in the DMV, the medial, and gelatinous subnuclei of the nucleus tractus solitarius and the area postrema. CB1R in the nodose ganglion and DMV are involved in the control of GI motility (Vianna et al., 2012). Additionally, the efferent fibers from the pontine Barrington nucleus project to the sacral spinal cord and terminate in the region of the preganglionic pelvic nerve. This pelvic nerve is also parasympathetic and projects to the distal colon and rectum. In rats, activation of this pelvic nerve regulates distal colonic and rectal motility under physiological conditions (Suda, Setoyam, Nanno, Matsumoto, & Kawai, 2013). To our knowledge, the ECS in these pelvic efferents to the distal gut has not been studied.

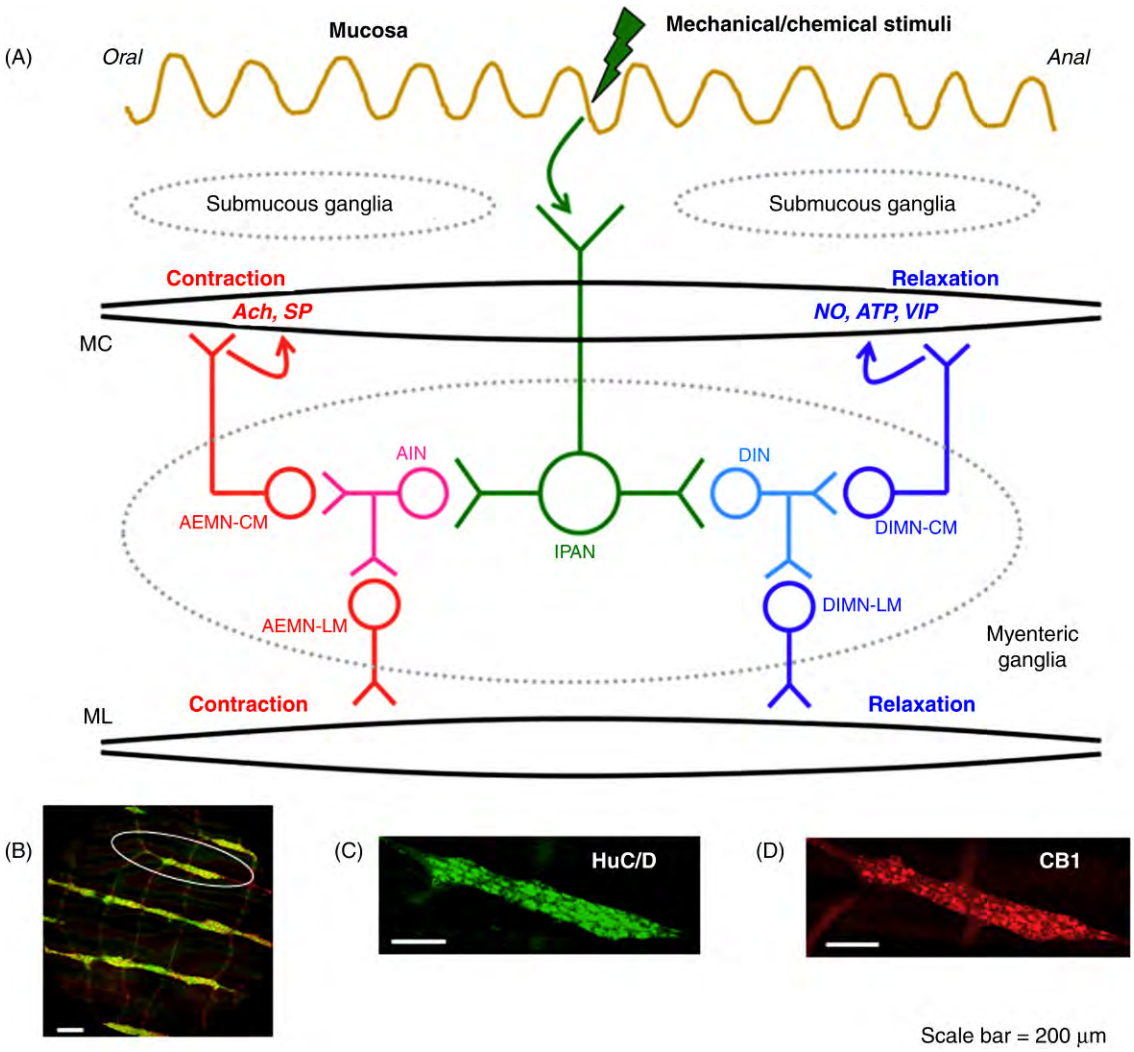


FIGURE 98.1 **Myenteric neurons and the endocannabinoid system.** (A) Schematic representation of the myenteric plexus and its main neural components involved in the intrinsic control of gastrointestinal motor function. Main myenteric neuronal populations are: intrinsic primary afferent neurons (IPAN) which detect mechanical (distension of the gut wall) and/or chemical stimuli acting upon the mucosa; ascending interneurons (AIN); ascending excitatory motor neurons to longitudinal and circular muscle (AEMN-LM and AEMN-CM, respectively), which induce contraction of the smooth muscle layers oral to the stimulus via the release of excitatory neurotransmitters (*Ach*, acetylcholine; *SP*, substance P); descending inhibitory interneurons (DIN); descending inhibitory motor neurons to longitudinal and circular muscle (DIMN-LM and DIMN-CM), which induce relaxation of the smooth muscle layers anal to the stimulus via the release of inhibitory neurotransmitters (*NO*, nitric oxide; *ATP*, adenosine triphosphate; *VIP*, vasoactive intestinal peptide). (B—D): Microphotographs of whole-mount preparations from guinea pig ileum processed immunohistochemically to show the neurons immunoreactive for the pan-neuronal marker HuC/D (C, *green*), the cannabinoid CB1 receptor (D, *red*) or both (B, *yellow*, due to merging of C and D). The myenteric ganglion encircled in B is shown in C and D. CB1R are present in most but not all myenteric neurons.

CANNABINOIDS AND GASTROINTESTINAL MOTILITY

Cannabinoid effects on GI motility have been reviewed previously (Abalo, Vera, López-Pérez, Martínez-Villaluenga & Martín-Fontelles, 2012; Izzo & Sharkey, 2010; Izzo & Coutts, 2005) and are summarized in Table 98.2.

Both central and peripheral cannabinoid receptors are likely to be involved in the regulation of GI motility. As shown in vitro for multiple species (mouse, rat, guinea pig, human) and different GI preparations, CB1R agonists inhibit GI muscle contraction via the presynaptic reduction of excitatory neurotransmitter release (acetylcholine, substance P) from the myenteric neurons (Roth, 1978; Abalo et al., 2012). Gastrointestinal CB1R appears to exert tonic control over ENS circuits, and operates as a "brake" for neural overreactivity (Schicho & Storr, 2011; Abalo et al., 2012). Furthermore, the selective GPR55 agonist O-1602 concentration-dependently

TABLE 98.1 Components of the Endocannabinoid System in the Main Myenteric Neuronal Populations Responsible for Control of Gastrointestinal Motor Function

Function	Chemical code	CB1R[a,b,c]	CB2R[d,e]	TRPV1[f,g]	FAAH[h]
Ascending excitatory motor neurons to circular muscle (AEMN-CM)	SP/ACh/±ENK/NFT	+	+	+	+
Ascending excitatory motor neurons to longitudinal muscle (AEMN-LM)	SP/ACh/CR	+	+	+	+
Descending Inhibitory motor neurons to circular muscle (DIMN-CM)	VIP/NOS/ATP/DYN/GRP/NFT	?	?	–	–
Descending inhibitory motor neurons to longitudinal muscle (DIMN-LM)	VIP/NOS	?	?	–	–
Ascending interneurons (AIN)	SP/ACh/ENK/NFT/CR	+	+	+	+
Descending interneurons (DIN)	5-HT/ChAT/NFT/SOM	+	+	–	+
Intrinsic primary afferent neurons (IPAN)	CALB/SP/ACh	+	+	+	+

For each myenteric neuronal population, its function, its characteristic chemical code (in the guinea pig ileum) and the presence of the main components of the endocannabinoid system are specified. ACh, Acetylcholine; ATP, adenosine triphosphate; CALB, calbindin; CB1R, CB2R, cannabinoid receptors 1 and 2; CR, calretinin; DYN, dynorphin; ENK, enkephalin; FAAH, fatty acid amidohydrolase; GRP, gastrin releasing peptide; 5-HT, serotonin; NFT, neurofilament triplet protein; NOS, nitric oxide synthase; SOM, somatostatin; SP, substance P; TRPV1, transient receptor potential vanilloid 1; VIP, vasoactive intestinal peptide.

[a] *Sibaev, A., Yuce, B., Kemmer, M., Van Nassauw, L., Broedl, U., Aiiescher, H. D., …, Storr, M. (2009) Cannabinoid-1 (CB1) receptors regulate colonic propfusion by acting at motor neurons within the ascending motor pathways in mouse colon.* American Journal of Physiological Gastrointestinal Liver Physiology, 296, *G119–G12B.*

[b] *Coutts, A. A., Irving, A. J., Mackie, K., Pertwee, R. G., Anavi-Goffer, S. (2002). Localisation of cannabinoid CB(1) receptor immunoreactivity in the guinea pig and rat myenteric plexus.* The Journal of Comparative Neurology, 448*(4), 410–422.*

[c] *Kulkarn˙-Nar˙aA, Brown, D. R. (2000). Localization of CBl-cannabinoid receptor immunoreactivity in the porcine enteric nervous system.* Cell Tissue Research, 302*(1), 73–80.*

[d] *Duncan, M.; Mouihate, A., Mackis, K., Keenan, C. M., Buckley, N. E., … , Davison, J. S. (2008). Cannabinoid CB2 receptors in the enteric nervous system modulate gastrointestinal contractility in lipopolysaccharide-treated rats.* American Journal of Physiology: Gastrointestinal and Liver Physiology, 295*(1), G73–GS7.*

[e] *Aviello, G., Romano, B., Izzo, A. A. (2008). Cannabinoids and gastrointestinal motility: animal and human studies.* European Review for Medical and Pharmacological Sciences., 12 *Suppl 1, 81–93.*

[f] *Anavi-Goffer, S., Coutts, A. A. (2003) Celfufar distribution of vani.foid VR1 receptor immunoreactivity rnthe guinea-p>g myenteric piexus. Eur J Pharmacol. 4-58, 61-71.*

[g] *Poonyachoti, S., Kulkarni-Narta. A., Brown, D. R. (2002). Chemical coding of neurons expressing delta- and kappa-opioid receptor and type I vanilloid receptor immunoreactivities in the porcine ileum.* Cell and Tissue Research, 307*(1), 23-33.*

[h] *Fichna, J., Sataga, M., Stuart, J., Saur, D., Sobczak, M., … , Storr, M. A. (2014). Selective inhibition of FAAH produces antidiarrheal and antinociceptive effect mediated by endocannabinoidsand cannabinoid-like fatty acid amides.* Neurogastroenterology and Motility, 26*(4), 47C–81.*

reduced evoked contractions in muscle strips from mouse colon and ileum. These effects were reversed by cannabidiol, but not by CB1R or CB2R antagonists (Li et al., 2013). Indirect elevation of endocannabinoids by pharmacological blockade of FAAH also inhibited contractility of the mouse colon (Fichna et al., 2014). In human colonic tissue in vitro, anandamide and 2-arachidonoylglycerol suppressed cholinergic contractility via a noncannabinoid receptor-mediated pathway, suggesting that cholinergic contraction may be tonically modulated by endocannabinoids and/or other products of arachidonate metabolism (Smid, Bjorklund, Svensson, Heigis, & Revesz, 2007).

In vivo, synthetic and natural CB1R agonists decrease intragastric pressure and inhibit gastric emptying, pyloric contraction, and intestinal motility (Izzo & Coutts, 2005; Abalo et al., 2012). In humans, Δ^9-THC significantly reduced gastric emptying of solid food (McCallum et al., 1999) and dronabinol decreased postprandial colonic tone and increased compliance (Esfandyari et al., 2007), but did not affect colonic transit (Esfandyari et al., 2006).

More recently, Abalo and colleagues (Abalo et al., 2009, Abalo, Cabezos, Vera, Fernández-Pujol, & Martin, 2010) analyzed the in vivo effects of the nonselective cannabinoid agonist WIN55,212-2 in the rat, using radiographic techniques. WIN55,212-2 dose-dependently reduced motility throughout the gut and this effect was sensitive to CB1R blockade (Abalo et al., 2009, 2010a). However, at doses capable of delaying gastric emptying (2–5 mg/kg), higher than those already capable of reducing intestinal transit (0.5–1 mg/kg), WIN55,212-2 induced significant central effects. These were not found after administration of the selective CB1R agonist AM841 at a dose also capable of delaying gastric emptying, suggesting that central CB1R are not essential to alter GI motility (Abalo et al., 2010b). Some effects of natural and synthetic cannabinoid agonists are nonetheless mediated throughout CNS-located receptors. These include, for example, contractions of the lower esophageal sphincter (LES) (Izzo & Sharkey, 2010).

CB1R antagonists increased motility, suggesting that GI motility is under tonic suppression by the ECS (Izzo & Sharkey, 2010). Moreover, it has been shown that

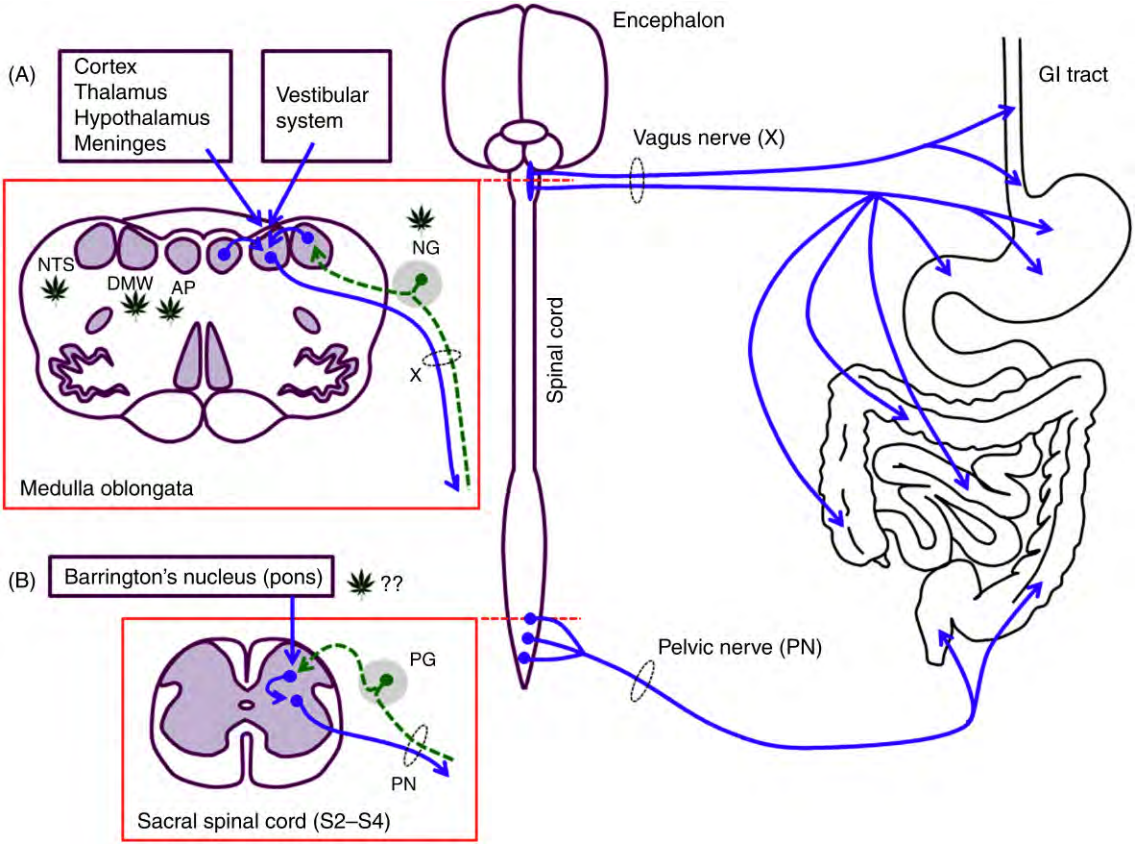


FIGURE 98.2 **Extrinsic neural control of gastrointestinal motor function and the endocannabinoid system.** Two main parasympathetic pathways control gastrointestinal motility: the vagus nerve (X cranial nerve) arises from the dorsal motor nucleus of the vagus (DMV) in the medulla oblongata, and innervates the stomach, small intestine, and proximal parts of the large intestine; the pelvic nerve (PN) arises from the sacral segments S2–S4 of the spinal cord, and innervates distal colon and rectum. (A) cross section of the medulla oblongata: the DMV receives inputs from other structures of the dorsal vagal complex, namely, the nucleus of the tractus solitarius (NTS, which receives inputs from the viscera, including the GI tract, via the nodose ganglion, NG) and area postrema (AP or chemoreceptor trigger zone, which detects emetogenic stimuli in the blood), as well as the vestibular system and other encephalic structures whose stimulation may induce an emetic reflex. CB1R have been demonstrated immunohistochemically in NTS, DMV, AP, and NG (marijuana leave symbol). (B) Cross section of the sacral spinal cord: the preganglionic fiber of the pelvic nerve receives inputs from colonic afferents with their somata in pelvic ganglia (PG), and also from the pontine Barrington nucleus. The involvement of the endocannabinoid system in this pathway has not been deeply investigated. *Blue lines*: parasympathetic efferents to the gut. *Green dotted lines*: parasympathetic afferents from the gut.

pharmacological blockade of FAAH delays whole GI transit and colonic propulsion in mice, and these effects are reversed by selective CB1R antagonists, suggesting the involvement of endogenous CB1R ligands in this effect (Fichna et al., 2014). The clinical trials using the CB1R antagonists rimonabant and taranabant, in patients with metabolic disorders and obesity, showed that diarrhea and other GI motor side effects like nausea and vomiting are observed frequently, though often transient (Ruilope et al., 2008; Despres, Golay & Sjostrom, 2005; Addy et al., 2008). From these trials, it seems that GI motility in humans is also under control of the ECS.

Besides CB1R, other "classical" and "nonclassical" cannabinoid receptors can regulate GI motility. Interestingly, endocannabinoids stimulate intestinal primary sensory neurons via TRPV1, resulting in enteritis and enhanced motility (Izzo & Coutts, 2005). In fact, exogenous and endogenous cannabinoids have an important role in GI inflammation (Izzo & Camilleri, 2009). On the other hand, CB2R stimulation may regulate motility in pathophysiologic conditions (Li et al., 2010). Other cannabinoid ligands also modulate gut motility in vivo. *N*-palmitoylethanolamine administered peripherally inhibited upper GI transit in mice, and this effect was not blocked by CB1R or CB2R antagonists (Capasso et al., 2001). The selective GPR55 agonist, O-1602, also decelerated whole gut transit. Interestingly, activation of GPR55 receptors was not associated with central sedation; thus it could be useful as a future target for the treatment of colonic motility disorders (Li et al., 2013).

Surprisingly, the effects of chronic administration of cannabinoid agonists on GI motor function have deserved relatively little attention. This is important, since cannabinoids are being proposed for the treatment of

TABLE 98.2 Effects of Drugs Targeting the Endocannabinoid System on Alterations in Gastrointestinal Motor Function

Pathology	↓CB tone	↑CB tone
GERD		*CB1 agonists* reduce transient lower esophageal sphincter relaxation in dogs and humans
Gastroparesis	*CB1 antagonists* increase gastric emptying in mice	*Δ^9-THC* reduces gastric emptying of solid food in humans *WIN 55,212-2* and other cannabinoid agonists reduce gastric emptying in rodents
Emesis	*CB1 antagonists* induce vomiting in humans, and conditioned taste avoidance and gaping in the taste reactivity test in rats	*CB1 and CB2 agonists, FAAH blockers,* reuptake inhibitors and MAGL inhibitors reduce emesis in animal models. *2-AG* decreases nausea-like behavior in rats *Nabilone, dronabinol and Sativex* are used as antiemetics in humans, intense use of *Cannabis* induces hyperemesis
Diarrhea	The *CB1 antagonists rimonabant-* and *taranabant-*induced diarrhea as a frequent side effect in clinical trials	*CBl and CB2 agonists, FAAH blockers* and *reuptake inhibitors* reduce gastrointestinal transit and hypermotility in mice and rats.
Constipation	*CB1 antagonists* increase gastrointestinal transit in mice	*Dronabinol* decreases postprandial colonic tone and increases compliance in humans
Ileus	*CB1 and CB2 antagonists* increase gastrointestinal transit in septic ileus in animal models *CBD* was also beneficial in a model of ileus in mice	Increased release of *endocannabinoids* is involved in development of ileus
IBS		*CB1 and CB2 agonists* reduce visceral pain, secretion, hypermotility and diarrhea in animal models *PEA* may decrease intestinal transit via CB1R and TRPVl *Δ^9-THC* delays gastric emptying in humans *Dronabinol* may reduce fasting colonic motility in patients with IBS-D or with IBS-A
IBD		*CB1 and CB2 agonists* reduce intestinal inflammation and visceral pain in mice and humans *FAAH blockers* and *reuptake inhibitors* reduce gastrointestinal inflammation in mice *Activation of QPR55 receptors* reduces colonic motility *Cannabis* use is common in patients with Crohn disease and subjectively improves pain and diarrheal symptoms

The increase or decrease in cannabinoid tone exerts the pharmacological effects indicated in the different motility alterations. See text for references. 2-AG, 2-Arachidonoylglycerol; CBD, cannabidiol; CB1R, cannabinoid receptor type 1; CB2R, cannabinoid receptor type 2; FAAH, fatty acid amide hydrolase; GERD, gastroesophageal reflux disease; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IBS-D, IBS with diarrhea; IBS-A, IBS alternating diarrhea and constipation; MAGL, monoacylglycerol lipase; PEA, palmitoylethanolamide; Δ^9-THC, Δ^9-tetrahydrocannabinol; TRPV1, transient receptor potential vanilloid 1.

different chronic diseases (including those affecting the gut), and also because of the high prevalence of chronic cannabinoid abuse. The effect of different patterns of repeated administration of WIN55,212-2 has been investigated radiographically in the rat. Upon daily administration, tolerance developed to the effect of WIN55,212-2 in the intestine, but gastric emptying was still delayed after the 14th systemic administration of the drug (Abalo et al., 2009). Interestingly, upon intermittent (weekly) administration, the gastric effect of WIN55,212-2 was enhanced (Abalo et al., 2011). CB1R were involved in both cases, but an additional, not yet identified receptor could also be implicated in WIN55,212-2-induced delayed gastric emptying upon weekly administration. In both studies, GI motility was normal again 1 week after the end of treatment, suggesting that the effect of cannabinoids on GI motility might not be long-lasting.

Finally, although generally considered efficient antiemetics (see later), cannabinoids (natural, such as Δ^9-THC; or synthetic, such as K2; Ukaigwe, Karmacharya & Donato, 2014) may induce paroxysmal vomiting or cannabinoid hyperemesis syndrome (CHS), first described by Allen et al. (2004). CHS is characterized by a striking pattern of cyclic nausea and vomiting among long-term marijuana users, which can be relieved by compulsive hot water bathing. Other symptoms include abdominal pain. CHS resolves with cannabis cessation, but recurs when patients resume the use of cannabinoids after hospital discharge (Simonetto, Oxentenko, Herman & Szostek, 2012). Different mechanisms have been suggested to contribute, such as central effects of long-term cannabis use on the hypothalamic-pituitary-adrenal axis, but also dysregulation of peripheral enteric nerves causing delayed gastric emptying and abdominal pain

(Simonetto et al., 2012; Izzo and Sharkey, 2010). Our results showing persistent or even aggravated gastric dysmotility upon chronic cannabinoid administration in the rat support this hypothesis.

POTENTIAL THERAPEUTIC APPLICATIONS OF CANNABINOIDS

Pharmacological manipulation of the ECS could have important applications in the treatment of GI motility alterations, such as, emesis, gastroesophageal reflux, paralytic ileus, or diarrhea.

Gastroesophageal Reflux Disease (GERD)

GERD is a common disorder with symptoms like heartburn, regurgitation, and retrosternal pain (Heading, 1999), resulting from prolonged acid exposure of the esophageal mucosa. The LES regulates the flow across the gastroesophageal junction by generating a tonic pressure to prevent reflux of gastric contents into the esophagus. Transient lower esophageal sphincter relaxations (TLESRs) are the main mechanism underlying gastroesophageal reflux and a potential pharmacological treatment target (Holloway, 2000). CB1R have been located in brain areas involved in the triggering of TLESRs in the ferret (Van Sickle et al., 2001) and the cannabinoid agonist WIN55,212-2 was shown to reduce the occurrence of TLESRs in dogs (Lehmann et al., 2002). Also, Δ^9-THC significantly reduced basal LES in humans (Beaumont et al., 2009). GERD and esophageal motility disorders occur in obesity; these alterations could be secondary to reduced endocannabinoids or CB1R expression (Mushref & Srinivasan, 2013).

Nausea and Emesis

Nausea and vomiting is an integral defense mechanism against toxin ingestion, but is an unpleasant side effect associated with medications (opiates, chemotherapeutics, interferon…). The introduction of 5-HT3 antagonists, together with the corticoid dexamethasone and aprepitant (a neurokinin 1 receptor antagonist), has largely controlled chemotherapy-induced nausea and vomiting (CINV). However, these conventional antiemetics are not as successful in treating nausea as they are in treating emesis (Abalo et al., 2012). *Cannabis* has been used for several centuries for the attenuation of nausea and vomiting. Moreover, cannabinoids were found to inhibit emesis in animal models (ferrets; least shrews, *Cryptotis parva*; house musk shrews, *Suncus murinus*…) of acute cisplatin-, morphine- and radiation-induced emesis, as well as in cisplatin-induced delayed emesis, and in motion sickness (Izzo & Sharkey, 2010; Parker, Rock, & Limebeer, 2011). Also, the FAAH inhibitor URB597 suppressed cisplatin- and nicotine-induced vomiting in the house musk shrew through CB1R (Parker et al., 2009).

Since rodents and mice lack the vomiting reflex, it is necessary to use other markers of nausea and emesis in order to evaluate the antiemetic potential of drug therapies in these species. There are two possibilities: first, conditioned taste avoidance and conditioned gaping, which are disgusting reactions to flavors or foods paired with illness; and pica, which is the consumption of nonnutritive substances (eg, kaolin clay) in response to nausea-inducing agents, a behavior which may represent an adaptive response to reduce the adverse effects of toxins in the organism. In addition, delayed gastric emptying is temporally related to pica induced by emetogenic stimuli in rodents (Cabezos et al., 2008; Cabezos, Vera, Martín-Fontelles, Fernández-Pujol, & Abalo, 2010). In rats, taste avoidance and gaping are suppressed by pretreatment with Δ^9-THC, HU-210, the FAAH blocker URB597, and cannabidiol (review in Abalo et al., 2012). However, the synthetic cannabinoid WIN55,212-2, administered in a weekly fashion together with the antineoplastic drug cisplatin in the rat at doses that prevented the development of neuropathic signs, was not able to reduce pica, anorexia or delayed gastric emptying, and small intestinal motility was even further delayed (Vera et al., 2007; Abalo, Cabezos, Vera, López-Pérez & Martín, 2013; see Fig. 98.3). This suggests that, although cannabinoids are used in humans to prevent CINV, gastric dysmotility is still likely to be present and may be aggravated when cannabinoids and antineoplastic drugs are combined.

In humans, nabilone (Cesamet), a synthetic analog of Δ^9-THC, was specifically licensed for the suppression of CNIV. Furthermore, the synthetic Δ^9-THC dronabinol is used as an antiemetic and an appetite stimulant (Pertwee, 2009). Dronabinol was found to be superior to neuroleptics (Machado Rocha, Stéfano, De Cássia Haiek, Rosa Oliveira, & Da Silveira, 2008) and similarly effective to ondansetron (Meiri et al., 2007) for the treatment of CINV. Unfortunately, the clinical usefulness of these cannabinoid agonists is restricted due to psychotropic side effects. A logical step is thus to examine the action of nonpsychotropic cannabinoids, such as cannabidiol. Sativex, a mixture of cannabidiol and Δ^9-THC, was effective in preventing delayed CINV in a phase II trial; unfortunately, one patient withdrew due to neuropsychiatric side effects (Duran et al., 2010).

Overall, the data suggest that—although cannabinoids are effective antiemetics—current formulations have unacceptable side effects.

Intestinal Inflammatory Conditions

This section includes irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), which have

similar symptoms, although their pathogenesis and treatment could differ.

IBS is a functional disorder characterized by abdominal discomfort, bloating, and altered bowel habits, including alterations in gut motility, visceral hypersensitivity, and bacterial overgrowth. IBS presents in three different forms: diarrhea-predominant (D-IBS), constipation-predominant (C-IBS), or alternating (A-IBS; episodes of either diarrhea or constipation). Due to their effects on motility and secretion, CB1 agonists may be useful to treat D-IBS, whereas CB1 antagonists could be useful to treat C-IBS. Activation of CB2R, which are overexpressed in the gut under inflammatory conditions, may also be used to treat D-IBS.

IBD includes Crohn disease (CD), ulcerative colitis (UC), and much less frequent diseases, for example, nonspecific colitis, collagenous colitis, and eosinophilic colitis. Therapeutic strategies target the underlying inflammatory process, but some patients continue to have chronic diarrhea. Experiments on human intestinal biopsies revealed increased expression of cannabinoid receptors and/or enhanced endocannabinoid levels in patients with UC and CD (Izzo & Camilleri, 2009). Many in vivo studies in various animal models of IBD demonstrated that administration of CB1 and/or CB2 agonists improved colitis (for review Izzo & Sharkey, 2010; Sałaga, Sobczak, & Fichna, 2013). Clinical trials have demonstrated that Δ^9-THC may be useful in the treatment of CD (Naftali et al., 2013). Anecdotal reports suggest that some UC and CD patients experience relief when smoking marijuana (Di Marzo & Izzo, 2006); accordingly, *Cannabis* use is common amongst patients with IBD for symptom relief (Lal et al., 2011). In contrast, in a recent study in CD patients, *Cannabis* use was associated with higher risk of surgery (Storr, Devlin, Kaplan, Panaccione & Andrews, 2014).

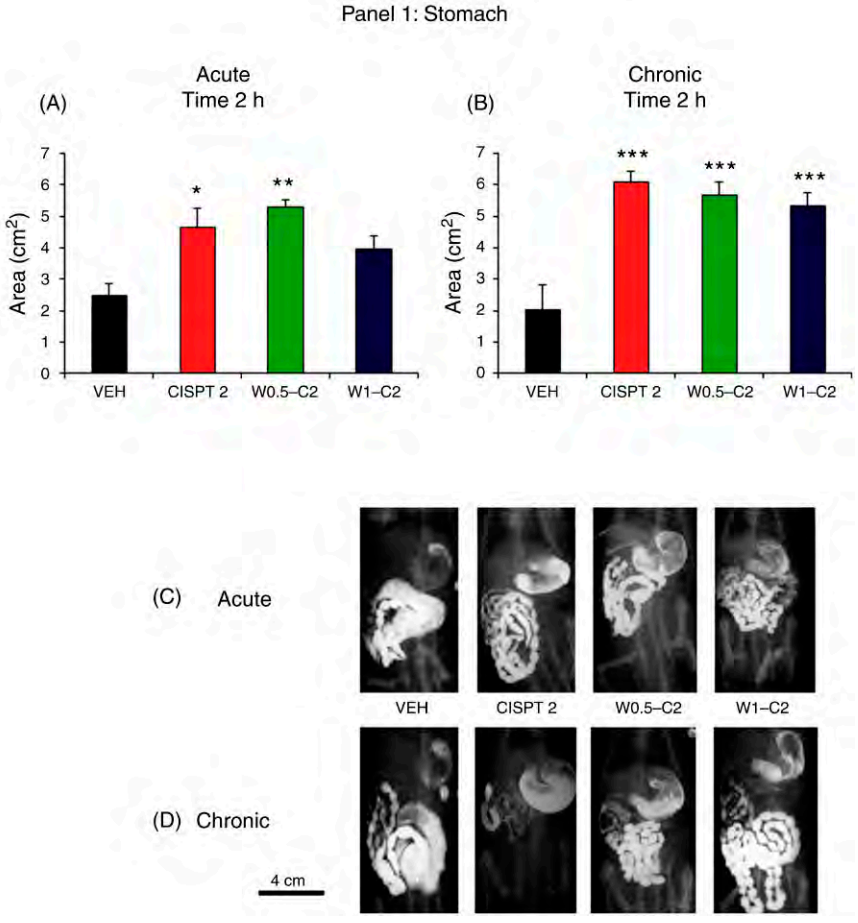


FIGURE 98.3 Effect of acute and chronic administration of the cannabinoid agonist WIN55,212-2 on gastrointestinal motor function in cisplatin-treated rats. Gastrointestinal motor function was analyzed using radiological methods. Stomach (Panel 1) and cecum (Panel 2) size were measured after contrast administration in rats treated once a week for 4 weeks with WIN55,212-2 (W; 0.5 or 1 mg/kg) or its vehicle, together with cisplatin (CISPT, 2 mg/kg) or saline. Barium sulfate was intragastrically administered immediately after the first (acute effect: A, C) or the last administration (chronic effect: B, D) and X-rays of the GI tract were obtained. No anesthesia was applied to avoid GI motility alterations. X-rays were digitized and morphometrically analyzed using an image processor (Image J; NIH). (A and B) Data represent mean ± SEM for images obtained 2 (Panel 1) or 4 (Panel 2) h after contrast administration. $N \geq 8$, each group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus vehicle (one-way ANOVA). (C and D) Representative X-rays obtained 2 (Panel 1) or 4 (Panel 2) h after contrast. Scale bar: 4 cm. Unpublished.

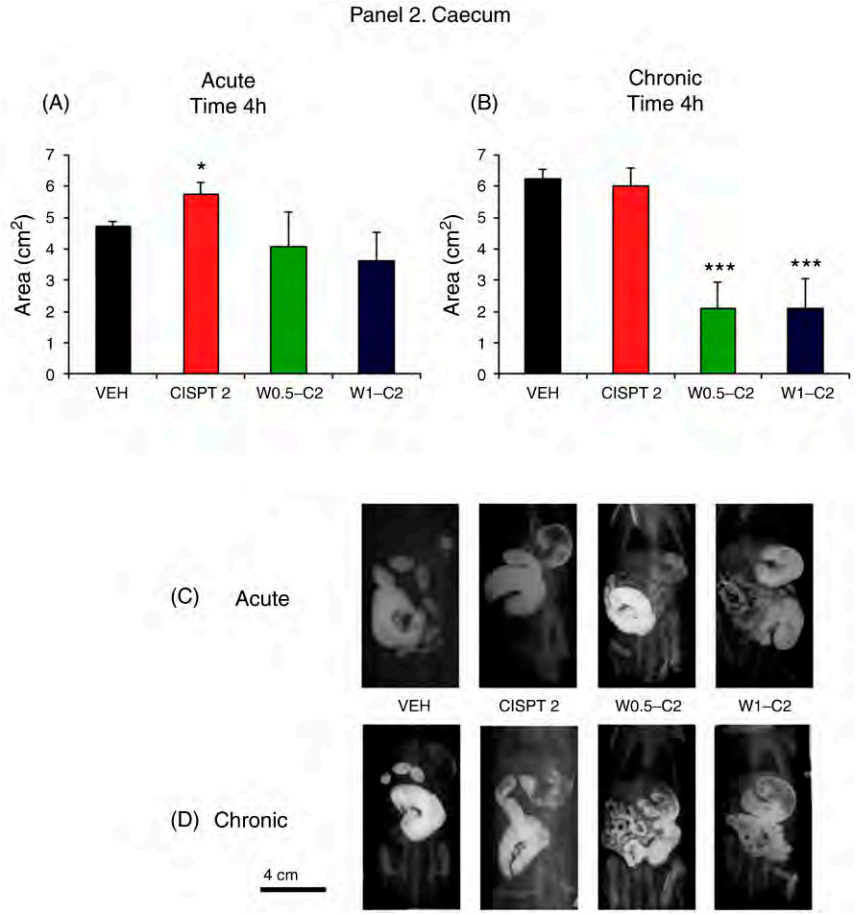


FIGURE 98.3 (*cont.*)

These inflammatory pathologies and ECS involvement have been more deeply reviewed in the chapter *Cannabis and Cannabinoids, and the Effects on Gastrointestinal Function: An Overview*.

Paralytic Ileus and Slow Transit Constipation

Paralytic ileus is defined as a long-lasting inhibition of GI motility in response to nociception initiated at the abdominal level. It may occur following peritonitis, surgery, or other noxious situations. Patients with paralytic ileus accumulate gas and secretions, leading to bloating, distension, emesis, and visceral pain (Di Carlo & Izzo, 2003). The intraperitoneal administration of acetic acid in mice, which mimics paralytic ileus, reduced intestinal motility and this occurred together with an increase in anandamide levels and overexpression of CB1R in the myenteric plexus; these effects were alleviated by rimonabant, but not by CB2R inactivation (Mascolo et al., 2002). In contrast, inactivation of either CB1R or CB2R led to normalization of motility in a model of septic ileus in the rat (Li et al., 2010). Cannabidiol was also beneficial in a mouse model of ileus (Lin et al., 2011), possibly via a reduction in nitric oxide production. In animal models, FAAH activity was significantly decreased (Camilleri & Katzka, 2012). In patients with constipation, the amounts of anandamide, 2-arachidonoylglycerol, and *N*-palmitoylethanolamine, which are negatively correlated with enzyme activity, were significantly higher in the constipation group (Zhang et al., 2014).

Thus the release of endogenous cannabinoids may be involved in the development of paralytic ileus and different strategies aimed at normalizing endocannabinoid levels could be therapeutically useful in these conditions.

CONCLUSIONS

The ECS within the gut is an important system involved in controlling motility. It is present both in the intrinsic and the extrinsic innervation of the GI system. Cannabinoid agonists and antagonists, as well as enzymes involved in the synthesis and degradation of the endocannabinoid ligands, have been demonstrated to exert prominent effects that could be useful to treat nausea/emesis, gastroesophageal reflux, paralytic ileus or diarrhea in different circumstances. Future studies should include strategies for reducing or abolishing the

adverse effects of cannabinoids and cannabinoid-related compounds without attenuating their beneficial therapeutic effects.

MINI-DICTIONARY

Area postrema Chemoreceptor trigger zone, lies outside the blood-brain barrier and detects chemical emetogenic stimuli in the blood.

Cannabinoid hyperemesis syndrome A disorder characterized by recurrent nausea, vomiting and abdominal pain; associated with chronic cannabis use in humans.

Chemotherapy-induced nausea and vomiting A common side effect of many cancer treatments.

Dorsal motor nucleus of the vagus It serves vagal fibers to the gastrointestinal tract, lungs, and other thoracic and abdominal viscera; related to the gastrointestinal tract, it receives inputs from different structures (including those detecting emetogenic stimuli) and innervates the stomach, small intestine, and proximal large intestine.

Dorsal vagal complex Group of nuclei in the medulla oblongata including the nucleus of the tractus solitarius, dorsal motor nucleus of the vagus, and area postrema.

Enteric nervous system One of the divisions of the peripheral nervous system that governs the function of the gastrointestinal system.

Gastroesophageal reflux disease A common disorder resulting from prolonged acid exposure of the esophageal mucosa due to increased relaxation of the lower esophageal sphincter.

Gastroparesis Delayed gastric emptying, a medical condition resulting in food remaining in the stomach for an abnormally long time.

Myenteric plexus Part of the ENS that controls gastrointestinal motility; it is located between the longitudinal and circular muscle layers in the gut.

Nucleus of the tractus solitarius It receives sensory information from the viscera, including the GI tract.

Paralytic ileus Disruption of the normal intestinal motility following surgery or noxious situations.

Pica The consumption of nonnutritive substances (eg, kaolin-clay) in response to nausea-inducing agents, typical in nonvomiting species like rodents.

References

Abalo, R., Cabezos, P. A., López-Miranda, V., Vera, G., González, C., Castillo, M., Fernández-Pujol, R., & Martín, M. I. (2009). Selective lack of tolerance to delayed gastric emptying after daily administration of WIN 55, 212-2 in the rat. *Neurogastroenterology and Motility*, *21*, 1002–1080.

Abalo, R., Cabezos, P. A., Vera, G., Fernández-Pujol, R., & Martín, M. I. (2010a). The cannabinoid antagonist SR 144528 enhances the acute effect of WIN 55, 212-2 on gastrointestinal motility in the rat. *Neurogastroenterology and Motility*, *22*, 694–e206.

Abalo, R., Chen, C., Vera, G., Castillo, M., Fichna, J., Thakur, G. A., Makriyannis, A., Martín, M. I., & Storr, M. (2010b). The cannabinoid-1 (CB1) agonist AM841 slows GI motility in the rat by peripheral actions. *Neurogastroenterology and Motility*, *22*, 14.

Abalo, R., Cabezos, P. A., Vera, G., López-Miranda, V., Herradón, E., & Martín-Fontelles, M. I. (2011). Cannabinoid-induced delayed gastric emptying is selectively increased upon intermittent administration in the rat: role of CB1 receptors. *Neurogastroenterology and Motility*, *23*, 457–467, e177.

Abalo, R., Vera, G., López-Pérez, A. E., Martínez-Villaluenga, M., & Martín-Fontelles, M. I. (2012). The gastrointestinal pharmacology of cannabinoids: focus on motility. *Pharmacology*, *90*, 1–10.

Abalo, R., Cabezos, P. A., Vera, G., López-Pérez, A. E., & Martín, M. I. (2013). Cannabinoids may worsen gastric dysmotility induced by chronic cisplatin in the rat. *Neurogastroenterology and Motility*, *25*, 373–382, e292.

Addy, C., Rothenberg, P., Li, S., Majumdar, A., Agrawal, N., Li, H., Zhong, L., Yuan, J., Maes, A., Dunbar, S., Cote, J., Rosko, K., Van Dyck, K., De Lepeleire, I., de Hoon, J., Van Hecken, A., Depré, M., Knops, A., Gottesdiener, K., Stoch, A., & Wagner, J. (2008). Multiple-dose pharmacokinetics, pharmacodynamics, and safety of taranabant, a novel selective cannabinoid-1 receptor inverse agonist, in healthy male volunteers. *Journal of Clinical Pharmacology*, *48*, 734–744.

Allen, J. H., de Moore, G. M., Heddle, R., & Twartz, J. C. (2004). Cannabinoid hyperemesis: cyclical hyperemesis in association with chronic cannabis abuse. *Gut*, *53*, 1566–1570.

Beaumont, H., Jensen, J., Carlsson, A., Ruth, M., Lehmann, A., & Boeckxstaens, G. (2009). Effect of delta-9-tetrahydrocannabinol, a cannabinoid receptor agonist, on the triggering of transient lower oesophageal sphincter relaxations in dogs and humans. *British Journal of Pharmacology*, *156*, 153–162.

Brookes, S., & Costa, M. (2002). *Innervation of the gastrointestinal tract*. London and New York: Taylor & Francis.

Cabezos, P. A., Vera, G., Castillo, M., Fernández-Pujol, R., Martín, M. I., & Abalo, R. (2008). Radiological study of gastrointestinal motor activity after acute cisplatin in the rat. Temporal relationship with pica. *Autonomic Neuroscience*, *141*, 54–65.

Cabezos, P. A., Vera, G., Martín-Fontelles, M. I., Fernández-Pujol, R., & Abalo, R. (2010). Cisplatin-induced gastrointestinal dysmotility is aggravated after chronic administration in the rat. Comparison with pica. *Neurogastroenterology and Motility*, *22*, 797–805, e224–e225.

Camilleri, M., & Katzka, D. A. (2012). Irritable bowel syndrome: methods, mechanisms, and pathophysiology. Genetic epidemiology and pharmacogenetics in irritable bowel syndrome. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *302*, G1075–G1084.

Capasso, R., Izzo, A. A., Fezza, F., Pinto, A., Capasso, F., Mascolo, N., & Di Marzo, V. (2001). Inhibitory effect of palmitoylethanolamide on gastrointestinal motility in mice. *British Journal of Pharmacology*, *134*, 945–950.

Despres, J. P., Golay, A., & Sjostrom, L. (2005). Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *New England Journal of Medicine*, *353*, 2121–2134.

Di Carlo, G., & Izzo, A. A. (2003). Cannabinoids for gastrointestinal diseases: potential therapeutic applications. *Expert Opinion on Investigational Drugs*, *12*, 39–49.

Di Marzo, V., & Izzo, A. A. (2006). Endocannabinoid overactivity and intestinal inflammation. *Gut*, *55*, 1373–1376.

Duran, M., Pérez, E., Abanades, S., Vidal, X., Saura, C., Majem, M., Arriola, E., Rabanal, M., Pastor, A., Farré, M., Rams, N., Laporte, J. R., & Capellà, D. (2010). Preliminary efficacy and safety of an oromucosal standardized cannabis extract in chemotherapy-induced nausea and vomiting. *British Journal of Clinical Pharmacology*, *70*, 656–663.

Esfandyari, T., Camilleri, M., Ferber, I., Burton, D., Baxter, K., & Zinsmeister, A. R. (2006). Effect of a cannabinoid agonist on GI transit and postprandial satiation in healthy human subjects: a randomized, placebo-controlled study. *Neurogastroenterology and Motility*, *18*, 831–838.

Esfandyari, T., Camilleri, M., Busciglio, I., Burton, D., Baxter, K., & Zinsmeister, A. R. (2007). Effects of a cannabinoid receptor agonist on colonic motor and sensory functions in humans: a randomized, placebo-controlled study. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, *293*, G137–G145.

Fichna, J., Sałaga, M., Stuart, J., Saur, D., Sobczak, M., Zatorski, H., Timmermans, J. P., Bradshaw, H. B., Ahn, K., & Storr, M. A. (2014).

Selective inhibition of FAAH produces antidiarrheal and antinociceptive effect mediated by endocannabinoids and cannabinoid-like fatty acid amides. *Neurogastroenterology and Motility*, *26*, 470–481.
Heading, R. C. (1999). Aims in the management of gastroesophageal reflux disease: a gastroenterologist's viewpoint. *Diseases of the Esophagus*, *12*, 1–6.
Holloway, R. H. (2000). Pathophysiology of gastro-oesophageal reflux: recent advances and therapeutic implications. *Digestive and Liver Disease*, *32*, S235–S238.
Izzo, A. A., & Camilleri, M. (2009). CB in intestinal inflammation and cancer. *Pharmacological Research*, *60*, 117–125.
Izzo, A. A., & Coutts, A. A. (2005). Cannabinoids and the digestive tract. *Handbook of Experimental Pharmacology*, *168*, 573–598.
Izzo, A. A., & Sharkey, K. A. (2010). Cannabinoids and the gut: new developments and emerging concepts. *Pharmacology and Therapeutics*, *126*, 21–38.
Kulkarni-Narla, A., & Brown, D. R. (2007). Localization of CB1-cannabinoid receptor immunoreactivity in the porcine enteric nervous system. *Cell and Tissue Research*, *302*, 73–80.
Kumar, R. N., Chambers, W. A., & Pertwee, R. G. (2001). Pharmacological actions and therapeutic uses of cannabis and cannabinoids. *Anaesthesia*, *56*, 1059–1068.
Lal, S., Prasad, N., Ryan, M., Tangri, S., Silverberg, M. S., Gordon, A., & Steinhart, H. (2011). Cannabis use amongst patients with inflammatory bowel disease. *European Journal of Gastroenterology and Hepatology*, *23*, 891–896.
Lehmann, A., Blackshaw, L. A., Brändén, L., Carlsson, A., Jensen, J., Nygren, E., & Smid, S. D. (2002). Cannabinoid receptor agonism inhibits transient lower esophageal sphincter relaxations and reflux in dogs. *Gastroenterology*, *12*, 1129–1134.
Li, Y. Y., Li, Y. N., Ni, J. B., Chen, C. J., Lv, S., Chai, S. Y., Wu, R. H., Yüce, B., & Storr, M. (2010). Involvement of cannabinoid-1 and cannabinoid-2 receptors in septic ileus. *Neurogastroenterology and Motility*, *22*, 350–388.
Li, K., Fichna, J., Schicho, R., Saur, D., Bashashati, M., Mackie, K., Li, Y., Zimmer, A., Göke, B., Sharkey, K. A., & Storr, M. (2013). A role for O-1602 and G protein-coupled receptor GPR55 in the control of colonic motility in mice. *Neuropharmacology*, *71*, 255–263.
Lin, X. H., Yüce, B., Li, Y. Y., Feng, Y. J., Feng, J. Y., Yu, L. Y., Li, K., Li, Y. N., & Storr, M. (2011). A novel CB receptor GPR55 and its ligands are involved in regulation of gut movement in rodents. *Neurogastroenterology and Motility*, *23*, 862–e342.
Machado Rocha, F. C., Stéfano, S. C., De Cássia Haiek, R., Rosa Oliveira, L. M., & Da Silveira, D. X. (2008). Therapeutic use of Cannabis sativa on chemotherapy-induced nausea and vomiting among cancer patients: systematic review and meta-analysis. *European Journal of Cancer Care*, *17*, 431–443.
Mascolo, N., Izzo, A. A., Ligresti, A., Costagliola, A., Pinto, L., Cascio, M. G., Maffia, P., Cecio, A., Capasso, F., & Di Marzo, V. (2002). The endocannabinoid system and the molecular basis of paralytic ileus in mice. *FASEB Journal*, *16*, 1973–1975.
McCallum, R. W., Soykan, I., Sridhar, K. R., Ricci, D. A., Lange, R. C., & Plankey, M. W. (1999). Delta-9-tetrahydrocannabinol delays the gastric emptying of solid food in humans: a double-blind, randomised study. *Alimentary Pharmacology and Therapeutics*, *13*, 77–80.
Meiri, E., Jhangiani, H., Vredenburgh, J. J., Barbato, L. M., Carter, F. J., Yang, H. M., & Baranowski, V. (2007). Efficacy of dronabinol alone and in combination with ondansetron versus ondansetron alone for delayed chemotherapy-induced nausea and vomiting. *Current Medical Research and Opinion*, *23*, 533–543.
Mushref, M. A., & Srinivasan, S. (2013). Effect of high fat-diet and obesity on gastrointestinal motility. *Annals of Translational Medicine*, *1*, 14.
Naftali, T., Bar-Lev Schleider, L., Dotan, I., Lansky, E. P., Sklerovsky Benjaminov, F., & Konikoff, F. M. (2013). Cannabis induces a clinical response in patients with Crohn's disease: a prospective placebo-controlled study. *Clinical Gastroenterology and Hepatology*, *11*, 1276–1280.
Parker, L. A., Limebeer, C. L., Rock, E. M., Litt, D. L., Kwiatkowska, M., & Piomelli, D. (2009). The FAAH inhibitor URB-597 interferes with cisplatin- and nicotine-induced vomiting in the Suncus murinus (house musk shrew). *Physiology and Behavior*, *97*, 121–124.
Parker, L. A., Rock, E. M., & Limebeer, C. L. (2011). Regulation of nausea and vomiting by cannabinoids. *British Journal of Pharmacology*, *163*, 1411–1422.
Pertwee, R. G. (2009). Emerging strategies for exploiting cannabinoid receptor agonists as medicines. *British Journal of Pharmacology*, *156*, 397–411.
Roth, S. H. (1978). Stereospecific presynaptic inhibitory effect of delta9-tetrahydrocannabinol on cholinergic transmission in the myenteric plexus of the guinea pig. *Canadian Journal of Physiology and Pharmacology*, *56*, 968–975.
Ruilope, L. M., Després, J. P., Scheen, A., Pi-Sunyer, X., Mancia, G., Zanchetti, A., & Van, Gaal, F. L. (2008). Effect of rimonabant on blood pressure in overweight/obese patients with/without comorbidities: analysis of pooled RIO study results. *Journal of Hypertension*, *26*, 357–367.
Sałaga, M., Sobczak, M., & Fichna, J. (2013). Inhibition of proteases as a novel therapeutic strategy in the treatment of metabolic, inflammatory and functional diseases of the gastrointestinal tract. *Drug Discovery Today*, *18*, 708–715.
Schicho, R., & Storr, M. (2011). Alternative targets within the endocannabinoid system for future treatment of GI diseases. *Canadian Journal of Gastroenterology*, *25*, 377–383.
Simonetto, D. A., Oxentenko, A. S., Herman, M. L., & Szostek, J. H. (2012). Cannabinoid hyperemesis: a case series of 98 patients. *Mayo Clinic Proceedings*, *87*, 114–119.
Smid, S. D., Bjorklund, C. K., Svensson, K. M., Heigis, S., & Revesz, A. (2007). The endocannabinoids anandamide and 2-arachidonoylglycerol inhibit cholinergic contractility in the human colon. *European Journal of Pharmacology*, *575*, 168–176.
Storr, M., Devlin, S., Kaplan, G. G., Panaccione, R., & Andrews, C. N. (2014). Cannabis use provides symptom relief in patients with inflammatory bowel disease but is associated with worse disease prognosis in patients with Crohn's disease. *Inflammatory Bowel Disease*, *20*, 472–480.
Suda, K., Setoyama, H., Nanno, M., Matsumoto, S., & Kawai, M. (2013). Involvement of parasympathetic pelvic efferent pathway in psychological stress-induced defecation. *World Journal of Gastroenterology*, *19*, 1200–1209.
Ukaigwe, A., Karmacharya, P., & Donato, A. (2014). A gut gone to pot: a case of cannabinoid hyperemesis syndrome due to K2, a synthetic cannabinoid. *Case Reports in Emergency Medicine*, 167098.
Van Sickle, M. D., Oland, L. D., Ho, W., Hillard, C. J., Mackie, K., Davison, J. S., & Sharkey, K. A. (2001). Cannabinoids inhibit emesis through CB1 receptors in the brainstem of the ferret. *Gastroenterology*, *121*, 767–774.
Vera, G., Chiarlone, A., Cabezos, P. A., Pascual, D., Martín, M. I., & Abalo, R. (2007). WIN 55, 212-2 prevents mechanical allodynia but not alterations in feeding behaviour induced by chronic cisplatin in the rat. *Life Sciences*, *81*, 468–479.
Vianna, C. R., Donato, J., Rossi, J., Scott, M., Economides, K., Gautron, L., Pierpont, S., Elias, C. F., & Elmquist, J. K. (2012). Cannabinoid receptor 1 in the vagus nerve is dispensable for body weight homeostasis but required for normal gastrointestinal motility. *Journal of Neuroscience*, *32*, 10331–10337.
Zhang, S. C., Wang, W. L., Su, P. J., Jiang, K. L., & Yuan, Z. W. (2014). Decreased enteric fatty acid amide hydrolase activity is associated with colonic inertia in slow transit constipation. *Journal of Gastroenterology and Hepatology*, *29*, 276–283.

CHAPTER

99

Potential Medical Uses of Cannabigerol: A Brief Overview

S. Deiana

CNS Diseases Research Department, Boehringer Ingelheim Pharma GmbH & Co. KG, Birkendorfer straße, Biberach an der Riss, Germany

SUMMARY POINTS

- This chapter focuses on medical uses of cannabigerol (CBG), a phytocannabinoid present in the *Cannabis* plant.
- CBG is a short-life precursor of tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabichromene (CBC), which explains its low concentration in the plant.
- CBG acts on serotoninergic, cannabinoid, peroxisome proliferator-activated receptors, α2-adrenoceptors, vanilloid, melastatin, and ankyrin channels.
- CBG modulates enzymes activity including monoacylglycerol lipase (MAGL), *N*-acylethanolamine acid amide (NAAA) hydrolase, cyclooxygenase (COX), phospholipase-A2 (PLA2), prostaglandin-E2 production (PGE2), and inhibits dopamine, norepinephrine, GABA, and serotonin reuptake.
- By inhibiting prostaglandin production at different levels, CBG owns antinflammatory and analgesic properties.
- CBG possesses antiproliferative effects and it has been suggested for cancer therapy.
- Its effects on preclinical models of mood disorders granted the filing of a patent for CBG use to treat mood disorders, particularly depression.
- CBG has been suggested for skin conditions, glaucoma, sex hormonal dysregulations, eating disorders, and bone healing.
- Due to its difficult availability, CBG has received attention only recently, and further research is required before its clinical efficacy can be demonstrated.

KEY FACTS OF CANNABIGEROL CONTENTS IN *CANNABIS* PLANT

- Cannabigerol (CBG) concentrations in the *Cannabis* plant are usually extremely low.
- This is due to its rapid conversion into tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabichromene (CBC).
- Recent genetic techniques allowed the inactivation of the devoted synthases to prevent the natural conversion of CBG into its metabolites.
- This permitted the selection of *Cannabis* plant strains with high CBG contents.

KEY FACTS OF FIRST MEDICAL USES OF *CANNABIS*

- First *Cannabis* uses are documented in the Chinese herbarium Pen-ts'ao (2800 BC), a pharmacopeia describing several drugs.
- Pen-ts'ao described *Cannabis* as "*ma,' meaning* chaotic.
- *Cannabis* pain-relieving, stupefying, hallucinogenic properties were described together with its use for malaria, menstrual irregularities, constipation, gout and rheumatism.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00115-0
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

5HT$_{1A}$R	5-Hydroxytryptamine-1A Receptors
BBB	Blood–brain barrier
BDS	Botanical drug substance
CB$_1$R, CB$_2$R	Cannabinoid receptors type 1 and 2
CBC	Cannabichromene
CBD	Cannabidiol
CBG	Cannabigerol
COX	Cyclooxygenase
DNBS	Dinitrobenzene sulfonic acid
EAE	Experimental autoimmune encephalomyelitis
IBD	Inflammatory bowel disease
iNOS	Inducible nitric oxide synthase
IOP	Intra ocular pressure
MAGL	Monoacyl glycerol lipase
MS	Multiple sclerosis
MSCs	Mesenchymal stem cells
NAAA	*N*-acylethanolamine acid amidase
NSAIDs	Nonsteroidal antiinflammatory drugs
PCCs	Prostate cancer cells
PGE2	Prostaglandin-E2
PLA2	Phospholipase-A2
PPARs	Peroxisome proliferator-activated receptors
THC	Tetrahydrocannabinol
TLM	Testosterone lowering medicaments
TMEV	Theiler's murine encephalomyelitis virus
TRP	Transient receptor potential
TRPA	Transient teceptor potential ankyrin
TRPM	Transient receptor potential melastatin
TRPV	Transient receptor potential vanilloid

INTRODUCTION

Medicinal use of *Cannabis* dates back to 2800 BC, with suggested use in treating constipation, malaria, gout, rheumatism, pain, asthma, and menstrual anomalies (Iversen, 2007).

The main constituent of the *Cannabis* plant, tetrahydrocannabinol (THC), possesses psychotropic effects, thus limiting its therapeutic use; attention now focuses on nonpsychotropic phytocannabinoids, such as cannabidiol (CBD), cannabichromene (CBC), and cannabigerol (CBG) as potential therapeutic agents for conditions ranging from cancer to inflammation and to mental disorders. For instance, CBD has been suggested to be beneficial for schizophrenia (Deiana, 2013; Zuardi et al., 2012), while the oromucosal spray Sativex (THC and CBD) is approved for the treatment of spasticity in multiple sclerosis (MS) (GW Pharmaceuticals, 2014).

Described for the first time in 1964 (Gaoni & Mechoulam, 1964), CBG is the least studied among phytocannabinoids (a Pubmed search using keyword "cannabigerol" results in 69 publications only), primarily due to its low availability in the *Cannabis* plant (~10% of the cannabinoid fraction) (de Meijer & Hammond, 2005). Specific synthases rapidly metabolize CBG into THC (Taura, Morimoto, Shoyama, & Mechoulam, 1995), CBD (Taura, Morimoto, & Shoyama, 1996) and CBC (Gaoni & Mechoulam, 1966) thus reducing CBG availability in the plant (Fig 99.1). Plants carrying a recessive allele which reduces the efficacy of these synthases contain higher CBG concentration (94% of the cannabinoid fraction) and genetic engineering allows the generation of CBG-predominant *Cannabis* plants (de Meijer, 2004; GW Pharmaceuticals, 2014), facilitating research into CBG pharmacology, and hence fostering the knowledge on its potentials as a therapeutic agent.

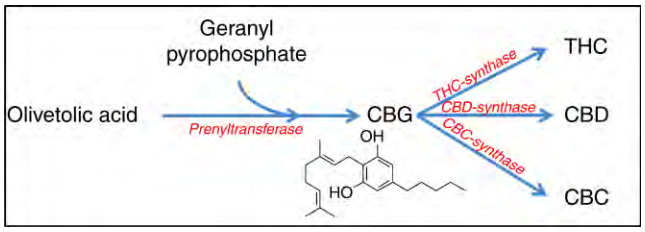


FIGURE 99.1 Biosynthesis and metabolism of cannabigerol. A prenyltransferase catalyzes the conversion of olivetolic acid into CBG, which is then converted into THC, CBD, or CBG by specific synthases. *Source: Information collected from Hill, Williams, Whalley, and Stephens (2012) and Marks et al. (2009).*

PHARMACOKINETICS AND PHARMACOLOGY OF CANNABIGEROL

The mechanism of action of CBG was until recently unknown. In recent times, novel studies revealed its polypharmacological profile, making it an interesting molecule for direct use as medicament itself or as a possible starting molecular structure for the development of more selective/potent chemical entities.

Pharmacodynamics and pharmacokinetic figures are fundamental for profiling and selecting a drug for medical use. Human CBG exposure and bioavailability are still unknown, but its pharmacokinetics in rodents have been described (Deiana et al., 2012). Plasma and brain pharmacokinetics of CBG (120 mg/kg) were studied in rats and mice, following acute oral and intraperitoneal administration (Table 99.1).

CBG readily crossed the blood–brain barrier (BBB) and, in general, in both species, intraperitoneal administration provided higher plasma and brain concentration being the difference only marginal in rats. Indeed, C_{max} values were similar in rats receiving oral or intraperitoneal CBG administration, whereas in mice, C_{max} values

TABLE 99.1 Pharmacokinetics of Cannabigerol

	Mouse				Rat			
	Plasma		Brain		Plasma		Brain	
Measure	po	ip	po	ip	po	ip	po	ip
C_{max} (μg/mL)	0.67	40.8	0.42	3.48	1.05	0.81	0.97	1.23
T_{max} (min)	30	120	60	120	30	60	120	60
Apparent elimination half-life (min)	193	176	≤121	252	100	560	96	447
$AUC_{0–6\,h}$ (μg/mL, g min)	57	4324	≤44	679	98	≤199	181	335
$AUC_{0–24\,h}$ (μg/mL, g min)	ID	5563	≤65	1103	ID	≤514	ID	884
$AUC_{0–\infty}$ (μg/mL, g min)	67	5571	≤86	1117	106	≤617	205	992

$n = 4$; po, *per os*; ip, intraperitoneal; ID, insufficient data available for analysis; C_{max}, maximal drug concentration in tissue; T_{max}, time to reach C_{max}; AUC, area under the curve. Mouse and rat pharmacokinetic values in plasma and brain following 0.5, 1, 2, 4, 6, and 24 h oral and intraperitoneal CBG (120 mg/kg) administration. Apparent elimination half-life (minutes) 2–6 h for po; 4–24 h for ip.
Data from Deiana et al. (2012).

were higher after CBG intraperitoneal compared to oral administration. Mice brain/plasma ratios were 0.77 and 0.15 for oral and intraperitoneal treatments, respectively, and in rats ratios were similar in the two administration routes (1.84 oral and 1.68 intraperitoneal).

Considerably broadening the knowledge of CBG pharmacology, Cascio, Gauson, Stevenson, Ross, and Pertwee (2010) conducted a set of experiments elucidating CBG ability to produce G-protein-coupled-receptor activation or blockade. CBG demonstrated partial agonism/antagonism at cannabinoid type-1 (CB_1R) and type-2 (CB_2R) receptors (Cascio et al., 2010; Gauson et al., 2007). Additionally, CBG has been demonstrated to act as an agonist for α2-adrenoceptors, but receptor subtypes selectivity awaits further elucidation. Like other α2-adrenoceptor ligands, CBG also binds at 5-hydroxytryptamine-1A receptors ($5HT_{1A}R$), acting as a neutral antagonist (Cascio et al., 2010).

At transient receptor potential (TRP) channels, CBG induced a TRPA1- but not TRPM8-mediated Ca^{++} elevation; however, it antagonized the menthol- or icilin-induced Ca^{++} elevation TRPM8-mediated (De Petrocellis et al., 2008). This effect was confirmed and expanded by pharmacological experiments using the botanical drug substance (BDS) CBG (De Petrocellis et al., 2011), derived from CBG-predominant genetically selected *Cannabis* plants. Notably, "CBG-free-CBG-BDS" was inactive, but when added to pure CBG, it became more potent and efficacious at antagonizing TRPM8 than CBG alone. This suggests a synergistic effect between CBG and noncannabinoids contained in its BDS. In cells overexpressing recombinant rat TRPV2 or human TRPV1, CBG also stimulated and then desensitized TRPV1 and TRPV2 (De Petrocellis et al., 2011).

CBG acts on several enzymes involved in prostaglandin production pathways. In an in vitro enzyme based cyclooxygenases (COX)-inhibition assay, it showed full inhibition of COX-1 and ~70% of COX-2. In tumor-necrosis-factor-α-stimulated human colon adenocarcinoma cell line HT29, CBG barely decreased prostaglandine-2 (PGE2) production (~5%) (Ruhaak et al., 2011). More potent activity has been seen in human rheumatoid synovial cells stimulated by the proinflammatory agent 12-0-tetradecanoylphorbol-13-acetate where CBG caused 90% inhibition of PGE2 release (Barrett, Gordon, & Evans, 1985).

CBG further modulates the inflammatory pathway by acting on phospholipase-A2 (PLA2), with high doses reducing its activity and low doses enhancing it (Evans, Formukong, & Evans, 1987). It is, therefore, hypothesized that CBG-mediated activation of PLA2, triggers the hydrolysis of phospholipids, inducing increase in arachidonic acid and subsequent inflammatory cascade (Fig 99.3). Vice versa, high doses of CBG may decrease the lipids metabolites, and hence PGE2 synthesis.

CBG-BDS was also shown to inhibit *N*-acylethanolamine acid amidase (NAAA), which hydrolyzes fatty-acid ethanolamides, such as *N*-palmitoylethanolamine (an endogenous antinociceptive and antiinflammatory fatty acid), anandamide cellular uptake, and monoacyl glycerol lipase (MAGL), which hydrolyze the endocannabinoid 2-arachidonoylglycerol and intracellular triglycerides to fatty-acids and glycerol. Pure CBG and the "CBG-free-CBG-BDS" were less potent than CBG-BDS, indicating that both cannabinoid and noncannabinoid substances present in the BDS might synergistically contribute to MAGL inhibition (De Petrocellis et al., 2011).

CBG was found to be active at L-type Ca^{++} and Na^{+} channels (Duncan et al., 2014), it demonstrated partial agonism at peroxisome proliferator-activated receptors (PPARγ), (Granja et al., 2012), and it inhibited the uptake of dopamine (Duncan et al., 2014), 5HT, norepinephrine (in rat hypothalamic synaptosomes) and GABA (in cerebrocortical synaptosomes) (Banerjee, Snyder, & Mechoulam, 1975); all these effects are not fully elucidated so far.

TABLE 99.2 Pharmacological Targets and Actions of Cannabigerol

Target	Action	Pharmacology	References
5HT uptake	Inhibition	K_i = 50 μM	Banerjee et al. (1975)
5HT1A	Antagonism	K_i = 50 μM	Cascio et al. (2010)
Anandamide cellular uptake	Inhibition	*(CBG-BDS)* IC50 = 11.3 μM	De Petrocellis et al. (2011)
CB1R	Partial agonism/antagonism	K_i = 440 nM; 381 nM	Gauson et al. (2007); Cascio et al. (2010)
CB2R	Partial agonism/antagonism	K_i = 337 nM; 2.6 μM	Gauson et al. (2007); Cascio et al. (2010)
COX1	Inhibition	*Not determined*	Ruhaak et al. (2011)
COX2	Inhibition	IC50 = $2.7{\cdot}10^{-4}$ M	Ruhaak et al. (2011)
Dopamine uptake	Inhibition		Duncan et al. (2014)
GABA uptake	Inhibition	K_i = 50 μM	Banerjee et al. (1975)
L Type Ca^{++}channels	Block	*Dihydropyridine site:* IC50 = 3.8 μM; K_i = 1.3 μM *Diltiazem site:* IC50 = 5.1 μM; K_i = 4.7 μM	Duncan et al. (2014)
MAGL	Inhibition	*(CBG-BDS)* IC50 = 24.6 μM	De Petrocellis et al. (2011)
Na^{++} channels	Block	*Site 2:* IC50 = 500 nM; K_i = 450 nM	Duncan et al. (2014)
NAAA	Inhibition	*(CBG-BDS)* IC50 = 18.3 μM	De Petrocellis et al. (2011)
NE uptake	Inhibition	K_i = 67 μM	Banerjee et al. (1975)
PGE2	Release inhibition	*Not determined*	Ruhaak et al. (2011); Barrett et al. (1985)
PLA2	High doses reduce activity; low doses enhance it	EC50 = 9.5 μM, IC50 = 55 μM	Evans et al. (1987)
PPARγ	Partial agonism	EC50 = 12.7 μM	Granja et al. (2012)
TRPA1	Stimulation + desensitization	EC50 = 0.7 μM; IC50 = 13 μM	De Petrocellis et al. (2011)
TRPM8	Antagonism	IC50 (against icilin 0.25 mM = 0.16 μM	De Petrocellis et al. (2011)
TRPV1	Stimulation + desensitization	EC50 = 1.3 μM; IC50 = 2.6 μM	De Petrocellis et al. (2011)
TRPV2	Stimulation + desensitization	EC50 = 1.72 μM; IC50 = 1.5 μM	De Petrocellis et al. (2011)
TRPV3	Stimulation + desensitization	EC50 = 1 μM; IC50 = 66.7 μM	De Petrocellis et al. (2012)
TRPV4	Stimulation + desensitization	EC50 = 5.1 μM; IC50 = 1.3 μM	De Petrocellis et al. (2012)
α2-Adrenoceptors	Agonism	EC50 = 0.2 nM	Cascio et al. (2010)

Cellular target, pharmacological action and values of CBG and/or CBG-BDS. BDS, Botanical drug substance; CBG, cannabigerol; CBR, cannabinoid receptor; EC50, half maximal effective concentration; IC50, half maximal inhibitory concentration; COX, cyclooxygenase; K_i, receptor-ligand inhibition constant; MAGL, monoacyl glycerol lipase; NAAA, *N*-acylethanolamine acid amidase; NE, norpinephrine; PPARs, peroxisome proliferator-activated receptors; PLA2, phospholipase-A2; PGE2, prostaglandin-E2; TRPA, transient receptor potential ankyrin; TRPM, transient receptor potential melastatin; TRPV, transient receptor potential vanilloid.
Data collected from several publications (see references in the table).

Table 99.2 summarizes CBG in vitro pharmacology as determined via different methodological approaches, which suggests caution in the direct comparison of individual data.

POTENTIAL MEDICAL USES OF CANNABIGEROL

Medical use of *Cannabis* has been recognized for millennia for the treatment of a multitude of conditions. Intrigued by its complex and diverse pharmacology, scientists are currently addressing renewed attention on CBG's therapeutic uses (Fig. 99.2).

Analgesia and Inflammation

There exists an increasing body of evidence from in vivo experiments showing that cannabinoids reduce sensitivity to pain.

Acting through inhibition of COX, nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely used drugs for the treatment of pain and inflammation. COXs catalyze the conversion of arachidonic acid into prostaglandins, hormone-like lipids responsible for homeostatic regulation of pathogenic mechanisms, including inflammatory response (Fig. 99.3). In this context, CBG may hold potential as an antiinflammatory agent by acting at different levels of

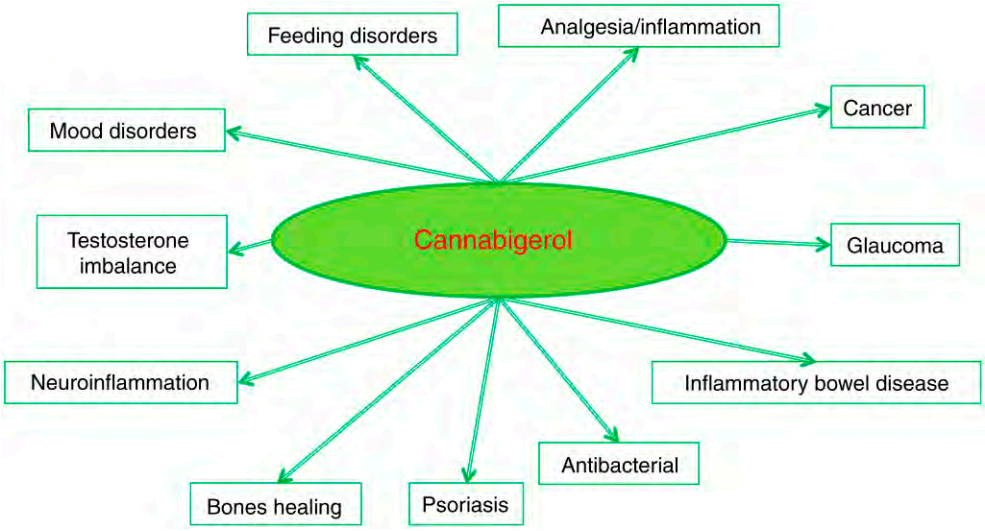


FIGURE 99.2 **Possible therapeutic applications of cannabigerol.** Cannabigerol has been suggested as a possible therapeutic agent for several conditions. To date, for most of these the exact mechanism of action awaits elucidation.

the prostaglandin synthesis pathway: by direct inhibition of PGE2 production, by inhibition of the upstream enzyme PLA2 and by unselective inhibition of the COX enzymes. Its inhibitory activity on MAGL, could also result in reduced prostaglandin synthesis by inhibiting 2-arachidonilglycerol conversion into arachidonic acid.

Data from in vivo experiments showed that CBG was more potent, although less maximally effective, than THC and aspirin in the phenyl benzoquinone (PBQ) model of peripheral inflammatory pain (Formukong, Evans, & Evans, 1988). CBG showed 61% maximum percent inhibition of PBQ-induced writhing behavior in mice with an ED50 = 1.26 mg/kg, a remarkable effect especially in comparison with Aspirin (65%, ED50 = 15 mg/kg) or THC (100%, ED50 = 25 mg/kg).

CBG agonism at α2-adrenoceptor triggered in vivo investigations of its analgesic effects. Comelli et al. (2012) integrated computational and pharmacological studies where the three-dimensional structures of α2A, α2B, and α2C isoforms of murine and human adrenoceptors were modeled by comparative techniques and molecular dynamics simulations. In this computational model, CBG affinity for the receptor appeared higher than that of the α2-adrenoceptor-agonist clonidine. Measured in behavioral models of acute inflammatory pain, CBG dose-dependently reduced the first and second nocifensive phases associated with intraplantar-injection of formalin and reduced λ-carrageenan-evoked hypersensitivity. Both models are α2-adrenoceptor-mediated, as they are blocked by the α2-adrenoceptor-antagonist yohimbine.

Although, to date, there are no supportive in vivo studies, it is interesting to note that CBG is active at the analgesic targets: $5HT_{1A}$Rs, TRPVA1, TRPM8, TRPV2, NAAA, and MAGL. Definitive analgesic efficacy of CBG through these pathways has not yet been reported (Fig. 99.3).

Notably, in 2012, at the 14th World Congress on Pain, CBG was highlighted as one of the phytocannabinoids that may have therapeutic effects on pain (Canadian Consortium for the Investigation of Cannabinoids, 2012).

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) describes a group of inflammatory conditions of the colon and intestine caused by dysregulated immune response. It primarily includes ulcerative colitis limited to the colon, and Crohn disease, which can affect any section of the gastrointestinal tract.

TRP channels play a role in gastrointestinal inflammation as they function as sensors of innocuous and noxious chemical/physical stimuli; indeed, they are involved in the antiinflammatory effects of phytocannabinoids in in vivo models of intestinal inflammation (De Petrocellis et al., 2012). In a model of intestinal inflammation induced by the irritant croton oil, CBG exerted a significant TRPV3-mediated elevation of intracellular Ca^{++} with an efficacy (determined by comparing its effect with the analogous effect observed with 4 μM ionomycin, a Ca^{2+} ionophore that mobilizes intracellular calcium stores), lower (18%) and a potency higher (EC50 = 1 μM) than that of typical TRPV3 agonist carvacrol (41.4% and EC50 = 547 μM). When administered 5 min earlier, CBG also desensitized TRPV3 to carvacrol-mediated cell stimulation (IC50 = 66.7 μM).

CBG induced a significant TRPV4-mediated elevation of intracellular Ca^{++} with low efficacy (23.7% of the effect of ionomycin) and medium-low potency (5.1 μM). Despite its low efficacy, CBG desensitized TRPV4 to the action of its agonist 4αPDD with an EC50 value lower than that at which it activates it at low efficacy (De Petrocellis et al., 2012).

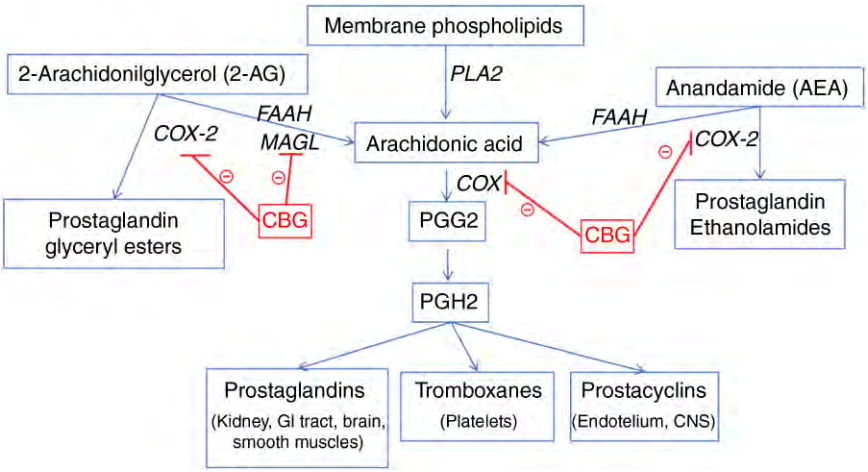


FIGURE 99.3 **Cannabigerol in prostaglandin biosynthesis.** In inflammatory processes arachidonic acid is produced by phospolipase-A2 (PLA2) from membrane phospholipids and it is then metabolized by COX into the instable prostaglandine-G2 (PGG2) which is then converted into prostaglandin-H2 (PGH2). Depending on the tissue, PGH2 is converted into prostaglandins, tromboxanes and prostacyclines. 2-Arachidonilglycerol and anandamide are also substrates of the COX-2, and are metabolized into prostaglandin-glycerol ester and prostaglandin-ethanolamide, respectively. Nonsteroidal antiinflammatory drugs (NSAIDs) and cannabigerol (CBG) inhibit COX and MAGL thereby reducing prostaglandin production. *GI*, gastrointestinal tract; *CNS*, central nervous system. *Source: Information collected from Alhouayek and Muccioli (2014).*

If also in vivo CBG desensitizes TRPV to endogenous stimuli and at doses lower than that required to activate them, its use as a TRPV desensitizer might be employed to develop novel therapies for diseases associated to altered TRPV activity.

In a dinitrobenzene sulfonic acid (DNBS) model of murine colitis, CBG showed protective and curative effects by reducing colon weight/length ratio (an indicator of the inflammatory response severity), it reduced DNBS-induced overexpression of the inducible nitric oxide synthase (iNOS), but not of COX-2. In lipopolysaccharide-activated macrophages, it inhibited nitric oxide production and iNOS protein overexpression. DNBS induced an increase in colonic interleukin-1β and interferon-γ and a decrease in interleukin-10 levels; these alterations were all counteracted by CBG. Finally, CBG restored the DNBS-induced decrease of superoxide-dismutases thus restoring antioxidant protection in intestinal epithelial cells (Borrelli et al., 2013).

Neuroinflammation in Multiple Sclerosis

Neuroinflammation is a component of disorders, such as multiple sclerosis (MS), Alzheimer and Parkinson diseases. It can be initiated as a response to traumatic brain injury, infections, aging, toxic metabolites, or autoimmunity. Acute neuroinflammation involves a rapid activation of microglia and no peripheral immunoresponse. In chronic inflammation, the microglia induces a sustained production of inflammatory modulators that may alter the integrity of the BBB, and recruit peripheral immune cells for inflammatory responses, leading to neuronal dysfunction/neurodegeneration.

In MS, a chronic inflammatory demyelinating disease, microglial activation contributes to the pathology through secretion of proinflammatory mediators.

Effects of CBG and its quinone derivate, VCE-003 (obtained by CBG oxidation), on neuroinflammation have been investigated and they both revealed neuroprotective action by preventing glutamate-induced cell viability decline. In the Theiler's murine encephalomyelitis virus (TMEV) model of MS, VCE-003 improved the motor functions, and locomotor activity was restored to normal levels. VCE-003 also reduced microglia reactivity in the spinal cord of TMEV-infected mice, it outperformed CBG in terms of capacity to inhibit cytokine release, and was as efficient as CBG at inhibiting the release of PGE2 (Granja et al., 2012).

Extending the studies on VCE-003, recently, the same group reported that in the autoimmune model of MS (EAE), VCE-003 attenuated EAE by activating CBR_2 and PPARs. A reduction of CD4+ and T cells infiltrates, inhibition of Th1 and Th17 (T-helper cells involved in different stages of the pathogenesis of autoimmune diseases, including MS) responses in the spinal cord of VCE-003-treated mice, a decreased microglial activation, a structural protection of myelin sheets and reduced axonal damage were reported (Carrillo-Salinas et al., 2014).

Taken together, these data support a potential therapeutic use of quinone derivate of CBG for the treatment of human immune diseases with inflammatory and autoimmune components, such as MS.

Cancer

Cannabinoids are used in the treatment of nausea and vomiting associated with chemotherapy. Their use in oncology is currently receiving increasing interest

as they inhibit the growth of various cancer cell types, inducing apoptosis, cell-cycle arrest, cell growth inhibition, and affecting angiogenesis, and cell migration (Guzman, 2003).

Like other phytocannabinoids, CBG was reported to reduce cell proliferation in several cancer cell lines, including human breast, prostate, colorectal carcinoma, gastric adenocarcinoma, C6-rat glioma, rat basophilic leukemia, and transformed thyroid cells (Ligresti et al., 2006). Interestingly, prostate carcinoma cells were found to be unresponsive to most phytocannabinoids, only CBD and CBG elicited antiproliferative effects.

Although the exact mechanism of action is not fully elucidated, TRPV1, TRPV2, and TRPM8 are involved in prostate cancer, all being CBG-sensitive channels. Specifically, TRPV1 agonist capsaicin induces antiproliferative and proapoptotic effects on prostate cancer cells (PCCs) (Diaz-Laviada, 2010), TRPV2 activation stimulates PCCs migration (Gkika & Prevarskaya, 2011), whereas TRPM8 is overexpressed in androgen-dependent PCCs, and TRPM8 antagonists have been suggested as being effective during the early androgen–responsive stage of prostate cancers (Liu & Qin, 2011). These findings suggest CBG as a relevant agent for prostate cancer since it antagonizes TRPM8 channels and activates/desensitize TRPV2 and TRPV1 (De Petrocellis et al., 2011). In line with this, together with other phytocannabinoids, CBG showed to inhibit PCCs viability in androgen receptors-positive and receptors-negative PCC lines (De Petrocellis et al., 2013). Moreover, CBG activated the proapoptotic proteases caspase3/7 under conditions of serum deprivation and it increased intracellular Ca^{++} (indicating endoplasmic reticulum stress) in PCC lines. It is therefore suggested that phytocannabinoids and, albeit at lower potency/efficacy, CBG can retard PCC proliferation, and cause apoptosis.

CBG showed the greatest growth-inhibitory activity against human oral epitheloid carcinoma cell lines and fibroblasts, when compared to olivetol, geraniol, and cannabinol-methyl-ether (Baek et al., 1998). Although CBG was not the most potent phytocannabinoid, it demonstrated antiproliferative action in human breast cancer cells (McAllister, Christian, Horowitz, Garcia, & Desprez, 2007) and mouse skin melanoma cells (Baek, Han, Yook, Kim, & Kwak, 1996).

The exact mechanism by which CBG acts against cancer remains to be elucidated, and further research is required; however, mounting evidence suggests that this phytocannabinoid possesses several properties, which may counteract cancer cell proliferation.

Mood Disorders

The endocannabinoid system has bidirectional influence on anxiety; its inhibition results in anxiogenic responses, while a moderate increase leads to anxiolytic effects. Anandamide itself possesses a bidirectional profile, acting as an anxiolytic agent at CB_1R, and an anxiogenic at TRPV1 (Rubino et al., 2008).

The CB_1R inverse agonist/antagonist, rimonabant, marketed in the past as an antiobesity drug, was withdrawn as chronic administration induced undesirable anxious and depressive-like states along with suicide (Janero & Makriyannis, 2009).

This discovery sparked interest in the role of cannabinoids in anxiety/depression and a number of studies addressed this subject.

O'Brien et al. (2013) recently investigated acute and chronic effects of CGB in rats in the light/dark immersion model of anxiety-like behavior and in a test of palatability processing for depression-like behavior, that is, the saccharin hedonic reactions in the taste reactivity test. While CBG revealed no effects in the light/dark test, its acute administration produced a slight enhancement of saccharin palatability, indicative of possible antidepressant effect.

El-Alfy et al. (2010) found 20 and 80 mg/kg CBG ineffective in mice locomotor activity, catalepsy, body temperature, and nociception and in the forced swimming test (assessing depression-like behavior). However, in a different study performed in mouse the tail suspension test, CBG (40 mg/kg) reduced the time spent immobile (which in this test is interpreted as a predictor of antidepressant activity), with effects comparable to the antidepressant imipramine (Musty & Deyo, 2006).

Following this finding, the same authors filed several US patents (the latest in 2014: *US20,140,039,043-A1*) claiming the use of CBG for mood disorders, preferably in depression.

Other Potential Medical Uses of Cannabigerol

Further expanding the knowledge on its potential medical uses, CBG effects on rats' feeding behavior have been investigated, revealing to be ineffective when tested at doses up to 17.6 mg/kg (Farrimond, Whalley, & Williams, 2012). In a later study, higher doses of CBG (120 and 240 mg/kg) induced a dose-dependent increase of food intake, number of meals, a decrease of latency to first meal and an enhanced locomotor activity suggesting that at higher doses, CBG stimulates appetite, and enhances consummatory feeding behavior (Brierley, Whalley, & Williams, 2013). Preliminary results also indicate that pure CBG (120 mg/kg) can attenuate weight loss induced by the chemotherapy agent cisplatin (Brierley et al., 2015). Given these findings, CBG was suggested to be useful for feeding disorders associated with chemotherapy but also for the treatment of weight loss in cancer anorexia-cachexia syndrome.

CBG was found to inhibit testosterone synthesis in rat testis Leydig cells more potently than THC (Jakubovic, McGeer, & McGeer, 1979; Burstein, Hunter, & Sedor, 1980). Testosterone lowering medicaments (TLM) are often prescribed for men with prostate cancer, hypersexuality, male contraception, gender reassignment. For women, TLM are prescribed for severe cases of acne, amenorrhea, seborrhea, hirsutism, androgenic alopecia, and hyperandrogenism. TLM are also administered in sex offender treatment, in addition to psychotherapy (Turner, Basdekis-Jozsa, & Briken, 2013).

CBG effects on skin conditions have also been explored. Psoriasis is an inflammatory skin disease characterized by epidermal keratinocyte hyperproliferation paralleled by increased expression of skin proinflammatory mediators. CBG inhibited keratinocyte proliferation in a CB_1/CB_2-receptors-independent manner, and with a greater potency than THC, suggesting a possible therapeutic use for psoriasis (Wilkinson & Williamson, 2007). Effects of CBG on the epigenetic regulation of epidermal differentiation genes have been investigated and CBG acted as transcriptional repressors controlling cell proliferation and differentiation by increasing DNA methylation of keratin-10 gene in a CBR-independent mechanism (Pucci et al., 2013).

Acting through an indirect mechanism regulated by accessory cells, CBG stimulated recruitment of quiescent mesenchymal stem cells (MSCs) in bone marrow (Scutt & Williamson, 2007). The induction of MSC migration by suitable agents, such as in this example CBG, could represent an attractive cell-based therapy to treat osteoporosis and bone regeneration issues by accelerating healing and/or regeneration.

A number of cannabinoids have been shown to reduce intra ocular pressure (IOP) in a CBR-mediated mechanism (Oltmanns et al., 2008). CBG's chronic and, although to a lesser extent, acute administration reduced ocular tension in cats such that IOP of CBG-treated eyes resulted in 4–8 mmHg lower than untreated contralateral eyes (Colasanti, Craig, & Allara, 1984). Additionally, CBG was devoid of ocular toxicity and neurotoxicity, it did not change overall brain electrical activity, but it prolonged the onset of sleep/REM sleep. Possessing such IOP lowering action, CBG may be a possible candidate for the treatment of glaucoma.

CBG possesses antibacterial properties (Mechoulam & Gaoni, 1965) with activity against gram-positive bacteria, mycobacteria, and fungi superior to that of THC, CBD, and CBC (Eisohly, Turner, Clark, & Eisohly, 1982). Additionally, CBG has potent activity against various methicillin-resistant *Staphylococcus aureus* strains of current clinical relevance (Appendino et al., 2008). Finally, and importantly, CBG (1, 3, 10 mg/kg) showed low potential abuse when assessed in a rat model of drug discrimination (Duncan et al., 2014).

CONCLUSIONS

CBG owns a broad pharmacological profile stimulating interest on this nonpsychotropic phytocannabinoid as a potential polydrug or as a starting molecular structure for chemical engineering aimed at developing more selective/potent drugs.

The expanding knowledge on its multifaceted pharmacology allowed intercepting numerous possible medical uses inspiring several attempts to patent its pharmaceutical use for a number of conditions. Among all, its antiinflammatory and anticancer properties stimulated tremendous interest pointing CBG as a possible candidate in the development of novel drugs to prevent, control, and treat conditions where pathological inflammatory responses and abnormal cell proliferation represent a threat.

While the recent research finding summarized in this chapter highlighted the vast potentials of CBG, it is clear that extensive work is needed before its clinical benefits can be definitively stated. Decisive is the fact that, currently, there is no material on CBG pharmacokinetics/pharmacodynamics in humans, and clinicians should remain cautious until more definite studies demonstrate the safety and efficacy of CBG. Further pharmacological and preclinical experiments, together with human toxicological and proof of concept studies, should be performed to confirm the number of reports suggesting CBG value in medical practice.

MINI-DICTIONARY

Botanical drug substance (BDS) Botanical unrefined material from the *Cannabis* plant where a specific cannabinoid, in this case CBG, has at least 95% purity. The amount of the main cannabinoid in the corresponding BDS, expressed as a %w/w of extract, is used to calculate the amount of BDS necessary to achieve the equimolar amount of the corresponding pure cannabinoid. Cannabinoid and noncannabinoid components of the BDS are always identified, and vary between different BDS. "CBG-free CBG BDS" is a BDS extracted from a CBG-predominant *Cannabis* plant virtually devoid (but it may still contain traces) of CBG.

Cyclooxygenase (COX) The enzyme that catalyzes the biosynthesis of prostaglandins from arachidonic acid, produced on demand from arachidonate through PLA2. Two isoforms of the COX exist: COX-1 is constitutively expressed in the gastrointestinal tract, kidneys, and platelets, and it regulates the prostaglandins responsible for the integrity of the gastric mucosa, for the normal platelet function and for the regulation of renal blood flow. COX-2 is expressed in immune system cells, and is inducible by inflammatory stimuli, thereby producing prostaglandins mediating inflammation, pain, and fever.

Transient receptor potential (TRP) Ligand-gated cation channels are a group of ion channels found in the plasma membrane of several cell types mediating a multiplicity of sensations including hotness, pain, warmth or coldness, tastes, pressure, and vision. Endogenous lipids modulate TRP channel activity by direct binding, acting as endogenous ligands. Endocannabinoids interact with several types of TRP channels.

TRP ankyrin 1 (TRPA1) Expressed in trigeminal and dorsal root ganglia neurons. It is activated by low temperatures (17°C), mustard oil, and environmental irritants, such as unsaturated aldehydes, causing painful burning sensation. Its function in cold transduction is still debated; it contributes to inflammatory hypersensitivity, vasodilation, and nociception to irritants.
TRP melastatin 8 (TRPM8) Expressed in nociceptive and nonnociceptive neurons and it is gated by medium–low (25°C) temperatures, as well as chemical cooling agents, such as spearmint, menthol, and eucalyptol. Anandamide binds to TRPM8. Above its role in nociception, which is to date still under discussion, TRPM8 channel has been linked with cancer cell proliferation.
TRP vanilloid type 1 (TRPV1) Activated by heat temperatures (42°C) and basic pH (5.9). Anandamide binds this channel, and CBG activates and desensitizes TRPV1 being this responsible for some of CBG analgesic and antiinflammatory actions.
TRPV type 2 Shares nearly 50% DNA sequence identity with TRPV1, but unlike TRPV1, it is not modulated by vanilloids. It is activated by high temperatures (~52°C), physical stimuli, such as mechanical stretch, osmotic swelling, and modulators, such as hormones, growth factors, chemotactic peptides, lysophospholipids, and cannabinoids. Its physiological role remains one of the most controversial. It has been proposed to be involved in the transduction of high-temperature heat responses in sensory ganglia and to function as a noxious heat thermosensor. However, TRPV2-knockout mice show normal sensory transduction, suggesting that TRPV2 does not function as a noxious heat and mechanical sensor and that it has other, as yet unknown, functions. *Cannabis* derivatives are the most potent TRPV2 activators.

References

Alhouayek, M., & Muccioli, G. G. (2014). COX-2-derived endocannabinoid metabolites as novel inflammatory mediators. *Trends in Pharmacological Sciences*, *35*, 284–292.

Appendino, G., Gibbons, S., Giana, A., Pagani, A., Grassi, G., Stavri, M., Smith, E., & Rahman, M. M. (2008). Antibacterial cannabinoids from *Cannabis sativa*: a structure-activity study. *Journal of Natural Products*, *71*, 1427–1430.

Baek, S. H., Han, D. S., Yook, C. N., Kim, Y. C., & Kwak, J. S. (1996). Synthesis and antitumor activity of cannabigerol. *Archives of Pharmacal Research*, *19*(3), 228–230.

Baek, S. H., Kim, Y. O., Kwag, J. S., Choi, K. E., Jung, W. Y., & Han, D. S. (1998). Boron trifluoride etherate on silica-A modified Lewis acid reagent (VII). Antitumor activity of cannabigerol against human oral epitheloid carcinoma cells. *Archives of Pharmacal Research*, *21*, 353–356.

Banerjee, S. P., Snyder, S. H., & Mechoulam, R. (1975). Cannabinoids: influence on neurotransmitter uptake in rat brain synaptosomes. *Journal of Pharmacology and Experimental Therapeutics*, *194*, 74–81.

Barrett, M. L., Gordon, D., & Evans, F. J. (1985). Isolation from *Cannabis* sativa L. of cannflavin—a novel inhibitor of prostaglandin production. *Biochemical Pharmacology*, *34*, 2019–2024.

Borrelli, F., Fasolino, I., Romano, B., Capasso, R., Maiello, F., Coppola, D., Orlando, P., Battista, G., Pagano, E., Di Marzo, V., & Izzo, A. A. (2013). Beneficial effect of the non-psychotropic plant cannabinoid cannabigerol on experimental inflammatory bowel disease. *Biochemical Pharmacology*, *85*, 1306–1316.

Brierley, D. I., Whalley, B. J., & Williams, C. M. (2013). Modulation of feeding behaviour by the non-psychoactive phytocannabinoid cannabigerol. *Proceedings of Pharmacology 2013*. London: British Pharmacological Society. Available from: http://www.pa2online.org/abstracts/vol11issue3abst067p.pdf

Brierley, D. I., Harman, J. R., Giallourou, N., Swann, J. R., Patel, K., Whalley, B. J., & Williams C. M. (2015). Cannabigerol attenuates muscle catabolism and anorexia In a pre-clinical model of chemotherapy-induced cachexia. *Twenty-fifth annual symposium of the international cannabinoid research society*. June 28 – July 3, 2015. P 10. Wolfville, Nova Scotia.

Burstein, S., Hunter, S. A., & Sedor, C. (1980). Further studies on the inhibition of Leydig cell testosterone production by cannabinoids. *Biochemical Pharmacology*, *29*, 2153–2154.

Canadian Consortium for the Investigation of Cannabinoids (2012). Cannabinoids & pain. The state of the art, the state of the science White Paper. *Official satellite symposium of the fourteenth world congress on pain*, August 25, 2012. Milan, Italy.

Carrillo-Salinas, F. J., Navarrete, C., Mecha, M., Feliu, A., Collado, J. A., Cantarero, I., Bellido, M. L., Muñoz, E., & Guaza, C. (2014). A cannabigerol derivative suppresses immune responses and protects mice from experimental autoimmune encephalomyelitis. *PLoS One*, *9*, e94733.

Cascio, M. G., Gauson, L. A., Stevenson, L. A., Ross, R. A., & Pertwee, R. G. (2010). Evidence that the plant cannabinoid cannabigerol is a highly potent alpha2-adrenoceptor agonist and moderately potent $5HT_{1A}$ receptor antagonist. *British Journal of Pharmacology*, *159*, 129–141.

Colasanti, B. K., Craig, C. R., & Allara, R. D. (1984). Intraocular pressure, ocular toxicity and neurotoxicity after administration of cannabinol or cannabigerol. *Experimental Eye Research*, *39*, 251–259.

Comelli, F., Filippi, F., Papaleo, E., De Gioia, L., Pertwee, R. G., & Costa, B. (2012). Evidence that the phytocannabinoid cannabigerol can induce antinociception by activating α2-adrenoreceptors: a computational and a pharmacological study. *Twenty-second annual symposium of the international cannabinoid research society* (p. 44), July 22–27, 2012. Freiburg im Breisgau, Germany.

de Meijer, E. P. (2004). The breeding of Cannabis cultivars for pharmaceutical end-uses. In G. W. Guy, B. A. Whittle, & P. J. Robson (Eds.), *The medicinal uses of Cannabis and cannabinoids* (pp. 55–69). London, UK: Pharmaceutical Press.

de Meijer, E. P., & Hammond, K. M. (2005). The inheritance of chemical phenotype in *Cannabis sativa* L. (II): cannabigerol predominant plants. *Euphytica*, *145*, 189–198.

De Petrocellis, L., Vellani, V., Schiano-Moriello, A., Marini, P., Magherini, P. C., Orlando, P., & Di Marzo, V. (2008). Plant-derived cannabinoids modulate the activity of transient receptor potential channels of ankyrin type-1 and melastatin type-8. *Journal of Pharmacology and Experimental Therapeutics*, *325*, 1007–1015.

De Petrocellis, L., Ligresti, A., Moriello, A. S., Allara, M., Bisogno, T., Petrosino, S., Stott, C. G., & Di Marzo, V. (2011). Effects of cannabinoids and cannabinoid-enriched *Cannabis* extracts on TRP channels and endocannabinoid metabolic enzymes. *British Journal of Pharmacology*, *163*, 1479–1494.

De Petrocellis, L., Orlando, P., Moriello, A. S., Aviello, G., Stott, C., Izzo, A. A., & Di Marzo, V. (2012). Cannabinoid actions at TRPV channels: effects on TRPV3 and TRPV4 and their potential relevance to gastrointestinal inflammation. *Acta Physiologica (Oxford)*, *204*, 255–266.

De Petrocellis, L., Ligresti, A., Schiano, M. A., Iappelli, M., Verde, R., Stott, C. G., Cristino, L., Orlando, P., & Di Marzo, V. (2013). Non-THC cannabinoids inhibit prostate carcinoma growth in vitro and in vivo: pro-apoptotic effects and underlying mechanisms. *British Journal of Pharmacology*, *168*, 79–102.

Deiana, S. (2013). Medical use of *Cannabis*. Cannabidiol: a new light for schizophrenia? *Drug Testing and Analysis*, *5*, 46–51.

Deiana, S., Watanabe, A., Yamasaki, Y., Amada, N., Arthur, M., Fleming, S., Woodcock, H., Dorward, P., Pigliacampo, B., Close, S., Platt, B., & Riedel, G. (2012). Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Δ^9-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive-compulsive behaviour. *Psychopharmacology (Berlin)*, *219*(3), 859–873.

Diaz-Laviada, I. (2010). Effect of capsaicin on prostate cancer cells. *Future Oncology*, *6*, 1545–1550.

Duncan, M., Cascio, M. G., Pertwee, R. G., Riedel, G., Amada, N., & Yamasaki, Y. (2014). Pharmacological properties of pure cannabigerol and botanical drug substance. *Twenty-fourth annual symposium*

of the international cannabinoid research society, 28 June–3 July 2014 (pp. P3–36). Baveno, Italy.

Eisohly, H. N., Turner, C. E., Clark, A. M., & Eisohly, M. A. (1982). Synthesis and antimicrobial activities of certain cannabichromene and cannabigerol related compounds. *Journal of Pharmaceutical Sciences*, *71*, 1319–1323.

El-Alfy, A. T., Ivey, K., Robinson, K., Ahmed, S., Radwan, M., Slade, D., Khan, I., ElSohly, M., & Ross, S. (2010). Antidepressant-like effect of delta9-tetrahydrocannabinol and other cannabinoids isolated from *Cannabis sativa* L. *Pharmacology Biochemistry and Behavior*, *95*, 434–442.

Evans, A. T., Formukong, E., & Evans, F. J. (1987). Activation of phospholipase A2 by cannabinoids. Lack of correlation with CNS effects. *FEBS Letters*, *211*, 119–122.

Farrimond, J. A., Whalley, B. J., & Williams, C. M. (2012). Cannabinol and cannabidiol exert opposing effects on rat feeding patterns. *Psychopharmacology (Berlin)*, *223*, 117–129.

Formukong, E. A., Evans, A. T., & Evans, F. J. (1988). Analgesic and antiinflammatory activity of constituents of *Cannabis sativa* L. *Inflammation*, *12*, 361–371.

Gaoni, Y., & Mechoulam, R. (1964). The structure and synthesis of cannabigerol, a new hashish constituent. *Proceedings of the Chemical Society*, 82–83.

Gaoni, Y., & Mechoulam, R. (1966). Cannabichromene, a new active principle in hashish. *Chemical Communications*, *1*, 20–21.

Gauson, L. A., Stevenson, L. A., Thomas, A., Baille, G. L., Ross, R. A., & Pertwee, R. G. (2007). Cannabigerol behaves as a partial agonist at both CB1 and CB2 receptors. *Symposium on the Cannabinoids*, 26 June–1 July, 2007 (pp. 206). St-Sauveur, Canada: International Cannabinoid Research Society.

Gkika, D., & Prevarskaya, N. (2011). TRP channels in prostate cancer: the good, the bad and the ugly? *Asian Journal of Andrology*, *13*, 673–676.

Granja, A. G., Carrillo-Salinas, F., Pagani, A., Gomez-Canas, M., Negri, R., Navarrete, C., Mecha, M., Mestre, L., Fiebich, B. L., Cantarero, I., Calzado, M. A., Bellido, M. L., Fernandez-Ruiz, J., Appendino, G., Guaza, C., & Muñoz, E. (2012). A cannabigerol quinone alleviates neuroinflammation in a chronic model of multiple sclerosis. *Journal of Neuroimmune Pharmacology*, *7*, 1002–1016.

Guzman, M. (2003). Cannabinoids: potential anticancer agents. *Nature Reviews Cancer*, *3*, 745–755.

GW Pharmaceuticals (2014). *GW Breeding programme*. http://www.gwpharm.com/cultivation.aspx

Hill, A. J., Williams, C. M., Whalley, B. J., & Stephens, G. J. (2012). Phytocannabinoids as novel therapeutic agents in CNS disorders. *Pharmacology and Therapeutics*, *133*, 79–97.

Iversen, L. (2007). *The science of* Cannabis (2nd ed.). Oxford, UK: Oxford University Press.

Jakubovic, A., McGeer, E. G., & McGeer, P. L. (1979). Effects of cannabinoids on testosterone and protein synthesis in rat testis Leydig cells in vitro. *Molecular and Cellular Endocrinology*, *15*, 41–50.

Janero, D. R., & Makriyannis, A. (2009). Cannabinoid receptor antagonists: pharmacological opportunities, clinical experience, and translational prognosis. *Expert Opinion on Emerging Drugs*, *14*, 43–65.

Ligresti, A., Moriello, A. S., Starowicz, K., Matias, I., Pisanti, S., De Petrocellis, L., Laezza, C., Portella, G., Bifulco, M., & Di Marzo, V. (2006). Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. *Journal of Pharmacology and Experimental Therapeutics*, *318*, 1375–1387.

Liu, Y., & Qin, N. (2011). TRPM8 in health and disease: cold sensing and beyond. *Advances in Experimental Medicine and Biology*, *704*, 185–208.

Marks, M. D., Tian, L., Wenger, J. P., Omburo, S. N., Soto-Fuentes, W., He, J., Gang, D. R., Weiblen, G. D., & Dixon, R. A. (2009). Identification of candidate genes affecting Delta9-tetrahydrocannabinol biosynthesis in *Cannabis sativa*. *Journal of Experimental Botany*, *60*, 3715–3726.

McAllister, S. D., Christian, R. T., Horowitz, M. P., Garcia, A., & Desprez, P. Y. (2007). Cannabidiol as a novel inhibitor of Id-1 gene expression in aggressive breast cancer cells. *Molecular Cancer Therapeutics*, *6*, 2921–2927.

Mechoulam, R., & Gaoni, Y. (1965). Hashish. IV. The isolation and structure of cannabinolic, cannabidiolic, and cannabigerolic acids. *Tetrahedron*, *21*, 1223–1229.

Musty, R. & Deyo, R. (2006). A cannabigerol extract alters behavioral dispair in an animal model of depression. *In Sixteenth annual symposium of the international cannabinoid research society*, (p. 32), 24–28. Tihany, Hungary.

O'Brien, L. D., Wills, K. L., Segsworth, B., Dashney, B., Rock, E. M., Limebeer, C. L., & Parker, L. A. (2013). Effect of chronic exposure to rimonabant and phytocannabinoids on anxiety-like behavior and saccharin palatability. *Pharmacology Biochemistry and Behavior*, *103*, 597–602.

Oltmanns, M. H., Samudre, S. S., Castillo, I. G., Hosseini, A., Lichtman, A. H., Allen, R. C., Lattanzio, F. A., & Williams, P. B. (2008). Topical WIN55212-2 alleviates intraocular hypertension in rats through a CB1 receptor mediated mechanism of action. *Journal of Ocular Pharmacology and Therapeutics*, *24*, 104–115.

Pucci, M., Rapino, C., Di, F. A., Dainese, E., D'Addario, C., & Maccarrone, M. (2013). Epigenetic control of skin differentiation genes by phytocannabinoids. *British Journal of Pharmacology*, *170*, 581–591.

Rubino, T., Realini, N., Castiglioni, C., Guidali, C., Vigano, D., Marras, E., Petrosino, S., Perletti, G., Maccarrone, M., Di Marzo, V., & Parolaro, D. (2008). Role in anxiety behavior of the endocannabinoid system in the prefrontal cortex. *Cerebral Cortex*, *18*, 1292–1301.

Ruhaak, L. R., Felth, J., Karlsson, P. C., Rafter, J. J., Verpoorte, R., & Bohlin, L. (2011). Evaluation of the cyclooxygenase inhibiting effects of six major cannabinoids isolated from *Cannabis sativa*. *Biological and Pharmaceutical Bulletin*, *34*, 774–778.

Scutt, A., & Williamson, E. M. (2007). Cannabinoids stimulate fibroblastic colony formation by bone marrow cells indirectly via CB2 receptors. *Calcified Tissue International*, *80*, 50–59.

Taura, F., Morimoto, Y., Shoyama, Y., & Mechoulam, R. (1995). First direct evidence for the mechanism of delta-1-tetrahydrocannabinolic acid biosynthesis. *Journal of the American Chemical Society*, *38*, 9766–9767.

Taura, F., Morimoto, S., & Shoyama, Y. (1996). Purification and characterization of cannabidiolic-acid synthase from *Cannabis sativa* L. Biochemical analysis of a novel enzyme that catalyzes the oxidocyclization of cannabigerolic acid to cannabidiolic acid. *Journal of Biological Chemistry*, *271*, 17411–17416.

Turner, D., Basdekis-Jozsa, R., & Briken, P. (2013). Prescription of testosterone-lowering medications for sex offender treatment in German forensic-psychiatric institutions. *Journal of Sexual Medicine*, *10*, 570–578.

Wilkinson, J. D., & Williamson, E. M. (2007). Cannabinoids inhibit human keratinocyte proliferation through a non-CB1/CB2 mechanism and have a potential therapeutic value in the treatment of psoriasis. *Journal of Dermatological Science*, *45*, 87–92.

Zuardi, A. W., Crippa, J. A., Hallak, J. E., Bhattacharyya, S., Atakan, Z., Martin-Santos, R., McGuire, P. K., & Guimarães, F. S. (2012). A critical review of the antipsychotic effects of cannabidiol: 30 years of a translational investigation. *Current Pharmaceutical Design*, *18*(32), 5131–5140.

Page left intentionally blank

CHAPTER

e16

Cannabis Use in Fibromyalgia

M. Farré[*], *A. Farré*[**], *J. Fiz*[†], *M. Torrens*[‡]

[*]Clinical Pharmacology Unit, Germans Trias i Pujol University Hospital—IGTP, and Human Pharmacology Unit, Hospital del Mar Medical Research Institute—IMIM, and Autonomous University of Barcelona, Barcelona, Spain

[**]Dual Disorder Unit, Addiction Program, Institute of Neuropsychiatry and Addiction—INAD, and Hospital del Mar Medical Research Institute—IMIM, Barcelona, Spain

[†]CEDIR, Center for Diagnosis and Rehabilitation, San Martín Santa Fe, Argentina

[‡]Addiction Program, Institute of Neuropsychiatry and Addiction—INAD, and Hospital del Mar Medical Research Institute—IMIM, Barcelona, Spain

SUMMARY POINTS

- This chapter focuses on the cannabis and fibromyalgia and begins with the diagnosis and treatment of this condition.
- Therapy includes nonpharmacological and pharmacological treatments.
- Some drugs have demonstrated efficacy in randomized controlled trials (pregabalin, duloxetine, milnacipran, amitriptyline, serotonin selective reuptake inhibitors, and cyclobenzaprine).
- Cannabis products are frequently used in patients with fibromyalgia. Different surveys showed that most of the patients who tried medical marijuana found it to be effective or very effective for the therapy of symptoms of the condition.
- Evidence of efficacy is based on some noncontrolled studies and two randomized-controlled clinical trials using oral nabilone for the treatment of pain and sleep difficulties in fibromyalgia patients.
- Nabilone appears to have beneficial effect with significant reduction in pain and functional improvement, and it is well tolerated. Nabilone is more effective than amitriptyline in improving sleep in fibromyalgia.
- More studies are needed to evaluate the efficacy and safety of cannabinoids in fibromyalgia.

KEY FACTS OF FIBROMYALGIA

- Fibromyalgia is a chronic disorder characterized by widespread pain and tenderness, often accompanied by fatigue, memory problems, and sleep disturbances.
- The origin of fibromyalgia is unknown but it seems to be related to a sensitization of the central nervous system.
- The diagnosis of fibromyalgia is based on clinical symptoms, and routine laboratory and diagnostic tests are normal.
- There is no specific treatment for fibromyalgia. Nonpharmacological and pharmacological therapies could help patients.
- Some drugs are often used in the treatment of fibromyalgia pain. Three of them have been approved for this indication (pregabalin, duloxetine, and milnacipran).
- Cannabinoids by oral or smoked route can be useful to alleviate pain in fibromyalgia. However, more studies are needed in order to recommend their use.

LIST OF ABBREVIATIONS

ACR	American College of Rheumatology
AEA	Anandamide
CB1/CB2	Cannabinoid receptor 1 and 2
CEDS	Clinical endocannabinoid deficiency syndromes

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00112-5
Copyright © 2017 Elsevier Inc. All rights reserved.

CNS	Central nervous system
EMA	European Medicines Agency
FDA	Food and Drug Administration
FIQ	Fibromyalgia impact questionnaire
GABA	Gamma-aminobutyric acid
LSEQ	Leeds sleep evaluation questionnaire
RCT	Randomized clinical trial
SR-MA	Systematic reviews with metaanalysis
SSNRI	Selective serotonin and norepinephrine reuptake inhibitor
SSRI	Selective serotonin reuptake inhibitor
TCA	Tricyclic antidepressants
THC	Δ^9-Tetrahydrocannabinol

INTRODUCTION

Fibromyalgia

Fibromyalgia is a chronic condition characterized by widespread pain and tenderness. It is the second most common rheumatologic disorder and approximately 2–8% of the world's population meets the 1990 and 2010 American College of Rheumatology (ACR) diagnostic criteria. Fibromyalgia is more common in women and can appear at any age with a similar worldwide prevalence. Most patients have had a previous medical history of chronic pain throughout the body or chronic pain experienced in their first-degree relatives. Roughly 10–30% of patients with fibromyalgia meet criteria for other chronic pain disorders such as osteoarthritis, rheumatoid arthritis, and lupus (Rahman, Underwood, & Carnes, 2014).

As in most illnesses, fibromyalgia has a genetic component, and some studies have focused on specific genetic polymorphisms associated with the risk of developing this disorder. Findings related to polymorphisms involved in pain neurotransmission, essentially those concerning serotonin receptors and transporters, and dopamine receptors, are, however, contradictory. In addition, environmental factors play a role in triggering development of the illness. It is known that infections (Epstein–Barr virus infection, hepatitis C, parvovirus infection, and Lyme disease) and psychological stress contribute to the precipitation of this condition (Clauw, 2014).

The pathophysiological bases for fibromyalgia pain are related to a sensitization of the central nervous system (CNS) which amplifies the peripheral nociceptive inputs and maintains chronic pain. Patients with fibromyalgia feel pain in conditions that nonsufferers perceive as touch, this signifies that the former have problems with augmented pain or sensory processing in the CNS. For this reason, they display diffuse hyperalgesia (increased pain from painful stimuli) and/or allodynia (pain from nonpainful stimuli). There are, however, no abnormalities in the region of the body where pain is experienced. A number of factors are related with an individual's sensitivity to pain. The neurotransmitters involved in pain control are also known to control other functions such as mood, fatigue, memory, and sleep, often altered in the illness. To date, it has been suggested that endogenous opioidergic activity may be normal or even increased while there is decreased activity in the endogenous serotonergic and noradrenergic systems. In addition, the glutamatergic system is also involved in the pathogenesis of fibromyalgia. Recent findings suggest an imbalance in the levels of the neurotransmitters that affect pain and sensory transmission: high levels of neurotransmitters increase pain transmission and/or low levels of neurotransmitters decrease it. Neuroimaging studies have contributed to demonstrating the neurotransmitter hypothesis, and to finding the structures implicated in fibromyalgia such as the insula. Moreover, sensitivity to different stimuli such as heat, cold, electrical stimuli, and auditory tones is also augmented (Kuttikat & Shenker, 2013; Clauw, 2014, 2015; Rahman et al., 2014)

The symptoms of fibromyalgia include musculoskeletal pain in various groups of muscles, especially in the proximal shoulder girdle muscles, which is poorly localized, of severe intensity, and difficult to ignore. It is present most of the day, appears every day, and for at least 3 months. Another characteristic of this disorder is the appearance of focal, triggered point tenderness in affected muscles. In addition, patients complain of fatigue, muscular weakness, sleep disturbance, mood disorders, and impairment of some cognitive domains such as concentration and alertness. Depending on the somatic and psychological symptoms, fibromyalgia can cause functional disability (Clauw, 2014; Rahman et al., 2014).

In 1999, the ACR published the classification criteria for fibromyalgia syndrome which have been used widely used in therapeutic clinical trials. These criteria had a sensitivity of 88% and a specificity of 81% in a patient population (Table e16.1). Subsequently, the ACR published in 2010 further preliminary diagnostic criteria, taking into account the relevance of somatic symptoms such as fatigue and cognitive difficulties associated with this syndrome. These new criteria developed for clinical use have a symptom severity scale which can be useful for longitudinal monitoring. One major difference is that they do not require a tender point count (Table e16.2). In addition, there is a patient self-report version (Clauw, 2014, 2015).

Routine laboratory and radiographic tests are normal, focused on excluding other diagnoses and evaluating pain generators and comorbid conditions. It is important to carry out a differential diagnosis because some symptoms can overlap with other chronic pain conditions such as headaches, facial/jaw pain, regional myofascial

TABLE e16.1 American College of Rheumatology 1990 Classification Criteria for Fibromyalgia

Criteria

A. History of widespread pain for at least 3 months. Pain is considered widespread when it is present at all of the following sites (b):
   - the left side of the body
   - the right side of the body
   - above the waist
   - below the waist
   - in the axial skeletal (cervical spine or anterior chest or thoracic spine or low back)

B. Pain on digital palpation in at least 11 of the 18 tender point sites; all sites bilateral (c):
   (a) For purposes of classification, patients will be said to have FMS if both criteria A and B are satisfied. The presence of a second clinical disorder does not exclude the classification of fibromyalgia
   (b) Pain in a patient in for instance the left shoulder, right buttock, and cervical spine is generalized, according to these criteria
   (c) Digital palpation should be performed with an approximate force of 4 kg. For a tender point to be considered positive, the palpation has to be painful. "Tender points" that are only tender are not tender points

TABLE e16.2 American College of Rheumatology 2010 Preliminary Diagnostic Criteria for Fibromyalgia

Criteria

A patient satisfies diagnostic criteria for FMS if the following three conditions are met:

1. Widespread pain index (WPI) ≥7 and symptom severity (SS) scale score ≥5, or WPI between 3 and 6 and Symptom Severity (SS) scale score ≥9
2. Symptoms have been present at a similar level for at least 3 months
3. The patient does not have a disorder that would otherwise explain the pain

Ascertainment

1. Widespread pain index (WPI)
   - Note the number of areas in which the patient has had pain over the last week. In how many areas has the patient had pain? Shoulder girdle (left, right); Hip (left, right); Jaw (left, right); Upper back: Lower back; Upper arm (left, right); Upper leg (left, right); Chest; Lower arm (left, right); Lower leg (left, right); Abdomen and Neck
     Score will be between 0 and 19
2. Symptom severity (SS) scale score: fatigue, waking unrefreshed, cognitive symptoms
   - For the each of the three symptoms above, indicate the level of severity over the past week using the following scale: 0 = no problem, 1 = slight or mild problems, generally mild or intermittent, 2 = moderate, considerable problems, often present and/or at a moderate level, 3 = severe: pervasive, continuous, life-disturbing problems
   - Considering somatic symptoms in general[a], indicate whether the patient has: 0 = no symptoms, 1 = few symptoms, 2 = a moderate number of symptoms, 3 = a great deal of symptoms
   - The somatic symptoms scale score is the sum of the severity of the three symptoms (fatigue, waking unrefreshed, cognitive symptoms) plus the extent (severity) of somatic symptoms in general
   - The final score is between 0 and 12

[a] *Somatic symptoms that might be considered muscle pain, irritable bowel syndrome, fatigue/tiredness, thinking or remembering problem, muscle weakness, headache, pain/cramps in the abdomen, numbness/tingling, dizziness, insomnia, depression, constipation, pain in the upper abdomen, nausea, nervousness, chest pain, blurred vision, fever, diarrhea, dry mouth, itching, wheezing, Raynaud's phenomenon, hives/welts, ringing in ears, vomiting, heartburn, oral ulcers, loss of/change in taste, seizures, dry eyes, shortness of breath, loss of appetite, rash, sun sensitivity, hearing difficulties, easy bruising, hair loss, frequent urination, painful urination, and bladder spasms.*

pain in the neck or back, and arthritis. Visceral pain complaints involving the gastrointestinal tract, bladder, and pelvic or perineal region are also often present (Crofford, 2013; Clauw, 2014, 2015).

Treatment of Fibromyalgia

Treatment strategies include nonpharmacological and pharmacological therapies including medicines and cannabis. It has been demonstrated that education, cognitive behavioral therapy, and exercise (passive and active) are effective in dealing with fibromyalgia with a response that usually surpasses pharmacological therapies. There is also limited evidence from a systematic review and a summary of reviews concerning the benefits of acupuncture, homeopathy, and mind-body therapies, including hypnotherapy, biofeedback, and stress reduction (Clauw, 2014; Ablin & Buskila, 2010; Häuser, Walitt, Fitzcharles, & Sommer, 2014; Rahman et al., 2014).

Pharmacological treatment includes drugs approved by the agencies of various countries; however, a wide number of medications are used in an off-label indication (Ablin & Buskila, 2010; Häuser et al., 2014). Three drugs (pregabalin, duloxetine, and milnacipran) have been passed by the United States Food and Drug Administration (FDA) but not by the European Medicines Agency (EMA). The use of cannabinoids is described in section cannabinoids and fibromyalgia (p. 1095).

Taking into account the moderate efficacy of the available pharmacological therapies, drug therapy is not

mandatory in all fibromyalgia cases. Mean efficacy from systematic reviews with metaanalysis (SR-MAs) pooled data found that 19% of patients had at least a 50% reduction in pain but that 11% stopped treatment due to adverse events. It is recommended to choose the drug according to the main symptoms (pain, fatigue, and sleep disorder), physical and psychological comorbidities, and side effects. In addition, it is crucial to monitor efficacy, tolerability, and safety in order to achieve responsible prescribing practices. In most cases, treatment includes a combination of drugs with different mechanisms of action (Rahman et al., 2014).

Gabapentinoids (pregabalin, gabapentin), serotonin norepinephrine reuptake inhibitors (duloxetine and milnacipran), tricyclic antidepressants (TCAs), and selective serotonin reuptake inhibitors (SSRIs) are currently prescribed as standard treatment for fibromyalgia. These options have been studied in randomized clinical trials (RCT); nevertheless, their clinical benefits remain limited for most patients (Häuser et al., 2014; Rahman et al., 2014).

Pregabalin

A gamma-aminobutyric acid (GABA) analog, that binds to an auxiliary subunit (alpha2-delta protein) of voltage-gated calcium channels in the CNS. Pregabalin is approved in Europe for the treatment of peripheral and central neuropathic pain in adults, as adjunctive therapy in adults with partial seizures, and for the treatment of generalized anxiety disorder (GAD) in adults. In the United States, pregabalin has the following approved indications: neuropathic pain associated with diabetic peripheral neuropathy, postherpetic neuralgia, adjunctive therapy for adult patients with partial onset seizures, fibromyalgia, and neuropathic pain associated with spinal cord injury. In the management of fibromyalgia, doses must be titrated to reach the recommended dose of 300–450 mg/day (divided in two administrations). Higher doses, such as 600 mg/day, do not confer additional benefit and are less well tolerated. Most common adverse effects are dizziness, somnolence, dry mouth, edema, blurred vision, weight gain, and difficulty concentration/attention (>5% patients). The efficacy of pregabalin in fibromyalgia has been demonstrated in several RCTs and SR-MA (Moore, Straube, Wiffen, Derry, & McQuay, 2009; Moore et al., 2012a; Üçeyler, Sommer, Walitt, & Häuser, 2013; Rahman et al., 2014).

Duloxetine

A selective serotonin and norepinephrine reuptake inhibitor (SSNRI) indicated in the United States for: major depressive disorder, GAD, diabetic peripheral neuropathic pain, chronic musculoskeletal pain, and fibromyalgia, but in Europe not approved for fibromyalgia. The recommended dose for fibromyalgia is 60 mg/day. Nausea, somnolence, insomnia, dry mouth, constipation, decreased appetite and dizziness, and sexual dysfunction are the main side effects (>5% of patients). Its efficacy in fibromyalgia has been demonstrated in several RCTs and SR-MAs (Häuser, Bernardy, Uçeyler, & Sommer, 2009; Häuser, Urrútia, Tort, Uçeyler, & Walitt, 2013, Lunn, Hughes, & Wiffen, 2014; Rahman et al., 2014).

Milnacipran

It is an SSNRI more selective for norepinephrine uptake. The recommended dose is 100 mg in two divided daily doses. Common side effect are nausea, headache, constipation, dizziness, insomnia, hot flush, hyperhidrosis, palpitations, dry mouth, and hypertension (>5% patients). Its efficacy in fibromyalgia has been demonstrated in several RCTs and SR-MAs (Häuser et al., 2009, 2013; Derry, Gill, Phillips, & Moore, 2012; Rahman et al., 2014).

Amitriptyline

Amitriptyline 10–50 mg daily is used in fibromyalgia to treat sleep problems and fatigue as an off-label indication. Common (>5% frequency) side effects include dizziness, headache, weight gain, hypotension, somnolence, sexual dysfunction, and dry mouth. Its efficacy in fibromyalgia has been demonstrated in several RCTs and SR-MAs (Häuser et al., 2009, 2014; Moore et al., 2012a,b).

Selective Serotonin Reuptake Inhibitors

There are some studies that found some evidence in favor of fluoxetine (12–80 mg/day), paroxetine (20–60 mg/day), and citalopram (20–40 mg/day) (Häuser et al., 2009, 2014; Rahman et al., 2014).

Cyclobenzaprine

A muscle relaxant marketed in the United States, but unavailable in most European countries. The recommended dose is 5 mg 3 times a day. Most common side effects were drowsiness (38% of patients), dry mouth (24%), and dizziness (10%). Its use in fibromyalgia is based on six RCTs (with small samples) and a SR-MA (Tofferi, Jackson, & O'Malley, 2004; Häuser et al., 2014).

Gabapentin

It is similar to pregabalin, and with approved use for adjunctive therapy of epilepsy and treatment of peripheral neuropathic pain/postherpetic neuralgia in adults. The recommended dose for pain varies between 1200–2400 mg/day (maximum 3600 mg/day). Gabapentin has shown similar efficacy and adverse effects as pregabalin in fibromyalgia, but there is less evidence from RCTs and SR-MA (Üçeyler et al., 2013; Moore, Wiffen, Derry, Toelle, & Rice, 2014).

Strong opioids (tramadol, oxycodone) and nonsteroidal antiinflammatory drugs, either as a prescription or on-demand, are not recommended as a strategy in

TABLE e16.3 Drug Classes That Failed to Show Superiority Over Placebo in Reducing Fibromyalgia Syndrome Symptoms

Antidepresssants (sertraline, venlafaxine)
Antiviral agents (valacyclovir)
Anxiolytics (alprazolam, bromazepam)
Dopamine agonists (pramipexole, ropinirole, terguride)
Hormones (calcitonin, dehydroepiandrosterone, prednisone)
Hypnotics (zolpidem)
Interferons
Ketamine (intravenous)
Local anesthesics (lidocaine, intravenous)
Neuroleptics (ritanserin, quetiapine)
Opioids (oxycodone, tramadol)
Serotonin receptor antagonists (tropisetron, ondansetron)
Sodium oxybate (gamma-hydroxybutyrate)

Based on data from Hauser et al. (2014).

fibromyalgia because of the lack of evidence from published RCTs and the potential risk of side effects (Häuser et al., 2014).

A number of other drugs have been studied for treating fibromyalgia, none of them; however, show any superiority with respect to placebo (Table e16.3). Recent research, based on few studies with small sample sizes, has shown efficacy of nabilone for pain and sleep disturbance (see section randomized controlled clinical trials, p. e164), growth hormones for pain and fatigue, and quetiapine or naltrexone to reduce pain and depression (Häuser et al., 2014).

CANNABINOIDS AND FIBROMYALGIA

Biological Basis

The endogenous cannabinoid system mainly includes the cannabinoid receptors CB1 and CB2, the ligands anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and their metabolic enzymes (fatty acid amide hydrolase 1 or FAAH, and monoacylglycerol lipase or MAGL). CB1 is the most abundant G protein-coupled receptor expressed in the brain, in nonneuronal cells such as adipocytes and hepatocytes, and in musculoskeletal tissues. CB2 is principally associated with immune cell function, although it may also be expressed in the CNS. Endocannabinoids have the particularity of act as retrograde messengers by binding to presynaptic CB1 receptors. Cannabinoids are neuromodulators of the glutamate, GABA, serotonin, dopamine, norepinephrine and acetylcholine release (Pacher, Bátkai, & Kunos, 2006; McPartland, Guy, & Di Marzo, 2014). The endocannabinoid system and its role are extensively reviewed in other chapters.

The endocannabinoid system has a number of physiological actions. It is implicated in the modulation of embryological development, neural plasticity, neuroprotection, immunity and inflammation, apoptosis and carcinogenesis, pain and emotional memory, stress response, neuroendocrine regulation and cognitive functions, alimentary behavior (hunger, feeding), and metabolism.

Various authors (Russo, 2008; Smith & Wagner, 2014) have suggested that some diseases are associated with the suboptimal functioning of the endocannabinoid system. Known collectively as clinical endocannabinoid deficiency syndromes (CEDS), these dysfunctions have been proposed as being responsible for migraine, fibromyalgia, irritable bowel syndrome, and related conditions. It has been suggested that interventions enhancing the endocannabinoid system—upregulating cannabinoid receptors, increasing ligand synthesis, and inhibiting ligand degradation—could be useful for CEDS (McPartland et al., 2014).

Kaufmann et al. (2008) evaluated the plasma concentrations of catecholamines, cortisol AEA, and neutrophil function in 22 patients with primary fibromyalgia and 22 age- and sex-matched healthy controls. The results showed that the fibromyalgia patients had significantly higher norepinephrine and AEA plasma levels than controls. Adhesion and phagocytosis capabilities of neutrophils correlated positively with AEA plasma levels.

Studies on the Use of Cannabinoids in the Treatment of Fibromyalgia

There is a limited number of clinical studies assessing the efficacy of cannabinoids (natural or synthetic) in the treatment of fibromyalgia symptoms. Most evidence comes from surveys and observational studies with a few RCTs (Lynch & Campbell, 2011; Lynch & Ware, 2015).

Surveys

In a study carried out at the University of Washington in a regional pain clinic (Aggarwal et al., 2009), the retrospective chart review of a total of 139 patients using medicinal cannabis showed that 19% were taking it for fibromyalgia pain.

A survey was performed in pain clinics in Germany where medicinal dronabinol was prescribed for pain in addition to the current treatment (Weber et al., 2009). Complete data were collected from 124 patients suffering from refractory central neuropathic pain and fibromyalgia. The subjects participated in a standardized, retrospective, telephone interview survey that included changes in pain intensity using the Numeric Rating Scale (NRS), the Pain Disability Index (PDI), the Medical Outcomes Short-Form (SF-12), the Quality of Life Impairment by Pain (QLIP), Hospital Anxiety Depression Scale (HADS), and concomitant pain medication. In the 32 fibromyalgia patients included, mean pain intensity prior

to dronabinol administration was on average 7.9 ± 1.5 units, which was reduced to 4.4 ± 1.5 units during/after THC-treatment. On average, maximum pain intensity was recorded at 9.3 ± 1.1 units, prior to dronabinol and 6.1 ± 2.1 thereafter. Other results of the survey were presented collectively from all the patients: opioid doses were reduced, and patients perceived THC therapy as effective, with relatively good tolerability (25% did not tolerate the treatment).

The prevalence of cannabinoid use in 457 fibromyalgia patients who had been referred to a tertiary care pain center was assessed in Canada (Ste-Marie, Fitzcharles, Gamsa, Ware, & Shir, 2012). The study included a retrospective chart review of all the patients. It was found that 1 in 8 (n = 59, 13%) were using cannabis, of these 80% consumed herbal cannabis (marijuana), 24% took prescription cannabinoids (n = 13 nabilone, and n = 1 dronabinol), and 3% took both herbal cannabis and prescription cannabinoids. All 47 patients using herbal cannabis declared smoking marijuana between 0.5 and 6 g/day, and 34 (72%) reported the use of 1 g or less a day. The clinical data of the 59 subjects employing cannabinoids were compared with the 308 nonusing patients. The cannabinoid users were more frequently male, took more opioids, and presented greater drug-seeking behavior. A subanalysis of the sample, taking into account only those reporting herbal cannabis administration (47 vs 410 subjects), was carried out. Herbal cannabis users presented more current unstable mental illness (36% vs 23%) and opioid drug-seeking behavior (17% vs 4%), and were more frequently male (26% vs 7%). The conclusions of the study, that herbal cannabis use is associated with negative psychosocial parameters, should be cautiously interpreted due to the design of the protocol (a retrospective medical chart review).

An online survey performed by the National Fibromyalgia and Chronic Pain Association (NFCPSA; Bener, 2014) obtained a response from 1200 of its members: 44% had tried marijuana or cannabis for their pain. Positive responses were obtained for sleep (8.4 points in a 0–10 scale), pain (8.2 points), and fatigue (6 points). 22% of the responders used marijuana on a daily basis for their pain. Most subjects did not report side effects (66%), but some described sedation (165), cognitive dulling (6%), anxiety (5%), weight gain (4%), and addictive craving (3%).

A recent survey from the National Pain Foundation showed that medical marijuana may top currently available medicines for treating fibromyalgia (Anson, 2014). The survey, which was conducted online, asked more than 1300 patients with fibromyalgia to rate the effectiveness of the treatments that they had used. Medical marijuana was compared with the three drugs approved by the FDA for fibromyalgia: milnacipran (Savella), pregabalin (Lyrica), and duloxetine (Cymbalta) (Table e16.4).

TABLE e16.4 Effectiveness of Some Pharmacological Treatments Rated by Fibromyalgia Patients in an Online Survey (n = 1300)

Drug	Very effective (%)	Helps a little (%)	Does not help at all (%)
Pregabalin (Lyrica)	10	29	61
Duloxetine (Cymbalta)	8	32	60
Milnacipran (Savella)	10	22	68
Medical marijuana	62	33	5

Patients rated the efficacy as "Very effective", "Helps a little," and "Does not help at all". Results are presented as percentages [based on data from Anson (2014)].

Only 8% of the patients that tried duloxetine, and 10% that tried pregabalin or milnacipran, found the drugs to be "very effective" for managing symptoms of the disorder. Over 60% of the patients reported that each of three drugs "does not help at all." On the other hand, 62% of the patients who tried medical marijuana found it to be "very effective." Another 33% said it "helps a little," and 5% felt that using cannabis for fibromyalgia "does not help at all."

Nonrandomized and Noncontrolled Clinical Trials

In Spain, Fiz, Durán, Capellà, Carbonell, and Farré (2011) conducted a case-control study in patients suffering from fibromyalgia, some were cannabis users (n = 28), and others not (n = 28). The participants completed a series of quality of life questionnaires including the Fibromyalgia Impact Questionnaire (FIQ), the Pittsburgh Sleep Quality Index (PSQI), and the Short Form 36 Health Survey (SF-36). They were further asked to record the perceived benefits of cannabis on a range of symptoms (pain, stiffness, relaxation, drowsiness, and well-being) using 100 mm VAS scales (VAS) prior to and at 2 h of cannabis consumption. Based on a list of symptoms, the occurrence and frequency of side effects were evaluated. Demographics and clinical variables were similar in both groups. Of the 28 fibromyalgia cannabis users, 11 (40%) reported a duration of cannabis consumption of less than 1 year, 9 (32%) between 1 and 3 years, and 8 (29%) more than 3 years. Only eight patients in the cannabis group had previously tried it recreationally. The cannabis derivate in every case was marijuana, and the route of administration was smoking (54%), oral (46%), and combined (43%). Patients employed cannabis not only to alleviate pain but for almost all the symptoms associated with fibromyalgia, and

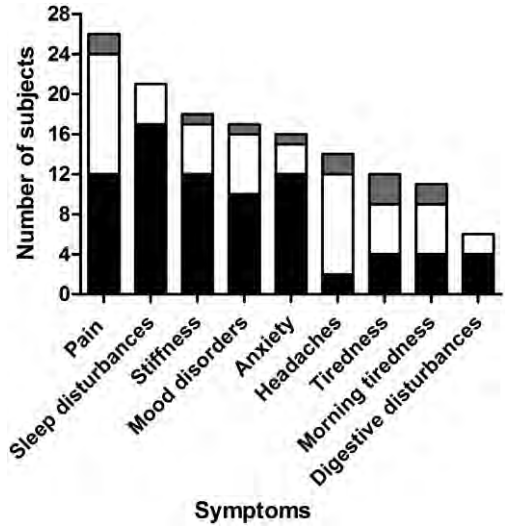


FIGURE e16.1 Symptoms and perceived relief reported by fibromyalgia patients using cannabis. Perceived relief was recorded using 5-point Likert scale (strong relief, mild relief, not change, slight worsening, great worsening). The histogram shows the number of subjects for each relief category (*black* color, strong relief; *gray* color, mild relief; *white* color, no change.). *Source: Based on data from Fiz et al. (2011).*

none reported worsening of symptoms. The proportion of patients who described strong relief ranged from 81% for sleep disorders to 14% for headache (Fig. e16.1). In relation to quality of life, only the mental health component summary score of the SF-36 questionnaire was slightly, but significantly higher, in the cannabis group than in the nonuser group. After 2 h of cannabis use, VAS scores showed a statistically significant reduction of pain and stiffness, enhancement of relaxation, and an increase in somnolence and feeling of well-being (Fig. e16.2). The

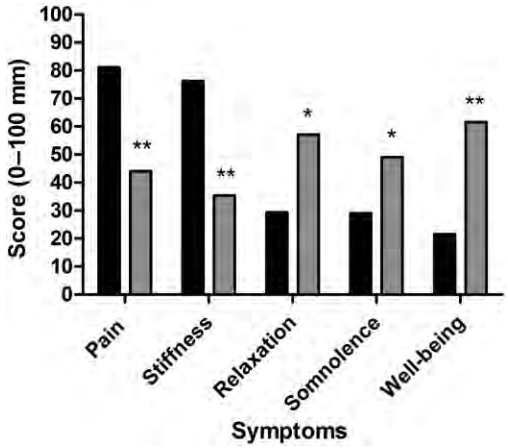


FIGURE e16.2 Perceived effects of cannabis self-administration in some symptoms (scores at baseline and 2 h after cannabis use). Perceived benefits of cannabis recorded by patients on a range of symptoms using 100-mm VAS scales before and at 2 h of cannabis consumption. *Gray* bars, pre-cannabis; *black* bars, post-cannabis. [**, $p < 0.001$; *, $p < 0.05$; *with permission from Fiz et al. (2011)*].

most frequent side effects were somnolence ($n = 18$), dry mouth ($n = 17$), sedation ($n = 12$), dizziness ($n = 10$), feeling high ($n = 9$), tachycardia ($n = 8$), conjunctival irritation ($n = 7$), and hypotension ($n = 6$).

In nine fibromyalgia patients, the effect of oral THC on electrically induced pain, axon reflex flare (vasodilatation), and psychometric variables was investigated (Schley et al., 2006). The study was noncontrolled, and subjects were compared before and after cannabinoid administration. The participants received a daily oral dose of 2.5–15 mg THC. Starting with 2.5 mg, the dosage was increased weekly by 2.5 mg THC, as long as no severe side effects were reported. Once a week, 24 h after the last THC medication and a day before any dose increase, an electrically induced pain was performed. The pain intensity was recorded daily by means of a numeric pain scale, from 0 (no pain) to 10 (maximum pain imaginable). In the four participants who completed the 3-month study, pain decreased on average by 67% (mean pain score difference –5.3 ± 2.3). All four experienced a pain reduction by more than 50% (mean pain score difference –2.8 ± 5). The experimentally induced pain was significantly reduced by THC at a dose of 10 and 15 mg. Daily recorded pain intensity was lowered on average from 8.1 at the beginning of the study, to 2.8 after 3 months. THC had no effect on the axon reflex flare, and no differences were found in the psychometric variables. Five of the nine participants dropped out of the study before achieving the maximum dose of 15 mg due to severe side effects (primarily sedation, dizziness, fatigue, and continuous tiredness).

Randomized Controlled Clinical Trials

In one of the few published RCTs, Skrabek, Galimova, Ethans, and Daryl (2008) carried out a double-blind, randomized, placebo-controlled clinical trial to analyze the effects of oral nabilone and placebo on pain and quality of life in patients with fibromyalgia. After 4 weeks of treatment (nabilone group = 0.5 mg once daily in week 1, 0.5 mg twice daily in week 2, 0.5 mg in the morning, and 1 mg in the evening in week 3, and 1 mg twice daily in week 4), patients who received nabilone ($n = 15$) experienced significant improvements in clinical pain, measured on a visual analog scale (–2.04 cm, $p < 0.02$), FIQ score (–12.07, $p < 0.02$) and the 10-point anxiety scale of the FIQ (–1.67, $p < 0.02$) in comparison to placebo. After a 4-week washout period at the end of the trial, all benefits were lost in the nabilone cohort, who returned to their baseline levels of pain and quality of life. Patients who received placebo ($n = 18$) experienced no change throughout the study. Side effects were more common in the nabilone treated subjects, compared with placebo controls at both 2 and 4 weeks of treatment. The most common side effects reported by subjects in the

nabilone group include drowsiness (7/15), dry mouth (5/15), vertigo (4/15), and ataxia (3/15). No serious adverse events appeared during the study.

A randomized, double-blind, active-control, equivalency crossover trial was carried out in fibromyalgia patients with chronic insomnia (Ware, Fitzcharles, Joseph, & Shir, 2010). The compared oral treatments were nabilone and amitriptyline (0.5–1.0 mg and 10–20 mg before bedtime, respectively). Subjects received each drug for 2 weeks with a 2 week washout period between the two treatment phases. The primary outcome was quality of sleep as measured by the Insomnia Severity Index and the Leeds Sleep Evaluation Questionnaire (LSEQ). Secondary outcomes included pain, mood, quality of life, and adverse events. A total of 31 subjects were included and 29 completed the study (26 women, mean age 49.5 years). Sleep was improved by both amitriptyline and nabilone, although nabilone was superior to amitriptyline (Insomnia Severity Index difference 3.2; 95% confidence interval 1.2–5.3). No significant differences were found in the LSEQ. No effects on pain, mood, or quality of life were observed. Preference at the end of the trial for nabilone was higher (41% of the subjects) than for amitriptyline (32%). Adverse events were mostly mild to moderate and were more frequent with nabilone. Fifty-three and ninety-one adverse events were deemed possibly or probably related to amitriptyline and nabilone therapy, respectively. Adverse events occurring in more than two subjects, and which were more common for nabilone, were dizziness ($n = 10$ subjects), nausea ($n = 9$), dry mouth ($n = 7$), drowsiness ($n = 6$), headache ($n = 4$), constipation ($n = 4$), insomnia ($n = 3$), and vomiting ($n = 3$). In the case of amitriptyline they were headache ($n = 6$ subjects), dizziness ($n = 4$), dry mouth ($n = 3$), and blurred vision ($n = 3$). The researchers concluded that "nabilone is effective in improving sleep in patients with fibromyalgia and is well tolerated. Low-dose nabilone given once daily at bedtime may be considered as an alternative to amitriptyline."

Health Professionals' Opinions on the Use of Cannabinoids

There are no specific studies about opinions or surveys concerning the use of cannabinoids in fibromyalgia pain. A recent online survey carried out with rheumatologists in Canada evaluated their confidence concerning their knowledge of cannabinoid molecules and mechanisms relevant to rheumatology, and their ability to advise patients about cannabinoid treatments (Fitzcharles et al., 2014b). Results showed that over three quarters of the 128 respondents lacked confidence in their knowledge with respect to cannabinoid molecules. A total of 45% of respondents believed there was no current role for cannabinoids in rheumatology patient care, and only 25% supported any use of herbal cannabis. Most of them had never previously prescribed or recommended any cannabinoid treatment (70%). Their uncertainty regarding good prescribing practices was very prevalent. The authors suggested that "Guidance is required to inform rheumatologists on the evidence regarding cannabinoids." The results were similar to a survey that was conducted among Canadian family doctors to determine the educational needs regarding the use of cannabis for therapeutic purposes (Ziemianski et al., 2015).

Fitzcharles, Clauw, Ste-Marie, and Shir (2014a) commented recently on the increasing hiatus among public advocacies for herbal cannabis as a therapeutic agent in rheumatic conditions. Shortfalls included uncertainty of compound content, unknown dosing, recommendations not to use by inhalation, and the indicators of harm, both in the acute as well as chronic setting, and the medical evidence for efficacy and side effects. They concluded that "Taking all factors into consideration, health care professionals should currently dissuade rheumatology patients from using herbal cannabis as a therapy."

CONCLUSIONS

In the near future, the worldwide increase of countries with authorized use of medicinal cannabis, and the augment in licenses for doctors to prescribe it, will raise the number of patients legally employing cannabis products as compassionate disease therapy.

In the case of fibromyalgia, evidence of cannabinoid use is very limited. There are only two short-term RCTs with a limited number of subjects experiencing positive results from oral dronabinol. Noncontrolled studies have shown positive effects in pain after the administration of natural cannabinoids by different administration routes. Patient surveys have reported that some of them employ cannabinoids, mainly herbal cannabis, to control pain and other symptoms in fibromyalgia.

The use of cannabinoid medicines in fibromyalgia pain must be assessed through standard scientific methods. More studies are needed to know the role of the endocannabinoid system in their pathogenesis, and to evaluate efficacy and safety of cannabinoids in fibromyalgia.

MINI-DICTIONARY

Allodynia A pain response from stimuli which do not normally provoke pain (eg, mild tactile pressure on a muscle).
Crossover clinical trial A study in which subjects receive more than one treatment, usually a sequence of two or more different treatments (or exposures).
Double-blind clinical trial A study in which neither the participants nor the researchers know which treatment the participants are taking.

Fibromyalgia A disorder characterized by chronic widespread pain, and a heightened and painful response to pressure, often accompanied by fatigue, memory problems, and sleep disturbances.
Hyperalgesia An increased pain response. Pain is more painful than it should be considering the stimuli.
Metaanalysis A statistical method for contrasting and combining results from different studies, usually randomized clinical trials, to resolve uncertainty when studies have a small number of subjects, or results from different studies do not concur.
Off-label use The use of drug agency-approved pharmaceutical drugs for different indications including an unapproved indication, an unapproved age group, unapproved dosage, and unapproved form of administration.
Randomized-controlled trial or Randomized-controlled clinical trial A type of scientific experiment in which the people being studied are randomly allocated to one (control) or other (test) of the different treatments under study. It is considered the gold standard for the efficacy evaluation of medical therapies. Sometimes the control group is placebo.

Acknowledgments

Role of funding source: Red de Trastornos Adictivos UE-FEDER 2012, RD12/0028/0009, RD12/0028/0006 and Instituto de Salud Carlos III-FIS-FEDER- PI14/00715.

References

Ablin, J. N., & Buskila, D. (2010). Emerging therapies for fibromyalgia: an update. *Expert Opinion on Emerging Drugs*, *15*, 521–533.
Aggarwal, S. K., Carter, G. T., Sullivan, M. D., ZumBrunnen, C., Morrill, R., & Mayer, J. D. (2009). Characteristics of patients with chronic pain accessing treatment with medical cannabis in Washington State. *Journal of Opioid Management*, *5*, 257–286.
Anson, P. (2014). Marijuana rated most effective for treating fibromyalgia. *The National Pain Report*. http://nationalpainreport.com/marijuana-rated-most-effective-for-treating-fibromyalgia-8823638.html
Bener J. (2014). *FMCPA-marijuana-cannabis survey preliminary analysis*. http://www.fmcpaware.org/nfmcpa-marijuana-cannabis-survey-prelim-analysis.html
Clauw, D. J. (2014). Fibromyalgia. A clinical review. *JAMA*, *311*, 1547–1555.
Clauw, D. J. (2015). Fibromyalgia and related syndromes. In M. C. Hochberg, A. J. Silman, J. S. Smolen, M. E. Weinblatt, & M. H. Weisman (Eds.), *Rheumatology* (6th ed., pp. 659–670). Philadelphia, PA: Mosby-Elsevier.
Crofford, L. J. (2013). Fibromyalgia. In A. Fauci (Ed.), *Harrison's rheumatology* (pp. 260–264). New York, NY: McGraw-Hill Education.
Derry, S., Gill, D., Phillips, T., & Moore, R. A. (2012). Milnacipran for neuropathic pain and fibromyalgia in adults. *Cochrane Database of Systematic Reviews*, *3*, CD008244.
Fitzcharles, M. A., Clauw, D. J., Ste-Marie, P. A., & Shir, Y. (2014a). The dilemma of medical marijuana use by rheumatology patients. *Arthritis Care and Research (Hoboken)*, *66*, 797–801.
Fitzcharles, M. A., Ste-Marie, P. A., Clauw, D. J., Jamal, S., Karsh, J., LeClercq, S., McDougall, J. J., Shir, Y., Shojania, K., & Walsh, Z. (2014b). Rheumatologists lack confidence in their knowledge of cannabinoids pertaining to the management of rheumatic complaints. *BMC Musculoskeletal Disorders*, *15*, 258.
Fiz, J., Durán, M., Capellà, D., Carbonell, J., & Farré, M. (2011). Cannabis use in patients with fibromyalgia: effect on symptoms relief and health-related quality of life. *PLoS One*, *6*, e18440.
Häuser, W., Bernardy, K., Uçeyler, N., & Sommer, C. (2009). Treatment of fibromyalgia syndrome with antidepressants: a meta-analysis. *JAMA*, *301*, 198–209.
Häuser, W., Urrútia, G., Tort, S., Uçeyler, N., & Walitt, B. (2013). Serotonin and noradrenaline reuptake inhibitors (SNRIs) for fibromyalgia syndrome. *Cochrane Database of Systematic Reviews*, *1*, CD010292.
Häuser, W., Walitt, B., Fitzcharles, M., & Sommer, C. (2014). Review of pharmacological therapies in fibromyalgia syndrome. *Arthritis Research and Therapy*, *16*, 201.
Kaufmann, I., Schelling, G., Eisner, C., Richter, H. P., Krauseneck, T., Vogeser, M., Hauer, D., Campolongo, P., Chouker, A., Beyer, A., & Thiel, M. (2008). Anandamide and neutrophil function in patients with fibromyalgia. *Psychoneuroendocrinology*, *33*, 676–685.
Kuttikat, A., & Shenker, N. (2013). Fibromyalgia and chronic widespread pain syndromes-adult onset. In R. Watts, P. G. Conaghan, C. Denton, H. Foster, J. Isaacs, & U. Müller-Ladner (Eds.), *Oxford textbook of rheumatology* (pp. 1373–1379). New York, NY: Oxford University Press.
Lunn, M. P., Hughes, R. A., & Wiffen, P. J. (2014). Duloxetine for treating painful neuropathy, chronic pain or fibromyalgia. *Cochrane Database of Systematic Reviews*, *1*, CD007115.
Lynch, M. E., & Campbell, F. (2011). Cannabinoids for treatment of chronic non-cancer pain; a systematic review of randomized trials. *British Journal of Clinical Pharmacology*, *72*, 735–744.
Lynch, M. E., & Ware, M. A. (2015). Cannabinoids for the treatment of chronic non-cancer pain: an updated systematic review of randomized controlled trials. *Journal of Neuroimmune Pharmacology*, *10*, 293–301.
McPartland, J. M., Guy, G. W., & Di Marzo, V. (2014). Care and feeding of the endocannabinoid system: a systematic review of potential clinical interventions that upregulate the endocannabinoid system. *PLoS One*, *9*, e89566.
Moore, A., Wiffen, P., & Kalso, E. (2012a). Antiepileptic drugs for neuropathic pain and fibromyalgia. *JAMA*, *312*, 182–183.
Moore, R. A., Derry, S., Aldington, D., Cole, P., & Wiffen, P. J. (2012b). Amitriptyline for neuropathic pain and fibromyalgia in adults. *Cochrane Database of Systematic Reviews*, *12*, CD008242.
Moore, R. A., Straube, S., Wiffen, P. J., Derry, S., & McQuay, H. J. (2009). Pregabalin for acute and chronic pain in adults. *Cochrane Database of Systematic Reviews*, *3*, CD007076.
Moore, R. A., Wiffen, P. J., Derry, S., Toelle, T., & Rice, A. S. (2014). Gabapentin for chronic neuropathic pain and fibromyalgia in adults. *Cochrane Database of Systematic Reviews*, *4*, CD007938.
Pacher, P., Bátkai, S., & Kunos, G. (2006). The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacological Reviews*, *58*, 389–462.
Rahman, A., Underwood, M., & Carnes, D. (2014). Fibromyalgia. *BMJ*, *348*, g1224.
Russo, E. B. (2008). Clinical endocannabinoid deficiency (CECD): can this concept explain therapeutic benefits of cannabis in migraine, fibromyalgia, irritable bowel syndrome and other treatment-resistant conditions? *Neuroendocrinology Letters*, *29*, 192–200.
Schley, M., Legler, A., Skopp, G., Schmelz, M., Konrad, C., & Rukwied, R. (2006). Delta-9-THC based monotherapy in fibromyalgia patients on experimentally induced pain, axon reflex flare, and pain relief. *Current Medical Research and Opinion*, *22*, 1269–1276.
Skrabek, R. Q., Galimova, L., Ethans, K., & Daryl, K. (2008). Nabilone for the treatment of pain in fibromyalgia. *Journal of Pain*, *9*, 164–173.
Smith, S. C., & Wagner, M. S. (2014). Clinical endocannabinoid deficiency (CECD) revisited: can this concept explain the therapeutic benefits of cannabis in migraine, fibromyalgia, irritable bowel syndrome and other treatment-resistant conditions? *Neuroendocrinology Letters*, *35*, 198–201.
Ste-Marie, P. A., Fitzcharles, M. A., Gamsa, A., Ware, M. A., & Shir, Y. (2012). Association of herbal cannabis use with negative psychosocial parameters in patients with fibromyalgia. *Arthritis Care and Research (Hoboken)*, *64*, 1202–1208.

Tofferi, J. K., Jackson, J. L., & O'Malley, P. G. (2004). Treatment of fibromyalgia with cyclobenzaprine: a meta-analysis. *Arthritis and Rheumatology*, *51*, 9–13.

Üçeyler, N., Sommer, C., Walitt, B., & Häuser, W. (2013). Anticonvulsants for fibromyalgia. *Cochrane Database of Systematic Reviews*, *10*, CD010782.

Ware, M. A., Fitzcharles, M. A., Joseph, L., & Shir, Y. (2010). The effects of nabilone on sleep in fibromyalgia: results of a randomized controlled trial. *Anesthesia and Analgesia*, *110*, 604–610.

Weber, J., Schley, M., Casutt, M., Gerber, H., Schuepfer, G., Rukwied, R., Schleinzer, W., Ueberall, M., & Konrad, C. (2009). Tetrahydrocannabinol (Delta 9-THC) treatment in chronic central neuropathic pain and fibromyalgia patients: results of a multicenter survey. *Anesthesiology Research and Practice*, *2009*, 1–9.

Ziemianski, D., Capler, R., Tekanoff, R., Lacasse, A., Luconi, F., & Ware, M. A. (2015). Cannabis in medicine: a national educational needs assessment among Canadian physicians. *BMC Medical Education*, *15*, 52.

SECTION VIII

SCREENING, DIAGNOSIS, AND TREATMENT

100 *The cannabis abuse screening test (CAST) and its applications* 971
101 *Screening of synthetic cannabinoids* 981
102 *On-site drug testing for cannabis* 998
103 *Cannabinoids in oral fluid: identification and interpretation of analytical results* 1007
104 *Cannabinoids in exhaled breath* 1018
105 *Barriers to treatment seeking for cannabis dependence* 1025
106 *Pharmacotherapies for cannabis use disorders* 1030
107 *Self-initiated cannabis use cessation in adolescents and emerging adults* 1036
108 *Treating cannabis-dependent adolescents with family therapy: the case of multidimensional family therapy* 1047
109 *Cognitive behavioral therapy in cannabis use disorder* 1056
110 *The cannabis withdrawal syndrome—symptoms and time course* 1066
111 *School-based cannabis prevention programs* 1074
112 *The capopus trial for cannabis use disorders* 1086
113 *Treating cannabis use disorders through technology-assisted interventions: the telephone and internet* 1093
114 *Reducing cannabis use with a real-time intervention using mobile technology* 1101

Page left intentionally blank

CHAPTER

100

The Cannabis Abuse Screening Test (CAST) and Its Applications

L. Bastiani, R. Potente, M. Scalese, V. Siciliano, L. Fortunato, S. Molinaro

Institute of Clinical Physiology, The Italian National Research Council (IFC-CNR), Pisa, Italy

SUMMARY POINTS

- This chapter focuses on self-report screening to detect the problematic use of cannabis (CAST).
- For a European standardized definition of problematic use of cannabis, the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) suggests the use of the Cannabis Abuse Screening Test (CAST).
- Cannabis Abuse Screening Test (CAST) is short and easy to administer.
- Not all European countries have tested and validated the CAST in the adolescent or general population.
- Few studies have investigated the association between the PCU detected by the CAST and other components relating to the subject and social and family environments.
- The positive relationship between problematic cannabis use and other risk behaviors, such as use of other substances (tobacco, alcohol and other, illegal substances), truancy at school and poor scholastic performance, and gambling for money.
- The protective variables regarding the PCU are high self-esteem and satisfaction with health status.
- These results must be taken into consideration when designing interventions of prevention and treatment targeting young people and families.

KEY FACTS OF DSM-5

- In the DSM-5, issued in May 2013 (www.dsm5.org), the diagnostic criteria for the identification of individuals with problematic substance use (now called Substance Use Disorders – SUD) underwent substantial changes.
- SUD is defined as a set of behavioral, cognitive, and physiological phenomena that arise after repeated substance use. They include typically a strong desire to consume the substance, difficulty controlling its use, persistence in its use despite harmful consequences, a preference for consumption with respect to other activities/duties, increased tolerance, and sometimes the onset of physical symptoms of abstinence.
- The changes in classification criteria of SUD include (1) omission of the criterion "recurrent legal problems," (2) addition of the criterion "craving," (3) combination of abuse and addiction.
- SUD is divided into light (two or three criteria met), moderate (four or five criteria), or severe (six or more criteria).
- The changes in the DSM-5 are the result of several international studies. The reason for the removal of the criterion "recurrent legal problems" is related to a low prevalence and a low discriminating power, in both the adult and adolescent populations. This criterion did not appear to be correlated highly with the other ones and would provide little information about the definition of SUD (Piontek, Kraus, Legleye, & Buhringer, 2011; Shmulewitz, Keyes, Wall, Aharonovich, & Aivadyan, 2011). Some studies,

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00117-4
Copyright © 2017 Elsevier Inc. All rights reserved.

such as Sinha (2011) and Weiss (2005), supported the addition of the criterion of "craving" for a better definition of SUD. Research on the association between abuse and addiction were performed by Hasin and Beseler (2009), Beseler and Hasin (2010) and specifically among adolescents, by Perron, Vaughn, Howard, Bohnert, & Guerrero (2010), Piontek et al. (2011) and Wu, Ringwalt, Yang, Reeve, & Pan (2009).

LIST OF ABBREVIATIONS

CAST	Cannabis abuse screening test
CI	Confidence interval
DSM	Diagnostic and Statistical Manual of Mental Disorders
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
LM	Last month
LY	Last year
NPCU	Nonproblematic cannabis use
OR	Odds ratio
PCU	Problematic cannabis use
SUD	Substance use disorders

THE CANNABIS ABUSE SCREENING TEST AND APPLICATIONS

Cannabis is the most widely used illegal psychoactive substance in the world, both in general and in the school population. Adolescence is a period of great change when teens may experiment with new behaviors, such as drug use. The 2012 report of the United Nations Office on Drugs and Crime (UNODC, 2012) indicates that cannabis use throughout Europe is concentrated particularly among young people aged 15–24 years, 14% of whom have used cannabis at least once a year.

According to the latest data provided by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), in Europe, in 2011 about 13% of students (aged 15–16 years) used cannabis at least once during the year preceding the survey (Hibell et al., 2013). Although most students are limited to a one-time use of the substance, the percentages of those using cannabis in "an intensive way", that is, 40 or more times during lifetime, ranges from 1% to 8% (Finland 1%, Germany 2%, Italy 4%, France 8%). Cannabis use 20 days or more in a month is defined as daily-use (or near-daily use), or frequent use reported by 1% of the students in Belgium and Germany, by 2% of Italian and 3% of French students.

A study involving 30 countries from Europe and North America was performed among 15-year-old students, who use cannabis in "an intensive way" (Ter Bogt, De Looze, Molcho, Godeau, & Hublet, 2014). In Western Europe, the frequency of cannabis use has decreased in recent years. This trend has not been observed in the developing countries of Eastern, Central, and South Europe. In the past decade, the use of cannabis has become less typical of rich countries (those with a high per capita gross domestic product) and of young people from wealthy families.

Family context has an enormous influence on teenagers. Parents play an important role in determining the risk of drug use. Laxity toward drug use and lack of parental monitoring are factors related to the use of cannabis. A recent study carried out in Spain (Becoña, Martínez, Calafat, Fernández-Hermida, & Juan, 2013) on a sample of students from Mallorca aged between 11 and 19 years shows that young people who perceive their parents as permissive are more likely to use cannabis.

A study performed among French teenagers shows that a higher family socioeconomic status has a protective effect against daily-use of cannabis (Legleye, Janssen, Beck, Chau, & Khlat, 2011). This study points out that, although adolescents from families with a high socioeconomic status have a higher risk of experimental cannabis use, they have a lower chance of using the substance in a problematic way.

Regarding problematic cannabis use, monitored through self-report screening, one in three students who used cannabis in the last year (LY) are classified as at high risk of developing problematic cannabis use. The prevalence of this use ranges from 1% to 9% in the various European countries, with an average of 5% (Hibell et al., 2013). A small number of studies have examined the notion of problematic cannabis use, comparing the frequent/daily use of the substance with and without addiction (Copeland, Swift, & Rees, 2001; Looby & Earleywine, 2007; Van der Pol, Liebregts, De Graaf, Ten Have, & Korf, 2013). According to the study population, between 25% and 50% of daily cannabis users met the criteria that define its problematic use (NIDA, 2014).

For cannabis and any other legal/illegal substances, any description of the forms of consumption is complicated due to the lack of a shared terminology, especially when the goal is the identification of subjects presenting a problematic use of the substance. In order to compare the various studies in different countries, and to effectively monitor phenomena related to substance use, the EMCDDA has established the criteria of substance consumption patterns approved by the scientific community, also referring to the recent version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), which substantially modifies the criteria for diagnosis of problematic use of substances (Substance Use Disorders – SUD) (see Key facts).

Although the evaluation of problematic cannabis use and of other substances is based on univocal criteria

shared by the scientific community, several tools for assessing this phenomenon can be used.

It is necessary to verify whether the problematic cannabis use is investigated in a clinical or in a general population.

In the first case, evaluations (clinical assessments) are to be performed by accredited and skilled professionals to issue a diagnosis and to investigate the severity of substance use. In the second case, the tools generally used are screenings for a preliminary evaluation, for example, self-report questionnaires, with the aim of detecting the use of problematic and potentially harmful cannabis use in a larger population.

Although screening does not allow a clinical diagnosis, it has the advantage of being simple, easy to administer, cheap, and able to involve very large populations.

There are many self-report screenings that are designed to detect the problematic use of cannabis. Many of these have been adapted from previous specific instruments for alcohol, for example, the 139 items of the problem-oriented screening instrument for teenagers (POSIT) or the drug abuse screening test (DAST-28). Many of these tools (and similar ones) are too long and complex, and inappropriate for investigating the phenomenon in nonclinical populations.

In recent years, the EMCDDA, in collaboration with several national experts, has developed methodologies for the monitoring of "intensive forms of drug use". In fact, for a standardized definition of problematic use of cannabis in the educated population, the EMCDDA suggests the use of the Cannabis Abuse Screening Test (CAST), developed by French researchers (Legleye, Karila, Beck, & Reynaud, 2007).

WHAT IS THE CANNABIS ABUSE SCREENING TEST?

The CAST questionnaire is composed of 6 items related to the past year:

1. Have you smoked cannabis before midday?
2. Have you smoked cannabis when you were alone?
3. Have you had memory problems when you smoked cannabis?
4. Have friends or family members told you that you should reduce or stop your cannabis consumption?
5. Have you tried to reduce or stop your cannabis use without succeeding?
6. Have you had problems because of your cannabis use (argument, fight, accident, poor results at school, etc.)?

All items are answered on a 5-point scale (0 "never," 1 "rarely," 2 "from time to time," 3 "quite often," and 4 "very often"). In the original version (Legleye et al., 2007), positive response thresholds vary across questions. The threshold was set at "from time to time" for the first two questions as they do not screen problems but frequencies of use in different contexts, and at "rarely" for the others. Using this algorithm, individual test scores can range from 0 to 6 and the cutoff points were ≥2.

Computing a score using the full range of items (Legleye et al., 2011) responses yield a total score ranging from 0 to 24 and the cutoff points ≥3.

In the Italian validated version (Bastiani et al., 2013), the scores were associated with different kinds of responses that vary attributing a different importance to the options of response and the cutoff points were ≥7 (Table 100.1).

TABLE 100.1 Computing a Score Using the Italian Validated Version

CAST	Never	Rarely	From time to time	Quite often	Very often
(1) Have you smoked cannabis before midday?	0	1	2	3	4
(2) Have you smoked cannabis when you were alone?	0	1	2	3	4
(3) Have you had memory problems when you smoked cannabis?	0	1	2	3	4
(4) Have friends or family members told you that you should reduce or stop your cannabis consumption?	0	2	3	4	4
(5) Have you tried to reduce or stop your cannabis use without succeeding?	0	2	3	3	4
(6) Have you had problems because of your cannabis use (argument, fight, accident, poor results at school, etc.)?	0	2	2	3	4

Original (Legleye et al., 2011) and Italian version of the CAST have the same questions, the content and order of items are equivalent. In relation to items characteristics, through the multiple correspondence analysis (MCA) (Gifi, 1990), we observed that the various response items of the five questions of CAST contributed with different weight to the total score (Bastiani et al., 2013).
Italian version scores were equal to the original version for the first three items [(1) consuming before midday; (2) consuming alone; and (3) memory problems]. On the contrary for the fourth, fifth, and sixth items, scores were associated with different kinds of response that vary attributing a particular and greater importance to the options of more moderate response (rarely) to these questions. So, we obtained the better score in relation to the importance of the response categories for each question of CAST. The score was obtained as the sum of items recoded after MCA results, and permitted maximizing item homogeneity from the scale and a best estimate of the phenomenon.

APPLICATION OF THE CAST: AN ITALIAN EXPERIENCE

Although several studies have investigated the factors associated with problematic use of cannabis, after a review of the scientific literature and to our knowledge, few of them aimed to investigate the association between the severity of cannabis use detected by the CAST and concurrently, components relating to the subject and the social and family environments. For this purpose, the Italian Epidemiology Group of the National Research Council – Institute of Clinical Physiology planned this study using data collected from the ESPAD Italia conducted in 2013. The survey is designed to monitor drug use among European youth, and in 2013, a total of 32,564 Italian adolescents (15–19 years) participated. The mean age of participants was 17 years, and 51.3% of them were males. Prior to the survey, participants were informed about the purpose of the study and were guaranteed complete anonymity; participation was voluntary. The response rate of schools participating in the survey was 85%. The present analyses were restricted to adolescents reporting cannabis use in the previous year ($n = 7915$) and shows that 24.7% of Italian students reported LY use (males = 30%; females = 19.2%).

The final sample comprised 6924 young cannabis users who completed the CAST in its entirety and with complete data on gender and age. These students were divided into two groups based on the CAST score: ≥7 is labeled as "problematic cannabis user" (PCU) and <7 as "nonproblematic cannabis user" (NPCU).

The prevalence of Italian students who are PCUs is 5.6% (22.8% of current users) and the rate is higher among males than females (7.7% vs 3.5%). It should be underlined that the average age at first use of cannabis is significantly lower among PCUs than NPCU students [14.4 years (d.s. 1.9) vs 15.6 years (d.s. 1.5); $p < 0.0001$] and that among PCU students the percentage of "frequent cannabis users" (20 or more times in the last month) is 39.7% (vs 5.5% among NPCUs).

In addition to variables regarding gender, age and age at first use of cannabis, we selected independent variables concerning specific topics (Fig. 100.1):

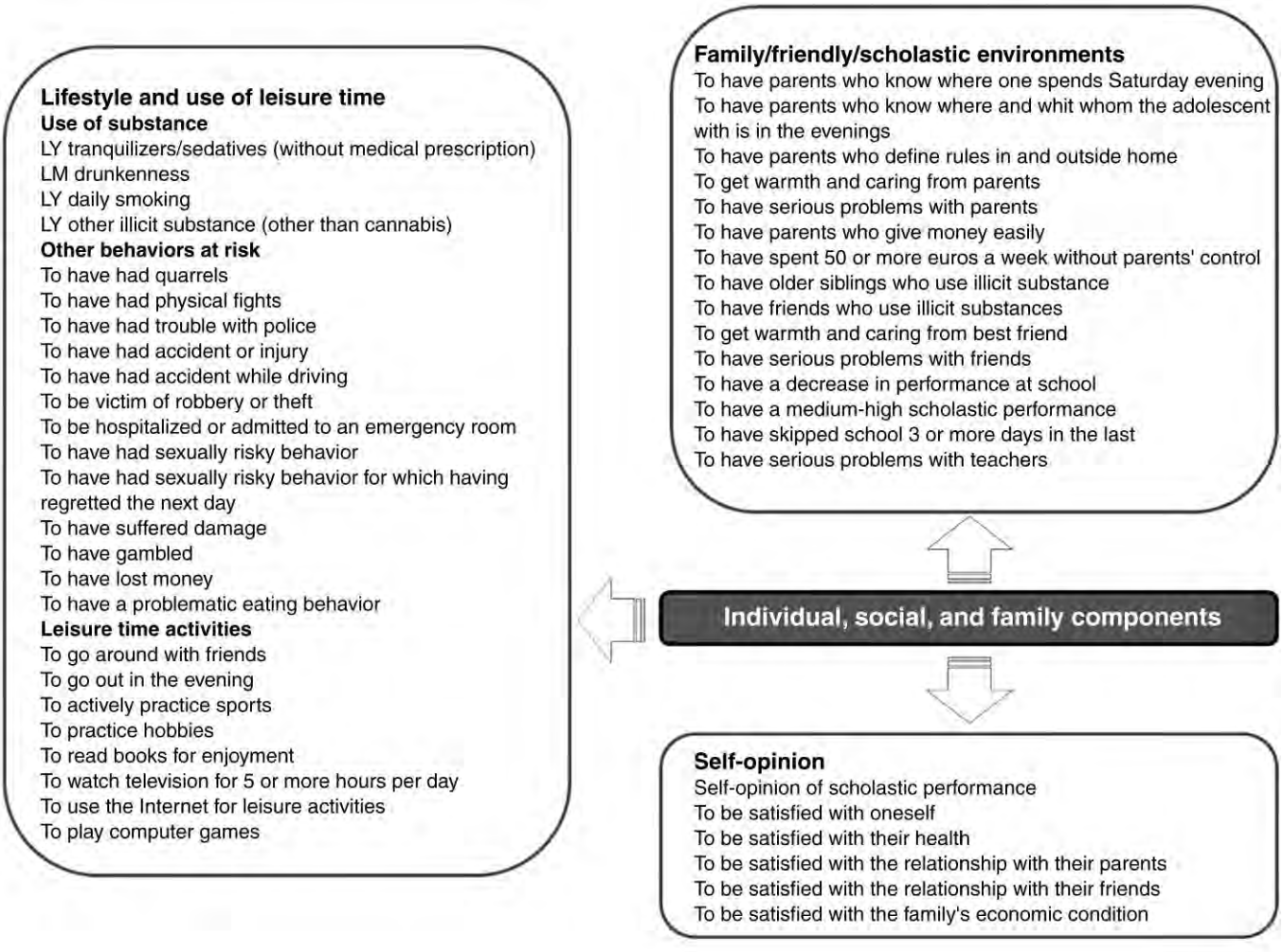


FIGURE 100.1 **Independent variables considered (except gender, age and age at first use of cannabis).** LY, last year; LM, last month.

1. Family/friendly/scholastic environments,
2. Lifestyle and use of leisure time,
3. Self-opinion.

The statistical analyses performed were divided into two phases. The first phase consists of univariate logistic regression analysis corrected by gender, age and age at first cannabis use. A significance of 0.05 was required for a variable to be included in the second stepwise analyses, consisting of a multivariate logistic regression model. We computed two separate multiple logistic regression analyses using respectively variables associated positively and inversely at univariate analysis. Results are reported as adjusted odds ratio (OR) with a 95% confidence interval. Statistical significance was set at $p < 0.05$.

THE MAIN EVIDENCE

First Phase: Univariate Logistic Regression

The univariate analysis shows association between single covariates and problematic use of cannabis (Table 100.2).

First, males were more likely to be PCU than females, while increasing age of initiation decreases the odds of being PCU. On the other hand, comparing students who have tried cannabis at the same age, it is highlighted that older students were more likely than younger ones to be PCU.

What the association with other covariates shows is covered in subsequent sections.

TABLE 100.2 Univariate Logistic Regression Correct by Gender, Age and Age of First Cannabis Use

Variables	Students with PUC	
	OR (95% CI)	*p* value
Sex: male versus female	1.46 (1.29–1.66)	<0.001
Age	1.12 (1.07–1.17)	<0.001
Age at first cannabis use	0.60 (0.58–0.63)	<0.001
Family/friendly/scholastic environments		
Having parents who know where one spends Saturday evening: always/often versus sometimes/usually don't know	0.42 (0.37–0.48)	<0.001
Having parents who know where and with whom the adolescent is in the evenings: almost always/often versus sometimes/seldom/almost never	0.48 (0.42–0.55)	<0.001
Having parents who define rules in and outside home: almost always/often versus sometimes/seldom/almost never	0.84 (0.74–0.94)	<0.004
Receiving personal warmth and care from parents: almost always/often versus sometimes/seldom/almost never	0.49 (0.43–0.57)	<0.001
Having serious problems with parents: yes versus no	2.86 (2.53–3.23)	<0.001
Having parents who give money easily: almost always/often verss sometimes/seldom/almost never	0.71 (0.63–0.80)	< 0.001
Spent 50 or more Euros a week without parents' control: yes versus no	3.15 (2.66–3.72)	<0.001
Having older siblings who use illicit substances: yes versus no	2.13 (1.81–2.51)	<0.001
Having friends who use illicit substances: yes versus no	1.04 (0.72–1.50)	ns
Receiving personal warmth and care from best friend: almost always/often versus sometimes/seldom/almost never	0.68 (0.59–0.79)	< 0.001
Having serious problems with friends: yes versus no	2.22 (1.95–2.53)	<0.001
Having a decrease in performance at school: yes versus no	2.20 (1.95–2.47)	<0.001
Having a medium-high scholastic performance: yes versus no	0.48 (0.40–0.56)	<0.001
Have skipped school 3 or more days in the last month: yes versus no	2.33 (2.04–2.65)	<0.001
Having serious problems with teachers: yes versus no	2.32 (2.05–2.63)	<0.001
Use of substance		
LY tranquilizers/sedative (without medical prescription): ≥1 time versus never	2.35 (2.02–2.74)	<0.001
LM drunkenness: ≥1 time versus never	2.44 (2.17–2.75)	<0.001
LY daily smoking: ≥1 cigarette/day versus <1 cigarette/day	4.40 (3.80–5.09)	<0.001
LY other illicit substances (other than cannabis): ≥1 time versus never	6.48 (5.64–7.44)	<0.001

TABLE 100.2 Univariate Logistic Regression Correct by Gender, Age and Age of First Cannabis Use (*cont.*)

Variables	Students with PUC	
	OR (95% CI)	*p* value
Other at risk behaviors		
Having quarrels: yes versus no	1.23 (1.05–1.44)	ns
Having physical fights: yes versus no	1.58 (1.41–1.79)	<0.001
Had trouble with police: yes versus no	3.84 (3.33–4.43)	<0.001
Had accident or injury: yes versus no	1.60 (1.42–1.80)	<0.001
Had accident driving vehicle: yes versus no	1.85 (1.61–2.13)	<0.001
Been victim of robbery or theft: yes versus no	1.67 (1.42–1.97)	<0.001
Been hospitalized or admitted to an emergency room: yes versus no	1.13 (0.99–1.29)	ns
Have performed sexually risky behavior: yes versus no	2.28 (2.02–2.58)	<0.001
Have performed sexually risky behavior and regretted the next day: yes versus no	2.30 (2.03–2.62)	<0.001
Have suffered damage: yes versus no	1.66 (1.47–1.86)	<0.001
Have gambled: almost every day/at least once a week versus never/few times a year/1–2 times a month	2.20 (1.88–2.58)	<0.001
Have lost money: yes versus no	1.79 (1.59–2.01)	<0.001
Have problematic eating behavior: yes versus no	1.89 (1.56–2.29)	<0.001
Leisure time activities		
Going around with friends: almost every day/at least once a week versus never/few times a year/ 1–2 times a month	0.96 (0.75–1.22)	ns
Going out in the evening: almost every day/at least once a week versus never/few times a year/ 1–2 times a month	1.39 (1.17–1.65)	<0.001
Actively practice sports: almost every day/at least once a week versus never/few times a year/ 1–2 times a month	0.64 (0.57–0.73)	<0.001
Practicing hobbies: almost every day/at least once a week versus never/few times a year/ 1–2 times a month	1.07 (0.96–1.20)	ns
Reading books for enjoyment: almost every day/at least once a week versus never few times a year/ 1–2 times a month	0.95 (0.82–1.10)	ns
Watching television for 5 or more hours per day: yes versus no	1.80 (1.44–2.24)	<0.001
Use of the Internet for leisure activities: almost every day/at least once a week versus never/few times a year/1–2 times a month	0.60 (0.45–0.80)	<0.001
Playing computer games: almost every day/at least once a week versus never/few times a year/ 1–2 times a month	0.98 (0.86–1.11)	ns
Self-opinion		
Self-opinion of scholastic performance: excellent/above average versus average/below average	0.79 (0.69–0.91)	<0.001
Being satisfied with oneself: very satisfied/satisfied versus not satisfied and not unsatisfied/not very satisfied/ not satisfied	0.76 (0.67–0.86)	<0.001
Being satisfied with their health: very satisfied/satisfied versus not satisfied and not unsatisfied/not very satisfied/not satisfied	0.51 (0.45–0.59)	<0.001
Being satisfied with the relationship with their parents: very satisfied/satisfied versus not satisfied and not unsatisfied/not very satisfied/not satisfied	0.68 (0.59–0.77)	<0.001
Being satisfied with the relationship with their friends: very satisfied/satisfied versus not satisfied and not unsatisfied/not very satisfied/not satisfied	0.69 (0.59–0.82)	<0.001
Being satisfied with the family's economic condition: very satisfied/satisfied versus not satisfied and not unsatisfied/not very satisfied/not satisfied	0.86 (0.76–0.97)	0.012

ns, Not significant.

Family/Friendly/Scholastic Environments

To be monitored by parents regarding leisure and Saturday evening activities, to have parents who establish rules of behavior in and outside the home and who often give money are variables negatively associated with being PCU. Similarly, students who experience warmth and caring by parents are less likely to be PCU.

On the other hand, to have had serious relational problems with parents and to have spent more than 50 Euros a week without their control, as well as having older siblings who use illegal substances, have a strong positive association with the odds of being PCU.

To feel accepted and emotionally supported by friends is negatively associated with being PCU, while relational problems with peers increase the odds of being PCU.

To have a medium-high scholastic performance is negatively associated with being PCU, while to have had serious problems with teachers, worse scholastic performance, and to have skipped school 3 or more days in the last month increase the probability of being PCU.

Lifestyle and Use of Leisure Time

The odds of being PCU are very high for students who in the LY had used other illegal substance other than cannabis, had used psychotropic drugs (tranquillizers/sedatives without a doctor's prescription) and had smoked cigarettes or gotten drunk in the past month.

Other at risk behaviors, such as having had physical fights and/or accident or injury, also when driving, having had trouble with police and/or sexually risky behaviors or for which having regretted the next day are variables positively associated with being PCU. Having been a victim of robbery or theft increases the odds of being PCU, such as having gambled and/or lost money often.

On the other hand, to be engaged in leisure time activities, such as active participation in sports or athletics and/or used the Internet for pleasure/recreation decrease the odds of being PCU

Instead, going out often in the evening (to a disco, cafe, party, etc.) and watching television for 5 or more hours a day are negatively associated with being PCU.

Last, having a problematic eating behavior (detected through the use of the screening test EAT-26) increases the odds of being PCU.

Self-Opinion

Students who have high self-esteem, who report being very satisfied with their health status, and/or with relationships with parents and friends, and/or with the economic situation of their families, have low probability of being PCUs. The low probability of being PCU is also highlighted among students who consider their scholastic performance above average.

SECOND PHASE: MULTIVARIATE MULTINOMIAL ANALYSIS

The variables described previously are included in a multivariate analysis for assessing the independent associations of each variable given the impact of the others. Figs. 100.2 and 100.3 present the results of the multivariate multinomial logistic regression separately by the variables negatively and positively associated with being PCU.

The variables that increase the odds of being PCU mainly concern the use of other illicit drugs (in addition to cannabis) in the past year, being a daily smoker of cigarettes, and getting drunk in the past month. A positive association with being PCU is frequent gambling, and having been in trouble with the police.

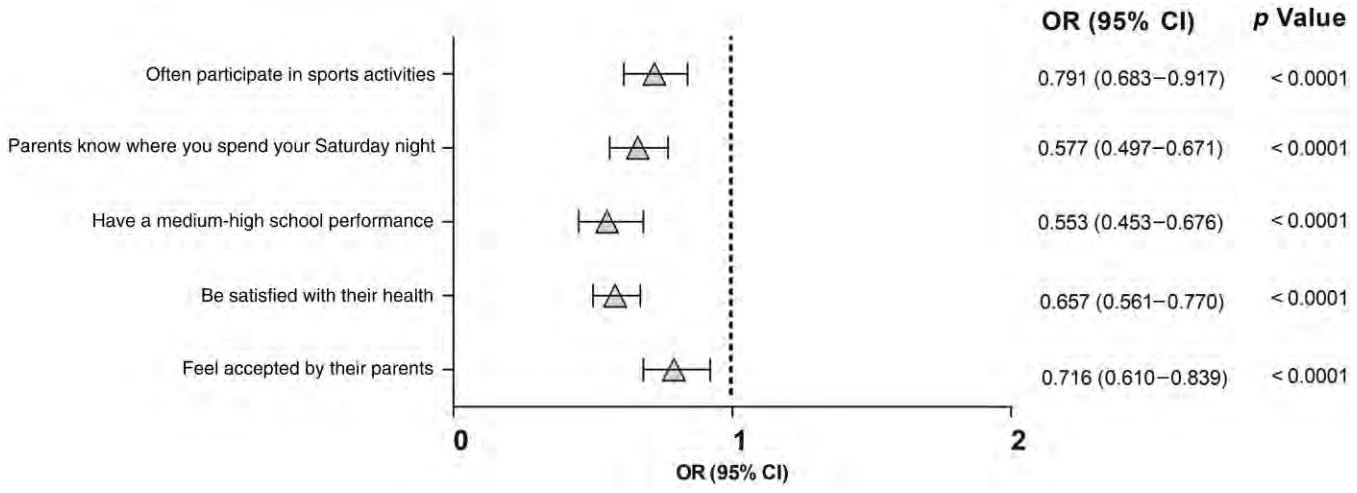


FIGURE 100.2 **Multivariate multinomial logistic regression: behaviors associated negatively with being PCU (OR < 1).**

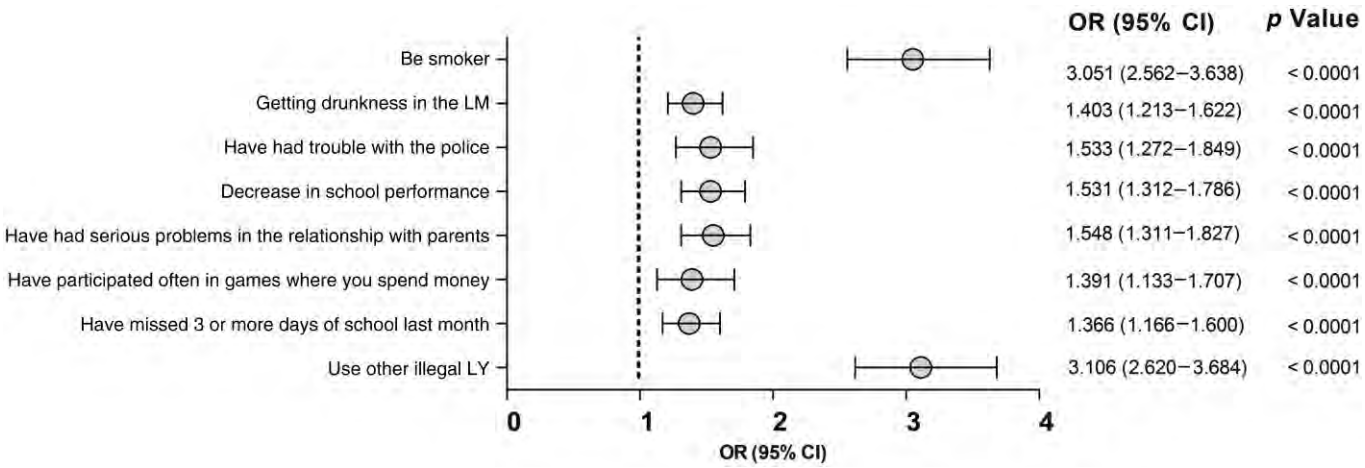


FIGURE 100.3 **Multivariate multinomial logistic regression: behaviors associated positively with being PCU (OR > 1).** LY, last year; LM, last month.

The students who have serious relational problems with the parents and/or have a poor scholastic motivation have a high probability of being PCU rather than NPCU.

On the other hand, for cannabis users who are monitored by parents and who experience warmth and caring from them the probability of being PCU is lower, as well as for the students who are engaged in sports or who use the Internet for fun and leisure.

Last, student cannabis users who have a good scholastic performance and who are satisfied with their health have a lower probability of being PCU rather than NPCU.

CONCLUSIONS

This study emphasizes the application of CAST regarding problematic cannabis use in a national survey that investigates adolescents' behaviors. In order to assess the problematic cannabis use in European adolescent school population, the 2007 ESPAD survey included the CAST as an optional module for the first time (Hibell et al., 2009). In 2007, the CAST module was used by 17 of 36 ESPAD countries and in 2011 by 13 of 35 countries. Although all of these countries have included CAST in their survey, not everyone has provided a validation study for this screening (Hibell et al., 2009, 2012). In order to test and validate this tool for the adolescent or general population, recent national studies, in France (Legleye et al., 2007; Legleye, Piontek, Kraus, Morand, & Falissard, 2013), Portugal (Cuenca-Royo et al., 2012; Cuenca-Royo, Sánchez-Niubó, Torrens, Suelves, & Domingo-Salvany, 2013), Spain (Thanki et al., 2013), Italy (Bastiani et al., 2013), Hungary (Gyepesi et al., 2014) and others are currently in progress or completed. The importance of a standardized tool to assess problematic-cannabis-use has also drawn attention at the European level, promoting, by EMCDDA, a validation study across European countries.

In ESPAD Italia, CAST has been included since 2009, while validation of the screening-tool was carried out in the 2011 Italian survey. In the Italian experience, the annual administration of this screening-test allows monitoring the evolution and characteristics of the phenomenon at the national level, as well as comparison with the other European countries. The application of CAST in the Italian context has allowed estimation of the proportion of young people who are at risk of developing problematic cannabis behavior or addiction and, at the same time, at the level of more limited geographic areas such as regional contexts, provides timely and relevant data for addressing future policies, programs and surveillance activities. The studies have provided statistical evidence useful in the planning and modulation of universal and selective prevention interventions aimed at adolescent and young people, significant adults and the general population.

Indeed, using a very large sample of adolescents aged 15–19 years, our study has analyzed the association of several psychosocial domains with PCU and has examined their simultaneous effects, using a multivariate approach, and not only the effects of each variable, as instead is done by many other studies.

Although several variables were significantly associated with PCU at the univariate level, only a few of them were significant in the multivariate model.

The multivariate analyses clearly pointed out the positive relationship between PCU and other risk behaviors, such as use of other substances (tobacco, alcohol and other illegal substances), truancy at school and low scholastic performance, gambling and being in trouble with

the police. This may be taken to indicate that the psychosocial environment underlying problematic cannabis consumption is similar to other problematic behaviors (often analyzed separately) and therefore responsible for their association.

Our results show that problematic-cannabis-use among secondary school students is strongly and significantly associated with consumption of other illegal substances and gambling during the past year and with well-established use of legal psychoactive substances, such as daily cigarette smoking and excessive alcoholic drinking in the last month, as shown in other studies (eg, Walther, Morgenstern, & Hanewinkel, 2012; Peters, Budney, & Carroll, 2012; Grevenstein & Kröninger-Jungaberle, 2014).

Regarding the family and friendly environments, in univariate analysis illicit substance use by friends, the quality of relationship and the emotion support by them, such as parental monitoring, the presence of rules, the relationship with parents and their emotional support, are all related to problematic-cannabis-use. However, in the multivariate model the friendly environment is not significantly associated, while only monitoring and emotional support by parents have a greater impact, highlighting the key role of the family in adolescent behavior. In fact, as pointed out by many recent as well long-standing studies, one of the risk factors for cannabis use and for PCU, is poor parental monitoring and a bad relationship with parents (Choquet, Hassler, Morin, Falissard, & Chau, 2007; Becoña et al., 2013; Maniglio & Innamorati, 2014).

A good school performance is negatively associated with PCU (Malhotra & Biswas, 2006; Iyalomhe, 2009; McCaffrey, Pacula, Han, & Ellickson, 2010; Brook, Lee, Finch, Koppel, & Brook, 2011; Grevenstein & Kröninger-Jungaberle, 2014); this could be related to the fact that adolescents with a good educational level are more knowledgeable about potential effects or risks associated with use of the substance. Furthermore, our results show that high self-esteem and satisfaction with their health status are protective variables regarding use of cannabis.

In conclusion, we can assume that PCU does not occur as an isolated and unique risk behavior, but is part of a pattern of risk behaviors; this aspect must be taken into consideration when designing interventions of prevention and treatment targeted at young people and families. Given the ease of administration and understanding, CAST has proved to be an efficient and valuable tool for evaluating the severity of cannabis use among adolescents and youth, and for this reason its large-scale application as early as possible may suggest intervention strategies for decreasing the involvement of adverse effects in young adults (eg, Swift et al., 2012) and reducing the costs associated with health care and social services (Degenhardt et al., 2013).

MINI-DICTIONARY

Cannabis Abuse Screening Test (CAST) The tool was developed by the French Monitoring Centre for Drug and Drug Addiction The CAST questionnaire is a 6-item scale screening for problematic patterns of cannabis use among adolescents and general population.

Craving (compulsion) An intense and uncontrollable desire, supported by an obsessive thought of taking the drug, the effects of which have already been previously tested and/or the absence of which results in a state of severe psychological and physical discomfort.

Diagnostic and Statistical Manual of mental disorders (DSM) The Manual was prepared by the American Psychiatric Association, which classifies all diseases or mental disorders, including abuse and dependence on psychoactive substances (including alcohol). Over the years it has been updated and improved several times in the following editions: DSM-I, DSM-II, DSM-III, DSM-III-R, DSM-IV, DSM-IV-TR, DSM-5 (last edition).

Eating attitude test-26 (EAT-26) The EAT-26 is a screening test that can detect a tendency towards anorexia, bulimia, and in general behaviors indicating a problematic relationship with food. The EAT-26 is a shortened version of the original EAT-40 and was first published in 1979.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) A decentralized agency of the European Union, the reference point for drugs and drug addiction information. The EMCDDA is located in Lisbon since 1995.

European School Survey Project on Alcohol and Other Drugs (ESPAD) A European study repeated every 4 years. It investigates tobacco, alcohol and substance use among European students aged between 15 and 16 years. 39 European countries participated in the last survey in 2011.

Frequent use It defines the consumption of cannabis repeated 20 or more times in the last month (also called "daily use" or "near daily use").

Odds ratio (OR) In epidemiology it is one of the indexes used to measure the association between two factors and therefore to quantify the relationship between exposure and a health outcome.

References

Bastiani, L., Siciliano, V., Curzio, O., Luppi, C., Gori, M., Grassi, M., & Molinaro, S. (2013). Optimal scaling of the CAST and of SDS Scale in a national sample of adolescents. *Addictive Behaviors*, *38*(4), 2060–2067.

Becoña, E., Martínez, Ú., Calafat, A., Fernández-Hermida, J. R., & Juan, M. (2013). Parental permissiveness, control, and affect and drug use among adolescents. *Psicothema*, *25*(3), 292–298.

Beseler, C. L., & Hasin, D. S. (2010). Cannabis dimensionality: dependence, abuse and consumption. *Addictive Behaviors*, *35*(11), 961–969.

Brook, J. S., Lee, J. Y., Finch, S. J., Koppel, J., & Brook, D. W. (2011). Psychosocial factors related to cannabis use disorders. *Substance Abuse*, *32*(4), 242–251.

Choquet, M., Hassler, C., Morin, D., Falissard, B., & Chau, N. (2007). Perceived parenting styles and tobacco, alcohol and cannabis use among French adolescents: gender and family structure differentials. *Alcohol and Alcoholism*, *43*(1), 73–80.

Copeland, J., Swift, W., & Rees, V. (2001). Clinical profile of participants in a brief intervention program for cannabis use disorder. *Journal of Substance Abuse Treatment*, *20*(1), 45–52.

Cuenca-Royo, A. M., Sánchez-Niubó, A., Forero, C. G., Torrens, M., Suelves, J. M., & Domingo-Salvany, A. (2012). Psychometric properties of the CAST and SDS scales in young adult cannabis users. *Addictive Behaviors*, *37*(6), 709–715.

Cuenca-Royo, A. M., Sánchez-Niubó, A., Torrens, M., Suelves, J. M., & Domingo-Salvany, A. (2013). The Cannabis Abuse Screening Test (CAST) for psychiatric disorders diagnosis in young cannabis users. *Adicciones*, *25*(1), 87–88In Spanish.

Degenhardt, L., Ferrari, A. J., Calabria, B., Hall, D. W., Norman, R. E., McGrath, J., et al. (2013). The global epidemiology and contribution of cannabis use and dependence to the Global Burden of Disease: results from the GBD 2010 Study. *PLoS One*, *8*(10), e76635.

Gifi, A. (1990). *Nonlinear multivariate analysis*. New York, NY: John Wiley & Sons (First edition 1981, Department of Data Theory, University of Leiden).

Grevenstein, D., & Kröninger-Jungaberle, H. (2014). Two patterns of cannabis use among adolescents: results of a 10-year prospective study using a growth mixture model. *Substance Abuse*, *36*(1), 85–89.

Gyepesi, A., Urbán, R., Farkas, J., Kraus, L., Piontek, D., Paksi, B., et al. (2014). Psychometric properties of the cannabis abuse screening test in Hungarian samples of adolescents and young adults. *European Addiction Research*, *20*(3), 119–128.

Hasin, D. S., & Beseler, C. L. (2009). Dimensionality of lifetime alcohol abuse, dependence and binge drinking. *Drug and Alcohol Dependence*, *101*(1-2), 53–61.

Hibell, B., Guttormsson, U., Ahlström, S., Balakireva, O., Bjarnason, T., Kokkevi, A., & Krauss, L. (2009). *The 2007 ESPAD report: Substance use among students in 35 European countries*. Stockholm, Sweden: The Swedish Council for Information on Alcohol and Other Drugs (CAN), and the Pompidou Group of the Council of Europe.

Hibell, B., Guttormsson, U., Ahlström, S., Balakireva, O., Bjarnason, T., Kokkevi, A., & Kraus, L. (2012). *The 2011 ESPAD Report: Substance use among students in 36 European Countries*. Stockholm, Sweden: The Swedish Council for Information on Alcohol and Other Drugs (CAN), and the Pompidou Group of the Council of Europe.

Hibell, B., Guttormsson, U., Ahlström, S., Balakireva, O., Bjarnason, T., Kokkevi, A., & Kraus, L. (2013). *A supplement to the 2011 ESPAD Report: Additional data from Bosnia and Herzegovina (Federation of Bosnia and Herzegovina), Kosovo (under UNSCR 1244) and the Netherlands*. Stockholm, Sweden: The Swedish Council for Information on Alcohol and Other Drugs (CAN), and the Pompidou Group of the Council of Europe.

Iyalomhe, G. B. (2009). Cannabis abuse and addiction: a contemporary literature review. *Nigerian Journal of Medicine*, *8*(2), 128–133.

Legleye, S., Karila, L., Beck, F., & Reynaud, M. (2007). Validation of the CAST, a general population Cannabis Abuse Screening Test. *Journal of Substance Use*, *12*, 233–242.

Legleye, S., Janssen, E., Beck, F., Chau, N., & Khlat, M. (2011). Social gradient in initiation and transition to daily use of tobacco and cannabis during adolescence: a retrospective cohort study. *Addiction*, *106*(8), 1520–1531.

Legleye, S., Piontek, D., Kraus, L., Morand, E., & Falissard, B. (2013). A validation of the Cannabis Abuse Screening Test (CAST) using a latent class analysis of the DSM-IV among adolescents. *International Journal of Methods in Psychiatric Research*, *22*(1), 16–26.

Looby, A., & Earleywine, M. (2007). Negative consequences associated with dependence in daily cannabis users. *Substance Abuse Treatment, Prevention and Policy*, *2*, 3.

Malhotra, A., & Biswas, P. (2006). Cannabis use and performance in adolescents. *Journal of Indian Association for Child and Adolescent Mental Health*, *2*(2), 59–67.

Maniglio, R., & Innamorati, M. (2014). Psychosocial correlates of adolescent cannabis use: data from the Italian subsample of the second International Self-Reported Delinquency study. *Journal of Addictive Diseases*, *33*(3), 210–220.

McCaffrey, D. F., Pacula, R. L., Han, B., & Ellickson, P. (2010). Marijuana use and high school dropout: the influence of unobservables. *Health Economics*, *19*(11), 1281–1299.

NIDA. (2014). *DrugFacts: Marijuana*. Washington, DC: National Institute on Drug Abuse, US Department of Health and Human Services, Available from: http://www.drugabuse.gov/publications/drugfacts/marijuana.

Perron, B. E., Vaughn, M. G., Howard, M. O., Bohnert, A., & Guerrero, E. (2010). Item response theory analysis of DSM-IV criteria for inhalant-use disorders in adolescents. *Journal of Studies on Alcohol and Drugs*, *71*(4), 607–614.

Peters, E. N., Budney, A. J., & Carroll, K. M. (2012). Clinical correlates of co-occurring cannabis and tobacco use: a systematic review. *Addiction*, *107*(8), 1404–1417.

Piontek, D., Kraus, L., Legleye, S., & Buhringer, G. (2011). The validity of DSM-IV cannabis abuse and dependence criteria in adolescents and the value of additional cannabis use indicators. *Addiction*, *106*(6), 1137–1145.

Shmulewitz, D., Keyes, K. M., Wall, M. M., Aharonovich, E., & Aivadyan, C. (2011). Nicotine dependence, abuse and craving: dimensionality in an Israeli sample. *Addiction*, *106*(9), 1675–1686.

Sinha, R. (2011). Modeling relapse situations in the human laboratory. *Current Topics on Behavioral Neurosciences*, *13*, 379–402.

Swift, W., Coffey, C., Degenhardt, L., Carlin, J. B., Romaniuk, H., & Patton, G. C. (2012). Cannabis and progression to other substance use in young adults: findings from a 13-year prospective population-based study. *Journal of Epidemiology and Community Health*, *66*(7), e26.

Ter Bogt, T. F., De Looze, M., Molcho, M., Godeau, E., & Hublet, A. (2014). Do societal wealth, family affluence and gender account for trends in adolescent cannabis use? A 30 country cross-national study. *Addiction*, *109*(2), 273–283.

Thanki, D., Salvany, A. D., Anta, G. B., Mañez, A. S., Aleixandre, N. L., Suelves, J. M., et al. (2013). The choice of screening instrument matters: the case of problematic cannabis use screening in Spanish population of adolescents. *International Scholarly Research Notices – Addiction*, *2013*, Article ID 723131.

UNODC. (2012). *Cannabis. A Short Review*. Vienna, Austria: United Nations Office on Drugs and Crime, Available from: http://www.unodc.org/documents/drug-prevention-and-treatment/cannabis_review.pdf.

Van der Pol, P., Liebregts, N., De Graaf, R., Ten Have, M., & Korf, D. J. (2013). Mental health differences between frequent cannabis users with and without cannabis dependence and the general population. *Addiction*, *108*(8), 1459–1469.

Walther, B., Morgenstern, M., & Hanewinkel, R. (2012). Co-occurrence of addictive behaviours: personality factors related to substance use, gambling and computer gaming. *European Addiction Research*, *18*(4), 167–174.

Weiss, F. (2005). Neurobiology of craving, conditioned reward and relapse. *Current Opinion in Pharmacology*, *5*(1), 9–19.

Wu, L. T., Ringwalt, C. L., Yang, C., Reeve, B. B., & Pan, J. J. (2009). Construct and differential item functioning in the assessment of prescription opioid use disorders among American adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry*, *48*(5), 563–572.

CHAPTER

101

Screening of Synthetic Cannabinoids

*E.L. Øiestad**, *R. Karinen**, *K. Haugland***, *Å.M.L. Øiestad**

**Norwegian Institute of Public Health, Division of Forensic Sciences, Oslo, Norway

***National Criminal Investigation Service (NCIS) Norway, Forensic Science Department, Oslo, Norway

SUMMARY POINTS

- Synthetic cannabinoids are increasingly important as drugs of abuse, and correspondingly important to include in drug screening panels.
- New synthetic cannabinoids are often first discovered in seized products.
- The number of available substances is increasing, making it difficult for screening methods to keep up.
- Concentrations in biological media are often low, making very sensitive screening methods important.
- Conventional screening assays for THC or THC acid will usually not give response for synthetic cannabinoids, as they are structurally very different from natural cannabinoids.
- Most analytical methods are based on separation by gas or liquid chromatography, followed by mass spectrometric detection, after conventional sample preparation as liquid–liquid or solid phase extraction.
- Some immunoassay kits for urine are on the market, but so far no kits covering all synthetic cannabinoids are available.
- High-resolution mass spectrometry is increasingly being used both for screening and metabolite elucidation.
- Several compounds may give the same metabolites, making the determination of the ingested compound difficult.
- Similar compounds (structural isomers) may pose both analytical problems and problems with interpretation, as all isomers are not necessarily on the prohibited list.

KEY FACTS OF CHROMATOGRAPHY

- The word chromatography is a combination of the Greek words "chroma," color, and "graphein," to write.
- The phrase was used first by the Russian–Italian botanist Mikhail Tsvet in two articles from 1906, describing the separation of a solution of chlorophyll in petroleum ether by adsorption to calcium carbonate in a column.
- Chromatography is used to separate and identify analytes in complex mixtures.
- Separation is based on the partitioning of analytes between a stationary phase and a mobile phase.
- The mobile phase will bring the analytes through the chromatographic column; compounds with little interaction with the stationary phase will elute early, and compounds with larger interaction will elute late.
- The time of elution, called retention time, can be used to identify the analyte by comparison with the retention time of a reference standard.

LIST OF ABBREVIATIONS

ELISA	Enzyme linked immunosorbent assays
FT–IR	Fourier transform infrared spectroscopy
GC–IRD	Gas chromatography with infrared detection
GC–MS	Gas chromatography mass spectrometry
HR–MS	High-resolution mass spectrometry
LC–MS/MS	Liquid chromatography tandem mass spectrometry
LLE	Liquid–liquid extraction
NMR	Nuclear magnetic resonance spectroscopy

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00118-6
Copyright © 2017 Elsevier Inc. All rights reserved.

SC	Synthetic cannabinoids
SPE	Solid phase extraction
SWGDRUG	Scientific Working Group for the Analysis of Seized Drugs
TOF	Time-of-flight ultra high performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS)
UNODC	United Nations Office on Drugs and Crime
UV	Ultra violet spectroscopy

INTRODUCTION

Synthetic cannabinoids are cannabimimetic compounds that interact with the endocannabinoid system, and are full cannabinoid receptor agonists with high potency and high efficacy (Wintermeyer et al., 2010). This makes them popular as alternatives to marijuana, and they are in many cases marketed as legal to use and distribute. The psychoactive effects of synthetic cannabinoids are perceived to be as strong as or stronger than cannabis (Griffiths, Sedefov, Gallegos, & Lopez, 2010), and adverse effects can be significant (Spaderna, Addy, & D'Souza, 2013). The need for screening for synthetic cannabinoids in seized products, intoxication cases, work place testing, driving under the influence cases, and general drug screening is therefore evident.

To circumvent legislation, new compounds are being produced continuously by clandestine laboratories, and rapid changes in methods for both biological media and plant material/solid substances are consequently required. Detection times in naïve users have been reported to be relatively short, 2–3 days for urine (de Jager, Warner, Henman, Ferguson, & Hall, 2012), hours to days for oral fluid (Coulter, Garnier, & Moore, 2011; Oiestad, Johansen, Christophersen, & Karinen, 2013), and 1–2 days in blood (Teske et al., 2010). The fact that estimated terminal elimination half-lives of up to 41 days for JWH-081, JWH-122, and JWH-210 with continuous decrease of serum concentration levels have been reported (Kneisel, Teske, & Auwärter et al., 2014), does however imply that as with THC accumulation in adipose tissue for heavy users could be possible. Analysis intended to determine new intakes of synthetic cannabinoids must therefore be evaluated by caution.

Only a few synthetic cannabinoids resemble the natural cannabinoids, as the classification is based on the binding to the cannabinoid receptors CB1 or CB2. An overview of the different classes of synthetic cannabinoids from an article by Barnes et al. (2014) can be found in Fig. 101.1. THC and other classical cannabinoids are dibenzopyranes, and the first synthetic cannabinoids were nonclassical cannabinoids like the cyclohexylphenoles. The naphthoylindoles JWH-018 and JWH-073 were among the first aminoalkylindoles to come on the market, followed by a large number of similar compounds. The creativity of clandestine drug manufacturers is clearly evident in the so-called 3rd, 4th, and 5th generation synthetic cannabinoids like UR-144, APINACA, and 5F-PB-22.

ANALYTICAL TECHNIQUES

For synthetic cannabinoids (SC), the analysis of plant material or pure substances is very important, as the higher concentrations compared to biological media facilitates the discovery of novel compounds. In May 2013, United Nations Office on Drugs and Crime (UNODC) published a manual for use by national drug analysis laboratories. This manual describes recommended methods for the identification and analysis of synthetic cannabinoid receptor agonists in seized materials (UNODC, 2013). In general, the identity of a controlled drug should be determined with at least two uncorrelated parameters, one of which should provide information on the chemical structure of the analyte (mass spectrometry (MS), infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy (NMR)) (UNODC, 2013). Also in 2013, minimum recommendations for method selection were published by Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG, 2013). Gas chromatography mass spectrometry (GC–MS) is a fast and sensitive technique that separates active compounds before each compound is identified due to its retention time and its fragmentation pattern (mass spectrum). It verifies the presence of new compounds (Choi et al., 2013; Dresen et al., 2010) as they occur and if reference spectra exist, this technique can give a positive identification.

For serum/blood and alternative matrixes as oral fluid or hair, chromatographic methods will usually be utilized. While GC has been a very common technique for drugs of abuse analysis, liquid chromatography (LC), which is directly compatible with aqueous biological samples, is increasingly being used for bioanalysis (Maurer, 2007, 2013). For forensic methods and many clinical applications, mass spectrometry is the preferred detector due to its high specificity.

Urine screening is commonly performed with either immunoassays or chromatographic analysis. Immunoassay screening is a very efficient technique for screening of large numbers of samples, especially when many of the samples are expected to be negative. SC do not cross-react on traditional marijuana immunoassay tests, as most of these cannabimimetic compounds are structurally unrelated to traditional cannabinoids. Kits for some SC have become commercially available. The rapid change of repertoire for SC is, however, a challenge as the development

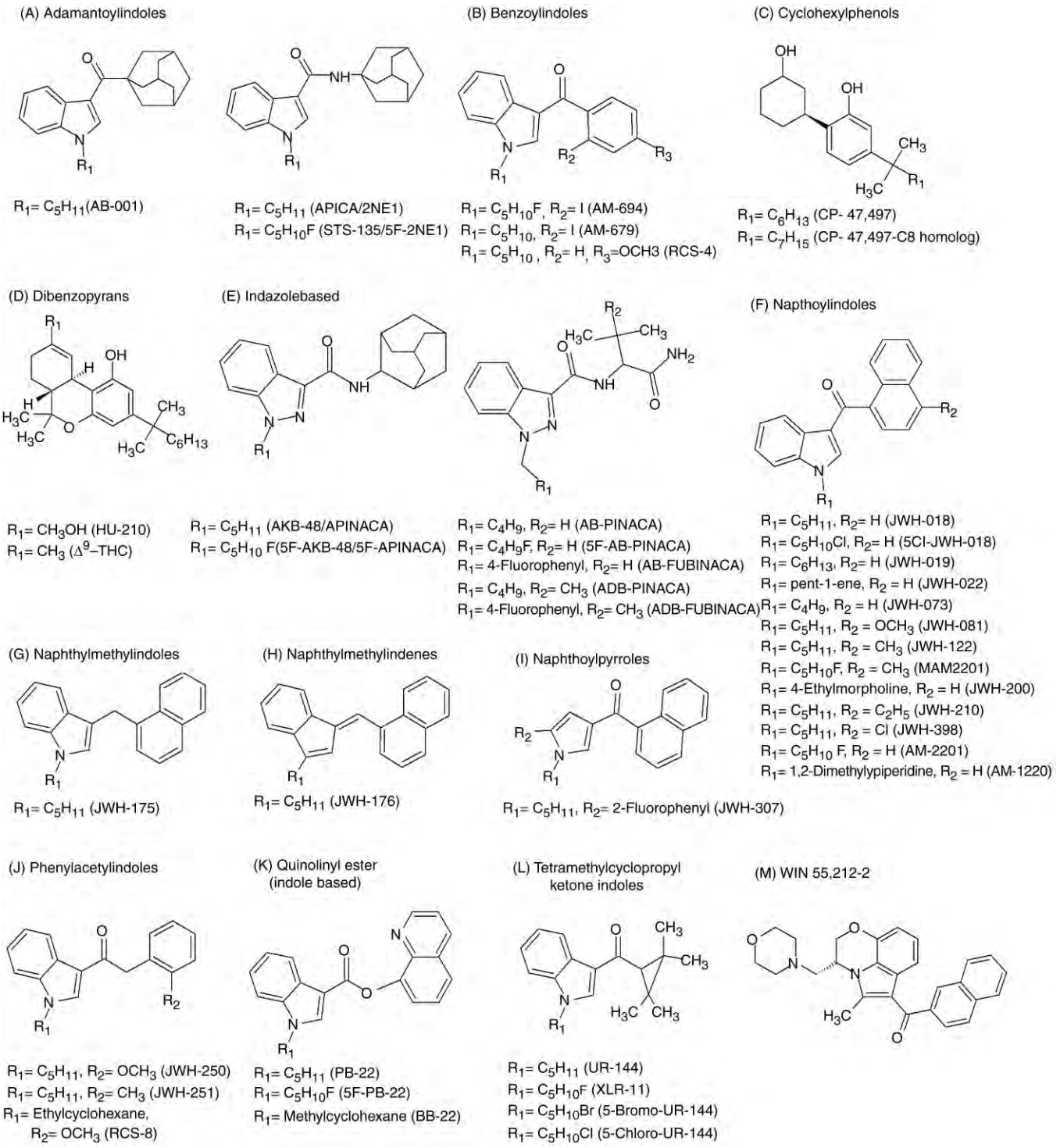


FIGURE 101.1 **Structures of synthetic cannabinoids.** General structures of synthetic cannabinoids from Barnes et al. (2014). Copyright (2014) reproduced by permission from Elsevier.

of new immunoassay kits is time-consuming. On the other hand, several SC have been found to have cross-reactivity with the commercially available assays, as the specificity of the antibodies used is not absolute.

To elucidate metabolic patterns of synthetic cannabinoids and identify possible urine biomarkers, high resolution mass spectrometry (HR-MS) has become an important tool (Gandhi et al., 2013; Holm, Pedersen, Dalsgaard, & Linnet, 2014; Hutter, Broecker, Kneisel, & Auwarter, 2012a). The high resolving power makes the distinction between ions with small mass differences possible. The instrument can in addition be used

both for screening and quantitative analysis (Favretto, Pascali, & Tagliaro, 2013). Another great advantage of HR-MS is the data-dependent acquisition of nontargeted compounds, enabling the recording of almost every ion generated. The data files can then be analyzed retrospectively. Lists of theoretically possible metabolites can be generated based on knowledge of the metabolism of earlier investigated SC of the same type or from knowledge of microsomal transformations (Peters & Meyer, 2011).

ANALYTICAL CHALLENGES

The number of SC is large and growing, and the ones most popular changes rapidly as substances become scheduled. In 2013, 29 new synthetic cannabinoids were reported by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA, 2014). Pure substances of new SC and metabolites are often unavailable, and it is difficult for the manufacturers of reference substances to be abreast of the development as metabolism studies of each substance might be necessary to find the right metabolites.

An increasing challenge is to determine position isomers. This is of great importance when only one form is a controlled substance. An example of position isomers are JWH-201 (para), JWH-250(ortho), and JWH-302 (meta). As demonstrated for GC–MS analysis by Harris, Hokanson, Miller, & Jackson (2014), these compounds have the exact same mass, the same fragmentation pattern, and similar retention times.

For alkylindole compounds analyzed with, for example, LC–MS/MS, the charge is retained commonly on the naphthalene part of the molecule after fragmentation. Fig. 101.2 shows two pairs of isobaric compounds

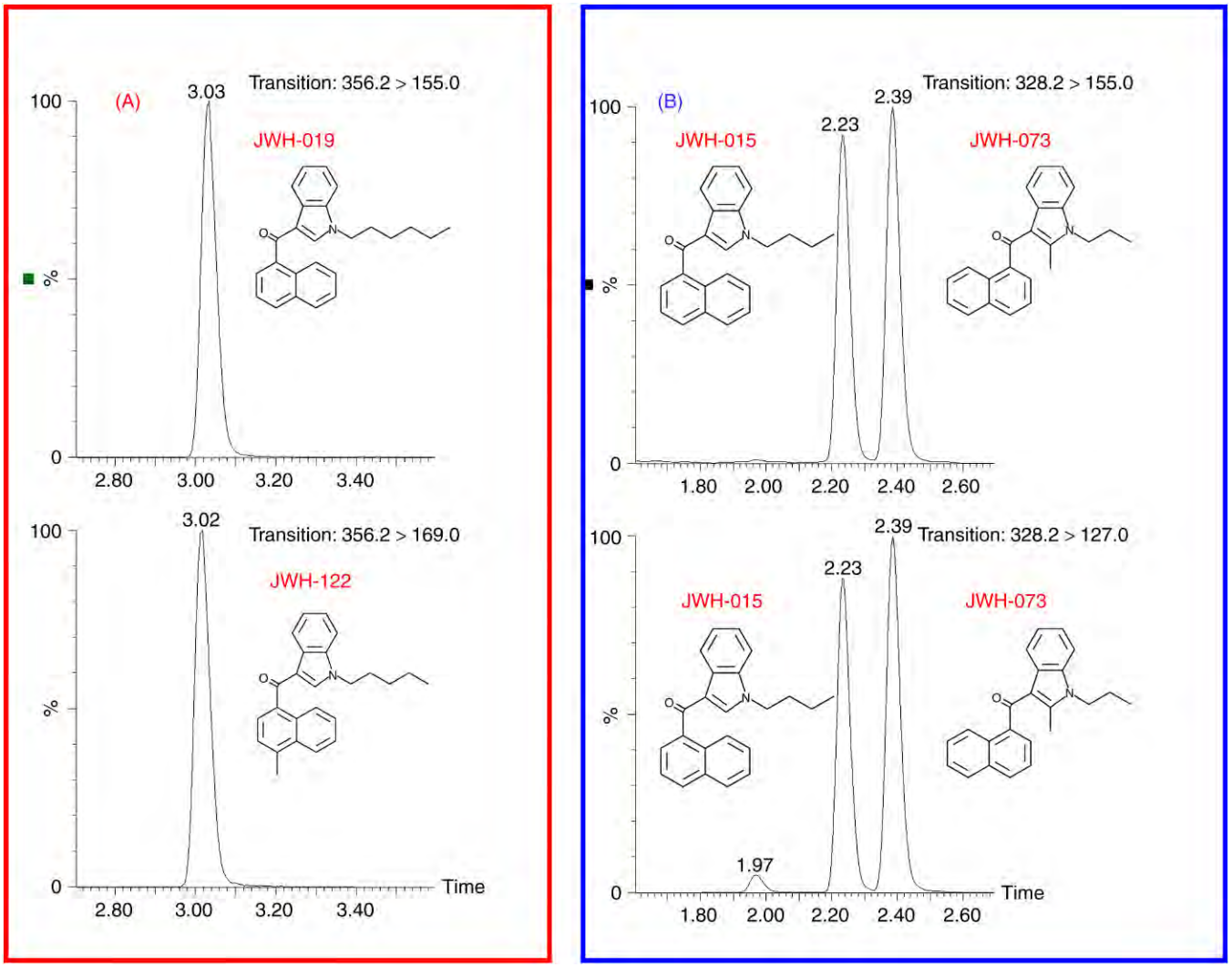


FIGURE 101.2 Separation of isobaric synthetic cannabinoids. (A) Coeluting isobaric compounds JWH-019 (upper panel) and JWH-122 (lower panel) separated due to different mass (B) Isobaric compounds JWH-015 and JWH-073 with identical fragments separated by different elution time.

(compounds with identical elemental composition) JWH-019 and JWH-122 (A) and JWH-015 and JWH-073 (B). As JWH-122 has an *N*-pentyl rather than *N*-hexyl group on the indole and correspondingly a methyl group on the naphthalene part, the mass of the resulting fragment is shifted from 155 for JWH-019 (Fig. 101.2A, upper panel) to 169 for JWH-122 (Fig. 101.2A, lower panel). For the isobaric compounds, JWH-015 and JWH-073, identical fragments are generated as the alkyl groups remain on the uncharged indole part of the molecule (Fig. 101.2B). The compounds are, however, chromatographically separated making the differentiation of the two compounds possible (Oiestad et al., 2013). If distinction is not possible either by chromatographic or mass separation, the two isomers cannot be discerned.

Ingested amounts of synthetic cannabinoids are usually in the low mg range, resulting in very low concentrations in biological media, Table 101.1. The lower range of concentrations are low compared to, for example, the guidelines of the Substance Abuse and Mental Health Services Administration (SAMHSA, 2014) for cannabis, suggesting screening cutoff concentrations of 50 ng/mL for marijuana metabolites and 15 ng/mL for confirmation of THC acid, or the recommended cutoff of 50 pg/mg for THC in hair (Salomone, Gerace, D'Urso, Di Corcia, & Vincenti, 2012). Although high concentrations for single cases can be seen, a majority of published papers demonstrate quite low median concentrations demonstrating the need for very sensitive instrumentation.

Extensive metabolism and no or only very low concentrations of the parent compounds in urine seem to be a common trait for most of the synthetic cannabinoids. In addition, the potency of these new drugs is as mentioned high, and the expected concentration of each of the many metabolites correspondingly low. This makes the detection of new compounds in urine difficult. Though hydroxylation on the alkyl chain have been found to be a major metabolite for many synthetic cannabinoids, such as JWH-015, JWH-018, AM-2201, etc.(Sobolevsky, Prasolov, & Rodchenkov, 2010, Sobolevsky, Prasolov, & Rodchenkov, 2012; Wintermeyer et al., 2010; Zhang, Ma, Cole, & Wang, 2006), other substances, such as APINACA and 5F-APINACA are hydroxylated on the adamantylgroup as the first step (Gandhi et al., 2013; Holm et al., 2014).The elucidation of the metabolism of these compounds illustrates the difficulty in suggesting metabolites. APINACA *N*-(4-hydroxypentyl) and 5F-APINACA *N*-(4-hydroxypentyl) are commercially available, but alas biologically less relevant. Structural similarities of metabolites further complicate the picture. The formation of identical metabolites from several compounds has been described in the literature (Hutter et al., 2012a; Moran et al., 2011), and care must be taken with interpretation.

ANALYSIS OF PLANT MATERIAL AND PURE SUBSTANCES

Synthetic cannabinoids are sold primarily as herbal smoking blends (EMCDDA, 2013, 2014) where one or more active substances are mixed together with or sprayed on to plant materials. The actual content of a product is hard to predict as the active substance are subject to changes over time (Dresen et al., 2010; Langer, Lindigkeit, Schiebel, Ludger, & Beuerle, 2013; Li & Lurie, 2014).

Synthetic cannabinoids are also sold as powder, in either bulk or as smaller amount of "research chemicals". The purity of research chemicals is almost the same as for reference materials (Ginsburg, 2012), while a study from Korea showed that bulk powders had a purity of 70–95% (Choi et al., 2013) Fig. 101.3 shows examples of seized products as bags with herbal products or powder (research chemicals).

Since the number of synthetic cannabinoids is increasing rapidly (EMCDDA, 2014), there is a great need for a universal screening method to verify the existence of a new substance whenever these occur. GC-MS is one of the most commonly used techniques for analysis of seized materials (Choi et al., 2013; Li & Lurie, 2014; Uchiyama, Kikura-Hanajiri, Ogata, & Goda, 2010) and is regarded as the golden standard for qualitative analysis (UNODC, 2013). If no GC–MS reference spectrum is available, the sample might be analyzed with LC–MS or LC–UV (Ernst, Krüger, Lindigkeit, Schiebel, & Beuerle, 2012; Li & Lurie, 2014; Uchiyama, Kawamura, Kikura-Hanajiri, & Goda, 2013; Uchiyama et al., 2010) to get a positive identification, GC–TOF (Ernst et al., 2012) can be used for exact mass determination or Fourier transform infrared spectroscopy (FT-IR) can be used to get information about functional groups. Common for all these techniques is the need for a reference spectrum, and reference spectrums are the key challenge for identification work. Some libraries are available commercially, some must be made in-house, while others are shared between different laboratories, examples are GC-MS libraries (NIST, MPW,ENFSI, SWGDRUG, Cayman Chemicals) and FT-IR libraries (ENFSI, SWGDRUG). If no such reference spectrums exist, NMR can be applied as a powerful tool in unambiguous structure elucidation (Ernst et al., 2012; Uchiyama et al., 2013). In order to distinguish types of compound, MS^n might be helpful (Smith, DeRuiter, Abdel-Hay, & Randall Clark, 2014). If not, FTIR is used for material containing pure substances (Smith et al., 2014). When the sample contains more than one compound, the separation power of GC is needed, making GC-IRD a valuable tool for determination of similar molecules.

TABLE 101.1 Synthetic Cannabinoid Concentrations

Medium	References	Unit	AM-694	AM-1220	AM-2201	AM2201-*N*-(4-hydrox-ypentyl)
Blood	Kronstrand, Roman, Andersson, & Eklund (2013)	ng/g ~	0.05–5.07 (n = 16)		0.05–7.85 (n = 451)	
		ng/mL				
Oral fluid	Kneisel, Auwärter, & Kempf (2013)	ng/mL	0.43 (n = 1)		0.33–22[a] (n = 6)	
Hair	Salomone et al. (2012)	pg/mg				
	Salomone et al. (2014)	pg/mg		1.3 (n = 1)		
Urine	Elian and Hackett (2014)	ng/mL				3.1 (n = 1)
	Lovett, Yanes, Herbelin, Knoerzer, & Levisky (2013)	ng/mL				
	ElSohly et al. (2011)					

Medium	References	Unit	JWH-073	JWH-073 butanoic acid	JWH-081	JWH-122
Blood	Kronstrand et al. (2013)	ng/g ~			0.06–1.85 (n = 15)	0.05–21.8 (n = 303)
		ng/mL				
Oral fluid	Kneisel et al. (2013)	ng/mL			<0.15–2.1[a] (n = 7)	<0.15–381[a] (n = 16)
Hair	Salomone et al. (2012)	pg/mg	0.5–413 (n = 8)			
	Salomone et al. (2014)	pg/mg	1.6–50.5 (n = 11)		8.0–194 (n = 5)	7.4–2800 n = 8
Urine	Elian & Hackett (2014)	ng/mL				
	Lovett et al. (2013)			0.3–305 (n = 46)		
	ElSohly et al. (2011)					

Examples of concentrations of synthetic cannabinoids and metabolites in biological media.
[a] *Oral fluid concentrations exceeding the highest calibrator were extrapolated.*

ANALYSIS OF BIOLOGICAL SAMPLES

Blood

The screening for synthetic cannabinoids in whole blood or serum has been important since the onset of the recreational use of synthetic cannabinoids, due to their potency and possibility for intoxication. From 2004, synthetic cannabinoids were sold as herbal mixtures "Spice" in European countries, and, in 2008/2009, Auwärter and coworkers poised the question: "'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs?" (Auwärter et al., 2009). Overdosing with these products lead to hospitalization, and different smoking blends were analyzed to find the substances responsible. Regular drug screening did not give any results, and a self-experiment with the smoking blend "Spice diamond" was therefore performed. The serum samples gave no result when screened with an 11-nor-9-carboxy-delta9-THC specific immunoassay. Analysis with GC-EI/MS after solid phase extraction did however reveal two steric isomers of a homologue of the nonclassical cannabinoid CP 47,497.

After this initial finding, several methods for synthetic cannabinoids in blood or serum have been published. In addition to evaluation of hospital admissions, the analysis of SC has become important in forensics, both for determination of driving under the influence and for post mortem case work. Chromatographic methods using GC and especially reversed phase LC coupled to mass spectrometry are the common choice for screening in blood or serum, and liquid-liquid extraction seems to be the preferred sample preparation method.

A comprehensive serum method was described by Dresen, Kneisel, Weinmann, Zimmermann, & Auwarter (2011). The method was applied to approximately

JWH-018	JWH-018 *N*-(4-hydroxy-pentyl)	JWH-018 *N*-(5-hydroxy-pentyl)	JWH018-5-Pentanoic acid	JWH018-6-OH-pentanyl	3-(3-(1-Naphthoyl)-1H-indol-1-yl) propanoic acid	JWH-019
0.05–11 (n = 115)						0.08–0.78 (n = 7)
<0.15–1.2 (n = 6)						<0.15–0.61 (n = 2)
0.6–70.5 (n = 9)						
3.1–17.3 (n = 3)						3.8–4.1 (n = 2)
	0.1–351 (n = 29)	0.4–706 (n = 33)	0.7–458 (n = 49)			
		10.2–5530 (n = 17)	2.1–27256 (n = 17)	3.1–36 (n = 6)	4.7–944 (n = 49)	

JWH-203	JWH-210	JWH-250	RCS4	MAM-2201	UR-144
	0.05–8.1 (n = 76)	0.14–0.73 (n = 3)	0.09–3.6 (n = 4)	0.05–21.3 (n = 151)	0.05–25.9 (n = 181)
<0.15–0.51 (n = 2)	<0.10–457[a] (n = 16)		0.1–5.3 (n = 3)	0.41 (n = 1)	
		1.5–729 (n = 8)			
		4.8–83 (n = 6)			

100 serum samples. JWH-081 was the most prevalent sample, followed by JWH-250, JWH-018, and JWH-073. Median concentrations of 0.88, 1.04, 0.64, and 0.42 ng/mL, respectively, demonstrate the need for sensitive screening methods. The method was later improved and extended (Kneisel & Auwärter, 2012) to cover 30 synthetic cannabinoids. Routine application of the method between August 2011 and January 2012 showed a dramatic change in user pattern during only few months.

For analysis in forensic cases whole blood, and not serum, will usually be the available material. In a method published by Kacinko et al. (2011), the authors present whole blood concentrations from a patient, who smoked a herbal incense blend known to contain JWH-018 and JWH-073. The samples were taken 19, 53, 107, and 199 min after smoking. JWH-018 and JWH-073 concentrations decreased from 4.8 and 4.2 to 0.2 ng/mL from the first sampling to the last sampling. Several authors have later published methods for whole blood. Examples of such methods with a summary of the included components and limits of quantitation can be found in Table 101.2.

The appearance of so called third generation synthetic cannabinoids, as UR-144 and XLR-11, and fourth generation synthetic cannabinoids, AKB-48 (APINACA), STS-135 etc., has made new methods or modification of previously published methods necessary. Adamowicz, Zuba, & Sekula (2013) have published a method for UR-144 and its pyrolysis product in blood, where they describe determination of UR-144 and its isomer in a blood sample, collected on admission to hospital. The measured UR-144 concentration was 6.1 ng/mL. Likewise the extensive method by Kneisel and Auwärter (2012) was extended to include AKB48, AKB48-5F, AM-1248, AM-2232, APICA, JWH-370, STS-135, and UR-144, as well as XLR-11 (Hermanns-Clausen, Kneisel, Hutter,

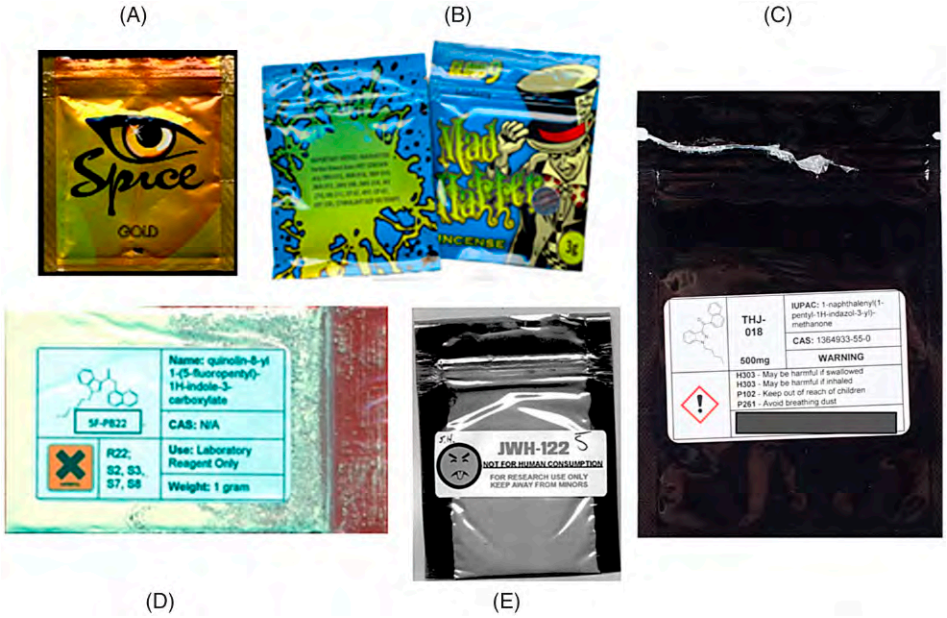


FIGURE 101.3 **Seized products and research chemicals.** Seizures from Norway; (A) a Spice Gold herbal blend package; (B) herbal blend "Mad Hatter" with the description "does not contain JWH-015, JWH-018, JWH-019, JWH-200, JWH-250, HU-210, HU-211, CP 49.497, Stimulant BZP, or TEMPP"; (C) Research chemical containing THJ-018 labelled with IUPAC name, CAS number, weight, molecular structure, and risk and safety statements; (D). Research chemical containing 5-fluoro-PB-22 labelled with IUPAC name, weight, molecular structure, and risk and safety statements; (E) bag containing JWH-122 marked not for human consumption.

Szabo, & Auwärter, 2013). The method was then used for the evaluation of acute intoxications.

Urine

Urine is historically the most common medium for drugs of abuse screening. Screening has often been performed using immunoassays; whereas, nowadays, chromatographic screening is also common. Methods for urine analysis that can easily be expanded with new compounds are advantageous as new SC appear on the market when others are scheduled. Chromatographic analysis is easier to adapt to this ever-changing regimen, and the majority of published urine methods are based on either GC–MS, LC–MS/MS, or LC–HR–MS. Commercial immunoassay kits have, however, become available for some SC metabolites. The antibodies can show cross-reactivity towards other structures than the targeted one, thus expanding the usefulness of the assays, and also enabling the detection of new SC.

Arntson et al. (2013) reported two enzyme-linked immunosorbent assays (ELISA) that were designed to detect the 5-OH metabolite of JWH-018 and the 4-OH metabolite of JWH-250 in urine. The JWH-018 assay had moderate to high cross-reactivity with 17 SC or SC metabolites. The JWH-250 assay had very limited cross-reactivity, with JWH-250-5-OH-pentyl and JWH-250-5-carboxypentyl metabolites at 50% being the only ones with a cross-reactivity >1%, compared to the JWH-250-4-OH calibrator. Compared to a confirmation method using LC–MS/MS, the specificities and sensitivities for both methods were more than 95%. Using the same immunalysis homogeneous enzyme immunoassay (HEIA) K2 Spice kit with JWH-018 *N*-pentanoic acid as calibrator, Barnes et al. (2014) and Kronstrand, Brinkhagen, Birath-Karlsson, Roman, & Josefsson (2014) presented sensitivity/specificity results of 92/98% and 87/82%, respectively. In total, 76 SC and metabolites were tested and of these 19 were classified as moderately to highly cross-reactive. Neither the HEIA nor ELISA assays showed cross-reactivity with the newer SC, such as UR-144 or XLR-11, or metabolites.

Many papers addresses the metabolism of different SC, as knowledge of the metabolites formed is crucial for the detection of the SC since the mother substances are usually not excreted in urine. In most of these papers, chromatographic methods based on GC–MS, LC–MS/MS, or LC–HR–MS, or combinations are utilized. In a few cases, volunteers had smoked or digested a SC and subsequent monitoring of urine allows the elucidation of different metabolites. More often, metabolite patterns are studied using mice models or in vitro experiments with either human liver microsomes or human hepatocytes. Several reports are also based on real samples from

TABLE 101.2 Quantitative Serum and Blood Methods

References	Analyte	Technique	Sample preparation	Sample amount (mL)	LOD (ng/mL)	LOQ (ng/mL)	Linearity (ng/mL)
Teske et al. (2010)	JWH-018	LC–MS/MS	LLE	0.2[S]	0.07	0.21	0.5–20
Kacinko et al. (2011); Yeakel and Logan (2013)	JWH-018	LC–MS/MS	LLE	0.2[B]	0.006	0.1	0.1–20
	JWH-019[a)]				0.010	0.1	0.1–20
	JWH-073				0.009	0.1	0.1–20
	JWH-250				0.016	0.1	0.1–20
	AM-220					0.1	
	AM-694					0.1	
	JWH-081					0.1	
	JWH-122					0.1	
	JWH-175					0.1	
	JWH-200					0.1	
	JWH-210					0.1	
	RCS-4					0.1	
	RCS-8					0.1	
(Dresen et al., 2011)	JWH-015	LC–MS/MS	LLE	1.0[S]		0.1	0.1–2.0
	JWH-018					0.1	0.1–2.0
	JWH-019[a]						0.1–2.0
	JWH-020[a]						0.1–2.0
	JWH-073					0.1	0.1–2.0
	JWH-081					0.1	0.1–2.0
	JWH-200					0.1	0.1–2.0
	JWH-250					0.1	0.1–2.0
	WIN 55,212-2					0.1	0.1–2.0
	Methanandamide					0.3	0.1–2.0
(Hermanns-Clausen et al., 2013; Kneisel & Auwärter, 2012)	JWH-007	LC–MS/MS	LLE	1.0[S]	0.03	0.1	0.1–2.0
	JWH-015				0.01	0.1	0.1–2.0
	JWH-018				0.02	0.1	0.1–2.0
	JWH-018 adamantyl derivative				0.80		—
					0.03	0.1	0.1–2.0
	JWH-019				0.03	0.1	0.1–2.0
	JWH-020				0.02	0.1	0.1–2.0
	JWH-073				0.02	0.1	0.1–2.0
	JWH-081				0.02	0.1	0.1–2.0
	JWH-122 5-fluoropentyl derivative				0.02	0.1	0.1–2.0
	JWH-200				0.05	0.1	0.1–2.0
	JWH-203				0.07	0.1	0.1–2.0
	JWH-210				0.03	0.1	0.1–2.0
	JWH-250				0.02	0.1	0.1–2.0
	JWH-251				0.02	0.1	0.1–2.0
	JWH-307				0.40	2.0	2.0–40
	JWH-387				0.10	0.5	0.5–10
	JWH-398				0.02	0.1	0.1–2.0
	JWH-412				0.03	0.5	0.5–10
	AM-694				0.02	0.1	0.1–2.0
	AM-1220				0.02	0.1	0.1–2.0
	AM-2201				0.02	0.1	0.1–2.0
	AM-2233				2.00	—	—
	CRA-13				0.50	0.5	0.5–10
	Methanandamide				0.02	0.1	0.1–2.0
	RCS-4				0.03	—	—
	RCS-4 ortho isomer				0.03	0.1	0.1–2.0
	RCS-8				0.02	0.1	0.1–2.0
	WIN 48,098				0.02	0.1	0.1–2.0

TABLE 101.2 Quantitative Serum and Blood Methods (*cont.*)

References	Analyte	Technique	Sample preparation	Sample amount (mL)	LOD (ng/mL)	LOQ (ng/mL)	Linearity (ng/mL)
	WIN 55,212-2				0.02		
	AKB48				0.02		
	AKB48-5F				0.02		
	AM-1248				0.02		
	AM-2232				0.02		
	APICA				0.02		
	JWH-370				0.02		
	STS-135				0.02		
	UR-144				0.02		
	XLR-11						
(Ammann, McLaren, Gerostamoulos, & Beyer, 2012)	WIN 48.098	LC–MS/MS	LLE	0.1[B]		0.5	0.5–100
	AM-1241					0.5	0.5–100
	AM-694					0.5	0.5–100
	WIN-55212-2					0.5	0.5–100
	RCS-4 C-4 homolog					0.5	0.5–100
	RCS-4 2-methoxy homolog					0.5	0.5–100
	JWH-030					0.5	0.5–100
	JWH-015					0.5	0.5–100
	JWH-302					0.5	0.5–100
	RCS-4					0.5	0.5–100
	RCS-4 3-methoxy homolog					0.5	0.5–100
	JWH-250					0.5	0.5–100
	JWH-073					0.5	0.5–100
	JWH-251					0.5	0.5–100
	JWH-203					0.5	0.5–100
	JWH-018					0.5	0.5–100
	JWH-081					0.5	0.5–100
	JWH-007					0.5	0.5–100
	CP 47,497					5.0	5.0–500
	JWH-019					0.5	0.5–100
	RCS-8					0.5	0.5–100
	CP 47,497 C-8 homolog					5.0	5.0–500
	JWH-398					0.5	0.5–100
	JWH-210					0.5	0.5–100
	HU-210					5.0	5.0–500
(Shanks, Dahn, & Terrell, 2012)	JWH-018	LC–MS/MS	LLE	0.5[B]	0.01	0.05	0.05–50
	JWH-073				0.01	0.05	0.05–50
(Adamowicz et al., 2013)	UR-144	LC–MS/MS	Protein precipitation with ACN	0.2[B]	0.15	0.5	0.5–100
(Karinen et al., 2013)	JWH-015	LC–MS/MS	LLE			0.06	0.06–3.25
	JWH-018					0.07	0.07–3.39
	JWH-019					0.07	0.07–3.53
	JWH-020					0.07	0.07–3.67
	JWH-073					0.06	0.06–3.25
	JWH-081					0.07	0.07–3.69
	JWH-122					0.07	0.07–3.53
	JWH-200					0.08	0.08–3.81
	JWH-210					0.07	0.07–3.67
	AM-2201					0.07	0.07–3.57
	JWH-250					0.07	0.07–3.33
	JWH-251					0.07	0.06–3.17
	AM-694					0.07	0.09–4.35
	WIN 55,212-2					0.09	0.09–4.27
	RCS-4					0.06	0.06–3.19
	RCS-4-C4					0.06	0.06–3.07

(*Continued*)

TABLE 101.2 Quantitative Serum and Blood Methods (*cont.*)

References	Analyte	Technique	Sample preparation	Sample amount (mL)	LOD (ng/mL)	LOQ (ng/mL)	Linearity (ng/mL)
Kronstrand et al. (2013)	JWH-007	LC–MS/MS	LLE	1[Bb]		0.05[c]	0.05–5.0[c]
	JWH-015					0.05[c]	0.05–5.0[c]
	JWH-018					0.05[c]	0.05–5.0[c]
	JWH-019					0.05[c]	0.05–5.0[c]
	JWH-020					0.05[c]	0.05–5.0[c]
	JWH-073					0.05[c]	0.05–5.0[c]
	JWH-073-methyl					0.05[c]	0.05–5.0[c]
	JWH-081					0.05[c]	0.05–5.0[c]
	JWH-098					0.05[c]	0.05–5.0[c]
	JWH-122					0.05[c]	0.05–5.0[c]
	JWH-147					0.05[c]	0.05–5.0[c]
	JWH-200					0.05[c]	0.05–5.0[c]
	JWH-203					0.05[c]	0.05–5.0[c]
	JWH-210					0.05[c]	0.05–5.0[c]
	JWH-250					0.05[c]	0.05–5.0[c]
	JWH-251					0.05[c]	0.05–5.0[c]
	JWH-398					0.05[c]	0.05–5.0[c]
	AB-001					0.05[c]	0.05–5.0[c]
	AM-694					0.05[c]	0.05–5.0[c]
	AM-1220					0.05[c]	0.05–5.0[c]
	AM-1241					0.05[c]	0.05–5.0[c]
	AM-2201					0.05[c]	0.05–5.0[c]
	AM-2233					0.05[c]	0.05–5.0[c]
	MAM-2201					0.05[c]	0.05–5.0[c]
	RCS-4					0.05[c]	0.05–5.0[c]
	RCS-4-ortho					0.05[c]	0.05–5.0[c]
	RCS-8					0.05[c]	0.05–5.0[c]
	UR-144					0.05[c]	0.05–5.0[c]
	WIN 55.212-2					0.05[c]	0.05–5.0[c]

Examples of quantitative methods for synthetic cannabinoids in serum or blood with analytical technique, sample amount, type of sample preparation and method characteristics.
[a]Qualitative; [b]g; [c]ng/g ; S, serum; B, blood

single cases, or more routine analyses. Several examples can be found in the mini-review by ElSohly, Gul, Wanas, & Radwan (2014). The formation of a large number of metabolites is a common finding, with the metabolic pathways usually involving hydroxylation and carboxylation, followed by glucuronidation.

Examples of quantitative methods with components, limits of quantitation and linear range are given in Table 101.3. In all these methods, urine samples are either chemically or enzymatically hydrolyzed previous to analysis, as the SC are known to form glucuronidated metabolites in urine. The predominant sample preparation methods used were LLE and SPE, with the exception of Moran et al. (2011) who diluted the urine samples with methanol and Yanes and Lovett (2012) who used a salting out technique (SALLE). For GC–MS methods, derivatization might be necessary before analysis (Emerson, Durham, Gidden, & Lay, 2013).

With the increasing number of synthetic cannabinoids on the market, urine methods are getting extensive; the LC–MS/MS method presented by Scheidweiler and Huestis (2014), targeting 53 compounds, is perhaps the most comprehensive quantitative urine SC method to-date. LC–HR–MS screening methods, such as presented by Kronstrand et al. (2014) and Sundström et al. (2013) are becoming more common, as they are able to screen for a large number of substances, but these methods are usually more qualitative than quantitative.

Other Matrixes

As with serum or blood the synthetic cannabinoid parent compounds can be found in oral fluid (Coulter et al., 2011), although in very low concentrations, making it an interesting medium for noninvasive screening of synthetic cannabinoids. Chapter 103 is dedicated to cannabinoids and oral fluid.

Hair is another matrix where the determination of parent drugs usually represents the target analyte (Salomone, Luciano, Di Corcia, Gerace, & Vincenti, 2014). Compounds are incorporated into hair from the blood stream or from sweat, and in some instances by external contamination (Auwärter, Wohlfarth, Traber, Thieme, & Weinmann, 2010). In addition hair analysis allows for retrospective analysis

TABLE 101.3 Quantitative Urine Methods

References	Analyte (metabolite)	Technique	Hydrolysis	Sample preparation	Sample amount (mL)	LOD (ng/mL)	LOQ (ng/mL)	Linearity (ng/mL)
Beuck et al. (2011)	JWH-018 *N*-(5-OH-pentyl)	LC–MS/MS	Yes	LLE	1	0.1	0.5	0.5–100
	JWH-018 *N*-pentanoic acid						0.5	0.5–100
de Jager et al. (2012)	JWH-018-*N*-(5-OH-pentyl)	LC–MS/MS	Yes	LLE	0.5		0.1	0.1–10
	JWH-073-NOH-butyl						0.1	0.1–10
	JWH-018-Npentanoic acid						0.1	0.1–10
	JWH-073-Nbutanoic acid						0.1	0.1–10
	JWH-398-NOH pentyl						0.1	0.1–10
	JWH-250- indole-OH						0.1	0.1–10
	JWH-122-OHpentyl						0.1	0.1–10
	JWH-250-COOH-pentyl						0.1	0.1–10
	JWH-019-OHindole						0.1	0.1–10
	RCS-4 OH-pentyl						0.1	0.1–10
	JWH-200-OHindole						0.1	0.1–10
Elian and Hackett (2014)	AM2201 *N*-(4-OH- pentyl)	LC–MS/MS	Yes	SPE	1	0.5	1	1–100
ElSohly et al. (2011)	JWH-018 Npentanoic acid	LC–MS/MS	Yes	LLE	2	0.1	1	1–50
	JWH-018 *N*-(5-OH-pentyl)					0.01	1	1–25
	JWH-018-6-OH-indole					0.01	1	1–25
Emerson et al. (2013)	JWH-018 *N*-(5-OH-pentyl)	GC-MS	Yes	SPE	1	2.8	9.2	9.2 > 500
	JWH-018 *N*-(4-OH-pentyl)					2.8	9.2	9.2 > 500
	JWH-018 *N*-pentanoic acid					2.8	9.2	9.2 > 500
Jang et al. (2013)	JWH-018-*N*-(5-OH-pentyl)	LC–MS/MS	Yes	SPE	1	0.025	2.5	2.5–75
	JWH-018 *N*-(4-OH-pentyl)					0.025	2.5	2.5–75
	JWH-018 *N*-pentanoic acid					0.1	2.5	2.5–75
	JWH-018 6-OH-indole					0.1	2.5	2.5–75
	JWH-073-*N*(4-OH-butyl)					0.025	2.5	2.5–75
	JWH-073-*N* (3-OH-butyl)					0.025	2.5	2.5–75
	JWH-073-Nbutanoic acid					0.1	2.5	2.5–75
	JWH-073-6-OH-indole					0.1	2.5	2.5–75
Yanes and Lovett (2012)	JWH-018 *N*-(4-OH-pentyl)	LC–MS/MS	Yes	SALLE	0.1		4	4–400
	JWH-018 *N*-pentanoic acid						4	4–400
	JWH-073-*N* 3-OH-butyl						4	4–400
	JWH-073-Nbutanoic acid						4	4–400

Moran et al. (2011)	JWH-018	LC–MS/MS	Yes	Dilution with MeOH		<2	2.7	2–100
	JWH-018 4-OH-indole					<2	3.6	2–100
	JWH-018 5-OH-indole					<2	2.4	2–100
	JWH-018 6-OH-indole					<2	3.3	2–100
	JWH-018 7-OH-indole					<2	2.4	2–100
	JWH-018 *N*-(5-OH-pentyl)					<2	1.8	2–100
	JWH-018 *N*-pentanoic acid					<2	3.3	2–100
	JWH-073					<2	6.3	2–100
	JWH-073 4-OH-indole					<2	4.8	2–100
	JWH-073 5-OH-indole					<2	2.4	2–100
	JWH-073 6-OH-indole					<2	10.8	2–100
	JWH-073 7-OH-indole					<2	1.8	2–100
	JWH-073 *N*-(4-OH-butyl)					<2	3.3	2–100
	JWH-073 *N*-butanoic acid					<2	6.0	2–100
Scheidweiler and Huestis (2014)	JWH-018	LC–MS/MS	Yes	SLE	0.2	0.2	0.2	0.2–50
	JWH-018 5-OH-indole					0.1	0.1	0.1–50
	JWH-018 6-OH-indole					0.1	0.1	0.1–50
	JWH-018 *N*-(5-OH-pentyl)					0.1	0.1	0.1–50
	JWH-018 *N*-pentanoic acid					0.1	0.1	0.1–50
	JWH-019					0.1	0.1	0.1–50
	JWH-019 5-OH-indole					0.1	0.1	0.1–50
	JWH-019 *N*-(6-OH-hexyl)					0.1	0.1	0.1–50
	JWH-073					0.1	0.1	0.1–50
	JWH-073 5-OH-indole					0.1	0.1	0.1–50
	JWH-073 6-OH-indole					0.1	0.1	0.1–50
	JWH-073 *N*-(4-OH-butyl)					0.1	0.1	0.1–50
	JWH-073 *N*-butanoic acid					0.1	0.1	0.1–50
	JWH-081					0.1	0.1	0.1–50
	JWH-081 *N*-(5-OH-pentyl)					0.1	0.1	0.1–50
	JWH-122					0.1	0.1	0.1–50
	JWH-122 *N*-(5-OH-pentyl)					0.1	0.1	0.1–50
	JWH-200					0.1	0.1	0.1–50
	JWH-200 5-OH-indole					1	1	1–100
	JWH-200 6-OH-indole					0.1	0.1	0.1–50
	JWH-203					0.5	0.5	0.5–50
	JWH-210					0.1	0.1	0.1–50
	JWH-210 5-OH-indole					0.2	0.2	0.2–50
	JWH-210 *N*(5-OH-pentyl)					0.1	0.1	0.1–50
	JWH-210 *N*-pentanoic acid					0.1	0.1	0.1–50
	JWH-250					0.2	0.2	0.2–50
	JWH-250 5-OH-indole					0.1	0.1	0.1–50
	JWH-250 *N*(5-OH-pentyl)					0.2	0.2	0.2–50
	JWH-250 *N*-pentanoic acid					0.2	0.2	0.2–50
	JWH-398					0.5	0.5	0.5–50
	JWH-398 *N*(5-OH-pentyl)					0.5	0.5	0.5–50
	JWH-398 *N*-pentanoic acid					1	1	1–100
	AM2201					0.1	0.1	0.1–50
	AM2201 6-OH indole					1	1	1–100

(*Continued*)

TABLE 101.3 Quantitative Urine Methods (*cont.*)

References	Analyte (metabolite)	Technique	Hydrolysis	Sample preparation	Sample amount (mL)	LOD (ng/mL)	LOQ (ng/mL)	Linearity (ng/mL)
	AM2201 *N*-(4-OH-pentyl)					0.1	0.1	0.1–50
	MAM2201					0.1	0.1	0.1–50
	MAM2201 *N*-(4-OH-pentyl)					0.1	0.1	0.1–50
	MAM2201 *N*-pentanoic acid					0.2	0.2	0.2–50
	AM694					0.1	0.1	0.1–50
	RCS-4					0.1	0.1	0.1–50
	RCS-4 *N*-(5-OH-pentyl)					0.2	0.2	0.2–50
	RCS-4 *N*-pentanoic acid					0.2	0.2	0.2–50
	RCS-4 M9 metabolite					0.2	0.2	0.2–50
	RCS-4 M10 metabolite					0.2	0.2	0.2–50
	RCS8					0.1	0.1	0.1–50
	UR-144 *N*-(5-OH-pentyl)					0.2	0.2	0.2–50
	UR-144 *N*-pentanoic acid					0.2	0.2	0.2–50
	XLR11					0.2	0.2	0.2–50
	CP 47,497-C7					0.5	0.5	0.5–50
	CP 47,497-C7 OH					0.5	0.5	0.5–50
	CP 47,497-C8					0.5	0.5	0.5–50
	CP 47,497-C8 OH					0.5	0.5	0.5–50
	HU210					0.5	0.5	0.5–50

Examples of quantitative methods for synthetic cannabinoids/metabolites in urine with analytical technique, sample amount, type of sample preparation, and method characteristics.

of compounds. This can be very beneficial for epidemiological studies, as the synthetic cannabinoids might appear and disappear from the market before the analytical laboratories are ready to analyze them.

Salomone et al. (2012) included five synthetic cannabinoids in a UHPLC–MS/MS method for analysis of THC, cannabidiol (CBD) and cannabinol (CBN) (Salomone, Gerace, D'Urso, Di Corcia, & Vincenti, 2012). The hair (50 mg) was washed with dichloromethane, digested by sodium hydroxide (NaOH), and extracted with *n*-hexane/ethylacetate 90: 10 (v/v). Quantitation limits (LOQ) ranged from 0.07 pg/mg for JWH-200 up to 18 pg/mg for CBD. Analysis of 179 real samples collected from cannabis consumers revealed 14 synthetic cannabinoid positive samples. Concentrations ranged from 0.60–70.5 pg/mg (Jwh-018), 0.50–413.3 pg/mg (JWH-073) and 1.50–729.4 pg/mg (JWH-250). Analysis of 179 real samples collected from cannabis consumers revealed 14 synthetic cannabinoid positive samples. The authors compared the concentrations with the internationally accepted cut-off for THC of 50 pg/mg, and found 6 of the 14 samples above this tentative limit. No such limit has however been set for synthetic cannabinoids, and thus further experience is needed to divide between chronic and occasional use. The same authors (Salomone et al., 2014) later expanded their method to 23 synthetic cannabinoids and reduced the amount of hair to 10 mg. The reported LOQ values varied between 0.7 and 4.3 pg/mg, except for HU-201 (80 pg/mg). The need for low detection limits is likewise illustrated in the paper by Gottardo et al. (Gottardo et al., 2014) reporting concentrations from 0.1 to 1.3 ng/mg in 100 mg hair.

While hair often is digested enzymatically or by use of NaOH for THC analysis, other compounds are often extracted from hair by incubation with buffers or organic solvents. A method for the analysis of 22 synthetic cannabinoids in hair presented by Hutter, Kneisel, Auwarter, & Neukamm (2012b), extracted the synthetic cannabinoids by incubation with ethanol, after washing with water, acetone, and petroleum ether. Concentrations in eight patient hair samples were reported to range from below 1 pg/mg to more than 1000 pg/mg. As rather poor correlation was found between self-stated consumption and the corresponding concentrations in hair samples; incorporation of the drugs in head hair via side-stream smoke condensation was offered as a possible explanation.

CONCLUSIONS

Synthetic cannabinoids are increasingly important as drugs of abuse, and effects as inebriation, severe intoxication, and even death have been reported. Consequently, inclusion of synthetic cannabinoids in drug screening panels is very important. Analysis of seized products are important to discover novel compounds, but incorporation of these compounds into standard drug tests are difficult due to the continuous appearance of new substances, and often low concentration in biological media. Seized products are mainly analyzed by GC–MS or IR; in addition NMR, LC–MS, LC–UV and high resolution mass spectrometry can be useful. Standard drug screening assays for cannabis will not test positive for these compounds, as they are mainly not structurally related to natural cannabinoids. Some immunoassay kits for urine are on the market, but so far no kits covering all synthetic cannabinoids are available, and gas or liquid chromatography coupled with mass spectrometry are commonly used for screening. Standard sample preparation includes liquid–liquid extraction or solid phase extraction. To analyze urine the relevant metabolites must first be discovered by analysis of drug user urine or experiments with microsomes or hepatocytes, and high resolution mass spectrometry has become an important tool in the elucidation of the structure of the multiple metabolites usually formed. The technique is in addition increasingly being used for screening. Definite determination of ingested compounds can be difficult, as several compounds may give the same metabolites. An added complication when analyzing these substances are the fact that structural isomers may pose both analytical problems and problems with interpretation. Several multicomponent methods for blood/serum, urine, oral fluid, and hair have been published, and are continuously being extended to keep up with the ingenuity of clandestine laboratories and the apparently increasing demand for recreational drugs of abuse.

MINI-DICTIONARY

Gas chromatography Separates analytes by interaction between a gaseous mobile phase (usually helium or nitrogen) and a stationary phase which often is a liquid film impregnated on the walls of a capillary column.

Immunoassay Screening technique based on the reaction between an antibody and an antigen. In immunoassay drug screenings the detection is usually not specific, but based on group analysis of, for example, opiates.

Liquid chromatography Separates analytes by interaction between a mobile liquid phase and a stationary solid phase.

Mass spectrometry A mass spectrometer is a very sensitive and specific detector, analyzing ions based on their mass to charge ratio.

References

Adamowicz, P., Zuba, D., & Sekula, K. (2013). Analysis of UR-144 and its pyrolysis product in blood and their metabolites in urine. *Forensic Science International*, *233*(1-3), 320–327.

Ammann, J., McLaren, J. M., Gerostamoulos, D., & Beyer, J. (2012). Detection and quantification of new designer drugs in human blood: part 1, synthetic cannabinoids. *Journal of Analytical Toxicology*, *36*(6), 372–380.

Arntson, A., Ofsa, B., Lancaster, D., Simon, J. R., McMullin, M., & Logan, B. (2013). Validation of a novel immunoassay for the detection of synthetic cannabinoids and metabolites in urine specimens. *Journal of Analytical Toxicology*, *37*(5), 284–290.

Auwärter, V., Dresen, S., Weinmann, W., Muller, M., Putz, M., & Ferreiros, N. (2009). 'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs? *Journal of Mass Spectrometry*, *44*(5), 832–837.

Auwärter, V., Wohlfarth, A., Traber, J., Thieme, D., & Weinmann, W. (2010). Hair analysis for Delta 9-tetrahydrocannabinolic acid A-New insights into the mechanism of drug incorporation of cannabinoids into hair. *Forensic Science International*, *196*(1-3), 10–13.

Barnes, A. J., Young, S., Spinelli, E., Martin, T. M., Klette, K. L., & Huestis, M. A. (2014). Evaluation of a homogenous enzyme immunoassay for the detection of synthetic cannabinoids in urine. *Forensic Science International*, *241*, 27–34.

Beuck, S., Moller, I., Thomas, A., Klose, A., Schlorer, N., Schanzer, W., & Thevis, M. (2011). Structure characterisation of urinary metabolites of the cannabimimetic JWH-018 using chemically synthesised reference material for the support of LC-MS/MS-based drug testing. *Analytical and Bioanalytical Chemistry*, *401*(2), 493–505.

Choi, H., Heo, S., Choe, S., Yang, W., Park, Y., Kim, E., Lee, J., et al. (2013). Simultaneous analysis of synthetic cannabinoids in the materials seized during drug trafficking using GC-MS. *Analytical and Bioanalytical Chemistry*, *405*(12), 3937–3944.

Coulter, C., Garnier, M., & Moore, C. (2011). Synthetic cannabinoids in oral fluid. *Journal of Analytical Toxicology*, *35*(7), 424–430.

de Jager, A. D., Warner, J. V., Henman, M., Ferguson, W., & Hall, A. (2012). LC-MS/MS method for the quantitation of metabolites of eight commonly-used synthetic cannabinoids in human urine—an Australian perspective. *Journal of Chromatography B*, *897*, 22–31.

Dresen, S., Ferreiros, N., Putz, M., Westphal, F., Zimmermann, R., & Auwärter, V. (2010). Monitoring of herbal mixtures potentially containing synthetic cannabinoids as psychoactive compounds. *Journal of Mass Spectrometry*, *45*(10), 1186–1194.

Dresen, S., Kneisel, S., Weinmann, W., Zimmermann, R., & Auwärter, V. (2011). Development and validation of a liquid chromatography-tandem mass spectrometry method for the quantitation of synthetic cannabinoids of the aminoalkylindole type and methanandamide in serum and its application to forensic samples. *Journal of Mass Spectrometry*, *46*(2), 163–171.

Elian, A. A., & Hackett, J. (2014). Analysis of AM-2201 and metabolites in a drugs and driving case. *Drug Testing and Analysis*, *6*(4), 389–395.

ElSohly, M. A., Gul, W., Elsohly, K. M., Murphy, T. P., Madgula, V. L. M., & Khan, S. I. (2011). Liquid chromatography-tandem mass spectrometry analysis of urine specimens for K2 (JWH-018) metabolites. *Journal of Analytical Toxicology*, *35*(7), 487–496.

ElSohly, M. A., Gul, W., Wanas, A. S., & Radwan, M. M. (2014). Synthetic cannabinoids: analysis and metabolites. *Life Sciences*, *97*(1), 78–90.

EMCDDA. (2013). *Perspectives on drugs: synthetic cannabinoids in Europe*. Lisbon, Portugal: EMCDDA, Available from http://www.emcdda.europa.eu/attachements.cfm/att_212361_EN_EMCDDA_POD_2013_Synthetic%20cannabinoids.pdf

EMCDDA. (2014). *EMCDDA European Drug Report 2014*. Lisbon, Portugal: EMCDDA, Available from http://www.emcdda.europa.eu/publications/edr/trends-developments/2014

Emerson, B., Durham, B., Gidden, J., & Lay, J. O. (2013). Gas chromatography-mass spectrometry of JWH-018 metabolites in urine samples with direct comparison to analytical standards. *Forensic Science International*, *229*(1-3), 1–6.

Ernst, L., Krüger, K., Lindigkeit, R., Schiebel, H. M., & Beuerle, T. (2012). Synthetic cannabinoids in "spice-like" herbal blends: first appearance of JWH-307 and recurrence of JWH-018 on the German market. *Forensic Science International*, *222*(1-3), 216–222.

Favretto, D., Pascali, J. P., & Tagliaro, F. (2013). New challenges and innovation in forensic toxicology: focus on the "New Psychoactive Substances". *Journal of Chromatography A*, *1287*, 84–95.

Gandhi, A. S., Zhu, M., Pang, S., Wohlfarth, A., Scheidweiler, K. B., Liu, H. F., & Huestis, M. A. (2013). First characterization of AKB-48 metabolism, a novel synthetic cannabinoid, using human hepatocytes and high-resolution mass spectrometry. *AAPS Journal*, *15*(4), 1091–1098.

Ginsburg, B. C. (2012). Purity of synthetic cannabinoids sold online for recreational use. *Journal of Analytical Toxicology*, *36*, 66–68.

Gottardo, R., Sorio, D., Musile, G., Trapani, E., Seri, C., Serpelloni, G., & Tagliaro, F. (2014). Screening for synthetic cannabinoids in hair by using LC-QTOF MS: a new and powerful approach to study the penetration of these new psychoactive substances in the population. *Medicine Science and the Law*, *54*(1), 22–27.

Griffiths, P., Sedefov, R., Gallegos, A. N. A., & Lopez, D. (2010). How globalization and market innovation challenge how we think about and respond to drug use: 'Spice' a case study. *Addiction*, *105*(6), 951–953.

Harris, D. N., Hokanson, S., Miller, V., & Jackson, G. P. (2014). Fragmentation differences in the EI spectra of three synthetic cannabinoid positional isomers: JWH-250, JWH-302, and JWH-201. *International Journal of Mass Spectrometry*, *368*, 23–29.

Hermanns-Clausen, M., Kneisel, S., Hutter, M., Szabo, B., & Auwärter, V. (2013). Acute intoxication by synthetic cannabinoids - four case reports. *Drug Testing and Analysis*, *5*(9–10), 790–794.

Holm, N. B., Pedersen, A. J., Dalsgaard, P. W., & Linnet, K. (2015). Metabolites of 5F-AKB-48, a synthetic cannabinoid receptor agonist, identified in human urine and liver microsomal preparations using liquid chromatography high-resolution mass spectrometry. *Drug Testing and Analysis*, *7*(3), 199–206.

Hutter, M., Broecker, S., Kneisel, S., & Auwärter, V. (2012a). Identification of the major urinary metabolites in man of seven synthetic cannabinoids of the aminoalkylindole type present as adulterants in 'herbal mixtures' using LC-MS/MS techniques. *Journal of Mass Spectrometry*, *47*(1), 54–65.

Hutter, M., Kneisel, S., Auwärter, V., & Neukamm, M. A. (2012b). Determination of 22 synthetic cannabinoids in human hair by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B*, *903*, 95–101.

Jang, M., Yang, W., Choi, H., Chang, H., Lee, S., Kim, E., & Chung, H. (2013). Monitoring of urinary metabolites of JWH-018 and JWH-073 in legal cases. *Forensic Science International*, *231*(1-3), 13–19.

Kacinko, S. L., Xu, A. L., Homan, J. W., McMullin, M. M., Warrington, D. M., & Logan, B. K. (2011). Development and validation of a liquid chromatography-tandem mass spectrometry method for the identification and quantification of JWH-018, JWH-073, JWH-019, and JWH-250 in human whole blood. *Journal of Analytical Toxicology*, *35*(7), 386–393.

Karinen, R., Johnsen, L., Andresen, W., Christophersen, A. S., Vindenes, V., & Oiestad, E. L. (2013). Stability study of fifteen synthetic cannabinoids of aminoalkylindole type in whole blood stored in Vacutainer® evacuated glass tubes. *Journal of Forensic Toxicology and Pharmacology*, *2:1*(1).

Kneisel, S., Teske, J., & Auwarter, V. (2014). Analysis of synthetic cannabinoids in abstinence control: long drug detection windows in serum and implications for practitioners. *Drug Testing and Analysis*, *6*(1–2), 135–136.

Kneisel, S., Auwärter, V., & Kempf, J. (2013). Analysis of 30 synthetic cannabinoids in oral fluid using liquid chromatography-electrospray ionization tandem mass spectrometry. *Drug Testing and Analysis*, *5*(8), 657–669.

Kronstrand, R., Brinkhagen, L., Birath-Karlsson, C., Roman, M., & Josefsson, M. (2014). LC-QTOF-MS as a superior strategy to immunoassay for the comprehensive analysis of synthetic cannabinoids in urine. *Analytical and Bioanalytical Chemistry*, *406*(15), 3599–3609.

Kronstrand, R., Roman, M., Andersson, M., & Eklund, A. (2013). Toxicological findings of synthetic cannabinoids in recreational users. *Journal of Analytical Toxicology*, *37*(8), 534–541.

Langer, N., Lindigkeit, R., Schiebel, H. M., Ludger, E., & Beuerle, T. (2013). Identification and quantification of synthetic cannabinoids

in 'spice-like' herbal mixtures: a snapshot of the German situation in the autumn of 2012. *Drug Testing and Analysis*, *6*(1–2), 59–71.

Li, L., & Lurie, I. S. (2014). Screening of seized emerging drugs by ultra-high performance liquid chromatography with photodiode array ultraviolet and mass spectrometric detection. *Forensic Science International*, *237*, 100–111.

Lovett, D. P., Yanes, E. G., Herbelin, T. W., Knoerzer, T. A., & Levisky, J. A. (2013). Structure elucidation and identification of a common metabolite for naphthoylindole-based synthetic cannabinoids using LC-TOF and comparison to a synthetic reference standard. *Forensic Science International*, *226*(1–3), 81–87.

Maurer, H. H. (2007). Current role of liquid chromatography-mass spectrometry in clinical and forensic toxicology. *Analytical and Bioanalytical Chemistry*, *388*(7), 1315–1325.

Maurer, H. H. (2013). What is the future of (ultra) high performance liquid chromatography coupled to low and high resolution mass spectrometry for toxicological drug screening? *Journal of Chromatography A*, *1292*, 19–24.

Moran, C. L., Le, V. H., Chimalakonda, K. C., Smedley, A. L., Lackey, F. D., Owen, S. N., Moran, J. H., et al. (2011). Quantitative measurement of JWH-018 and JWH-073 metabolites excreted in human urine. *Analytical Chemistry*, *83*(11), 4228–4236.

Oiestad, E. L., Johansen, U., Christophersen, A. S., & Karinen, R. (2013). Screening of synthetic cannabinoids in preserved oral fluid by UPLC-MS/MS. *Bioanalysis*, *5*(18), 2257–2268.

Peters, F. T., & Meyer, M. R. (2011). In vitro approaches to studying the metabolism of new psychoactive compounds. *Drug Testing and Analysis*, *3*(7–8), 483–495.

Salomone, A., Gerace, E., D'Urso, F., Di Corcia, D., & Vincenti, M. (2012). Simultaneous analysis of several synthetic cannabinoids, THC, CBD and CBN, in hair by ultra-high performance liquid chromatography tandem mass spectrometry. Method validation and application to real samples. *Journal of Mass Spectrometry*, *47*(5), 604–610.

Salomone, A., Luciano, C., Di Corcia, D., Gerace, E., & Vincenti, M. (2014). Hair analysis as a tool to evaluate the prevalence of synthetic cannabinoids in different populations of drug consumers. *Drug Testing and Analysis*, *6*(1–2), 126–134.

SAMHSA. (2014). *Drug Cut-off Concentrations*. Washington, DC: SAMHSA, Available from http://www.samhsa.gov/sites/default/files/workplace/2010GuidelinesAnalytesCutoffs.pdf

Scheidweiler, K. B., & Huestis, M. A. (2014). Simultaneous quantification of 20 synthetic cannabinoids and 21 metabolites, and semi-quantification of 12 alkyl hydroxy metabolites in human urine by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A*, *1327*, 105–117.

Shanks, K. G., Dahn, T., & Terrell, A. R. (2012). Detection of JWH-018 and JWH-073 by UPLCMS-MS in postmortem whole blood casework. *Journal of Analytical Toxicology*, *36*(3), 145–153.

Smith, F. T., DeRuiter, J., Abdel-Hay, K., & Randall Clark, C. (2014). GC–MS and FTIR evaluation of the six benzoyl-substituted-1-pentylindoles: Isomeric synthetic cannabinoids. *Talanta*, *129*, 171–182.

Sobolevsky, T., Prasolov, I., & Rodchenkov, G. (2010). Detection of JWH-018 metabolites in smoking mixture post-administration urine. *Forensic Science International*, *200*(1–3), 141–147.

Sobolevsky, T., Prasolov, I., & Rodchenkov, G. (2012). Detection of urinary metabolites of AM-2201 and UR-144, two novel synthetic cannabinoids. *Drug Testing and Analysis*, *4*(10), 745–753.

Spaderna, M., Addy, P., & D'Souza, D. (2013). Spicing things up: synthetic cannabinoids. *Psychopharmacology*, *228*(4), 525–540.

Sundström, M., Pelander, A., Angerer, V., Hutter, M., Kneisel, S., & Ojanpera, I. (2013). A high-sensitivity ultra-high performance liquid chromatography/high-resolution time-of-flight mass spectrometry (UHPLC-HR-TOFMS) method for screening synthetic cannabinoids and other drugs of abuse in urine. *Analytical and Bioanalytical Chemistry*, *405*(26), 8463–8474.

SWGDRUG (2013). SWGDRUG Recommendations, Edition 7.0. Available from http://www.swgdrug.org/Documents/SWGDRUG%20Recommendations%20Version%207-0.pdf

Teske, J., Weller, J. P., Fieguth, A., Rothamel, T., Schulz, Y., & Troger, H. D. (2010). Sensitive and rapid quantification of the cannabinoid receptor agonist naphthalen-1-yl-(1-pentylindol-3-yl)methanone (JWH-018) in human serum by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B*, *878*(27), 2659–2663.

Uchiyama, N., Kawamura, M., Kikura-Hanajiri, R., & Goda, Y. (2013). URB-754: a new class of designer drug and 12 synthetic cannabinoids detected in illegal products. *Forensic Science International*, *227*(1–3), 21–32.

Uchiyama, N., Kikura-Hanajiri, R., Ogata, J., & Goda, Y. (2010). Chemical analysis of synthetic cannabinoids as designer drugs in herbal products. *Forensic Science International*, *198*(1–3), 31–38.

UNODC. (2013). *Recommended methods for the Identification and Analysis of Synthetic Cannabinoid Receptor Agonists in Seized Materials*. Vienna, Austria: UNODC, Available from http://www.unodc.org/documents/scientific/STNAR48_Synthetic_Cannabinoids_ENG.pdf

Wintermeyer, A., Möller, I., Thevis, M., Jübner, M., Beike, J., Rothschild, M., & Bender, K. (2010). In vitro phase I metabolism of the synthetic cannabimimetic JWH-018. *Analytical and Bioanalytical Chemistry*, *398*(5), 2141–2153.

Yanes, E. G., & Lovett, D. P. (2012). High-throughput bioanalytical method for analysis of synthetic cannabinoid metabolites in urine using salting-out sample preparation and LC-MS/MS. *Journal of Chromatography B*, *909*, 42–50.

Yeakel, J. K., & Logan, B. K. (2013). Blood synthetic cannabinoid concentrations in cases of suspected impaired driving. *Journal of Analytical Toxicology*, *37*(8), 547–551.

Zhang, Q., Ma, P., Cole, R., & Wang, G. (2006). Identification of in vitro metabolites of JWH-015, an aminoalkylindole agonist for the peripheral cannabinoid receptor (CB2) by HPLC-MS/MS. *Analytical and Bioanalytical Chemistry*, *386*(5), 1345–1355.

CHAPTER

102

On-Site Drug Testing for Cannabis

A.A. Fernández

Forensic Laboratory Institute of Legal Medicine of Catalonia, Barcelona, Spain

SUMMARY POINTS

- On-site devices have been developed to detect and analyse cannabis use in populations.
- On-site devices are based in immunoassays.
- Performance of the tests must be assessed based on sensitivity, specificity and accuracy of results.
- Immunoassay is only applicable and valid for drug screening. Confirmation of results are required to corroborate presence of drug.
- On-site devices testing for cannabis use fluids collected from a subject, such as urine, blood, or oral fluid.
- Use of on-site cannabis devices: roadside scenarios, workplaces, drug treatment centres, law enforcements, emergency departments, primary health care, schools and forensic autopsies.
- On-site drug testing for cannabis is useful to obtain results in a short period of time.

KEY FACTS OF ON-SITE DRUG TESTING FOR CANNABIS

- Cannabis is the most prevalent drug of abuse around the world.
- Cannabis is an illegal drug and addicted people consider it as not dangerous for their wealth.
- On-site devices were developed to detect cannabis and another drugs by a simple and rapid proof of immunoassay.
- When results are positive for the presence of the drug, it is necessary to confirm the results by a more exact analysis as the chromatography gas/mass or chromatography liquid/mass.
- Urine, blood and oral fluid are the main samples specimens collected from a subject to conduct a cannabis drug test.
- On-site tests for cannabis are useful to have rapid results in clinical settings, emergency departments, primary health care, driving under the influence of drugs scenarios, forensic autopsies and schools.
- Gas-Chromatography-Mass Spectrometry (GC/MS) and Liquid Chromatography-Mass Spectrometry (LC/MS) are analytical techniques used to separate, identify and quantify drugs and medicaments. These techniques use a gas or a liquid solvent to carry the substances to be analyzed. Both of them are confirmatory and specific techniques to identify substances in samples. Cannabis and other drugs may be confirmed by these methods after the screening tests provide positive results for the drug.
- National Institute on Drug Abuse (NIDA) is an institution providing all information on drug abuse and addiction detecting and responding to emerging drug abuse trends. NIDA supports research and training on causes and consequences of drug abuse, on prevention and treatment of drugs, relationship to mental illness, genetic and social mechanisms, as well as epidemiological aspects.

LIST OF ABBREVIATIONS

DRUID	Driving under the influence of drugs
GC-MS	Gas chromatography-mass spectrometry
LC-MS	Liquid chromatography-mass spectrometry
NIDA	National Institute on Drugs Abuse
ng/mL	Nanogram per millilitre
ROSITA	Roadside testing assessment
THC	Δ^9-Tetrahydrocannabinol
THC COOH	11-nor-9-carboxi-Δ^9-tetrahydrocannabinol

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00119-8
Copyright © 2017 Elsevier Inc. All rights reserved.

ON-SITE DRUG TESTING FOR CANNABIS

Rapid on-site drug tests or point-of-collection (POC) drug tests are used to obtain samples for the rapid detection of cannabis and other drugs of abuse by immunoassay both from living subjects and cadavers in the fields of toxicology, legal and forensic medicine, and clinical medicine. These tests use antibody and antigen complexes to measure the presence of a specific analyte in a sample by competitive binding to an antibody, between the toxic substance present in the sample, which acts as an antigen, and that same toxic substance/antigen, which forms a complex with its corresponding antibody. One of the two components of this complex is labeled for detection and quantification when it is liberated from the complex. Immunoassays are classified as competitive or noncompetitive by measuring technique and as homogeneous or heterogeneous depending on the medium in which the measurement is made (Repetto & Repetto, 2009). The use of immunoassays for initial screening of cannabis remains the most cost-effective and efficient tool to eliminate "negative specimens" and to "identify those that require further confirmation" (Substance Abuse & Mental Health Services Administration, 1994).

On-site drug tests feature easy-to-follow protocols and provide preliminary results immediately. Two samples are required to conduct these tests and the stability, chain of custody, and storage conditions of samples must be ensured since the results require confirmation testing. On-site testing devices have evolved with time. On-site urine tests are the oldest, and they range from the simple ones with visual urine test strips to the more complex ones that include automated readers. As of 1990, year in which the first oral fluid drug tests were marketed, the effectiveness of urine drug testing was questioned, since the confirmation of results showed that their sensitivity and specificity for the detection of cannabis and other drugs of abuse were low. However, with time, the new technologies have enabled the development of new kits with enhanced performance (George & Braithwaite, 1995). Subsequent comparative studies have shown that there were significant differences between results and between the detection limits and cutoff levels set by different manufacturers due to the lack of standard specifications (Langel et al., 2008). According to several comparative studies, in general, many tests are effective for detecting other drugs of abuse, but are not reliable for detecting cannabis (Walsh, Flegel, Crouch, Cangianelli, & Baudys, 2003).

DRUG DETECTION KITS

There is a wide variety of drug detection kits in the market and new versions are being released on an ongoing basis. Many of them are testing kits that include a sample collection device and an analyzer of test results. Other test kits feature visual interpretation of results only, without the analyzer providing results analysis. An independent evaluation of the sensitivity and specificity, the positive and negative predictive values, and the accuracy of the method used is of technical and scientific interest. However, this is rarely done. It requires confirmation by gas chromatography–mass spectrometry (GC/MS) or liquid chromatography–mass spectrometry (LC/MS) in order to identify false positive and false negative results, estimate the statistical parameters, evaluate test performance, and compare the findings with the data supplied by the manufacturer. Most controlled studies of the performance of on-site tests for cannabis and drugs of abuse do not reveal these data, they only provide the number of accurate results, and the false positive and false negative rates. The sample size in the published studies providing independent confirmation of results is not always large enough to provide adequate reliability, and to allow to extrapolate results and to determine sensitivity and specificity parameters.

MATRICES

The matrix of choice for cannabis on-site testing is urine, in which 11-*nor*-9-carboxy-Δ^9-tetrahydrocannabinol (THC—COOH) is detected as a metabolite of Δ^9-tetrahydrocannabinol (THC), which is the primary constituent of cannabis. The presence of THC—COOH in urine indicates recent use but does not determine whether an individual is under the effect of the drug. Cannabis is one of the most widely used illegal drugs, and its detection is important for the assessment of the prevalence of its use. It is known that chronic use increases cannabinoid detection time in urine. According to several studies in the forensic medicine literature, the advantages of urine testing for cannabis use are that urine collection is easier than blood sampling, and that it significantly decreases the number of unnecessary blood analyses (Raes & Verstraete, 2005). Blood is also used as a matrix for cannabis testing; however, it is less applicable to rapid screening because blood sampling is invasive.

The utility of oral fluid as a sample matrix for the detection of cannabis abuse is largely due to collection advantages, since it can be used in various settings and situations, such as roadside drug testing (Speedy et al., 2007; Quintela, Crouch, & Andrenyak, 2006). It is widely recognized as a non-invasive and tamper-resistant screening method, which can identify drug use as accurately as blood testing, given the high correlation between the two fluids. However, from a technical point of view, recovery of the sample from the collection device is a major problem, especially for lipophilic drugs such as cannabinoids, although several authors report 94.5

and 91.4% THC recovery from different devices (Speedy, Baldwin, Jowett, Gallina, & Jehanli, 2007).

Oral fluid testing has some disadvantages. For example, the volume of specimen collected may be too low for testing and for subsequent laboratory confirmation testing. Moreover, contamination of the sample by food, beverages, or other adulteration agents may pose additional problems to this analytical method. Smoked cannabis may cause contamination of the oral cavity, increasing concentrations and disrupting correlations with blood concentrations. THC is detected in oral fluid. It has been observed that plasma THC concentrations drop at 30 min postsmoking while oral fluid concentrations are much higher. Issues related to detection times for marijuana have prevented wide acceptance of oral fluid drug test devices. Detection of THC in oral fluid has been reported 34 h after administration of smoked cannabis 20–25 mg (Niedbala et al., 2001). Several experimental performance studies have focused on oral fluid/plasma cannabinoid ratios at different times and at various concentrations (r = 0.84) in groups of marijuana smokers, taking into account THC contamination of the oral cavity (Ramaekers et al., 2006). In addition, oral fluid test results may be influenced by exposure to second hand marijuana smoke, yielding a positive result. However, many studies have shown that positive passive cannabis exposure results are not statistically significant (Huestis & Cone, 2004).

In Europe, many efforts have been made to develop roadside drug testing devices for cannabis and other drugs of abuse. At the end of the 1990s, the European Union launched the Roadside Testing Assessment (ROSITA) project (López-Rivadulla, 2002), involving an extensive evaluation of nine commercial brands of on-site testing devices. The performance of six of the devices was modest, with a malfunction rate of 25%, the highest percentage being related to screening for cannabis use. The ROSITA-I assessment evaluated the sensitivity and specificity of the devices; some on-site urine tests showed sensitivity and specificity of over 90% and an accuracy rate in excess of 95% compared with reference methods; however, none had good overall results. Sensitivity values for cannabis screening ranged from 0% to 97%, and specificity from 70% to 100%. Belgian police officers reported that, in their opinion, the devices were unsuitable for cannabis (Laloup et al., 2006).

Cannabis drug testing is of great interest, and a broad range of studies has evaluated which testing devices and matrices are most effective for detecting drug-related impairment of driving ability. For roadside testing, oral fluid has been found to be more effective than urine in correlation between drug detection in oral fluid and serum and in driver impairment symptoms (Toennes, Kavert, Steinmeyer, & Moeller, 2005). Several European countries have addressed the issue of road safety and cannabis abuse detection. In another assessment, German police officers collected specimens of body fluids (blood, saliva/sweat, and urine) in cases of suspected driving under the influence of drugs of abuse. Roadside drug test results yielded a positive predictive value of 79%. In view of this, these tests were seen as effective drug use screening tools and as evidence for legal proceedings (Steinmeyer, Ohr, Maurer, & Moeller, 2001).

DETECTION LIMITS

In on-site drug screening, detection limits refer to the lowest amount of cannabis or other drugs of abuse in a sample that can be detected by a given test. They are set by the laboratory or the device manufacturer and there are significant differences between them. As a rule, the detection limit values used are those set by the National Institute on Drug Abuse (NIDA), and the screening cutoff level in the NIDA guidelines for THC is 50 ng/mL. In the ROSITA project, the SAMHSA guidelines recommend a cutoff level of 4 ng/mL and a confirmation cutoff level of 2 ng/mL for THC in oral fluid (Verstraete, 2005). An assessment of four drug-testing devices applying the SAMHSA cutoff level for THC reported that their performance was not satisfactory (Walsh et al., 2003). Table 102.1 shows the cutoff levels set by different device manufacturers.

TABLE 102.1 Cutoffs for Cannabis in Different Devices

Device	Cutoff (ng/mL)
Drugwipe II	30
Orallab (Varian)	100
Oralscreen drugmeter (Avitar)	250–500
Oral Fluid Drug Screen (Acon)	12
OralStat (AmericanBiomedic Corporation)	25
Oratec II (Branan Medical Corporation)	40
Cozart Rapiscan (Cozart Bioscience Ltd)	150
Salicheck (Bamburg Marrsh LLC)	12
Dräger Drug test (Orasure Technologies)	20
Multiline Twist (Acon Lab)	100
Oraline (Sun Biomedical)	4
Saliva Screen 5 (Ultimed Products)	2
Alere DDS V Test (Fig. 102.1)	25

Unpublished table: Data adapted from Raes and Verstraete (2005), ROSITA-2 Project. Cannabis cutoffs were assessed by the manufacturers of different devices. The matrix tested was oral fluid. A great variability was observed among the devices

FIGURE 102.1 On-site Alere DDS V Test for detection of cannabis and other drugs in oral fluid. Results appear on the screen of the device. *Source: Alere Toxicology, Abingdon, United Kingdom.*

ON-SITE URINE TESTING

Recent studies conducted in Germany evaluated the performance of screening tests for drugs of abuse in urine with a cutoff level of 10 ng/mL for THC—COOH showed that accuracy rates were high (Agius, Nadulski, & Moore, 2012). Urine samples were screened by CEDIA immunoassay. Average relative sensitivities and relative specificities were 99.7 and 98.4%, respectively for ELISA, higher than those for CEDIA (Agius, Nadulski, Kahl, & Dufaux, 2013). Moody and collaborators compared two on-site testing devices to discriminate urine samples for cannabinoids and other drugs of abuse. The respective accuracies in respect to confirmation results were 94.4 and 95% for cannabinoids (Moody, Fang, Andrenyak, Monti, & Jones, 2006). A field study was performed at two police agencies to evaluate the accuracy of five commercial on-site urine analysis drug-testing devices. For marijuana, the laboratory confirmation cutoff level was 4 ng/mL and all devices had ≤0.25% false-positive rates, which were slightly higher for other drugs. However, it was concluded that the devices were useful in DRUID investigations (Crouch, Hersch, Cook, Frank, & Walsh, 2002). Another study of several on-site drug-testing devices for the detection of drugs of abuse in urine specimens reported that all devices determined the presence of THC—COOH and that results differed upon confirmation at different cutoff levels (Peace, Tarnai, & Poklis, 2000). In addition, recent studies evaluated the effectiveness of several rapid urine-screening tests for detection of cannabis, other drugs of abuse, and psychoactive substances at the workplace in Italy. Positivity rates were lower than expected due to the application of Italian legislation for workplace drug testing (Rosso, 2013). Table 102.2 shows assessment results for several cannabis screening devices.

TABLE 102.2 Sensitivity and Specificity for Cannabis in Different On-Site Testing Devices

Devices	Sensitivity	Specificity
LUCIO-direct ELISA (Agius et al., 2012)	99.7	98.4
Triage Plus TCA CB1 panel of Drugs (Peace et al., 2000)	90.5	90.8
QuickScreen (Peace et al., 2000)	97.5	78.5
Syva Rapid d.a.u.5 and d.a.u 2 (Peace et al., 2000)	97.5	92.4
Rapid Drug Screen (Peace et al., 2000)	97.5	83.3
Drug Screen NAL (Musshoff, 2014)	93	39
Syva Rapid Test d.a-u 10 (Attema-de Jonge, 2012)	>93	>93
Triage TOX Drug (Attema-de Jonge, 2012)	>93	>93
Test Tcard 9 (Attema-de Jonge, 2012)	>88	>93

Table unpublished. Data adapted from different articles published in specialized journals. Table includes recent studies. The matrix tested was urine

ON-SITE ORAL FLUID TESTING

A large number of published evaluations of on-site oral fluid testing focus on roadside testing for drug driving. Many points-of-collection test (POCT) devices have been assessed although many of them are not available any more or have been modified to enhance performance (Bosker & Huestis, 2009). A comparison of results is difficult due to the fact that the experimental methods in current use in this field have not been validated. Devices with absorbing components may cause analyte absorption into the collection device and the percentage of analyte recovery is low. The recovery of the analyte being measured is, therefore, a very important parameter in the evaluation (Langel et al., 2008), since absorption may lead to false-negative results (Gallardo & Queiroz, 2008). This was not known when the first devices came out, so data sensitivity was low. Device manufacturers tried to eliminate the problem by eluting the sample with buffers, thereby reducing sample thickness. Moreover, they had to comply with regulatory requirements during product development in order to achieve marketing approval for the new medical device (Kadehjian, 2005; Biermann et al., 2004; Blencowe et al., 2011). An ineffective sample collection method or errors in the reading of results may produce biased results. Malfunctions have been reported in some devices at temperatures lower than 10°C, or due to excessive test duration, or to extremely low sample volume. In European countries such as Germany, Denmark, Finland, Norway, and Sweden, researchers have assessed the efficacy of oral fluid drug testing devices for roadside testing. The assessments revealed that 75% of the results were accurate, although testing for marijuana yielded more false-positive results and test sensitivity to cannabis was not satisfactory in eight of the devices (Biermann, Schwarze, Zedler, & Betz, 2004). Another roadside study of three oral fluid screening devices reported that the devices were able to detect about 70% of all cannabis users in a roadside setting and that one showed a sensitivity of 93% (Wille, Samyn, Ramírez-Fernández, De Boeck, & de Boeck, 2010). Table 102.3 shows the sensitivity and specificity values in some devices.

It is important to compare the results for cannabis with oral fluid drug testing devices versus other matrices, such as blood and urine, with variable confirmation test results. Tables 102.4 and 102.5 show comparative data for two devices from a study conducted in Wisconsin, and in Belgium within the ROSITA project.

TABLE 102.3 Sensitivity and Specificity for Cannabis in Oral Fluid in Different On-Site Devices

Devices	Sensitivity (%)	Specificity (%)
Cozart DDS 801 (Arroyo et al., 2013)	87	86
Drugwipe 5 (Wille et al., 2010)	71	50
Rapistat (Wille et al., 2010)	71	51
Draeger DrugTest 5000 test (Desrosiers et al., 2014)	86–90	75–77
Drugtest 5000 A	72	93
Drugtest 5000 B (Wille et al., 2010)	50	71
Druggertest 5000 (Vanstechelman, 2012)	81	>88
Cozart DDS (Vanstechelman, 2012)	23	>88
Mavan Rapid STAT (Vanstechelman, 2012)	43	>88
Innovacon ORAlert (Vanstechelman, 2012)	28	>88
Drugwipe 5 (Pehrsson et al., 2008)	52	91
Drugwipe (ROSITA 2, Belgium)	41	96
Drager Drug test (ROSITA Belgium)	47	95
Cozart Rapiscan (Verstraete, 2005)	16	94
Varian Orallab[a] (Goessaert et al., 2010)	41	99
Varian Orallab[b] (Goessaert et al., 2010)	16	99
OraLine (Cirimele et al., 2006)	69	91
Avitar Oral Screen (Verstraete, 2005)	50–	84–
Oral screen (Yacoubian, 2006)	100	94
RapidStat (Rohrich, 2010)	85	87
RapSTAt (Musshoff, 2014)	91	–
DrugWipe 5/5+ (Musshoff, 2014)	71	29
Dräger Drug Test 5000 (Musshoff, 2014)	87	47
Alere DDS V Test	99	100
Oralab Varian (ROSITA Spain, 2004)	93	98
Dräger (ROSITA Spain, 2004)	58	89

Unpublished table. Data adapted from various articles published in specialized literature. Matrix tested: oral fluid. Old and new versions of devices in the market throughout the years up to this date. It is noteworthy that even very recent studies indicate that there are significant sensitivity and specificity differences for detection of cannabis in oral fluid

[a] Cutoff 50 ng/mL.

[b] Cutoff 1 ng/mL.

USE OF ON-SITE TESTING

A number of recent studies have focused on the evaluation of on-site testing devices in both urine and saliva matrices, indicating a growing interest in these devices

TABLE 102.4 Comparison of Results of On-Site Devices for Cannabis in Three Matrices

Oralab	Oral fluid (%)	Blood (%)	Urine (%)
Sensibility	37.5	50	25
Specificity	100	100	100
Accuracy	58	71	45
RapiScan	**Oral fluid (%)**	**Blood (%)**	**Urine (%)**
Sensibility	65	75	66
Specificity	70	73	90
Accuracy	67	74	74

Unpublished table. Data adapted from ROSITA-2 Project, Wisconsin (2004–2006). The devices were tested in different matrices, oral fluid, blood, and urine, and the results per matrix were compared

TABLE 102.5 Comparison of Results of On-Site Devices for Cannabis in Two Matrices

Dräger Drug Test	Oral fluid (%)	Plasma (%)
Sensibility	47	50
Specificity	95	84
Accuracy	53	59
Drugwipe	**Oral fluid (%)**	**Plasma (%)**
Sensibility	45	50
Specificity	96	84
Accuracy	51	56

Unpublished table. Data adapted from ROSITA-2 Project, Belgium (2004–2006). The devices were tested in oral fluid and plasma and the results per matrix were compared

and its relevance to road traffic (Strano-Rossi et al., 2011; Pehrsson et al., 2011) and roadside enforcement activities, in which the detection time for marijuana in oral fluid becomes a major issue (Desrosiers et al., 2014). Urine testing devices have been longer in the market and have shown many advantages in terms of sensitivity, accuracy, and precision. Reagent test strips allow on-site testing of urine specimens in all types of settings, and are useful for the monitoring and control of marijuana use in large populations; however, they are not commonly used for roadside testing. According to recent reports, the introduction of new derivatives of cannabis, the synthetic cannabinoids, has prompted researchers to study these substances for detection by immunoassay (Barnes et al., 2014). Oral fluid testing devices are far from being able to deliver optimal performance in terms of the parameters discussed in this chapter, but they hold promise and their use is discussed in many reports that provide valuable data on this technology and on their performance levels. Furthermore, on-site testing devices should detect cannabis at low cutoff levels to allow for optimal detection.

The main use for cannabis drug testing is in roadside driving-under-the-influence-of-drugs (DRUID) scenarios. In Spain, a project was launched using the Cozart DDS 801 on-site drug test device, which was evaluated in the Laboratory of Institute of Legal Medicine of Catalonia (Barcelona). The parameters to evaluate the efficacy of this device were determined through the analysis of 2180 oral fluid samples. The results showed that the device is effective for detecting cannabis use and its use has continued (Arroyo et al., 2013).

These devices can also be useful in other settings, although drug-testing policies may differ from country to country. Such settings are:

- workplace,
- primary health care/health care,
- emergency departments/psychiatric emergency departments,
- schools,
- home use (children-parents),
- antidoping,
- forensic autopsies.

Schools

Adolescents are more vulnerable to drug abuse than any other age group because their bodies and brains are not yet fully formed. In Spain, about 26.6% of students aged 14–18 years have used cannabis, according to the State Survey of Drug Use in High Schools 2012–2013, and the age of onset of drug use is 14.9 years. (Delegación del Gobierno, 2013). In the United States, some schools have implemented drug-testing programs based on the Federal Workplace Drug-Testing Programs, including random testing and reasonable suspicion/cause testing (NIDA, 2014). A study conducted in 2005 and supported by the NIDA estimated the proportion of US public schools districts that have high school grades in which random drug testing was conducted. Almost all districts randomly tested athletes and 65% randomly tested students engaged in extracurricular activities; however, 28% randomly tested all students (Ringwalt et al., 2008).

Emergency Departments

Marijuana was involved in 376,467 emergency department visits, amounting to 122.6 visits/100,000 people. The rates for emergency department visits were higher for males, especially among individuals aged 18–20 years (Substance Abuse & Mental Health Services Administration, 2010). About 25% of patients admitted to emergency departments are hidden drug users. On-site drug testing should be performed when there is a reasonable suspicion of drug use (Sanjurjo et al., 2005). On-site drug testing is also used in clinical settings. In

a study involving patients admitted to acute psychiatric treatment, initial evaluation included oral fluid collection with an on-site device and whole blood collection. The median oral fluid/blood drug concentration ratio for cannabis was 4.7 (Gjerde, Morland, Christophersen, & Bramness, 2010). Another study evaluated an on-site oral fluid test device in patients admitted to a psychiatric emergency unit; the accuracy was 94.7% for cannabis (Bagøien, Morken, Zahlsen, Aamo, & Spigset, 2009). The results of yet another study of a routine on-site testing in clinical psychiatric practice were 55% sensitivity and 82% specificity (Mordal, Holm, Morland, & Bramness, 2010).

Among patients with major trauma, initial screening of urine for drugs revealed a prevalence of 13% for cannabinoids (Carrigan, Field, Illingworth, Gaffney, & Hamer, 2000). A study was conducted in Spain to describe the prevalence of recent use of alcohol, medication, and illegal drugs in patients admitted to emergency departments for injuries due to external causes. The results revealed that the prevalence of cannabinoid use in trauma patients was 15.9% for males and 5.2% for females under 40 years of age. The prevalence of cannabinoid use according to cause of injury for male and female patients was found to be: violence 18.1/6.5%, falls 10.9/6.7%, domestic 13.8/0%, sport 8.8/10.5%, road traffic 21.9/4.5%, and workplace 7.9/6.7%, respectively (Pérez et al., 2009). A descriptive retrospective study of substance abuse in under-age patients admitted to emergency departments reported that the prevalence of cannabis use, determined by urine drug testing, was 13.5% in patients with a median age of 16.2 years, 33.8% of which had a psychiatric history. Most emergency admissions occurred during weekends, and the most frequent causes of admission were altered state of consciousness and agitation.

Primary Health Care

On-site testing for cannabis use can be an important clinical tool in patient management, if used correctly. The use of on-site testing devices contributes to medication safety by identifying the risk of overdose and serious drug interactions.

- Aids the physician in evaluating the efficacy of the therapeutic treatment plan, in chronic pain management, and in treatment of substance abuse disorders.
- Ensures medication safety, and reduces the risk of potentially harmful drug interactions during surgery or other medical procedures.
- Helps in the detection, evaluation, and diagnosis of substance abuse disorders, even though on-site testing has limited diagnostic value for such disorders.
- Detects cannabis drug use in pregnant women, or those who are planning to get pregnant.
- Identifies newborns at risk.
- Monitors abstinence, or identifies relapse in patients with known addiction to cannabis.
- Checks the validity of the information provided by the patient and/or relatives regarding the patient's drug use.

Forensic Autopsies

On-site testing devices are used in autopsy rooms for forensic toxicology. The results of an assessment of on-site testing for drugs of abuse in postmortem blood were substandard for the detection of cannabis (Moriya & Hashimoto, 1996). Although no cases of overdose deaths from cannabis have been reported (unlike other drugs of abuse, such as opioids and cocaine), it has been demonstrated that smoking marijuana is a rare trigger of acute myocardial infarction: "the risk of myocardial infarction onset was elevated 4.8 times over baseline (95% confidence interval, 2.4–9.5) in the 60 minutes after marijuana use, although the elevated risk rapidly decreased thereafter." (Mittleman, Lewis, Maclure, Sherwood, & Muller, 2001). In accordance with these findings, recent studies indicate that there is a link between cannabis consumption and myocardial infarction (Hartung, Kauferstein, Ritz-Timme, & Daldrup, 2014).

CONCLUSIONS

In conclusion, on-site testing for cannabis has been proved to be useful in a wide variety of settings, and there are many types of on-site tests available. As shown in this chapter, the most commonly used biological matrices for drug testing are: urine, blood, hair, oral fluid, sweat, and, recently, exhaled breath (Himes et al., 2013). Given that testing methods vary in terms of costs and reliability, the method of choice is that which best fits the needs of each setting.

MINI-DICTIONARY

Accuracy The closeness of a quantity of measurement to the true value.
Cut-off limit of detection Lowest detectable level permitted by the detection system used.
Gas chromatography–mass spectrometry (GC–MS) A technology by which many drugs and substances may be analyzed and identified according to a specific method using a gas to separate different compounds.
Immunoassay device A drug-testing device which incorporates a simple sample preparations step and an immunoassay.
Lipophilic To describe chemical entity which dissolves well is lipid-based system rather than aqueous-based systems as cannabis.
Liquid chromatography–mass spectrometry (LC–MS) Using a liquid to separate different compounds.

Matrix Different fluids collected from a subject as blood, urine, and oral fluid.
ng/mL Nanogram per millilitre, unit of concentration used to express the concentration of drug in a particular biological specimen. 1ng/mL is equivalent to 1 part per billion (ppb).
On-site test A test that can be conducted at the point of collection.
Oral fluid Fluid produced by glands in the mouth which contains a mixture of saliva, proteins, and other materials.
Sensitivity The ability of a particular test to determine a positive test result when it should. This is often expressed as a percentage—100% is completely sensitive, 0% is completely insensitive.
Specificity The ability of particular test to correctly determine a negative result when it should. This is often expressed as a percentage—100% is completely specific, 0% is completely unspecific.
THC COOH The principal metabolite of cannabinol without clinical effect.
THC Δ^9-Tetrahydrocannabinol The principal active substance of cannabis. It has clinical effects in the subject.

References

Agius, R., Nadulski, T., & Moore, C. (2012). Validation of LUCIO-Direct-ELISA kits for the detection of drugs of abuse in urine: application to the new German driving license regranting guidelines. *Forensic Science International*, *218*, 3–9.

Agius, R., Nadulski, T., Kahl, H. G., & Dufaux, B. (2013). Comparison of LUCIO®-direct ELISA with CEDIA immunoassay for 'zero tolerance' drug screening in urine as required by the German re-licensing guidelines. *Drug Testing and Analysis*, *5*, 390–399.

Arroyo, A., Sanchez, M., Barberia, E., Barbal, M., Marrón, M. T., & Mora, A (2013). Comparison of the Cozart DDS 801 on-site drug test device and gas chromatography/mass spectrometry (GC/MS) confirmation results of cannabis and cocaine in oral fluid specimens. *Australian Journal of Forensic Sciences*, *46*(3), 272–281.

Attema-de Jonge, M. E., Peeters, S. Y., & Franssen, E. J. (2012). Performance of three point-of-care urinalysis test devices for drugs of abuse and therapeutic drugs applied in the emergency department. *The Journal of Emergency Medicine*, *42*(6), 682–691.

Bagøien, G., Morken, G., Zahlsen, K., Aamo, T., & Spigset, O. (2009). Evaluation of a urine on-site drugs of abuse screening test in patients admitted to a psychiatric emergency unit. *Journal Clinical Psychopharmacology*, *29*, 248–254.

Barnes, A., Young, S., Spinelli, E., Martin, T., Klette, K., & Huestis, M. (2014). Evaluation of a homogenous enzyme immunoassay for the detection of synthetic cannabinoids in urine. *Forensic Science International*, *241*, 27–34.

Biermann, T., Schwarze, B., Zedler, B., & Betz, P. (2004). On-site testing of illicit drugs: the use of the drug-testing device "Toxiquick". *Forensic Science International*, *143*, 21–25.

Blencowe, T., Pehrsson, A., Lillsunde, P., Vimpari, K., Houwing, S., Smink, B., Mathijssen, R., Van der Linden, T., Legrand, S. A., Pil, K., & Verstraete, A. (2011). An analytical evaluation of eight on-site oral fluid drug screening devices using laboratory confirmation results from oral fluid. *Forensic Science International*, *208*, 173–179.

Bosker, W. M., & Huestis, M. A. (2009). Oral fluid testing for drugs of abuse. *Clinical Chemistry*, *55*, 1910–1931.

Carrigan, T. D., Field, H., Illingworth, R. N., Gaffney, P., & Hamer, D. W. (2000). Toxicological screening in trauma. *Journal of Accident and Emergency Medicine*, *17*, 33–37.

Cirimele, V., Villain, M., Mura, P., Bernard, M., & Kintz, P. (2006). Oral fluid testing for cannabis: on-site OraLine IV s.a.t. device versus GC/MS. *Forensic Science International*, *161*(2–3), 180–184.

Crouch, D. J., Hersch, R. K., Cook, R. F., Frank, J. F., & Walsh, J. M. (2002). A field evaluation of five on-site drug-testing devices. *Journal of Analytical Toxicology*, *26*, 493–499.

Delegación del Gobierno para el Plan Nacional sobre Drogas. (2013). *Encuesta estatal sobre uso de drogas en enseñanzas secundarias (ESTUDES)*. Madrid, Spain: Ministerio de Sanidad, Servicios Sociales e Igualdad, In Spanish. Available from http://www.pnsd.msc.es/Categoria2/observa/pdf/PresentESTUDES2012_2013.pdf

Desrosiers, N., Milman, G., Mendu, D., Lee, D., Barnes, A., Gorelick, D., & Huestis, M. (2014). Cannabinoids in oral fluid by on-site immunoassay and by GC-MS using two different oral fluid collection devices. *Analytical and Bioanalytical Chemistry*, *406*(17), 4117–4128.

Gallardo, E., & Queiroz, J. A. (2008). The role of alternative specimens in toxicological analysis. *Biomedical Chromatography*, *22*, 795–821.

George, S., & Braithwaite, R. A. (1995). A preliminary evaluation of five rapid detection kits for on site drugs of abuse screening. *Addiction*, *90*, 227–232.

Gjerde, H., Morland, J., Christophersen, A., & Bramness, J. (2010). Comparison of drugs concentrations in blood and oral fluid collected with Intercept sampling device. *Journal of Analytical Toxicology*, *34*, 204–209.

Goessaert, A. S., Pil, K., Veramme, J., & Verstraet, A. (2010). Analytical evaluation of a rapid on-site oral fluid drug test. *Analytical and Bioanalytical Chemistry*, *396*(7), 2461–2468.

Hartung, B., Kauferstein, S., Ritz-Timme, S., & Daldrup, T. (2014). Sudden unexpected death under acute influence of cannabis. *Forensic Science International*, *237*, e11–e13.

Himes, S. K., Scheidweiler, K. B., Beck, O., Gorelick, D. A., Desrosiers, N. A., & Huestis, M. A. (2013). Cannabinoids in exhaled breath following controlled administration of smoked cannabis. *Clinical Chemistry*, *59*, 1780–1789.

Huestis, M. A., & Cone, E. J. (2004). Relationship of Delta 9-tetrahydrocannabinol concentrations in oral fluid and plasma after controlled administration of smoked cannabis. *Journal of Analytical Toxicology*, *28*, 394–399.

Kadehjian, L. (2005). Legal issues in oral fluid testing. *Forensic Science International*, *150*, 151–156.

Laloup, M., Ramirez Fernandez, M., Wood, M., De Boeck, G., Maes, V., & Samyn, N. (2006). Correlation of Delta9-tetrahydrocannabinol concentrations determined by LC-MS-MS in oral fluid and plasma from impaired drivers and evaluation of the on-site Dräger DrugTest. *Forensic Science International*, *161*, 175–179.

Langel, K., Engblom, C., Pehrsson, A., Gunnar, T., Ariniemi, K., & Lillsunde, P (2008). Drug testing in oral fluid-evaluation of sample collection devices. *Journal of Analytical Toxicology*, *32*, 393–401.

López-Rivadulla, M. (2002). Estado actual del uso de dispositivos on site para el control de drogas en el tráfico rodado. *Revista de Toxicología*, *19*, 14–97, In Spanish.

Mittleman, M. A., Lewis, R. A., Maclure, M., Sherwood, J. B., & Muller, J. E. (2001). Triggering myocardial infarction by marijuana. *Circulation*, *102*, 2805–2809.

Moody, D. E., Fang, W. B., Andrenyak, D. M., Monti, K. M., & Jones, C. A. (2006). Comparative evaluation of the instant-view 5-panel test card with OnTrak TesTcup Pro 5: comparison with gas chromatography-mass spectrometry. *Journal of Analytical Toxicology*, *30*, 50–56.

Mordal, J., Holm, B., Morland, J., & Bramness, J. G. (2010). Recent substance intake among patients admitted to acute psychiatric wards: physicians assessment and on-site urine testing compared with comprehensive laboratory analysis. *Journal Clinical Psychopharmacology*, *30*, 455–459.

Moriya, F., & Hashimoto, Y (1996). Application of the Triage panel for drugs of abuse to forensic blood samples. *Nihon Hoigaku Zasshi*, *50*, 50–56.

Musshoff, F., Hokamp, E. G., Bott, U., & Madea, B. (2014). Performance evaluation of on-site oral fluid drug screening devices in normal police procedure in Germany. *Forensic Science International*, *238*, 120–124.

National Institute on Drug Abuse (NIDA). (2014). *Frequently asked questions about drug testing in schools*. Bethesda, MD: NIDA, Available from http://www.drugabuse.gov/related-topics/drug-testing/faq-drug-testing-in-schools

Niedbala, R. S., Kardos, K. W., Fritch, D. F., Kardos, S., Fries, T., & Waga, J (2001). Detection of marijuana use by oral fluid and urine analysis following single-dose administration of smoked and oral marijuana. *Journal of Analytical Toxicology*, *25*, 289–303.

Peace, M. R., Tarnai, L. D., & Poklis, A. (2000). Performance evaluation of four on-site drug-testing devices for detection of drugs of abuse in urine. *Journal of Analytical Toxicology*, *24*, 589–594.

Pehrsson, A., Blencowe, T., Vimpari, K., Langel, K., Engblom, C., Lillsunde, P., & Pehrsson, A. (2011). An evaluation of on-site oral fluid drug screening devices DrugWipe 5+ and Rapid STAT using oral fluid for confirmation analysis. *Journal of Analytical Toxicology*, *35*, 211–218.

Pehrsson, A., Gunnar, T., Engblom, C., Seppä, H., Jama, A., & Lillsunde, P. (2008). Roadside oral fluid testing: comparison of the results of drugwipe 5 and drugwipe benzodiazepines on-site tests with laboratory confirmation results of oral fluid and whole blood. *Forensic Science International*, *175*, 140–148.

Pérez, K., Santamariña-Rubio, E., Rodríguez-Martos, A., Brugal, M. T., Ricart, I., Suleves, J. M., de la Torre, R., Pujadas, M., Ariza, C., Díez, E., Nebot, M., Ramos, P., Martínez Beneyto, V., & Plasència, A. (2009). Substance use among non-fatally injured patients attended at emergency departments in Spain. *Drug and Alcohol Dependence*, *105*, 194–201.

Quintela, O., Crouch, D. J., & Andrenyak, D. M. (2006). Recovery of drugs of abuse from the Immunalysis Quantisal oral fluid collection device. *Journal of Analytical Toxicology*, *30*, 614–616.

Raes, E., & Verstraete, A. G. (2005). Usefulness of roadside urine drug screening in drivers suspected of driving under the influence of drugs (DUID). *Journal of Analytical Toxicology*, *29*, 632–636.

Ramaekers, J. G., Moeller, M. R., van Ruitenbeek, P., Theunissen, E. L., Schneider, E., & Kauert, G. (2006). Cognition and motor control as a function of Delta9-THC concentration in serum and oral fluid: limits of impairment. *Drug and Alcohol Dependence*, *85*, 114–122.

Repetto, M., & Repetto, G. (2009). *Toxicología fundamental* (4th ed.). Barcelona, Spain: Díaz de Santos (Chapter 15).

Ringwalt, C., Vincus, A., Ennett, S. T., Hanley, S., Bowling, M., Yacoubian, G. S., Jr., & Rohrbahc, L. A. (2008). Random Drug Testing in US Public School Districts. *American Journal of Public Health*, *98*, 826–828.

Röhrich, J., Zörntlein, S., Becker, J., & Urban, R. (2010). Detection of Delta9-tetrahydrocannabinol and amphetamine-type stimulants in oral fluid using the Rapid Stat point-of-collection drug-testing device. *Journal of Analytical Toxicology*, *34*, 155–161.

Rosso, G. L. (2013). Analysis of tools, methods and results of toxicological screening for detection of drug abuse in Italian professional drivers. *La Medicina del Lavoro*, *104*, 30–43.

Substance Abuse and Mental Health Services Administration (SAMHSA). (1994). *Mandatory guidelines for federal workplace drug testing programs*. Rockville, MD: SAMHSA, Fed. Reg. 59,29908; Available from http://workplace.samhsa.gov/fedprograms/MandatoryGuidelines/HHS09011994.pdf

Substance Abuse and Mental Health Services Administration, Center for Behavioral Health Statistics and Quality (2010). The DAWN Report: Highlights of the 2009 Drug Abuse Warning Network (DAWN) Findings on Drug-Related Emergency Department Visits. Rockville, MD: SAMHSA.

Sanjurjo, E., Cámara, M., Nogué, S., Negredo, M., García, S., To-Figueras, J., Miró, O., & de Pablo, J (2005). Urgencias por consumo de drogas de abuso: confrontación entre los datos clínicos y los analíticos. *Emergencias*, *17*, 26–31, In Spanish.

Speedy, T., Baldwin, D., Jowett, G., Gallina, M., & Jehanli, A (2007). Development and validation of the Cozart® DDS oral fluid collection device. *Forensic Science International*, *170*, 117–120.

Steinmeyer, S., Ohr, H., Maurer, H. S., & Moeller, M. R. (2001). Practical aspects of roadside tests for administrative traffic offences in Germany. *Forensic Science International*, *121*, 33–36.

Strano-Rossi, S., Castrignanò, E., Anzillotti, L., Serpelloni, G., Mollica, R., Tagliaro, F., Pascali, J. P., di Stefano, D., Sgalla, R., & Chiarotti, M. (2011). Evaluation of four oral fluid devices (DDS®, Drugtest 5000®, Drugwipe 5 + ® and RapidSTAT®) for on-site monitoring drugged driving in comparison with UHPLC-MS/MS analysis. *Forensic Science International*, *221*, 70–76.

Toennes, S., Kavert, G. F., Steinmeyer, S., & Moeller, M. P. (2005). Driving under the influence of drugs-evaluation of analytical data of drugs in oral fluid serum and urine and correlation with impairment symptoms. *Forensic Science International*, *152*, 149–155.

Vanstechelman, S., Isalberti, C., Van der Linden, T., Pil, K., Legrand, S. A., & Verstraete, A. G. (2012). Analytical evaluation of four on-site oral fluid drug testing devices. *Journal of Analytical Toxicology*, *36*, 136–140.

Verstraete, A. G. (2005). Oral fluid testing for driving under the influence of drugs: history, recent progress and remaining challenges. *Forensic Science International*, *150*, 143–150.

Walsh, J. M., Flegel, R., Crouch, D. J., Cangianelli, L., & Baudys, J. (2003). An evaluation of rapid point-of-collection oral fluid drug-testing devices. *Journal of Analytical Toxicology*, *27*, 429–439.

Wille, S. M., Samyn, N., Ramírez-Fernández, M., De Boeck, G., & de Boeck, G (2010). Evaluation of on-site oral fluid screening using Drugwipe-5(+), RapidSTAT and Drug Test 5000 for the detection of drugs of abuse in drivers. *Forensic Science International*, *198*, 2–6.

Yacoubian, G. S., Eric, D., & Wish, E. (2006). A comparison between instant and laboratory oral fluid analysis among arrestees. *Journal of Psychoactive Drugs*, *38*, 207–210.

CHAPTER

103

Cannabinoids in Oral Fluid: Identification and Interpretation of Analytical Results

C. Moore

Toxicology Research and Development, Immunalysis Corporation, Pomona, CA, United States

SUMMARY POINTS

- The detection of marijuana in biological specimens for numerous applications is of significant interest. Oral fluid is becomingly increasingly preferred as a test matrix because of its ease of collection, improved analytical techniques, and more scientifically-based interpretative value than urine. The window of drug detection represented by oral fluid for active drugs is relatively short; therefore it is an ideal specimen for collection in situations such as "for-cause" testing and traffic accident investigations.
- There are important variables surrounding oral fluid collection and analysis. Areas affecting the analytical result include: collection of a known volume of sample; characterization of the collection system; cannabinoid recovery and pad residence time; stability of cannabinoids in the transportation system, and storage conditions.
- The analysis of cannabinoids in oral fluid is a relatively routine procedure in most test facilities because of improved sensitivity of laboratory instrumentation. Problematic metabolites are 11-hydroxy-delta 9-tetrahydrocannabinol (11—OH—THC) and 11-nor-9-carboxy-delta 9-tetrahydrocannabinol (THC—COOH), present in such low concentration that their identification requires highly efficient extraction protocols, and sensitive analytical methods.
- Following marijuana smoking, which is still the most common route of cannabis self-administration, the active component tetrahydrocannabinol is predominantly detected in oral fluid. Other plant constituents such as cannabinol (CBN) and cannabidiol (CBD) are also present; other metabolites are detectable in low concentration.
- The interpretive value of oral fluid analysis depends on the reason for the test. Recent marijuana intake may be established by detection of tetrahydrocannabinol; historical use by metabolite identification at low detection levels. The addition of cannabinol and cannabidiol to the confirmation panel can assist in identifying recent smoking of marijuana; a closer look at cannabinoid ratios may give information on the use of medicinal preparations. The reliable interpretation of cannabinoid concentrations in oral fluid requires further research, but preliminary data is encouraging.

KEY FACTS OF ORAL FLUID ANALYSIS

- The main advantages of testing oral fluid for illicit or medicinal drugs include fast, easy, noninvasive, observed collection; as well as potential detection of an active compound, indicating recent intake.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00120-4
Copyright © 2017 Elsevier Inc. All rights reserved.

- The identification of recent drug use, particularly marijuana, is a key component in "for cause" testing or traffic accident investigations.
- The development of valid procedures for the quantification of drugs in oral fluid must include an awareness and understanding of the variability of collection devices, measurement of drug recovery from collection systems, evaluation of pad residence time (if appropriate), and determination of drug stability during transportation and storage.
- Potential positive results for marijuana (THC) as a result of passive exposure to cannabis may occur; the metabolite 11-nor-9-carboxy-delta 9-tetrahydrocannabinol (THC—COOH) should be included in analytical procedures to minimize this possibility.
- At this time, there is no definitive THC concentration in oral fluid that is indicative of causing impairment; however, research is continuing in this area.

LIST OF ABBREVIATIONS

11—OH—THC	11-Hydroxy-delta 9-tetrahydrocannabinol
CBD	Cannabidiol
CBN	Cannabinol
GC/MS	Gas chromatography–mass spectrometry
LC–MS/MS	Liquid chromatography with tandem mass spectrometry
OF	Oral fluid
THC	Δ^9-Tetrahydrocannabinol
THC—COOH	11-Nor-9-carboxy-delta 9-tetrahydrocannabinol

INTRODUCTION

Marijuana cultivation, transport, and use are common throughout the world. According to the World Health Organization (2014), about 147 million people, or 2.5% of the world population, consume cannabis. It used to be classified as the world's most prevalent illicit drug, but increasingly it is being legalized for medical and recreational use. While it remains largely illegal in most countries, many jurisdictions have decriminalized the possession of small quantities; however, laws on cultivation, transport, and sale are distinct from laws on possession and personal use.

Oral Fluid

The biochemical composition of saliva has been described (Schenkels, Veerman, & Nieuw Amerongen, 1995). Oral fluid (OF) is a combination of gingival crevicular fluid, and fluid from salivary parotid, submandibular, and sublingual glands. It consists predominantly of water (> 95%), proteins, electrolytes, mucin, enzymes, epithelial cells, cholesterol, and vitamins. Composition can vary with the time of day, diet, age, and artificial stimulation; a lack of saliva production which is often caused by drug use can result in issues with infection, swallowing, chewing and tooth decay. Oral fluid in healthy individuals is pH 6.5 (range 6.0–7.8); therefore drugs that have entered into the blood stream are more easily diffused into saliva, if they have basic properties rather than acidic; drugs such as amphetamine and cocaine accumulate well into oral fluid, while derivatives of carboxylic acids do not. As marijuana use becomes more socially acceptable throughout the world, its detection in biological specimens remains of high importance in areas of traffic and workplace safety, where recent use is suspected. In the 2007 National Roadside Survey (National Highway Traffic Safety Administration, 2010) randomly stopped drivers were tested for drug use using oral fluid as a sample matrix. Overall, 8.6% of the drivers were positive for tetrahydrocannabinol (THC), the active component in marijuana. The results showed that OF analysis provides data on drug use by drivers that is as least as reliable as blood, in terms of window of detection, which was collected simultaneously.

The convenience of oral fluid collection compared to other matrices is a distinct benefit. MacCall, Ritchie, & Sood (2013) studied patient preferences for drug testing collections. 85% of patients opted for OF rather than urine; 15% preferred to donate urine, citing a reluctance to try something new.

An observed collection makes it difficult to adulterate the specimen, and the identification of active drug reflects recent drug use, an advantage in "for cause" testing. Several publications have reviewed OF as a drug testing matrix (Drummer, 2006; Bosker & Huestis, 2009; Moore & Crouch, 2013) and discussed the current knowledge regarding cannabinoids in OF (Lee & Huestis, 2014).

COLLECTION DEVICES, MARIJUANA RECOVERY, AND DRUG STABILITY

Collection Devices

While saliva can be collected via expectoration (ie, "spitting"), the use of a device is preferred for hygienic reasons, as well as improved drug stability when buffers are included in the collection system. A significant variable in OF analysis is the collection device; it has been well documented that the method of collection affects

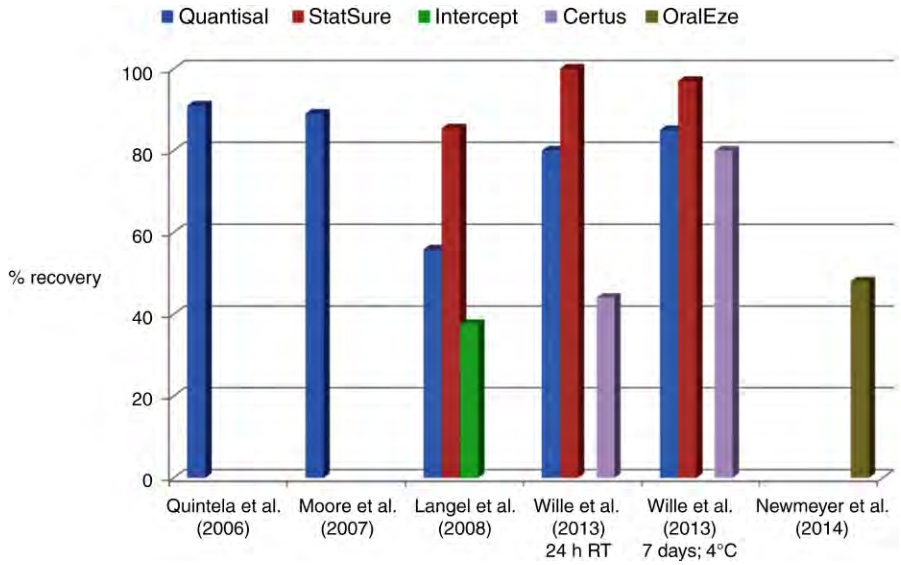


FIGURE 103.1 THC recovery from oral fluid collection devices. THC recovery from different commercially available OF collection devices is shown, based on data from peer-reviewed publications. Not all collection devices were evaluated by all authors.

the analytical result (Crouch, 2005; Drummer, 2008). The most effective and widely used devices have the following desirable characteristics:

- Collection pad (preferably with no added stimulants) and transportation buffer
- Examples include Quantisal, Omni-Sal, OralEze, Intercept, Certus
- Relatively fast, consistent collection
- Indication of adequate specimen volume
- Assessment of drug recovery from the collection pad under realistic conditions
- Documented stability of drugs during storage and transportation

THC Recovery

The adhesion of parent THC to many surfaces is a general concern in analytical techniques, therefore recovery assessment of THC within a collection system must be part of any validated assay development. Crouch, Day, & Baudys (2004) studied several collection devices in an attempt to determine whether oral fluid was suitable as an alternative to urine for workplace testing. The residence time of the pads in the transportation buffers in order to release drug into the liquid is of critical importance. As an example, the minimum residence time for the pad in the Quantisal system is 4 h; for Oral Eze ≥12 h are recommended. Quintela, Crouch, & Andrenyak (2006) reported high THC recovery for the Quantisal device; Langel et al. (2008) compared the recovery of several drugs, including THC, from nine different oral fluid collection devices. Some were simply unacceptable; but many of the currently commercially available devices were reported to have reasonable marijuana recovery. The authors acknowledge that the residence time for some of the pads was less than recommended by the manufacturers. More recently, Wille, Di Fazio, Ramirez-Fernandez, Kummer, & Samyn (2013) reported THC recovery from three devices under different storage conditions. Published recovery of THC from various devices by numerous authors is shown in Fig. 103.1. It should also be noted that there are considerable improvements with collection systems from most manufacturers so published recoveries may not be currently reflective of the efficiency of oral fluid THC recovery.

THC Stability

In contrast to other biological specimens, THC is relatively stable in oral fluid collection devices because of the composition of the transportation buffers. While fluorescent light and exposure to plastic surfaces will degrade THC, the overall stability particularly when refrigerated is acceptable (Moore et al., 2006a). More recently, Lee et al. (2012a) indicated the importance of device selection particularly when considering stability by stating: *"… because each collection device contains proprietary stabilizing buffers, cannabinoid stability in OF must be determined for each type of collection device, limiting generalization of results to other collection devices, and strongly supporting the need for additional stability studies. These data emphasize the importance of oral fluid collection device design."*

ANALYTICAL ISSUES

THC

Parent drugs are generally detected in higher concentration than metabolites in oral fluid (Allen, 2011). While the identification of THC in oral fluid has historically been considered difficult, recent improvements in analytical techniques have allowed its detection to become routine and there are numerous publications reporting its determination in oral fluid using immunoassay screening (Schwope, Milman, & Huestis, 2010; Moore & Crouch, 2013); confirmatory techniques using gas chromatography–mass spectrometry (GC/MS); liquid chromatography with tandem mass spectrometry (LC–MS/MS) or time of flight (TOF) detection (Bylda, Leinenbach, Thiele, Kobold, & Volmer, 2012; Lee, Chang, Lin, & Chang. 2012; Quintela, Andrenyak, Hoggan, & Crouch, 2007).

Immunoassays

Immunoassays have been developed for oral fluid targeting parent drugs because, as noted previously, they tend to be higher in concentration than metabolites. Immunoassay developments have also included improved sensitivity in homogeneous formats, to assist in laboratory throughput (Moore, 2011); but they also suffer the same drawbacks as immunoassays for other specimen types, such as the potential for false positive results caused by similarly structured compounds; or false negative results caused by inadequate sensitivity or interference.

Mass Spectrometry (MS)

MS-based detection systems are used when specific identification or quantitation of a drug/metabolite is needed. Disadvantages are mainly associated with ion suppression, or isobaric compounds having identical transitions, similar to those encountered with other matrices (Vogeser & Seger, 2010; Tuyay, Coulter, Rodrigues, & Moore, 2012). As long as drugs are efficiently extracted from transportation buffers to avoid injection of stabilizers and surfactants directly into instruments, then many issues can be avoided.

Other Cannabinoids

In addition to the parent compound, there are other naturally occurring cannabinoids, such as cannabidiol (CBD), cannabinol (CBN) and the precursor 2-carboxy-tetrahydrocannabinol that may be detected in oral fluid (Moore, Rana, & Coulter, 2007a). The metabolites 11-hydroxy-delta 9-tetrahydrocannabinol (11—OH—THC) and 11-nor-9-carboxy-delta 9-tetrahydrocannabinol (THC—COOH) have also been detected in very low concentration. In order to maximize the detection of THC—COOH, a hydrolysis procedure may be incorporated into the analysis, because THC—COOH has been found to be conjugated in oral fluid (Day, Kuntz, Feldman, & Presley, 2006; Moore, Coulter, Rana, Vincent, & Soares, 2006; Moore et al., 2007b). Various LC–MS/MS procedures which achieve the required sensitivity for the detection of THC—COOH in oral fluid have recently been published (He, Kozak, & Nimkar, 2012; Coulter, Garnier, & Moore, 2012; Scheidweiler, Himes, Chen, Liu, & Huestis, 2013).

There are fewer reports of 11—OH—THC being identified in oral fluid, but the detection of THC—COOH is a key issue in the potential differentiation between passive exposure to marijuana smoke and actual cannabis intake. THC—COOH is not present in cannabis smoke and in recent research was not measurable in OF collected from subjects passively exposed to cannabis in a Dutch "coffee-shop." THC was detectable in all specimens taken 3 h after realistic exposure to marijuana smoke (Moore et al., 2011).

DISPOSITION OF CANNABINOIDS IN ORAL FLUID

Several studies have attempted to characterize the disposition of THC and its metabolites in oral fluid, and to understand the relationship between THC concentrations in OF and plasma/serum. THC is deposited in the buccal cavity after smoking, and contamination may result in high OF concentrations, with C_{max} reported in most publications 0.25–0.5 h after smoking. Cannabinoid measurement in both blood and OF following controlled dosing has given some insight into OF:plasma ratios, although inter-subject variability is widely documented. Cannabis can be administered in several ways – most commonly smoking, but oral (Marinol) and oromucosal formulations (Sativex) are also available. There is a great deal of interest in determining a meaningful testing concentration for THC in oral fluid that correlates with blood THC and/or impairment; however, to date, such a concentration has not been well established. As this would be particularly useful in cases of driving under the influence of drugs, many projects have focused on traffic safety. Van der Linden, Silverans, & Verstraete (2014) noted that self-reported cannabis use is largely underestimated by drivers and, therefore the analysis of biological specimens is necessary. Gjerde, Langel, Favretto, & Verstraete (2014) recently reported the estimation of equivalent cutoff concentrations in OF and blood for 12 commonly used drugs, including THC in oral fluid, collected with the Statsure device. Multiplying the cutoff thresholds in

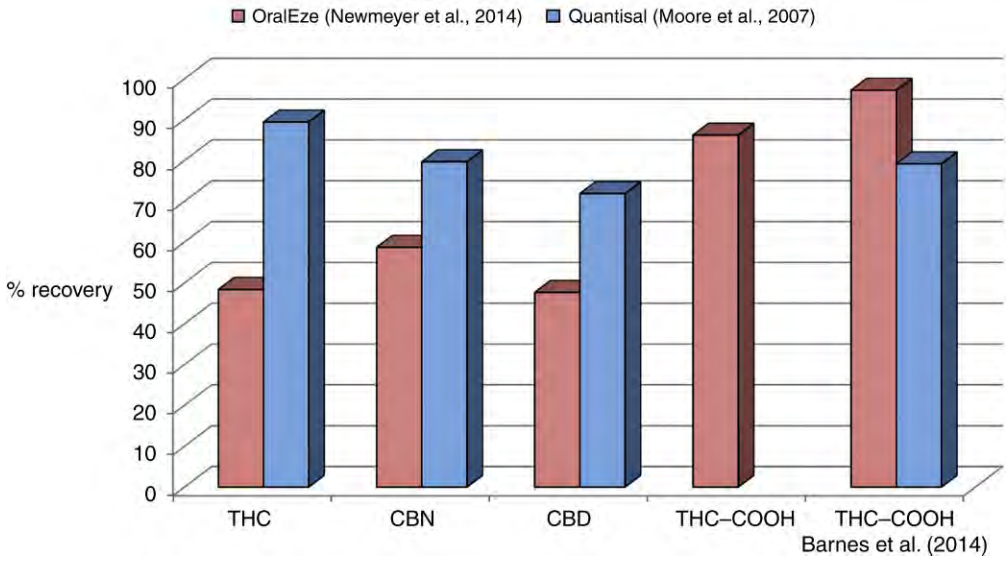


FIGURE 103.2 Cannabinoid recovery from oral fluid collection devices. Cannabinoid recovery from two different OF collection devices is shown based on data from peer-reviewed publications. Moore et al. (2007) did not include recovery data for THC—COOH; Barnes et al. (2014) only reported on THC—COOH.

blood with the average and median OF:blood ratios to estimate equivalent cutoffs in oral fluid were investigated. Using a THC concentration of 1 ng/mL in blood, an equivalent cutoff concentration in OF was estimated to be 44 ng/mL (range 27–90 ng/mL).

Marijuana Smoking

Kauert, Ramaekers, Schnieder, Moeller, & Toennes (2007) collected blood and oral fluid samples for 6 h from 10 participants, following low and high dosage cigarette smoking. Mean oral fluid THC concentrations in specimens collected after 15 min were 900 ng/mL (±589; median 797) and 1041 ng/mL (± 652; median 894), respectively. The OF:serum ratios were extremely high: 46 ± 27 (low dosage) and 36 ± 20 (high dosage); the authors concluded that kinetics of THC in oral fluid are not yet satisfactorily understood.

In subjects admitted to a psychiatric facility, Gjerde, Mordal, Christophersen, Bramness, & Morland (2010) reported a median OF:blood ratio for THC of 4.7. In a later publication, some of the same authors reported a median OF:blood ratio of 14, based on suspected drug users in Europe (Langel et al., 2014); the oral fluid was taken with a different collection device.

Anizan et al. (2013) studied chronic and occasional marijuana smokers. They compared THC and metabolites in OF collected with the Statsure device from 19 h before smoking a marijuana cigarette, to 30 h afterward. No significant differences between the two groups (chronic and occasional smokers) were noted. All OF specimens were THC-positive up to 13.5 h after smoking; CBD was detected up to 4 h after smoking; CBN up to 8 h. THC—COOH not surprisingly provided a longer detection window (>30 h) than THC in all frequent smokers.

Fabritius et al. (2013) collected both whole blood and OF from subjects before and up to 3.5 h after smoking marijuana. OF specimens were analyzed for THC, THC—COOH, CBN, and 2-carboxy-tetrahydrocannabinol. Measured THC concentrations in frequent smokers ranged from 19 to 3170 ng/mL, and in occasional smokers from 367 to 3110 ng/mL, with the highest levels being reached within 30 min of intake.

In a controlled study, Newmeyer et al. (2014) reported cannabinoid disposition in OF after cannabis smoking in frequent and occasional smokers, using saliva collected with the OralEze device. Specimens were analyzed for THC, 11—OH—THC, THC—COOH, CBN, and CBD. While the recovery of THC from collection devices is often reported (Fig. 103.1), efficiency for the other cannabinoids is not always included in research papers. The recovery of CBD and CBN from OralEze or Quantisal devices has only been reported in two publications (Newmeyer et al., 2014; Moore et al., 2007b, respectively); however, a recent paper (Barnes, Scheidweiler, & Huestis, 2014) compared the recovery of THC-COOH from two different devices (Fig. 103.2). Newmeyer et al. (2014) determined that frequent cannabis smokers had significantly higher concentrations of THC—COOH in their OF, compared to occasional smokers, and were positive for longer periods after smoking. The concentration of cannabinoids in OF collected from frequent marijuana smokers is shown in Table 103.1; the median concentration of THC, CBD, and CBN in OF taken from frequent

TABLE 103.1 Concentration of Cannabinoids in Oral Fluid From Frequent Marijuana Smokers

	Median (range) after marijuana smoking								
	THC (ng/mL)		**CBN (ng/mL)**		**CBD (ng/mL)**		**THC—COOH (pg/mL)**		**OF device**
Time after smoking	0.25 h	0.5 h	0.25 h	0.5 h	0.25 h	0.5 h	0.25 h	0.5 h	
Anizan et al. (2013) (n = 14)		517 (189–6508)		37 (16–476)		21 (4.5–255)		126 (59–430)	StatSure
Milman et al. (2012) (n = 9)	2629 (265–22,370)	527 (311–1524)	230 (0–1964)	40 (5.8–45)	121 (8.8–1000)	22 (3.6–49)	148 (0–468)	82 (0–157)	None
Fabritius et al. (2013) (n = 23)		636 (19–3170)		81 (6–310)				300 (100–2400)	Quantisal
Kauert et al. (2007) (n = 10)	894 (247–>2500)								Intercept
Newmeyer et al. (2014) (n = 14)		803 (135–4146)		32 (3.3–284)		14.8 (1.4–162)		168 (70–1107)	OralEze

The median reported range of cannabinoid concentrations in OF collected from frequent marijuana smokers at different time points is shown; not all researchers measured all cannabinoids.

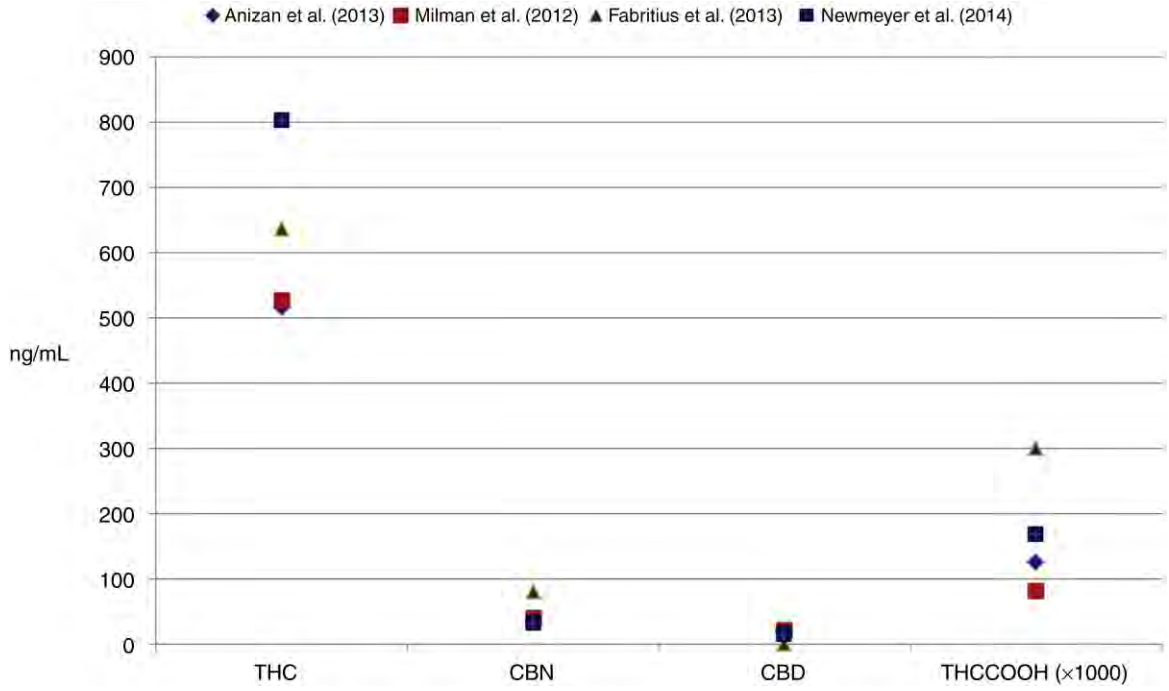


FIGURE 103.3 Median concentration of cannabinoids in OF from frequent marijuana smokers. Median concentrations of THC, CBD, and CBN in OF specimens taken from frequent marijuana smokers 30 min after the end of smoking sessions. Results published by different researchers are shown; the concentrations are remarkably similar.

marijuana smokers 30 min after the end of a smoking session has been reported by different research groups. While the range of concentrations was wide, the medians were remarkably similar (Fig. 103.3).

Smoking and Oral Administration

Lee et al. (2013a) reported OF:plasma ratios following both controlled oral THC administration and smoked cannabis in eleven chronic cannabis smokers. OF and plasma specimens were analyzed for THC, 11—OH—THC, THC—COOH, CBN, and CBD. Following cannabis smoking, the OF:plasma THC ratios extended over a wide range (median, 6.1; range, 0.2–348.5), likely due to oral contamination from the smoke. As the oral contamination dissipated over time, the median (2.1) and range (0.1–20.7) OF:plasma ratios decreased.

Milman, Schwope, Gorelick, & Huestis (2012) measured THC and metabolites in expectorated OF, following controlled cannabis smoking (n = 10). THC (>0.25 ng/mL) was detected in 95.2% of the OF samples; THC—COOH (> 5 pg/mL) was present in 94.7%; CBN (> 1 ng/mL) in 62.3%; CBD (> 0.25 ng/mL) in 69.3%; and 11—OH—THC (>0.25 ng/mL) in 2.9%. No information on plasma concentrations was given.

The consensus from these various studies is that there is a wide variation in THC concentrations and in OF:blood ratios following marijuana smoking.

Oral Administration

Oral administration shows a somewhat different disposition of cannabinoids in oral fluid. Over 9 days, Milman et al. (2010) administered 20 mg doses of oral THC to 10 participants reporting daily cannabis smoking, and cannabis use within 24 h of entering the study. OF samples (n = 440) were collected with Quantisal devices before, during, and after the dosing schedule. THC concentrations in OF actually decreased and reflected previously smoked cannabis, rather than orally administered Marinol. In contrast, the metabolite THC—COOH was present in 98.3% of specimens collected on days 2–7. CBD and CBN were in less than 2% of specimens; 11—OH—THC was not identified. Expectorated OF specimens gave similar results (Milman et al., 2011a).

The same research group of Milman et al. (2011b) collected OF and plasma samples from 10 daily cannabis smokers who were given synthetic THC in oral dosage form (dronabinol, Marinol) for 8 days. While the concentration of THC in plasma increased throughout daily dosing, the OF THC concentration generally decreased. THC—COOH concentrations increased in both matrices. The authors concluded that the time courses in OF and plasma were different, oral fluid monitoring did not reliably detect oral marijuana intake, and there was considerable interindividual variation.

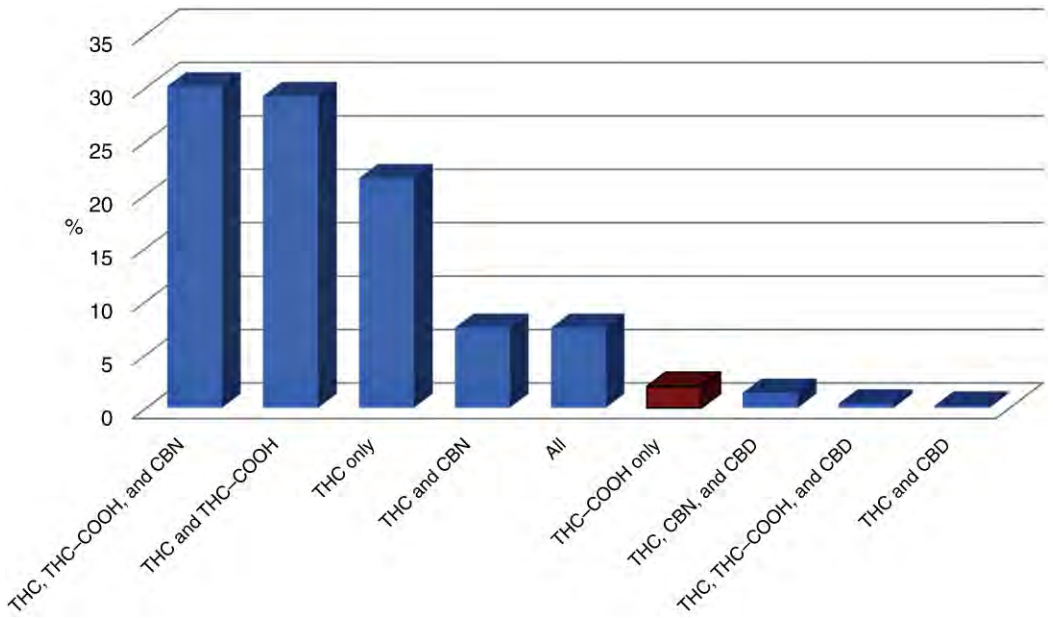


FIGURE 103.4 **Cannabinoid composition in marijuana positive oral fluid samples.** A breakdown of the composition of THC, THC—COOH, CBD, and CBN in oral fluid specimens positive for marijuana is depicted. 98% of samples contained the parent, active drug THC; only 2% showed the presence of the metabolite THC—COOH alone.

INTERPRETATION OF RESULTS

Variability in THC oral fluid concentrations especially after recent smoking can skew the data, making interpretation of concentrations problematic. The window of detection for marijuana intake depends on the route of administration, and the sensitivity of the analytical procedure. Lee et al. (2011) discussed the use of seven different cutoffs that, when applied, would give insight into the last use of marijuana. 28 frequent cannabis smokers were recruited and resided on a closed ward for approximately 30 days. Oral fluid specimens were collected periodically throughout the research period; the specimens were analyzed for THC, THC—COOH, CBD, and CBN. From a subset of 21 subjects, THC was detected for 24 h in the oral fluid of 9 participants (42%); for 48 h in 5 subjects (24%); and in no participants after 72 h. CBD and CBN were detected only at admission. However, THC—COOH in some cases was detectable for as long as 29 days (median 13 days). Correlation between THC and THC—COOH levels improved over time, as biological equilibrium likely occurred. In the remaining seven subjects, two were completely negative; one was positive at 48 h, but not at 24 h. One was positive at admission and on day 4, but not in between; others were negative on admission, and then positive several days later—data which are not easily explained in a closed monitored ward situation—a drawback acknowledged by the researchers. However, the concept of using different cutoff criteria to interpret oral fluid drug concentrations has some merit, and can be applied to different test scenarios:

Recent Use

For situations in which marijuana use or exposure is required to be recent (eg, driving incidents, "for-cause" testing), Lee et al. (2011) concluded that four of their seven studied cutoffs decreased the window of detection, thereby increasing the identification of recent smoking. The four criteria suggested were:

1. THC ≥ 2 ng/mL, and THC—COOH ≥ 20 pg/mL
2. THC—COOH (pg):THC (ng) ratio ≤ 4
3. THC ≥ 2 ng/mL, and CBD ≥ 0.5 g/mL
4. THC ≥ 2 ng/mL, and CBN ≥ 1 ng/mL

THC ≥ 1 ng/mL, plus CBD ≥ 1 ng/mL or CBN ≥ 1 ng/mL suggests cannabis intake within the previous 13.5 h (Anizan et al., 2013). CBN and/or CBD indicates recent use as these dissipate from oral fluid very quickly (<8 h).

Intuitively, a higher THC concentration indicates more recent drug exposure; therefore adoption of a specific cutoff concentration relating to cannabis intake within the last 2–3 h would be helpful for driving situations. Use within 8 h is arguably not impairing in individuals who have developed tolerance to the effects of marijuana.

TABLE 103.2 Median cannabinoid ratios in oral fluid from Sativex users and drivers

	CBD:THC	CBN:THC
Specimens collected after Sativex administration (Lee et al., 2013b)	0.82–1.34	0.04–0.06
Specimens from drivers involved in a research study (unpublished data)	0.014 (0.002–0.815)	0.039 (0.003–1.00)

A potential ratio of CBD:THC was established by Lee et al. (2013b) to differentiate Sativex patients from marijuana smokers. Based on the application of that ratio to CBD and THC concentrations detected in OF from research study specimens, none of the drivers would be considered Sativex users

Proof of Use

For applications where the proof of actual marijuana intake is required, as opposed to potential environmental exposure (workplace testing; child custody, etc.), the detection of the metabolite THC—COOH is advocated.

Historical Use

The lower the cutoff concentration the longer the window of detection of a drug, therefore historical use may be indicated if lower limits of quantitation are used analytically. The presence of THC—COOH in oral fluid from chronic users during abstinence has been reported for up to 29 days (median 13 days).

Medicinal Use

Marinol and Sativex are legal formulations of marijuana. Researchers have shown that THC does not accumulate well into oral fluid, after oral THC administration, but THC—COOH concentrations increase. Sativex is a cannabinoid medicine for the treatment of spasticity due to multiple sclerosis, and it is legally approved for use in over 25 countries. It was recently granted "Fast Track" designation by the US Food and Drug Administration (FDA) for the treatment of pain in patients with advanced cancer. The active compounds in Sativex are THC and CBD, and it is administered as an oromucosal spray. The possibility of differentiating the use of prescription Sativex from illicit marijuana smoking by measuring the ratio of CBD to THC in oral fluid has been addressed by two research groups:

1. Molnar, Fu, Lewis, Allsop, & Copeland (2014) analyzed oral fluid collected from ten participants, following high or low doses of Sativex. Results showed high concentrations of THC (5,356–15,468 ng/mL) and CBD (3,826–17,233 ng/mL) in the oral fluid up to 20 min after dosing. The oral fluid remained positive for THC and CBD for 2 h after the Sativex dose was given. CBN was also detected in the oral fluid of participants.
2. Lee et al. (2013b) characterized cannabinoid disposition in oral fluid following the administration of dronabinol (Marinol) or Sativex in low and high dosage forms. Following oral administration, CBD and CBN were rarely detected, and the THC concentration declined; in contrast following Sativex intake, THC, CBD, and CBN all increased with maximum concentrations 15–60 min after administration. The median CBD:THC ratio was 0.82–1.34; median CBN:THC ratio was 0.04–0.06. The authors reported that lack of measurable THC, CBD, and CBN following oral THC administration and high CBD:THC ratios after Sativex use can distinguish the mode of intake from marijuana smoking.

A retrospective study was performed on a subset of marijuana positive specimens collected from drivers involved in a research study (unpublished data). THC (>1 ng/mL) was detected in 97.9% of the oral fluid samples; THC-COOH (>20 pg/mL) was present in 69.3%; CBN (>0.2 ng/mL) in 46.6%; and CBD (>1 ng/mL) in only 9.5% of the samples (Fig. 103.4). An analytical confirmatory procedure detecting THC and THC—COOH would have confirmed all the specimens; however, the information provided by the CBD and CBN concentrations in proportion to the THC appears to have interpretive value (Table 103.2).

While the CBN:THC ratio was not markedly different between the two groups, the CBD: ratio in oral fluid from drivers was 0.014 (0.002–0.815; n = 52), compared to 0.82–1.34 (as described by Lee et al., 2013b). CBN and CBD are present to varying degrees in naturally occurring marijuana, so their presence in oral fluid is likely after recently smoking cannabis. If the median ratio of CBD:THC in Sativex users is 0.82–1.34, then none of the drivers was likely to be a Sativex user, as the concentration of CBD in the oral fluid is either zero (90.5%) or far lower than would be expected from use of a 1:1 CBD:THC (Sativex) medicinal preparation. Further, the median THC—COOH (pg) to THC (ng) ratio in the drivers was 4, implying that 50% of the drivers had smoked marijuana recently (Lee et al., 2011).

MINI-DICTIONARY

Cannabinoids Cannabinoids are naturally occurring substances which are found in the plant *Cannabis Sativa*. Δ^9-Tetrahydrocannabinol (THC) is primarily responsible for the psychoactive effects of cannabis; other cannabinoids within the plant are thought to moderate its effects.

Immunoassays Immunoassays are generally employed as screening techniques within a laboratory for the analysis of compounds in biological specimens. They tend to be used as an inexpensive first stage of analysis to identify samples that do not contain the drug of interest (negative specimens). Samples that are presumptively positive using the immunoassay should then be confirmed using a more specific, usually quantitative technique, such as mass spectrometry.

Mass spectrometry Mass spectrometers are complex, relatively expensive laboratory instruments, used for the detection of compounds in biological extracts, generally following separation through a gas or liquid chromatographic technique. Mass spectral detection methods are used as quantitative measures for drugs in biological samples, and are more specific than immunoassay techniques.

Oral fluid "Oral fluid" is the term used for the clear, viscous fluid predominantly excreted from salivary parotid, submaxillary, and sublingual glands. While the amount of saliva produced by an individual can vary, in healthy adults the reported volume is between 0.75 and 1.5 L per day. Drugs can be detected in oral fluid through buccal contamination or via diffusion from blood depending upon the chemical properties of the compound.

FINANCIAL DISCLOSURES

Christine Moore is employed by Immunalysis Corporation that manufactures the Quantisal oral fluid collection device and immunoassay reagents for oral fluid analysis.

References

Allen, K. R. (2011). Screening for drugs of abuse: which matrix, oral fluid or urine? *Annals of Clinical Biochemistry*, *48*, 531–541.

Anizan, S., Milman, G., Desrosiers, N., Barnes, A. J., Gorelick, D. A., & Huestis, M. A. (2013). Oral fluid cannabinoid concentrations following controlled smoked cannabis in chronic frequent and occasional smokers. *Analytical and Bioanalytical Chemistry*, *405*(26), 8451–8461.

Barnes, A. J., Scheidweiler, K. B., & Huestis, M. A. (2014). Quantification of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol in human oral fluid by gas chromatography-tandem mass spectrometry. *Therapeutic Drug Monitoring*, *36*(2), 225–233.

Bosker, W., & Huestis, M. H. (2009). Oral fluid testing for drugs of abuse. *Clinical Chemistry*, *55*, 1910–1931.

Bylda, C., Leinenbach, A., Thiele, R., Kobold, U., & Volmer, D. A. (2012). Development of an electrospray LC-MS/MS method for quantification of D9-tetrahydrocannabinol and its main metabolite in oral fluid. *Drug Testing and Analysis*, *4*(7-8), 668–674.

Coulter, C., Garnier, M., & Moore, C. (2012). Analysis of tetrahydrocannabinol and its metabolite, 11-nor-d9-tetrahydrocannabinol-9-carboxylic acid, in oral fluid using liquid chromatography with tandem mass spectrometry. *Journal of Analytical Toxicology*, *36*(6), 413–417.

Crouch, D. J. (2005). Oral fluid collection: the neglected variable in oral fluid testing. *Forensic Science International*, *150*(2-3), 165–173.

Crouch, D. J., Day, J., & Baudys, J. (2004). *Evaluation of saliva/oral fluid as an alternative drug testing specimen—Final report (NISTR 7139)*. Gaithersburg, MD: National Institute of Standards and Technology, 1–70.

Day, D., Kuntz, D. J., Feldman, M., & Presley, L. (2006). Detection of THCA in oral fluid by GC-MS-MS. *Journal of Analytical Toxicology*, *30*(7), 645–650.

Drummer, O. H. (2006). Drug testing in oral fluid. *Clinical Biochemistry Review*, *27*(3), 147–159.

Drummer, O. H. (2008). Introduction and review of collection techniques and applications of drug testing of oral fluid. *Therapeutic Drug Monitoring*, *30*(2), 203–206.

Fabritius, M., Chtioui, H., Battistella, G., Annoni, J. -M., Dao, K., Favrat, B., Fornari, E., Lauer, E., Maeder, P., & Giroud, C. (2013). Comparison of cannabinoid concentrations in oral fluid and whole blood between occasional and regular cannabis smokers prior to and after smoking a cannabis joint. *Analytical and Bioanalytical Chemistry*, *405*, 9791–9803.

Gjerde, H., Mordal, J., Christophersen, A. S., Bramness, J. G., & Morland, J. (2010). Comparison of drug concentrations in blood and oral fluid collected with the Intercept sampling device. *Journal of Analytical Toxicology*, *34*(4), 204–209.

Gjerde, H., Langel, K., Favretto, D., & Verstraete, A. G. (2014). Estimation of equivalent cutoff thresholds in blood and oral fluid for drug prevalence studies. *Journal of Analytical Toxicology*, *38*(2), 92–98.

He, X., Kozak, M., & Nimkar, S. (2012). Ultra-sensitive measurements of 11-nor-Δ(9)-tetrahydrocannabinol-9-carboxylic acid in oral fluid by microflow liquid chromatography-tandem mass spectrometry using a benchtop quadrupole/orbitrap mass spectrometer. *Analytical Chemistry*, *84*(18), 7643–7647.

Kauert, G. F., Ramaekers, J. G., Schnieder, E., Moeller, M. R., & Toennes, S. W. (2007). Pharmacokinetic properties of delta9-tetrahydrocannabinol in serum and oral fluid. *Journal of Analytical Toxicology*, *31*(5), 288–293.

Langel, K., Engblom, C., Pehrsson, A., Gunnar, T., Ariniemi, K., & Lillsunde, P. (2008). Drug testing in oral fluid—evaluation of sample collection devices. *Journal of Analytical Toxicology*, *32*, 393–401.

Langel, K., Gjerde, H., Favretto, D., Lillsunde, P., Øiestad, E. L., Ferrara, S. D., & Verstraete, A. G. (2014). Comparison of drug concentrations between whole blood and oral fluid. *Drug Testing and Analysis*, *6*(5), 461–471.

Lee, D., & Huestis, M. A. (2014). Current knowledge on cannabinoids in oral fluid. *Drug Testing and Analysis*, *6*, 88–111.

Lee, D., Milman, G., Barnes, A. J., Goodwin, R. S., Hirvonen, J., & Huestis, M. A. (2011). Oral fluid cannabinoids in chronic, daily cannabis smokers during sustained, monitored abstinence. *Clinical Chemistry*, *57*(8), 1127–1136.

Lee, D., Milman, G., Schwope, D. M., Barnes, A. J., Gorelick, D. A., & Huestis, M. A. (2012a). Cannabinoid stability in authentic oral fluid after controlled cannabis smoking. *Clinical Chemistry*, *58*(7), 1101–1109.

Lee, D., Schwope, D. M., Milman, G., Barnes, A. J., Gorelick, D. A., & Huestis, M. A. (2012b). Cannabinoid dispositionin oral fluid after controlled smoked cannabis. *Clinical Chemistry*, *58*(4), 748–756.

Lee, P. D., Chang, Y. J., Lin, K. L., & Chang, Y. Z. (2012c). Simultaneous determination of D9-tetrahydrocannabinol and 11-nor-9-carboxy-D9-tetrahydrocannabinol in oral fluid using isotope dilution liquid chromatography tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, *402*, 851–859.

Lee, D., Vandrey, R., Milman, G., Bergamaaschi, M., Mendu, D. R., Murray, J. A., Barnes, A. J., & Huestis, M. A. (2013a). Oral fluid/plasma cannabinoid ratios following controlled oral THC and smoked cannabis administration. *Analytical and Bioanalytical Chemistry*, *405*(23), 7269–7279.

Lee, D., Karschner, E. L., Milman, G., Barnes, A. J., Goodwin, R. S., & Huestis, M. A. (2013b). Can oral fluid cannabinoid testing monitor medication compliance and/or cannabis smoking during oral THC and oromucosal Sativex administration? *Drug and Alcohol Dependence*, *130*(1-3), 68–76.

MacCall, C. A., Ritchie, G., & Sood, M. (2013). Oral fluid testing as an alternative to urine testing for drugs of abuse in inpatient forensic settings: giving patients' choice. *Scottish Medical Journal*, *58*(2), 99–103.

Milman, G., Barnes, A. J., Schwope, D. M., Schwilke, E. W., Darwin, W., Goodwin, R. S. D., Kelly, D. L., Gorelick, D. A., & Huestis, M. A. (2010). Disposition of cannabinoids in oral fluid after controlled around-the-clock oral THC administration. *Clinical Chemistry*, *56*(8), 1261–1269.

Milman, G., Barnes, A. J., Schwope, D. M., Schwilke, E. W., Goodwin, R. S., Kelly, D. L., Gorelick, D. A., & Huestis, M. A. (2011a). Cannabinoids and metabolites in expectorated oral fluid after 8 days of controlled around-the-clock oral THC administration. *Analytical and Bioanalytical Chemistry*, *401*(2), 599–607.

Milman, G., Schwope, D. M., Schwilke, E. W., Darwin, W. D., Kelly, D. L., Goodwin, R. S., Gorelick, D. A., & Huestis, M. A. (2011b). Oral fluid and plasma cannabinoid ratios after around-the-clock controlled oral Δ9-tetrahydrocannabinol administration. *Clinical Chemistry*, *57*(11), 1597–1606.

Milman, G., Schwope, D. M., Gorelick, D. A., & Huestis, M. A. (2012). Cannabinoids and metabolites in expectorated oral fluid following controlled smoked cannabis. *Clinica Chimica Acta*, *413*(7-8), 765–770.

Molnar, A., Fu, S., Lewis, J., Allsop, D. J., & Copeland, J. (2014). The detection of THC, CBD and CBN in the oral fluid of Sativex® patients using two on-site screening tests and LC-MS/MS. *Forensic Science International*, *238*, 113–119.

Moore, C. (2011). Oral fluid and hair in workplace drug testing programs: new technology for immunoassays. *Drug Testing and Analysis*, *3*(3), 166–168.

Moore, C., & Crouch, D. (2013). Oral fluid for the detection of drugs of abuse using immunoassay and LC–MS/MS. *Bioanalysis*, *5*(12), 1555–1569.

Moore, C., Vincent, M., Rana, S., Coulter, C., Agrawal, A., & Soares, J. (2006a). Stability of Δ9-tetrahydrocannabinol (THC) in oral fluid using the Quantisal™ collection device. *Forensic Science International*, *164*(2-3), 126–130.

Moore, C., Coulter, C., Rana, S., Vincent, M., & Soares, J. (2006b). Analytical procedure for the determination of the marijuana metabolite, 11-nor-Δ^9-tetra-hydrocannabinol-9-carboxylic acid (THCA), in oral fluid specimens. *Journal of Analytical Toxicology*, *30*(7), 409–412.

Moore, C., Rana, S., & Coulter, C. (2007a). Simultaneous identification of 2-carboxy-tetrahydrocannabinol, tetrahydrocannabinol, cannabinol and cannabidiol in oral fluid. *Journal of Chromatography (Biomedical Applications)*, *852*, 459–464.

Moore, C., Rana, S., Coulter, C., Day, D., Vincent, M., & Soares, J. (2007b). Detection of conjugated 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid in oral fluid. *Journal of Analytical Toxicology*, *31*(4), 187–194.

Moore, C., Coulter, C., Uges, D., Tuyay, J., van der Linde, S., van Leuwen, A., Garnier, M., & Orbita, J. (2011). Cannabinoids in oral fluid following passive exposure to marijuana smoke. *Forensic Science International*, *212*(1-3), 227–230.

Newmeyer, M. H., Desrosiers, N. A., Lee, D., Mendu, D. R., Barnes, A. J., Gorelick, D. A., & Huestis, M. A. (2014). Cannabinoid disposition in oral fluid after controlled cannabis smoking in frequent and occasional smokers. *Drug Testing and Analysis*, *6*(10), 1002–1010.

National Highway Safety Transportation Administration (NHTSA). (2014). *2007 National Roadside Survey Methodology of Alcohol and Drug Use by Drivers*. Washington, DC: NHTSA. Available from www.nhtsa.gov/staticfiles/nti/pdf/811237.pdf

Quintela, O., Crouch, D. J., & Andrenyak, D. (2006). Recovery of drugs of abuse from the Immunalysis Quantisal™ oral fluid collection device. *Journal of Analytical Toxicology*, *30*, 614–616.

Quintela, O., Andrenyak, D. M., Hoggan, A. M., & Crouch, D. J. (2007). A validated method for the detection of delta 9-tetrahydrocannabinol and 11-nor-9-carboxy- delta 9-tetrahydrocannabinol in oral fluid samples by liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry. *Journal of Analytical Toxicology*, *31*(3), 157–164.

Scheidweiler, K. B., Himes, S. K., Chen, X., Liu, H. F., & Huestis, M. A. (2013). 11-Nor-9-carboxy-Δ9-tetrahydrocannabinol quantification in human oral fluid by liquid chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, *405*(18), 6019–6027.

Schenkels, L. C. P. M., Veerman, E. C. I., & Nieuw Amerongen, A. V. (1995). Biochemical composition of human saliva in relation to other mucosal fluids. *Critical Reviews in Oral Biology and Medicine*, *6*(2), 161–175.

Schwope, D. M., Milman, G., & Huestis, M. A. (2010). Validation of an enzyme immunoassay for detection and semi-quantification of cannabinoids in oral fluid. *Clinical Chemistry*, *56*(6), 1007–1014.

Tuyay, J., Coulter, C., Rodrigues, W., & Moore, C. (2012). Disposition of opioids in oral fluid: Importance of chromatography and mass spectral transitions in LC-MS/MS. *Drug Testing and Analysis*, *4*(6), 395–401.

Van der Linden, T., Silverans, P., & Verstraete, A. G. (2014). Comparison between self-report of cannabis use and toxicological detection of THC/THCCOOH in blood and THC in oral fluid in drivers in a roadside survey. *Drug Testing and Analysis*, *6*(1,2), 137–142.

Vogeser, M., & Seger, C. (2010). Pitfalls associated with the use of liquid chromatography–tandem mass spectrometry in the clinical laboratory. *Clinical Chemistry*, *56*(8), 1234–1244.

Wille, S. M., Di Fazio, V., Ramirez-Fernandez Mdel, M., Kummer, N., & Samyn, N. (2013). Driving under the influence of cannabis: pitfalls, validation, and quality control of a UPLC-MS/MS method for the quantification of tetrahydrocannabinol in oral fluid collected with StatSure, Quantisal, or Certus collector. *Therapeutic Drug Monitoring*, *35*(1), 101–111.

WHO. (2014). *Substance abuse facts*. Geneva, Switzerland: WHO. Available from http://www.who.int/substance_abuse/facts/cannabis/en/

CHAPTER

104

Cannabinoids in Exhaled Breath

O. Beck

Department of Laboratory Medicine, Section of Clinical Pharmacology, Karolinska Institutet, Stockholm, Sweden

SUMMARY POINTS

- Tetrahydrocannabinol (THC) is present in exhaled breath for a short time after smoking cannabis.
- THC is the principal cannabinoid present in exhaled breath.
- Nonvolatile compounds exit lung as part of aerosol particles.
- The aerosol particles are formed from the airway lining fluid during normal breathing.
- The aerosol particles in exhaled breath can be sampled by a simple procedure.
- Analytical investigations must be done in a laboratory using sophisticated instrument.
- Exhaled breath is a specimen that can be used for drug testing.

KEY FACTS OF BREATH DRUG TESTING

- Nonvolatile substances exit lung as part of aerosol particles.
- Cannabis, as well as other drugs of abuse, contaminates the airway system following exposure.
- Following cannabis exposure tetrahydrocannabinol is present in the exhaled particles.
- Technology exists to collect a specimen of the exhaled particles.
- The collection of an exhaled breath specimen is considered as noninvasive.
- Methodology exists to examine the exhaled particles for drug content.

LIST OF ABBREVIATIONS

CBN	Cannabinol
LC	Liquid chromatography
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
MS	Mass spectrometry
THC	Tetrahydrocannabinol
THCCOOH	Tetrahydrocannabinol carboxylic acid

INTRODUCTION

The idea that cannabinoids can be present in exhaled breath is very obvious since cannabis is administered by inhalation of smoke. The consequence of cannabis smoking is that lung tissue becomes heavily contaminated with cannabinoids from the inhaled smoke. It is therefore likely that the remains of this smoke will be part of the exhaled breath for at least some time. However, one complicating circumstance for this idea is that once the cannabinoids become deposited in the lung, it is unlikely that they will reenter the gas phase again. Support of this conception is the experience from bioanalytical work that shows tetrahydrocannabinol (THC) extracts can be dried by blowing with hot nitrogen, without any significant loss of THC.

The first indication that THC is present in exhaled breath is from the study of Manolis, McBurney, & Bobbie (1983) who were able to detect THC for 10–12 min following termination of the smoking session. After this work was published in 1983, no study has been reported on this subject for any abused drug until amphetamine was more recently reported to be present in exhaled breath after recovery from intoxication (Beck, Leine, Palmskog, & Franck, 2010).

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00121-6
Copyright © 2017 Elsevier Inc. All rights reserved.

This new observation has triggered renewed interest in exhaled breath as a possible specimen for drugs of abuse testing and for toxicology investigations in general (Beck, 2013). As detection of cannabis use is a major objective of drug testing, a series of studies have now examined the detectability of cannabinoids in exhaled breath.

MANOLIS STUDY FROM 1983

Manolis et al. (1983) studied the presence of THC, 11-hydroxy-THC, and THC carboxylic acid (THC-COOH) in exhaled breath, as part of a larger trial aimed at documenting effects from cannabis smoking on psychomotor function. Their interest in pursuing this task was based on a desire of developing a noninvasive test for marijuana exposure. At that point in time, urine drug testing was about to be developed for use on a larger scale, for both clinical and forensic applications, which is today's routine worldwide. The work was also based on earlier observations using more simple analytical technologies that had suggested but not been able to provide convincing evidence of the presence of THC in exhaled breath (McCarthy & van Zyl, 1972; Hauck & Moll, 1974; Valentine, Bryant, Gutshall, Owen, & Niu, 1979). Of concern for Manolis et al. (1983) was the possibility that any sample of exhaled breath collected through the mouth could potentially have become contaminated with saliva.

The study of Manolis et al. (1983) used experienced marijuana smokers classified as healthy male volunteers. Subjects were exposed to placebo cannabis material fortified (or not) with THC standard substance. After the 30 min smoking session, subjects rinsed their mouth and teeth. Components in exhaled breath were collected either using a Tenax tube (a polymer material used for trapping volatiles from air) or by bubbling the air through a collection solution. The collection using Tenax tube provided best recovery and results but suffered from the fact that individuals had problems applying high enough pressure to blow through the tube. Using a gas chromatographic-mass spectrometric method modified after a plasma analysis procedure, the authors were able to detect THC but not the two metabolites (11-hydroxy-THC and THC-COOH) in exhaled breath. The results were convincing from the fact that nonfortified cigarettes were used for control. Moreover, analytical reliability was high, and measures were taken to protect the specimen from saliva contamination. One disappointing fact was that THC was only detected in samples collected between 10 and 12 min after termination of smoking. THC was detected in 7 of 8 individuals using the Tenax collection method, and the amount measured was 0.32–5.7 ng/L ($n = 7$). At the time-point 20 min the amounts had decreased more than 10-fold to below the detection limit.

Manolis et al. (1983) concluded that since a longer detection time than 10–12 min is needed, it would require improvement in analytical sensitivity and in effectiveness of sample collection before exhaled breath can be used for detecting cannabis smoking.

EXHALED BREATH FOR DRUG TESTING

Development of bioanalytical methodology based on liquid chromatography-mass spectrometry (LC-MS) has been rapid during the past two decades and has triggered interest in alternative specimens for drug testing (Gallardo & Queiroz, 2008). Therefore a project was started in pursuing the possibility to use exhaled breath for drugs-of-abuse testing and making it as convenient as alcohol testing. Beck et al. (2010) were able to construct a sampling procedure for exhaled breath using a solid-phase extraction filter that is known to trap amphetamine from water solution and collected exhaled breath for 10 min, from 12 individuals recovered from amphetamine intoxication. Analytical investigation of extracts was done with an ultrasensitive LC-tandem MS (LC-MS/MS) method. The study demonstrated successfully that amphetamine was present in exhaled breath as long as 24 h after drug intake. No amphetamine was detected in controls and the detection of amphetamine in exhaled breath agreed with self-reports as well as urine and plasma analytical data.

Following this promising work, exhaled breath has now been shown to be of general interest for detecting exposure of abused drugs (Beck, Stephanson, Sandqvist, & Franck, 2013). In addition, a new technology for sample collection has been developed, which is even more rapid and convenient.

PARTICLES IN BREATH

From studies of exhaled breath condensate, it is known that nonvolatile components can exit the lung with breath (Montuschi, 2007; Kuban & Foret, 2013). Components like lipids and proteins have been measured in this specimen. Exhaled breath contains aerosol particles that are formed from the airway lining fluid during the normal breathing process (Papiniemi & Rosenthal, 1997; Almstrand et al., 2010). These particles are of micrometer size (median diameter 0.3 μm) (Schwarz, Biller, Windt, Koch, & Holhfeld, 2014), and are a possible vehicle for nonvolatile components to exit lung. The sampling device developed for drug testing using exhaled breath selectively collects the aerosol particles using electrostatic filter technology (Fig. 104.1), with a sampling time of 1–3 min.

FIGURE 104.1 Pictures of the exhaled breath sampling device that has been developed for the purpose of drug testing. (A) Components of the kit come in two plastic bags. (B) One plastic bag is mounted on the filter manifold together with the mouth piece. The plastic bag is used for controlling the length of the sampling process. The mouth piece protects the filter from being contaminated with saliva. (C) Device with filled bag after sampling, which takes 1–3 min. The sampling procedure is finished when the bag is full. Meanwhile, most of the exhaled air is going through the filter manifold, and aerosol particles from the lung are trapped on the filter. The sample donor can breathe normally since the back pressure from the device is low. (D) The filter manifold is sealed, labeled, and sent to laboratory for analysis.

STUDIES ON CANNABINOIDS IN BREATH

A first confirmation that cannabis smoking can be detected in drug testing using exhaled breath was obtained in a study on 10 occasional cannabis users (Beck, Sandqvist, Dubbelboer, & Franck, 2011). The subjects smoked their regular dose and volunteered to donate a breath sample 1–12 h later. In this study, the exhaled breath specimen was collected by passing the air through a 47 mm C18 Empore disk for 10 min by using underpressure from a pump. The THC collected on the filter was extracted using organic solvent and analyzed using LC-MS/MS. One hour after smoking, the mean value of THC in breath was 35 pg/min (n = 7), after 2 h it was 40 pg/min (n = 2), and after 12 h it was 9 pg/min (n = 1). Traces of the main urinary metabolite THCCOOH were detected in three samples.

A controlled administration experiment was performed at the National Institute on Drug Abuse (NIDA, National Institutes of Health, United States) involving 13 chronic and 11 occasional cannabis smokers (Himes et al., 2013). Participants smoked all or parts of a prepared cigarette with 54 mg THC for 10 min duration. Exhaled breath was collected for 3 min using the SensAbues collection device at several time-points during 0.5–21 h after termination of smoking. In addition to THC, the analytical investigation comprised THCCOOH and cannabinol (CBN) and analysis performed with LC-MS/MS.

THC was detected in the exhaled breath in 23 out of the 24 subjects (96%). THCCOOH was not detected at all and CBN was detected in one sample. The longest detection time of THC was 4 h and the detection rate at the 2.4 h time point in the chronic user group was 54%. A graphical presentation of results overtime is given in Fig. 104.2.

In another study, 13 university students having occasional cannabis smoking habits were recruited for an experiment where the subject smoked self-supplied cannabis cigarettes (estimated to contain 13–52 mg THC) (Coucke, Massarini, Ostjin, Beck, & Verstraete (2016). The collection procedure of exhaled breath used the SensAbues collection device and followed the instructions to fill a plastic bag (1–3 min). Repeated sampling was done in the time interval 15–180 min postsmoking. The analytical investigation used LC-MS/MS.

The results from this study deviated from the two previous ones. Four subjects reporting recent smoking had high levels of THC at time zero (before smoking). One of these had smoked 6 h before, two the day before and one a week before. Levels of THC were generally one to two orders of magnitude higher than seen before. All subjects had detectable THC at the longest time-point studied, 180 min. The mean value at 60 min was 4.2 ng/filter. A graphical presentation is seen in Fig. 104.3. No THCCOOH was detected.

Exhaled breath testing for cannabis was evaluated in a clinical setting where patients with cannabis smoking habits were in out-patient treatment with the goal of achieving a better life style (Skoglund, Hermansson, & Beck, 2015). Drug testing was part of the treatment model in order to validate self-reported drug use or abstinence. Samples of urine, plasma, and exhaled breath were taken in parallel. In 35 cases, the presence of THCCOOH in urine was analytically confirmed. In 14 of those cases, THC was detected in plasma indicating a recent intake and in 11 of these THC was detected in

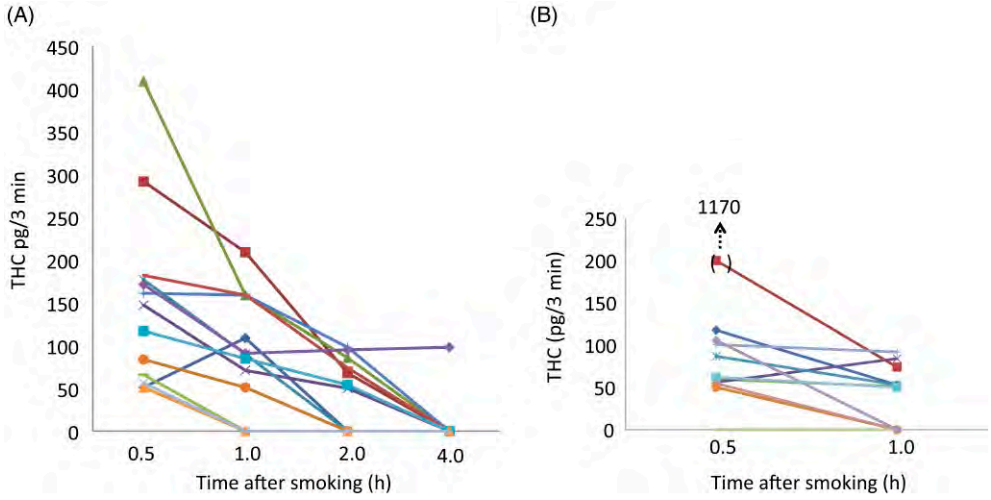


FIGURE 104.2 **Individual concentrations of THC in exhaled breath after controlled smoking of THC by (A) 13 chronic and (B) 11 occasional cannabis users.** *Source: Data from Himes et al. (2013).*

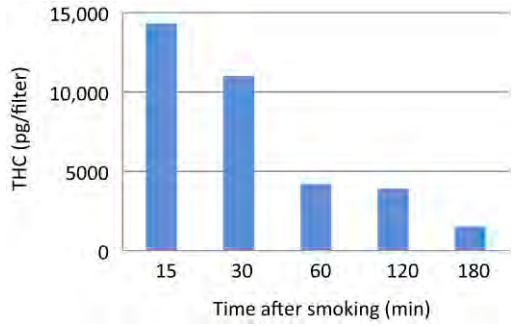


FIGURE 104.3 Mean concentration of THC in exhaled breath collected from 13 occasional cannabis users after smoking self-supplied cannabis. *Source: Data from Coucke et al. (2016).*

exhaled breath (79%). The results suggested that presence of THC in breath is a promising marker for recent cannabis exposure with sensitivity and detection rate similar to plasma.

Drug testing using exhaled breath was used in a few workplaces in Sweden for about a year with good results regarding acceptance of the sampling procedure from a practical perspective and acceptance of reported positive results. When comparing the rate of positive samples for cannabis during the same time period, it was 0.4% with oral fluid testing, 0.8% with exhaled breath testing and 1.3% with urine (Stephanson, Sandqvist, Shafaati Lambert, & Beck, 2014).

A study evaluating the feasibility of exhaled breath drug testing was performed on patients treated for acute intoxication (Beck et al., 2013). The patients were sampled after recovery from intoxication which was estimated to be 12–24 after drug intake. Twenty of the patients had THCCOOH detected in urine. Nine of those had THC detected in plasma, whereof eight had THC present in exhaled breath (89%). There was no possibility that these patients had been smoking cannabis for at least 12 h before sampling since they were inpatients at an emergency treatment clinic.

SAMPLING OF EXHALED BREATH

The main attraction of using exhaled breath as a biological specimen is the noninvasive nature of the sampling procedure. It is the experience from alcohol breath testing that this sampling procedure is well accepted both by donor and collector.

There are several procedures that can be used for sampling of exhaled breath (Buszewski, Kesy, Ligor, & Amann, 2007). These are developed mainly and used for collecting volatile components. For nonvolatile components, exhaled breath condensate is the most commonly used (Kuban & Foret, 2013), and that is known to work also for drugs of abuse (Beck, Sandqvist, Eriksen, Franck, & Palmskog, 2011). However, the procedure requires a special instrument, the collection time is often 10 min, and the collected sample is more complex as it contains not only the nonvolatile fraction, but also volatiles and condensed water. Another alternative is to use selective collection of particles separated by impactor technology (Almstrand et al., 2009), but this again requires an instrument and might need a longer sampling time due to low recovery. A very simple and disposable device that has been used in the above cited recent work is to use an aerosol particle filter (Fig. 104.1) that is of low cost and provides a rapid sampling that has been found to be well accepted by addiction patients and prison inmates (Beck, 2014; Skoglund et al., 2015).

ANALYTICAL ASPECTS

The amounts of drugs in exhaled breath are low, and analytical work needs to be performed using highly sensitive state-of-the-art technology. The work so far has been done using exclusively liquid chromatography-mass spectrometry that provides not only the sensitivity needed, but also provides secure identifications needed for forensic applications and for confident scientific results (Fig. 104.4). The LC-MS/MS technique has the advantage of providing selectivity that enables a simple sample preparation procedure to be applied (Beck et al., 2013; Stephanson et al., 2014) (Fig. 104.5). For further development of the field of exhaled breath drug testing, alternative detection instruments might be needed for enabling instant results on-site as for alcohol testing. One such possibility is to use surface enhanced Raman spectroscopy (SERS) that is known to provide the required sensitivity (Stiles, Dieringer, Shah, & van Duyne, 2008).

MINI-DICTIONARY

Exhaled breath condensate This specimen is collected by letting exhaled air pass through a cold trap. The specimen collected consists of a mixture of volatile and nonvolatile substances. The main component is condensed water. During a collection period of 10 min about 1 mL of sample is produced.

Gas chromatography-mass spectrometry This hyphenated technology was the most selective and sensitive bioanalytical technique of small molecules before LC-MS was developed. Chromatographic separation is achieved in the gas phase and detection with mass spectrometry working in high vacuum.

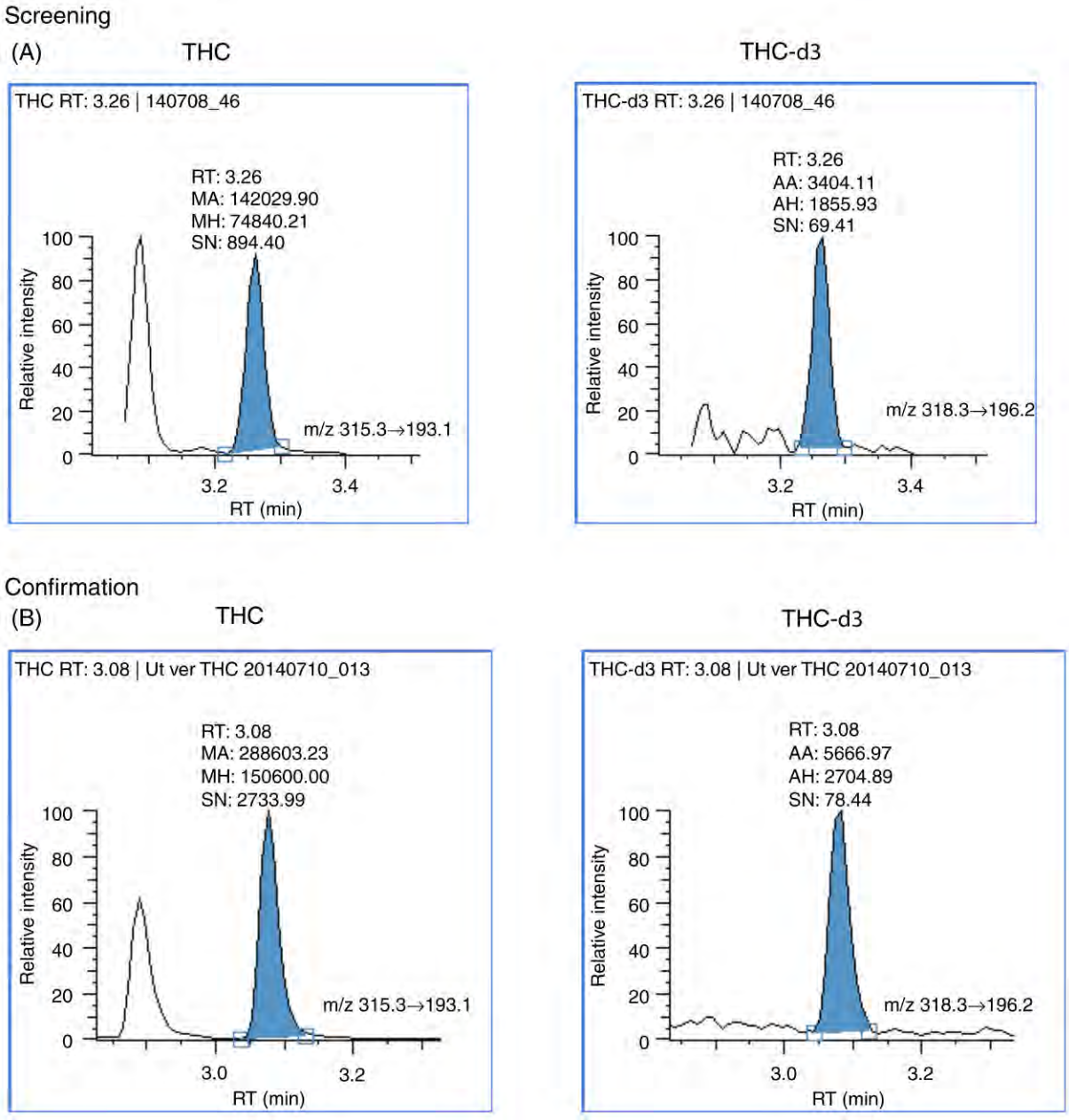


FIGURE 104.4 **Chromatograms from the analysis of THC in a sample collected from a patient in treatment for drug addiction disorder. The sample was taken for purpose of drug testing.** Panel (A) shows results from the screening analysis. THC-d3 is the internal standard added for securing exact quantification. Panel (B) shows results from the confirmation analysis. Both procedures use liquid chromatography-tandem mass spectrometry (LC-MS/MS) in selected reaction monitoring mode. Two product ions are monitored for securing identification fulfilling forensic standard.

Workflow of exhaled breath drug testing:

- Sampling of breath at site, 1–3 min, in controlled environment
- Seal device and send to laboratory with request form
- In laboratory, the device is extracted with solvent using a simple procedure
- Prepared extract is analyzed for many drugs with LC-MS/MS
- Final result reported

FIGURE 104.5 **Short summary of the workflow for using exhaled breath in routine drug testing.**

Impactor technology This collection technique of small particles collects fractions of particles according to size after the air flow is passed through a cascade of impactor stages. Several different designs exist but they are all based on the fact that large particles deviate from the air flow when directed toward a surface while small particles follow the air stream.

Liquid chromatography-mass spectrometry This is a hyphenated technology that has provided unique simplicity and performance to the field of bioanalysis when it was put into practice in the 1990s. The aqueous mobile phase containing water is introduced to the mass spectrometry system, working in high vacuum, using an electrospray interface. The tandem MS

technology for detection has provided additional selectivity and sensitivity.

Specimen for drug testing The most common specimen for drug testing is urine. Alternative specimens are of interest mainly because of the risk for adulteration that a supervised collection procedure may cause inconvenience for both the donor and collector. Alternative matrices are hair, oral fluid, blood, nails, sweat, and exhaled breath.

Tetrahydrocannabinol The main psychoactive component of cannabis and the substance targeted in most laboratory investigation except for urine.

Tetrahydrocannabinolcarboxylic acid The main metabolite of tetrahydrocannabinol. This metabolite is excreted in urine in a conjugated form and the primary target compound in urine drug testing.

References

Almstrand, A. C., Bake, B., Ljungström, E., Larsson, P., Bredberg, A., Mirgorodskaya, E., & Olin, A. C. (2010). Effect of airway opening on production of exhaled particles. *Journal of Applied Physiology*, *108*, 584–588.

Almstrand, A. C., Ljungström, E., Lausmaa, J., Bake, B., Sjövall, P., & Olin, A. C. (2009). Airway monitoring by collection and mass spectrometric analysis of exhaled particles. *Analytical Chemistry*, *81*, 662–668.

Beck, O. (2014). Exhaled breath for drugs of abuse testing—evaluation in criminal justice settings. *Science and Justice*, *54*, 57–60.

Beck, O. (2013). Is there a place for exhaled breath analysis in clinical pharmacology and toxicology? *Compass IATDMCT*, *June issue*, 4–6.

Beck, O., Leine, K., Palmskog, G., & Franck, J. (2010). Amphetamines detected in exhaled breath from drug addicts: a new possible method for drugs-of-abuse testing. *Journal of Analytical Toxicology*, *34*, 233–237.

Beck, O., Sandqvist, S., Dubbelboer, I., & Franck, J. (2011a). Detection of Δ9-tetrahydrocannabinol in exhaled breath collected from cannabis users. *Journal of Analytical Toxicology*, *35*, 541–544.

Beck, O., Sandqvist, S., Eriksen, P., Franck, J., & Palmskog, G. (2011b). Determination of methadone in exhaled breath condensate by liquid chromatography-tandem mass spectrometry. *Journal of Analytical Toxicology*, *35*, 129–133.

Beck, O., Stephanson, N., Sandqvist, S., & Franck, J. (2013). Detection of drugs of abuse in exhaled breath using a device for rapid collection—comparison with plasma, urine and self-report in 47 drug users. *Journal of Breath Research*, *7*(2), 026006.

Buszewski, B., Kesy, M., Ligor, T., & Amann, A. (2007). Human exhaled air analytics: biomarkers of diseases. *Biomedical Chromatography*, *21*, 553–566.

Coucke, L., Massarini, E., Ostjin, Z., Beck, O., & Verstraete, A. (2016). Δ(9)-Tetrahydrocannabinol concentrations in exhaled breath and physiological effects following cannabis intake—a pilot study using illicit cannabis. *Clinical Biochemistry*, *49*, 1072–1077.

Gallardo, E., & Queiroz, J. A. (2008). The role of alternative specimens in toxicological analysis. *Biomedical Chromatography*, *22*, 795–821.

Hauck, G., & Moll, H. R. (1974). Versuche zum nachweis von Cannabis-inhaltstoff in der ausatenluft. *Beitrage Gerichtlichen Medizin*, *23*, 221–226.

Himes, S. K., Scheidweiler, K. B., Beck, O., Gorelick, D. A., Desrosiers, N., & Huestis, M. A. (2013). Cannabinoids in exhaled breath following controlled administration of cannabis. *Clinical Chemistry*, *59*, 1780–1789.

Kuban, P., & Foret, F. (2013). Exhaled breath condensate: determination of non-volatile compounds and their potential for clinical diagnosis and monitoring. *Analytica Chimica Acta*, *805*, 1–18.

Manolis, A., McBurney, L. J., & Bobbie, B. A. (1983). The detection of Δ^9-tetrahydrocannabinol in the breath of human subjects. *Clinical Biochemistry*, *16*, 229–233.

McCarthy, T. J., & van Zyl, J. D. (1972). Breath analysis of cannabis smokers. *Journal of Pharmacy and Pharmacology*, *24*, 489–490.

Montuschi, P. (2007). Analysis of exhaled breath condensate in respiratory medicine: methodological aspects and potential clinical applications. *Therapeutic Advances in Respiratory Disease*, *1*, 5–23.

Papineni, R. S., & Rosenthal, F. S. (1997). The size and distribution of droplets in the exhaled breath of healthy subjects. *Journal of Aerosol Medicine*, *10*, 105–116.

Schwarz, K., Biller, H., Windt, H., Koch, W., & Holhfeld, J. M. (2014). Characterization of exhaled particles from the human lungs in airway obstruction. *Journal of Aerosol Medicine and Pulmonary Drug Delivery*, *27*, 1–7.

Skoglund, C., Hermansson, U., & Beck, O. (2015). Clinical trial of a new technique for drugs of abuse testing: a new possible sampling technique. *Journal of Substance Abuse Treatment*, *48*(1), 132–136.

Stephanson, N., Sandqvist, S., Shafaati Lambert, M., & Beck, O. (2014). Validated and applied method for drugs of abuse testing using exhaled breath and liquid chromatography–tandem mass spectrometry. *Drug Testing and Analysis (submitted)*.

Stiles, P. L., Dieringer, J. A., Shah, N. C., & van Duyne, R. P. (2008). Surface-enhanced Raman spectrometry. *Annual Review of Analytical Chemistry*, *1*, 601–626.

Valentine, J. L., Bryant, P. J., Gutshall, P. L., Owen, H. M. G., & Niu, H. C. (1979). Detection of Δ^9-tetrahydrocannabinol in human breath following marijuana smoking. *Analytical Letters*, *12*, 867–880.

CHAPTER

105

Barriers to Treatment Seeking for Cannabis Dependence

P. Gates, J. Copeland

National Cannabis Prevention and Information Centre, University of New South Wales, Sidney, NSW, Australia

SUMMARY POINTS

- This chapter focuses on the barriers to seeking treatment for managing cannabis use disorder (CUD).
- CUD is a psychiatric diagnosis of clinical importance relating to dependence on cannabis. It is characterized typically by a lack of control overuse, or use despite known harms.
- Although treatment is thought to be effective in assisting cannabis use reduction and abstinence, few individuals with cannabis use disorder seek treatment.
- Barriers to cannabis treatment may help explain this underutilization of services. The most commonly cited barriers include a lack of a perceived need for treatment and a desire to reduce use without assistance.
- Other important barriers include a desire to avoid negative views from the community or employers and the belief that treatment is ineffective.
- In contrast, cannabis treatment seeking may be facilitated if information on the effectiveness of treatments, and how they can be accessed, are promoted and the treatments are easily accessible.
- Cannabis treatment services must address the concerns of cannabis users who are likely to lack information on their services, and feel that they are better off reducing their use without assistance should they believe that is required.

KEY FACTS OF CANNABIS USE DISORDER

- Approximately 9–15% of cannabis users will develop a cannabis use disorder at some point in their lives.
- The risk of developing cannabis use disorder is further increased by early onset use, chronic use patterns and other substance use.
- Although some individuals with cannabis use disorder are able to quit using cannabis, failure to do so is associated with being a male of younger age, being unmarried, obtaining low educational achievement, and having mental health concerns.

KEY FACTS OF CANNABIS TREATMENT SEEKING

- Treatment seeking for cannabis use problems is particularly low, compared to other illicit drugs, even among those meeting criteria for cannabis use disorder.
- The rates of individuals seeking treatment have increased over the last two decades in the United States, Europe, and Australia.
- Due to the prevalence of cannabis use, just over one in five of substance use treatment episodes were principally for cannabis use. This is despite the proportionately few cannabis users who seek treatment.

KEY FACTS OF CANNABIS USE DISORDER TREATMENT

- Those cannabis users who do seek treatment often have a long history of use over many years, and a record of previous failed quit attempts.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00123-X
Copyright © 2017 Elsevier Inc. All rights reserved.

- Although some pharmacotherapies have shown promise, none is approved currently to treat cannabis use disorder.
- There is growing evidence for the effectiveness of counseling, particularly the combination of cognitive behavioral therapy and motivational enhancement therapy over multiple sessions, for assisting with cannabis use reductions.

LIST OF ABBREVIATIONS

CUD Cannabis use disorder
UNODC United Nations Office on Drugs and Crime

INTRODUCTION

Most individuals who try using cannabis do so experimentally; however, those who go on to develop a cannabis use disorder (CUD) cease their use typically after encountering undesirable consequences over time (Von Sydow et al., 2001; Swift, Coffey, Carlin, Degenhardt, & Patton, 2008; Ellingstad, Sobell, Sobell, Eickleberry, & Golden, 2006). In contrast, those cannabis users who report heavy, persistent, and early-onset use are more likely to require intervention to achieve long-term abstinence (Swift et al., 2008). Despite a need for intervention, rates of cannabis-related treatment seeking are low even among the most entrenched users. Indeed, before seeking treatment, the average cannabis user is likely to first attempt to abstain from use without assistance at least 6 times and report using cannabis almost daily for more than 10 years on average (Stephens, Roffman, & Simpson, 1993), and meet criteria for CUD for at least 3–5 years (Stinson, Ruan, Pickering, & Grant, 2006).

Moreover, there are clear differences among those individuals with CUD who do and don't seek treatment. Large samples of treatment seekers in the United States have revealed that those seeking treatment for cannabis use are more likely to be male, single, failed to graduate high school, previously attended treatment for mental health or other substance use problems (particularly alcohol use disorder), and report comorbid major depression, and alcohol dependence (Strike, Urbanoski, & Rush, 2003; Agosti & Levin, 2004). Similarly, in Australia, mandatory reporting data from publicly funded substance use treatment services show that cannabis treatment seekers are typically male (69.4%), with a mean age of 29 years (Copeland & Allsop, 2014). The cannabis treatments which are most commonly delivered across these services were counseling services (34.1%; with 11.6% receiving counseling at a cannabis-specific clinic) (Copeland & Allsop, 2014).

Rates of cannabis treatment-seeking are increasing and existing treatments have shown efficacy in assisting with reducing use (Copeland, Clement, & Swift, 2014). Despite this, a large gap remains between the proportions of those who are in need of cannabis treatment (that is, those who meet criteria for CUD) and the numbers who actually seek treatment. Specifically, current estimates report that approximately 4% of the global population aged 14–65 years used cannabis in the past 12 months (UNODC, 2013), and approximately 1 in 10 of these individuals are likely to meet criteria for a CUD (Hall & Pacula, 2003). According to data from the US National Epidemiologic Survey on Alcohol and Related Conditions, among those with a CUD, only between 6.4% and 18.1% report accessing treatment. This chapter explores the literature on the barriers to cannabis treatment, and why so few individuals in need of such treatment actually report accessing treatment.

BARRIERS TO SEEKING CANNABIS TREATMENT

Several studies on the barriers to substance use treatment have been conducted, although only a handful are specific to cannabis treatment (Gates, Copeland, Swift, & Martin, 2012; Ellingstad et al., 2006; Vendetti et al., 2002; Fernández-Artamendi, Fernández-Hermida, García-Fernández, Secades-Villa, & García-Rodríguez, 2013; Mariani et al., 2011; Stephens et al., 1993; van der Pol et al., 2013). As shown in Fig. 105.1, the prevailing barriers to substance use treatment are a low perceived need for treatment, a desire to make improvements without help among those who recognize a need for treatment, concerns regarding negative views from the community (including employers) and concerns regarding treatment availability and cost (Andrade et al., 2014; Mojtabai, Chen, Kaufmann, & Crum, 2014; Chen, Strain, Crum, & Mojtabai, 2013; Wisdom, Cavaleri, Gogel, & Nacht, 2011; Mojtabai & Crum, 2013). Also important is a lack of belief in the effectiveness of treatment (Mojtabai & Crum, 2013).While these attitudinal barriers to treatment are likely to be shared by cannabis users, recent research has identified some beliefs to be particularly important for these individuals.

An initial study of interest in cannabis-specific treatment identified a significant demand for such treatment, but that those who reported interest were significantly less likely to be abusing other substances (Stephens et al., 1993). The authors concluded that such individuals may be less interested in treatments which group all illicit drug using clients together (Stephens et al., 1993). Building on this research, Vendetti et al. (2002) investigated a sample of nontreatment seeking, adult daily cannabis users who showed interest in a large cannabis

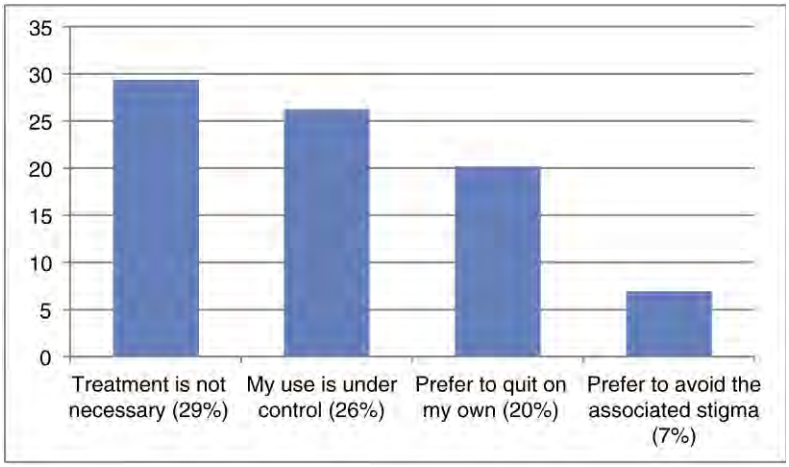


FIGURE 105.1 Barriers to cannabis treatment reported by cannabis users (n = 1021). This pie-chart depicts the percentage of participants reporting the most commonly described barriers to treatment from a pooled sample of the studies reviewed in this chapter (Ellingstad et al., 2006, n = 25; Fernández-Artamendi et al., 2013, n = 261; Gates et al., 2012, n = 494; van der Pol et al., 2013, n = 241).

treatment trial but did not go on to participate (n = 363) and those that did participate (n = 450). This comparison focused on factors associated with *pretreatment* dropout. Factors which predicted this kind of dropout were a lack of self-perceived dependence on cannabis, membership in Asian or Native American ethnic groups, social instability (not married, unemployed, low education), young age, and use of other drugs (sedatives and cocaine). Without directly assessing barriers to cannabis treatments, these two studies were important in identifying that cannabis treatment was not uniformly appealing to all those who may benefit from such treatment.

In the first study to directly investigate barriers to cannabis-specific treatment, 25 individuals who reported at least 1 year of recent abstinence from long-term daily cannabis use were interviewed regarding their past substance use, their successful quit attempt and any perceived barriers to treatment (Ellingstad et al., 2006). As it is common among other illicit substance users, the participants most commonly reported the belief that their cannabis use was not enough of a problem to warrant treatment (80%). In addition, the participants also commonly reported that treatment was not sought due to wanting to quit without treatment (76%), and the desire to avoid the stigma of being labeled a drug user (48%). Importantly, given the sample consisted of individuals who had successfully quit their cannabis use on their own, it is not surprising that support for treatment would be low.

Subsequently, a larger study of different samples of regular cannabis users throughout Australia also investigated beliefs regarding treatment barriers (Gates et al., 2012). Specifically, the sample included cannabis users who were in treatment services in New South Wales (CT; n = 100), others who were not in treatment in the same area (NT; n = 100), and a separate group participating from throughout Australia via online survey (IS; n = 294). In line with earlier research on illicit drug treatment, the most commonly reported barrier from the total sample was the feeling that treatment is not necessary to reduce cannabis use. This study also compared the responses between the sample groups, and investigated the importance of the participants' demographic characteristics. The belief that treatment is unnecessary was reported significantly more frequently by those not in treatment, compared to those in treatment (44% of NT, and 61% of IS, compared to 36% of CT), and by females compared to males (58% compared to 48%). The next most commonly reported barrier was the opinion that cannabis users are not likely to be ready to stop their use. This barrier was mentioned significantly more frequently by those in treatment, compared to not in treatment (34% of CT compared to 14% of NT, and 23% of IS), and by participants aged 24–29 years, compared to older age groups. Other commonly reported barriers included a lack of awareness of treatment options (14% of all participants), and a preference for avoiding the stigma associated with being labeled a drug user in need of treatment (12% of all participants) (Gates et al., 2012). Again; however, this study did not specify that the participants were in need of treatment (that is, were diagnosed with a CUD) and so, as with Ellingstad and colleagues (2006), such results could be expected.

A more recent study addressed this concern by recruiting individuals who were dependent on cannabis use and had not sought treatment [n = 241; including subsamples of those who felt they would "probably not" seek "specialized addiction treatment" for their cannabis use (n = 160) and those who felt "the need for professional help with their cannabis problem" in the past year (n = 30)] (van der Pol et al., 2013). In line with previous

studies, the most commonly reported barrier was a desire to be self-reliant (47.8%; with little difference between the subsamples). Also common was a preference to seek informal help (19.4%), and a feeling that treatment is not needed (17.0%). Notably, there was little difference in these more commonly cited barriers between participant groups.

Recognizing that previous studies on barriers to cannabis treatment recruited only adult participants, an additional study investigated the opinions of 261 high-school students who reported past-year cannabis use (selected from nine high-schools in Spain) (Fernández-Artamendi et al., 2013). The participants were presented with a list of previously cited barriers (and two not previously used due to the nature of the sample: "ignorance of resources available" and "fear of the family becoming involved"), and were asked to rate their influence in preventing treatment seeking on a Likert-type scale. In addition, the participants' intention to change their cannabis use was assessed. Consistent with previous research, the barriers to treatment which were most frequently cited by the young people were a lack of perceived problem (50.6%), the desire to solve one's problems alone (27.6%), and fear of family finding out (24.9%). In contrast, feelings of shame (11.5%) and financial cost (13.3%) were less commonly cited. Notably, the desire to solve one's problems alone was particularly relevant to those who reported high motivation to change their cannabis use.

IMPLICATIONS OF THE BARRIERS TO TREATMENT

These commonly cited attitudinal barriers to cannabis treatment (lack of treatment need and a desire to reduce use without assistance) stand in contrast to barriers to treatment for other illicit drug use that more commonly include concerns regarding negative views from the community and employers, and the inconvenience and cost of accessing services (Mojtabai et al., 2014; Wisdom et al., 2011). Treatment services can address these attitudinal barriers to treatment that are expressed by cannabis users in two main ways. First, available services may attempt to minimize the professional contact involved, so as to appeal to those resisting formal assistance, and those concerned about any negative views from the community. As such, self-guided interventions, such as, computer-based interventions that minimize the roles of therapists—sometimes operating completely without face-to-face contact—may be appropriate. Indeed, early research has supported the efficacy of such interventions (Budney et al., 2011; Kay-Lambkin, Baker, Lewin, & Carr, 2009; Rooke, Copeland, Norberg, Hine, & Mccambridge, 2013; Tossmann, Jonas, Tensil, Lang, & Struber, 2011) and they are currently freely available online (for example, www.reduceyouruse.org.au). In addition, the utility of smartphone apps to assist with substance use monitoring is beginning to get recognized by users (Savic, Best, Rodda, & Lubman, 2013), and pilot work on an initial smartphone app to assist with cannabis use reductions has been completed (Norberg et al., 2013). Further research is required to assess the appropriateness of implementing these low threshold interventions.

Second, given the prevalence of cannabis use, it is desirable that services extend their accessibility so as to reach the minority of users interested in accessing professional help. This could be done by promoting knowledge of treatment and by improving the accessibility of treatment. Indeed, the particular influence of advertising cannabis treatments has been demonstrated in an initial investigation on the impact of promoting substance use treatment. That is, a study of 489 individuals reporting substance use problems found that those seeking treatment for cannabis use were significantly more likely, compared to other illicit substance users, to report that they would seek treatment upon hearing or seeing an advertisement regarding treatment. Moreover, in the study by Gates et al. (2012) of almost 500 cannabis users in the community and in treatment, the participants were asked to report on any facilitating factors that may help to increase rates of entry into cannabis treatment. The most commonly reported facilitator was the improvement of available information regarding treatments (30%). Notably, other reported facilitators included increasing the availability or accessibility of cannabis specific services (27%), and introducing additional options, such as, telephone counseling (12%).

Importantly, existing treatments for cannabis use are known to be effective—particularly, treatments based on cognitive behavioral therapies and motivational enhancement therapies for adults, and family systems approaches for youths (Copeland et al., 2014). In addition, the prevalence of cannabis use is such that cannabis is the most likely drug of concern among those in illicit drug treatment services (UNODC, 2013). As such, the important focus may no longer be developing appropriate interventions, but rather addressing the barriers to treatment with the aim of increasing the likelihood that those in need of treatment will access the treatments that are available.

CONCLUSIONS

Despite increasing rates of cannabis treatment seeking (UNODC, 2013), there remains an important proportion of cannabis users who lack control over their use, but yet do not feel treatment is necessary or desirable to stop using. As treatment is known to be effective for these individuals (Copeland et al., 2014), it is necessary

for treatment services to increase their awareness and accessibility. Consistent with this need are highly accessible and easily promoted computer-based interventions. Future work is required to establish a large and long-term evidence-base for these interventions; however, early work is promising.

MINI-DICTIONARY OF TERMS

Cannabis use disorder Refers to a psychiatric diagnosis of clinical importance relating to mild to severe dependence on cannabis which is typically characterized by a lack of control over use, or use despite known harms.

Early onset cannabis use Refers to the use of cannabis during adolescence. There is a well-established link between early onset cannabis use and an increased risk of harms to both physical and mental health, as well as decreased odds of high-school completion.

Stigma Refers to a mark of disgrace or shame which is associated with a particular circumstance or quality. For example, an individual entering treatment for substance use may feel stigma upon being labeled a drug user, and thus avoid treatment.

United Nations Office on Drugs and Crime (UNODC) Refers to an extensive network of field offices throughout the world dedicated to the fight against illicit drugs and international crime. Its operations include field-based technical operation and normative work, as well as research and analytical work.

References

Agosti, V., & Levin, F. R. (2004). Predictors of treatment contact among individuals with cannabis dependence. *American Journal of Drug and Alcohol Abuse*, *30*, 121–127.

Andrade, L. H., Alonso, J., Mneimneh, Z., Wells, J. E., Al-Hamzawi, A., Borges, G., Bromet, E., Bruffaerts, R., De Girolamo, G., De Graaf, R., Florescu, S., Gureje, O., Hinkov, H. R., Hu, C., Huang, Y., Hwang, I., Jin, R., Karam, E. G., Kovess-Masfety, V., Levinson, D., Matschinger, H., O'Neill, S., Posada-Villa, J., Sagar, R., Sampson, N. A., Sasu, C., Stein, D. J., Takeshima, T., Viana, M. C., Xavier, M., & Kessler, R. C. (2014). Barriers to mental health treatment: results from the WHO World Mental Health surveys. *Psychological Medicine*, *44*, 1303–1317.

Budney, A. J., Fearer, S., Walker, D. D., Stanger, C., Thostenson, J., Grabinski, M., & Bickel, W. K. (2011). An initial trial of a computerized behavioral intervention for cannabis use disorder. *Drug and Alcohol Dependence*, *115*, 74–79.

Chen, L. Y., Strain, E. C., Crum, R. M., & Mojtabai, R. (2013). Gender differences in substance abuse treatment and barriers to care among persons with substance use disorders with and without comorbid major depression. *Journal of Addiction Medicine*, *7*, 325–334.

Copeland, J., & Allsop, D. (2014). Dedicated 'cannabis only' treatment clinics in New South Wales, Australia: client and treatment characteristics and associations with first-time treatment seeking. *Drug and Alcohol Review*, *33*(5), 567–571.

Copeland, J., Clement, N., & Swift, W. (2014). Cannabis use, harms and the management of cannabis use disorder. *Neuropsychiatry*, *4*, 55–63.

Ellingstad, T. P., Sobell, L. C., Sobell, M. B., Eickleberry, L., & Golden, C. J. (2006). Self-change: a pathway to cannabis abuse resolution. *Addictive Behaviours*, *31*, 519–530.

Fernández-Artamendi, S., Fernández-Hermida, J. R., García-Fernández, G., Secades-Villa, R., & García-Rodríguez, O. (2013). Motivation for change and barriers to treatment among young cannabis users. *European Addiction Research*, *19*, 29–41.

Gates, P., Copeland, J., Swift, W., & Martin, G. (2012). Barriers and facilitators to cannabis treatment. *Drug and Alcohol Review*, *31*, 311–319.

Hall, W., & Pacula, R. L. (2003). *Cannabis use and dependence: public health and public policy*. Cambridge, UK: Cambridge University Press.

Kay-Lambkin, F. J., Baker, A. L., Lewin, T. J., & Carr, V. J. (2009). Computer-based psychological treatment for comorbid depression and problematic alcohol and/or cannabis use: a randomized controlled trial of clinical efficacy. *Addiction*, *104*, 378–388.

Mariani, J. J., Cheng, W. Y., Bisaga, A., Sullivan, M., Carpenter, K., Nunes, E. V., & Levin, F. R. (2011). Comparison of clinical trial recruitment populations: treatment-seeking characteristics of opioid, cocaine-, and cannabis-using participants. *Journal of Substance Abuse Treatment*, *40*, 426–430.

Mojtabai, R., Chen, L. -Y., Kaufmann, C. N., & Crum, R. M. (2014). Comparing barriers to mental health treatment and substance use disorder treatment among individuals with comorbid major depression and substance use disorders. *Journal of Substance Abuse Treatment*, *46*, 268–273.

Mojtabai, R., & Crum, R. M. (2013). Perceived unmet need for alcohol and drug use treatments and future use of services: results from a longitudinal study. *Drug and Alcohol Dependence*, *127*, 59–64.

Norberg, M. M., Rooke, S. E., Albertella, L., Copeland, J., Kavanagh, D. J., & Lau, A. Y. S. (2013). The first mHealth app for managing cannabis use: gauging its potential helpfulness. *Addictive Behaviors, Therapy and Rehabilitation*, *3*.

Rooke, S., Copeland, J., Norberg, M., Hine, D., & Mccambridge, J. (2013). Effectiveness of a self-guided web-based cannabis treatment program: randomized controlled trial. *Journal of Medical Internet Research*, *15*, e26.

Savic, M., Best, D., Rodda, S., & Lubman, D. I. (2013). Exploring the focus and experiences of smartphone applications for addiction recovery. *Journal of Addictive Diseases*, *32*, 310–319.

Stephens, R. S., Roffman, R. A., & Simpson, E. E. (1993). Adult marijuana users seeking treatment. *Journal of Consulting and Clinical Psychology*, *61*, 1100–1104.

Stinson, F. S., Ruan, W. J., Pickering, R., & Grant, B. F. (2006). Cannabis use disorders in the USA: prevalence, correlates and co-morbidity. *Psychological Medicine*, *36*, 1447–1460.

Strike, C. J., Urbanoski, K. A., & Rush, B. R. (2003). Who seeks treatment for cannabis-related problems? *Canadian Journal of Public Health*, *94*, 351–354.

Swift, W., Coffey, C., Carlin, J. B., Degenhardt, L., & Patton, G. C. (2008). Adolescent cannabis users at 24 years: trajectories to regular weekly use and dependence in young adulthood. *Addiction*, *103*, 1361–1370.

Tossmann, H. P., Jonas, B., Tensil, M. D., Lang, P., & Struber, E. (2011). A controlled trial of an internet-based intervention program for cannabis users. *Cyberpsychology, Behavior and Social Networking*, *14*, 673–679.

UNODC. (2013). *World drug report 2013*.

van der Pol, P., Liebregts, N., de Graaf, R., Korf, D. J., van den Brink, W., & van Laar, M. (2013). Facilitators and barriers in treatment seeking for cannabis dependence. *Drug and Alcohol Dependence*, *133*, 776–780.

Vendetti, J., Mcree, B., Miller, M., Christiansen, K., & Herrell, J. The Marijuana Treatment Project Research Group. (2002). Correlates of pre-treatment drop-out among persons with marijuana dependence. *Addiction*, *97*, 125–134.

Von Sydow, K., Lieb, R., Pfister, H., Hofler, M., Sonntag, H., & Wittchen, H. U. (2001). The natural course of cannabis use, abuse and dependence over four years: A longitudinal community study of adolescents and young adults. *Drug and Alcohol Dependence*, *64*, 347–361.

Wisdom, J. P., Cavaleri, M., Gogel, L., & Nacht, M. (2011). Barriers and facilitators to adolescent drug treatment: Youth, family, and staff reports. *Addiction Research and Theory*, *19*, 179–188.

CHAPTER

106

Pharmacotherapies for Cannabis Use Disorders

A.L. McRae-Clark

Medical University of South Carolina, Charleston, SC, United States

SUMMARY POINTS

- This chapter reviews medication development efforts in the area of cannabis use disorders.
- Medications have been evaluated targeting reduction of cannabis withdrawal symptoms, abstinence initiation, and relapse prevention.
- Promising medications for treatment for cannabis withdrawal include agents targeting sleep disturbances and agonist therapy.
- *N*-acetylcysteine and gabapentin have shown preliminary utility in treatment of cannabis dependence in randomized, placebo-controlled trials.
- Medications have also been evaluated for treatment of cannabis dependence and comorbid psychiatric disorders without positive findings.
- To date, there are no approved medications for treatment of either cannabis withdrawal or cannabis dependence.

KEY FACTS OF CANNABIS TREATMENT

- Nearly 1 million Americans seek treatment for cannabis use disorders annually.
- Psychosocial treatments for cannabis use disorders include relapse prevention, cognitive behavioral therapy, motivational enhancement therapy, and motivational incentives.
- Range of 1-year abstinence rates was between 10% and 30 % for the various behavioral approaches.
- Other mental disorders commonly cooccur with cannabis dependence, including alcohol dependence, antisocial personality disorder, conduct disorder, and anxiety and mood disorders, and may lead to a poorer prognosis for treatment.
- Although an area of active research, there are no currently approved pharmacotherapy treatments for cannabis use disorders.

LIST OF ABBREVIATIONS

ADHD	Attention deficit hyperactivity disorder
FDA	US food and drug administration
GABA	Gamma-aminobutyric acid
NAC	*N*-acetylcysteine
THC	Δ^9-Tetrahydrocannabinol

INTRODUCTION

In 2012, nearly 1 million Americans received treatment for cannabis-related problems (SAMHSA, 2013). Although a high demand for effective interventions obviously exists, few specific treatments have been developed for cannabis use disorders. Further, the treatments that have been examined have had limited efficacy, with few individuals achieving abstinence (Dennis et al., 2004; Kadden, Litt, Kabela-Cormier, & Petry, 2007; McRae, Budney, & Brady, 2003; Nordstrom & Levin, 2007; Vandrey & Haney, 2009). In the Marijuana Treatment Project, the largest controlled trial of treatment

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00124-1
Copyright © 2017 Elsevier Inc. All rights reserved.

for cannabis dependence to date, the highest abstinence rate achieved was 23% (Marijuana Treatment Project Research Group, 2004). As such, there is significant interest in the development of new treatment approaches for cannabis dependence. One potential avenue is exploring the role of pharmacological interventions, either targeting withdrawal symptoms in early cannabis abstinence, or as complements to psychosocial treatments in abstinence initiation, or relapse prevention (Hart, 2005; Vandrey & Haney, 2009). Further, medications may also play a role in treatment of individuals with comorbid cannabis dependence and other psychiatric disorders.

PHARMACOTHERAPY FOR THE TREATMENT OF CANNABIS WITHDRAWAL SYNDROME

A valid and reliable cannabis withdrawal syndrome has been documented in both controlled laboratory and clinical evaluations (see Budney & Hughes, 2006 for review). Common symptoms associated with cannabis withdrawal include irritability, anxiety, restlessness, appetite changes, and sleep disturbances. A number of studies have been conducted evaluating the potential use of medications to treat cannabis withdrawal, on the presumption that withdrawal symptoms may contribute to difficulty achieving or maintaining abstinence (Table 106.1).

Antidepressants

An initial human laboratory study evaluated the effects of sustained-release bupropion, an antidepressant with norepinephrine and dopamine reuptake inhibition properties that has been approved for cigarette smoking cessation, in nontreatment seeking, heavy cannabis smokers. During an inpatient abstinence period, participants receiving bupropion reported increased irritability, depressed mood, and trouble sleeping compared to participants receiving placebo (Haney et al., 2001). However, a recent preliminary outpatient study reported more promising results with a paradigm similar to cigarette smoking cessation guidelines (Penetar, Looby, Ryan, Maywalt, & Lukas, 2012). In this study, withdrawal symptoms were greater in the placebo-treated participants, and cannabis craving increased in participants receiving placebo, but not in those receiving bupropion.

The effects of nefazodone, a norepinephrine and serotonin reuptake inhibitor, have also been evaluated in an inpatient laboratory model (Haney, Hart, Ward, & Foltin, 2003). Nefazodone decreased reports of anxiety and muscle pain during withdrawal, but did not reduce feelings of irritability or improve sleep quality, compared to placebo. The antidepressant mirtazapine had no impact on cannabis withdrawal symptoms (Haney et al., 2010).

TABLE 106.1 Medications Evaluated to Treat Cannabis Withdrawal Symptoms

Positive	Negative	Mixed data
Oral THC	Nefazodone	Bupropion SR
Oral THC/Lofexidine	Divalproex	
Zolpidem	Mirtazapine	
	Baclofen	
	Lithium	
	Quetiapine	

Summary of results from published studies evaluating medication effects on cannabis withdrawal symptoms. Data is from published studies.

Cannabinoid Agonists

Several studies have evaluated the utility of the oral cannabinoid agonist Δ^9-tetrahydrocannabinol (THC) on ameliorating cannabis withdrawal symptoms. An initial inpatient human laboratory investigation found that oral THC 10 mg administered five times daily decreased both withdrawal symptoms and cannabis craving (Haney et al., 2004). A subsequent outpatient trial also found oral THC at doses of 10 mg three times daily, and 30 mg three times daily to be effective in suppressing cannabis withdrawal symptoms (Budney, Vandrey, Hughes, Moore, & Bahrenburg, 2007). Of note, a follow-up human laboratory study found that administration of oral THC 20 mg three times daily reduced only a subset of cannabis withdrawal symptoms, and did not attenuate cannabis craving (Haney et al., 2008). The alpha$_2$-receptor agonist lofexidine was also evaluated, and did not decrease cannabis craving or most mood symptoms of withdrawal; however, when coadministered, oral THC and lofexidine significantly improved measures of withdrawal symptomatology, as well as decreased relapse in a laboratory model.

Mood Stabilizers

Divalproex has also been tested in a laboratory model of cannabis withdrawal, as divalproex has been used previously to treat mood lability and irritability (Haney et al., 2004). Although divalproex was shown to decrease cannabis craving during abstinence, its use was also associated with increased ratings of anxiety, irritability, tiredness, and "bad effect." In addition, divalproex administration worsened subject performance on psychomotor tasks. A small, open-label evaluation suggested some utility for lithium carbonate for management of cannabis withdrawal; however, the lack of a placebo-control condition limited interpretation (Winstock, Lea,

& Copeland, 2009). A recent randomized trial failed to show an effect of lithium treatment on withdrawal symptoms (Johnston et al., 2014).

Sedative Hypnotics

Sleep disturbances are reported commonly in cannabis withdrawal, and recent research has examined the utility of hypnotic medication targeting abstinence-induced sleep disruptions. In a within-subject crossover study, extended-release zolpidem attenuated the effects of cannabis abstinence on sleep architecture, and also normalized sleep efficiency scores (Vandrey, Smith, McCann, Budney, & Curran, 2011). No differences in withdrawal severity or cannabis craving were noted between placebo-abstinence and zolpidem-abstinence periods.

Other Medication Classes

The $GABA_B$ receptor agonist baclofen had limited impact on cannabis withdrawal symptoms in an inpatient human laboratory evaluation (Haney et al., 2010). Quetiapine, an atypical antipsychotic, improved some symptoms of cannabis withdrawal, but increased cannabis craving and relapse (Cooper et al., 2013). As discussed previously, lofexidine, an $alpha_2$-receptor, did not demonstrate utility on its own for cannabis withdrawal, but may act synergistically with oral THC to ameliorate symptoms (Haney et al., 2008).

PHARMACOTHERAPY FOR THE TREATMENT OF CANNABIS USE DISORDERS

As with other dependencies, pharmacotherapy clinical trials for cannabis use disorders also incorporate generally other treatment modalities, such as cognitive behavioral, motivational enhancement, and contingency management therapies. The inclusion of a behavioral platform has several advantages, including alleviating ethical concerns of providing a placebo or nonproven pharmacotherapy intervention, as all patients receive some form of psychosocial counseling. Further, a behavioral platform may improve study retention and compliance, particularly in studies of medications that may take several weeks to reach therapeutic blood levels or effects, and may increase the statistical power for an intention-to-treat analysis by decreasing the variability and protocol deviations commonly associated with pharmacotherapy trials (Carroll, Kosten, & Rounsaville, 2004). Medications evaluated for cannabis disorder treatment have included antidepressants and anxiolytics, agonist therapy, and agents targeting other specific neurotransmitters thought to be involved in cannabis addiction.

TABLE 106.2 Medications Evaluated to Treat Cannabis Dependence

Positive	Negative
N-Acetylcsyteine	Divalproex
Gabapentin	Oral THC
	Nefazodone
	Bupropion SR
	Escitalopram
	Atomoxetine
	Buspirone

Summary of results from published studies evaluating medication effects on cannabis dependence. Data is from published studies.

Antidepressants and Anxiolytics

Several antidepressant medications have been evaluated for the treatment of cannabis dependence (Table 106.2). Nefazodone and sustained-release bupropion were studied in a placebo-controlled trial of cannabis-dependent adults; neither medication had a significant effect on cannabis use or cannabis withdrawal symptoms (Carpenter, McDowell, Brooks, Cheng, & Levin, 2009). A recent evaluation of escitalopram in an adult population also failed to find a positive effect of treatment on abstinence or withdrawal outcomes (Weinstein et al., 2014).

Preclinical evidence suggests a role of the 5-HT1A receptor in the pharmacological actions of cannabinoids. Buspirone, a nonbenzodiazepine anxiolytic, is a partial 5-HT1A agonist, and showed limited preliminary evidence as a potential treatment for cannabis use disorders (McRae, Brady, & Carter, 2006; McRae-Clark et al., 2009). In a pilot, randomized controlled trial, buspirone combined with motivational enhancement therapy was associated with a trend for reduced positive urine drug screens, as compared to placebo; when only protocol completers were included, the difference reached statistical significance (McRae-Clark et al., 2009). However, no significant differences between treatment groups were observed in the intent to treat analysis.

Cannabinoid Agonists

Based on the proven utility of agonist medication in other addictions and data in cannabis withdrawal, oral THC was evaluated in a large randomized, placebo-controlled trial for treatment of cannabis dependence (Levin et al., 2011). Although oral THC was shown to improve retention in the trial and to reduce withdrawal symptoms, there was not a medication effect on cannabis use.

Glutamatergic Agents

Glutamate has recently emerged as a promising neurochemical target for addictive disorders medication development. *N*-Acetylcysteine (NAC) is an *N*-acetyl prodrug of the naturally occurring amino acid cysteine. NAC administration has been shown to increase nonsynaptic glial release of glutamate via stimulation of the cystine-glutamate exchange that becomes dysregulated after chronic drug use. As a result of normalization of this exchange process, NAC has been shown to reduce the reinstatement of drug seeking in animal models across multiple substances. An open-label trial of NAC in young (18–21 years old) cannabis users demonstrated reductions in self-report of cannabis use and cannabis craving (Gray, Watson, Carpenter, & Larowe, 2010). A follow-up placebo-controlled study showed that NAC, when paired with contingency management (CM) to promote abstinence, and brief counseling, doubled the odds of negative urine cannabinoid tests during treatment among cannabis-dependent adolescents (Gray et al., 2012).

Other Medication Classes

Atomoxetine, a nonstimulant medication approved for treatment of attention deficit disorder (ADHD), increases levels of both dopamine and norepinephrine in the prefrontal cortex, but does not appear to increase dopamine in subcortical areas where there are few noradrenergic nerve terminals. An open-label study of atomoxetine reported a trend toward reduction in cannabis use on self-report; however, this finding was not supported with urine drug screen results (Tirado, Goldman, Lynch, Kampman, & Obrien, 2008). Further, adverse events were commonly reported, with 77% of subjects experiencing mild to moderate gastrointestinal side effects.

Levin et al. (2004) conducted a double-blind, placebo-controlled, crossover study of the mood stabilizer divalproex in cannabis dependent adults. Both treatment groups reported less cannabis craving and drug use, over the course of the study; however, there were no significant differences between treatment groups on psychological or cannabis use outcomes.

Gabapentin is an analogue of the neurotransmitter gamma-aminobutyric acid (GABA), and is FDA-approved for the treatment of epilepsy and neuropathic pain. Although the precise mechanism of action is unclear, gabapentin binds to the alpha-2-delta subunit of voltage-gated calcium channels (VGCCs), and changes their conformation, reducing calcium influx at nerve terminals. Gabapentin also reduces the release of several neurotransmitters, including glutamate, noradrenaline, and substance P, and increases central GABAergic activity. Cannabis withdrawal is associated with increased CRF release in the amygdala, and gabapentin normalizes CRF-induced GABA activation in the amygdala, providing a basic science rationale for the use of gabapentin in cannabis dependence (Rodriguez de Fonseca, Carrera, Navarro, Koob, & Weiss, 1997). Mason et al. (2012) conducted a 12-week, placebo-controlled trial exploring the use of gabapentin in 50 cannabis-dependent individuals. The gabapentin-treated group demonstrated significantly less cannabis use by both self-report and urine toxicology, significantly decreased cannabis withdrawal symptoms, and greater improvement in executive function.

PHARMACOTHERAPY FOR THE TREATMENT OF COMORBID DISORDERS IN INDIVIDUALS WITH CANNABIS USE DISORDERS

The comorbidity of psychiatric and substance use disorders, including cannabis disorders, is associated with poor treatment prognosis. Patients with both disorders have been shown to have higher rates of substance use and more psychosocial impairment than patients without this comorbidity, as well as a more complicated course of treatment (Rounsaville, Weissman, Crits-Christoph, Wilber, & Kleber, 1982; Rounsaville et al., 1991; Strakowski, Tohen, Stoll, Faedda, & Goodwin, 1992). Importantly, improved substance abuse outcomes have been reported with pharmacological treatment of cooccurring psychiatric disorders such as depression, anxiety disorders, and bipolar disorder (Kranzler et al., 1994; Brady, Sonne, Anton, & Ballenger, 1995; Nunes et al., 1998). Data from the National Comorbidity Survey found that 90% of cannabis-dependent individuals had a lifetime psychiatric disorder, compared with 55% of individuals without cannabis dependence (Agosti, Nunes, & Levin, 2002).

A limited number of studies have evaluated the pharmacological treatment of cannabis use disorders in individuals with other psychiatric diagnoses. The selective serotonin receptor inhibitor fluoxetine significantly reduced cannabis use in depressed, adult alcohol-dependent individuals (Cornelius et al., 1999). A recent trial in adolescents and young adults with comorbid major depression and cannabis use disorders did not find a significant effect of fluoxetine on cannabis-related outcome measures (Cornelius et al., 2010); lack of effect may have been attributable to the strong psychosocial platform used (cognitive behavioral therapy plus motivational enhancement therapy), as there were significant improvements in both depressive and cannabis symptoms in both the placebo and fluoxetine-treated groups.

A recent trial examined the utility of venlafaxine-extended release combined with cognitive behavioral

therapy for cooccurring cannabis dependence and major depressive disorder or dysthymia (Levin et al., 2013). Venlafaxine was not better than placebo in reducing depressive symptoms. Further, participants receiving venlafaxine were less likely to reduce their cannabis use, or become abstinent, than participants receiving placebo.

An evaluation of atomoxetine combined with motivational enhancement therapy on the symptoms of ADHD and cannabis use in cannabis-dependent adults found greater improvement in some ADHD measurements, but not on cannabis use outcomes (McRae-Clark et al., 2010). These results, taken together with the atomoxetine aforementioned study, suggest that atomoxetine does not reduce cannabis use in this population.

CONCLUSIONS

It is clear that more research on the pharmacotherapy of cannabis use disorders is necessary. Some promising results have been reported in the treatment of cannabis withdrawal symptoms; however, these findings have not yet translated to improved clinical outcomes. Encouraging findings have also recently been reported in clinical trials of NAC and gabapentin. However, to date, there is no FDA-approved medication for either cannabis withdrawal or cannabis dependence. As cannabis use disorders frequently occur in individuals with other psychiatric disorders, more research is also needed in the area of dual diagnosis. With increased recognition of cannabis abuse and dependence as a significant public health concern, more attention should be focused on the development of effective treatments for this disorder.

MINI-DICTIONARY

Agonist therapy Use of a medication that stimulates the same brain receptors as a drug of abuse.
Cannabis use disorders A problematic pattern of cannabis use leading to clinically significant impairment or distress; specific criteria are defined in the fifth edition of the Diagnostic and Statistical Manual.
Cannabis withdrawal Signs and symptoms developing after cessation of heavy, prolonged cannabis use including irritability, anxiety, difficulty sleeping, reduced appetite, restlessness, and depressed mood.
Comorbidity A psychiatric disorder coexisting with a substance and/or alcohol use disorder.
Pharmacotherapy Use of a medication to treat a disease or symptom.

References

Agosti, V., Nunes, E., & Levin, F. (2002). Rates of psychiatric comorbidity among U.S. residents with lifetime cannabis dependence. *American Journal of Drug and Alcohol Abuse*, *28*, 643–652.

Brady, K. T., Sonne, S. C., Anton, R., & Ballenger, J. C. (1995). Valproate in the treatment of acute bipolar affective episodes complicated by substance abuse: a pilot study. *Journal of Clinical Psychiatry*, *56*, 118–121.

Budney, A. J., & Hughes, J. R. (2006). The cannabis withdrawal syndrome. *Current Opinion in Psychiatry*, *19*, 233–238.

Budney, A. J., Vandrey, R. G., Hughes, J. R., Moore, B. A., & Bahrenburg, B. (2007). Oral delta-9-tetrahydrocannabinol suppresses cannabis withdrawal symptoms. *Drug and Alcohol Dependence*, *86*, 22–29.

Carpenter, K. M., McDowell, D., Brooks, D. J., Cheng, W. Y., & Levin, F. R. (2009). A preliminary trial: double-blind comparison of nefazodone, bupropion-SR, and placebo in the treatment of cannabis dependence. *American Journal of Addiction*, *18*, 53–64.

Carroll, K. M., Kosten, T. R., & Rounsaville, B. J. (2004). Choosing a behavioral therapy platform for pharmacotherapy of substance users. *Drug and Alcohol Dependence*, *75*, 123–134.

Cooper, Z. D., Foltin, R. W., Hart, C. L., Vosburg, S. K., Comer, S. D., & Haney, M. (2013). A human laboratory study investigating the effects of quetiapine on marijuana withdrawal and relapse in daily marijuana smokers. *Addiction Biology*, *18*, 993–1002.

Cornelius, J. R., Salloum, I. M., Haskett, R. F., Ehler, J. G., Jarrett, P. J., Thase, M. E., & Perel, J. M. (1999). Fluoxetine versus placebo for the marijuana use of depressed alcoholics. *Addictive Behaviors*, *24*, 111–114.

Cornelius, J. R., Bukstein, O. G., Douaihy, A. B., Clark, D. B., Chung, T. A., Daley, D. C., Wood, D. S., & Brown, S. J. (2010). Double-blind fluoxetine trial in comorbid MDD-CUD youth and young adults. *Drug and Alcohol Dependence*, *112*, 39–45.

Dennis, M., Godley, S. H., Diamond, G., Tims, F. M., Babor, T., Donaldson, J., Liddle, H., Titus, J. C., Kaminer, Y., Webb, C., Hamilton, N., & Funk, R. (2004). The Cannabis Youth Treatment (CYT) Study: main findings from two randomized trials. *Journal of Substance Abuse Treatment*, *27*, 197–213.

Gray, K. M., Watson, N. L., Carpenter, M. J., & Larowe, S. D. (2010). N-acetylcysteine (NAC) in young marijuana users: an open-label pilot study. *American Journal of Addiction*, *19*, 187–189.

Gray, K. M., Carpenter, M. J., Baker, N. L., DeSantis, S. M., Kryway, E., Hartwell, K. J., McRae-Clark, A. L., & Brady, K. T. (2012). A double-blind randomized controlled trial of *N*-acetylcysteine in cannabis-dependent adolescents. *American Journal of Psychiatry*, *169*, 805–812.

Haney, M., Ward, A. S., Comer, S. D., Hart, C. L., Foltin, R. W., & Fischman, M. W. (2001). Bupropion SR worsens mood during marijuana withdrawal in humans. *Psychopharmacology (Berlin)*, *155*, 171–179.

Haney, M., Hart, C. L., Ward, A. S., & Foltin, R. W. (2003). Nefazodone decreases anxiety during marijuana withdrawal in humans. *Psychopharmacology (Berlin)*, *165*, 157–165.

Haney, M., Hart, C. L., Vosburg, S. K., Nasser, J., Bennett, A., Zubaran, C., & Foltin, R. W. (2004). Marijuana withdrawal in humans: effects of oral THC or divalproex. *Neuropsychopharmacology*, *29*, 158–170.

Haney, M., Hart, C. L., Vosburg, S. K., Comer, S. D., Reed, S. C., & Foltin, R. W. (2008). Effects of THC and lofexidine in a human laboratory model of marijuana withdrawal and relapse. *Psychopharmacology (Berlin)*, *197*, 157–168.

Haney, M., Hart, C. L., Vosburg, S. K., Comer, S. D., Reed, S. C., Cooper, Z. D., & Foltin, R. W. (2010). Effects of baclofen and mirtazapine on a laboratory model of marijuana withdrawal and relapse. *Psychopharmacology (Berlin)*, *211*, 233–244.

Hart, C. L. (2005). Increasing treatment options for cannabis dependence: a review of potential pharmacotherapies. *Drug and Alcohol Dependence*, *80*, 147–159.

Johnston, J., Lintzeris, N., Allsop, D. J., Suraev, A., Booth, J., Carson, D. S., Helliwell, D., Winstock, A., & McGregor, I. S. (2014). Lithium carbonate in the management of cannabis withdrawal: a randomized placebo-controlled trial in an inpatient setting. *Psychopharmacology (Berlin)*, *231*, 4623–4636.

Kadden, R. M., Litt, M. D., Kabela-Cormier, E., & Petry, N. M. (2007). Abstinence rates following behavioral treatments for marijuana dependence. *Addictive Behaviors*, *32*, 1220–1236.

Kranzler, H. R., Burleson, J. A., Del Boca, F. K., Babor, T. F., Korner, P., Brown, J., & Bohn, M. J. (1994). Buspirone treatment of anxious alcoholics. A placebo-controlled trial. *Archives of General Psychiatry*, *51*, 720–731.

Levin, F. R., McDowell, D., Evans, S. M., Nunes, E., Akerele, E., Donovan, S., & Vosburg, S. K. (2004). Pharmacotherapy for marijuana dependence: a double-blind, placebo-controlled pilot study of divalproex sodium. *American Journal of Addiction*, *13*, 21–32.

Levin, F. R., Mariani, J. J., Brooks, D. J., Pavlicova, M., Cheng, W., & Nunes, E. V. (2011). Dronabinol for the treatment of cannabis dependence: a randomized, double-blind, placebo-controlled trial. *Drug and Alcohol Dependence*, *116*, 142–150.

Levin, F. R., Mariani, J., Brooks, D. J., Pavlicova, M., Nunes, E. V., Agosti, V., Bisaga, A., Sullivan, M. A., & Carpenter, K. M. (2013). A randomized double-blind, placebo-controlled trial of venlafaxine-extended release for co-occurring cannabis dependence and depressive disorders. *Addiction*, *108*, 1084–1094.

Marijuana Treatment Project Research Group. (2004). Brief treatments for cannabis dependence: findings from a randomized multisite trial. *Journal of Consulting and Clinical Psychology*, *72*, 455–466.

Mason, B. J., Crean, R., Goodell, V., Light, J. M., Quello, S., Shadan, F., Buffkins, K., Kyle, M., Adusumalli, M., Begovic, A., & Rao, S. (2012). A proof-of-concept randomized controlled study of gabapent: effects on cannabis use, withdrawal and executive function deficits in cannabis-dependent adults. *Neuropsychopharmacology*, *37*, 1689–1698.

McRae, A. L., Budney, A. J., & Brady, K. T. (2003). Treatment of marijuana dependence: a review of the literature. *Journal of Substance Abuse Treatment*, *24*, 369–376.

McRae, A. L., Brady, K. T., & Carter, R. E. (2006). Buspirone for treatment of marijuana dependence: a pilot study. *American Journal of Addiction*, *15*, 404.

McRae-Clark, A. L., Carter, R. E., Killeen, T. K., Carpenter, M. J., Wahlquist, A. E., Simpson, S. A., & Brady, K. T. (2009). A placebo-controlled trial of buspirone for the treatment of marijuana dependence. *Drug and Alcohol Dependence*, *105*, 132–138.

McRae-Clark, A. L., Carter, R. E., Killeen, T. K., Carpenter, M. J., White, K. G., & Brady, K. T. (2010). A placebo-controlled trial of atomoxetine in marijuana-dependent individuals with attention deficit hyperactivity disorder. *American Journal of Addiction*, *19*, 481–489.

Nordstrom, B. R., & Levin, F. R. (2007). Treatment of cannabis use disorders: a review of the literature. *American Journal of Addiction*, *16*, 331–342.

Nunes, E. V., Quitkin, F. M., Donovan, S. J., Deliyannides, D., Ocepek-Welikson, K., Koenig, T., Brady, R., McGrath, P. J., & Woody, G. (1998). Imipramine treatment of opiate-dependent patients with depressive disorders. A placebo-controlled trial. *Archives of General Psychiatry*, *55*, 153–160.

Penetar, D. M., Looby, A. R., Ryan, E. T., Maywalt, M. A., & Lukas, S. E. (2012). Bupropion reduces some of the symptoms of marihuana withdrawal in chronic marihuana users: a pilot study. *Substance Abuse*, *6*, 63–71.

Rodriguez de Fonseca, F., Carrera, M. R., Navarro, M., Koob, G. F., & Weiss, F. (1997). Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. *Science*, *276*, 2050–2054.

Rounsaville, B. J., Weissman, M. M., Crits-Christoph, K., Wilber, C., & Kleber, H. (1982). Diagnosis and symptoms of depression in opiate addicts. Course and relationship to treatment outcome. *Archives of General Psychiatry*, *39*, 151–156.

Rounsaville, B. J., Anton, S. F., Carroll, K., Budde, D., Prusoff, B. A., & Gawin, F. (1991). Psychiatric diagnoses of treatment-seeking cocaine abusers. *Archives of General Psychiatry*, *48*, 43–51.

SAMHSA. (2013). *Results from the 2012 National Survey on Drug Use and Health: Volume 1. Summary of National Findings*. Rockville, MD: SAMHSA, Department of Health and Human Services, Office of Applied Studies.

Strakowski, S. M., Tohen, M., Stoll, A. L., Faedda, G. L., & Goodwin, D. C. (1992). Comorbidity in mania at first hospitalization. *American Journal of Psychiatry*, *149*, 554–556.

Tirado, C. F., Goldman, M., Lynch, K., Kampman, K. M., & Obrien, C. P. (2008). Atomoxetine for treatment of marijuana dependence: a report on the efficacy and high incidence of gastrointestinal adverse events in a pilot study. *Drug and Alcohol Dependence*, *94*, 254–257.

Vandrey, R., & Haney, M. (2009). Pharmacotherapy for cannabis dependence: how close are we? *CNS Drugs*, *23*, 543–553.

Vandrey, R., Smith, M. T., McCann, U. D., Budney, A. J., & Curran, E. M. (2011). Sleep disturbance and the effects of extended-release zolpidem during cannabis withdrawal. *Drug and Alcohol Dependence*, *117*, 38–44.

Weinstein, A. M., Miller, H., Bluvstein, I., Rapoport, E., Schreiber, S., Bar-Hamburger, R., & Bloch, M. (2014). Treatment of cannabis dependence using escitalopram in combination with cognitive-behavior therapy: a double-blind placebo-controlled study. *American Journal of Drug and Alcohol Abuse*, *40*, 16–22.

Winstock, A. R., Lea, T., & Copeland, J. (2009). Lithium carbonate in the management of cannabis withdrawal in humans: an open-label study. *Journal of Psychopharmacology*, *23*, 84–93.

CHAPTER

107

Self-Initiated Cannabis Use Cessation in Adolescents and Emerging Adults

*J. Tsai**, *M. Little***, *S. Sussman*†

**Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, United States

***Center for Population Sciences, Department of Preventive Medicine, University of Tennessee Health Science Center, Memphis, TN, United States

†Departments of Preventive Medicine and Psychology, and School of Social Work, Institute for Health Promotion and Disease Prevention Research, University of Southern California, Los Angeles, CA, United States

SUMMARY POINTS

- This chapter focuses on key predictors of self-initiated cannabis cessation in adolescents and emerging adults using the triadic theory of influence (TTI) as a means of organizing empirical studies.
- Predictors are categorized as intrapersonal (biological), cultural/attitudinal (socio-environmental), or interpersonal (social context) factors related to cannabis cessation.
- Intrapersonal predictors of self-initiated cessation include neurobiological disposition (eg, positive and negative affect), self-image, deviant behaviors, and cognitive processing (eg, decision-making).
- Cultural and attitudinal predictors of self-initiated cessation include one's larger social environment (eg, neighborhood/community approval or disapproval of cannabis use, neighborhood safety), acculturation, emerging adulthood, cannabis-related consequences attitudes, and pro-drug use myths.
- Interpersonal predictors of self-initiated cessation include peer social norms, peer approval or disapproval of marijuana cessation, and familial interactions.
- Strategies discussed thus far pertaining to self-initiated marijuana cessation include "distraction" and "stimulus removal."
- These predictors and strategies can and should be used in future cannabis formal and nonformal (self-initiated, naturally occurring) prevention and intervention programs, along with a better understanding of their long-term impacts.

KEY FACTS OF TRIADIC THEORY OF INFLUENCE

- A theory developed by Petraitis, Flay, and Miller in 1995 (Petraitis, Flay, & Miller, 1995).
- TTI was originally used to look at predictors for experimental substance use (ESU).
- Petraitis et al. (1995) examined 14 multivariate theories associated with ESU and combined the main constructs of these theories into a more comprehensive theoretical framework.
- ESU predictors are separated into three causal streams: intrapersonal, cultural/attitudinal, and interpersonal.
- These streams can be further delineated as ultimate, distal, and proximal influences on behavior; describing a "distance" from engagement in the behavior.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00125-3
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

EA Emerging adulthood
THC Δ^9-Tetrahydrocannabinol
TTI Theory of triadic influence

SELF-INITIATED CANNABIS CESSATION IN ADOLESCENTS AND EMERGING ADULTS[a]

Adolescent prevention and intervention programs have the ability to delay onset, decrease the frequency of cannabis use, facilitate quitting, and limit passive exposure to cannabis smoke and risky contexts of use (Sussman & Ames, 2008). These programs aim to reduce the likelihood of prolonged cannabis use in an individual's lifetime by targeting and counteracting effects of antecedents associated with substance use behavior. Unfortunately, the number of studies among adolescents looking at antecedents associated with cannabis cessation pale in comparison to the number of studies that look at antecedents associated with cannabis initiation (Sussman, Stacy, Dent, & Simon, 1996), leading to development of prevention (but not cessation) programming. Furthermore, research on evidence-based cannabis cessation programs are tailored toward heavy and dependent cannabis users (Boyd et al., 2005), and most of those studies focus on adults (eg, Davis et al., 2014).

Most cannabis users have attempted to quit at least once on their own and have failed (Copersino et al., 2006; Weiner, Sussman, McCuller, & Lichtman, 1999). While cessation rates among regular cannabis users trying to quit is similar to other substances (only around 25% after three months on average; Sussman et al., 1996), research has shown that prolonged cannabis use can induce some negative general health, psychological, and behavioral consequences (Van den Bree & Pickworth, 2005). For youth, the decision to quit using cannabis may not typically occur until a formalized institutional program is forced to intervene.

Further discussion is warranted regarding the differences between those who choose to quit on their own (self-initiated) and those who quit via more structured programs. Experimentation with cannabis oftentimes begins at adolescence, and becomes habitual by early adulthood. Research that compares adolescents who continue prolonged use of cannabis with other users that stop using cannabis will help tailor more effective adolescent cannabis intervention programs, and will create a better understanding of how social contextual variables might assist in cessation. Relatedly, perhaps the success and failures of cessation are not only dependent on the context of how cannabis intervention programs are implemented, but also on the characteristics of individuals and their surrounding environment.

The present chapter focuses on identifying the antecedents associated with self-initiated cannabis cessation in adolescents and emerging adults (ages 11–25). Self-initiated cessation or "self-managed change" can be defined as individuals who do not seek any sort of formal treatment (eg, clinical, school-based interventions, internet interventions), but rather, attempt to quit in the real-world setting (Rooke, Norberg, & Copeland, 2011; Hughes, Peters, Callas, Budney, & Livingston, 2008). We will discuss the intrapersonal and extrapersonal factors that contribute to why some adolescents are more likely to quit cannabis on their own using the theory of triadic influence (TTI) as a guide. Furthermore, we will describe the implications of these factors on formal cannabis cessation programming and on sustained cessation.

THEORY OF TRIADIC INFLUENCE AND CANNABIS CESSATION

The theory of triadic influence (TTI) was first developed to explain experimental substance use, including cannabis use (Petraitis et al., 1995). TTI posits there are three levels (or streams) of causation that influence behavior: intrapersonal, cultural/attitudinal, and interpersonal levels (Little et al., 2013; Petraitis et al., 1995). These three levels of causation are further delineated as ultimate causes (ie, factors beyond the individual's immediate control), distal factors (ie, factors dealing with the interaction between the individual and his immediate social environment), and proximal factors (ie, factors directly related to the immediate decision-making process of a particular behavior, which are usually less stable and more interchangeable; Flay, Snyder, & Petraitis, 2009). The constituents of this theory may be viewed from the lens of facilitating or deterring cannabis use. This theory is used as a heuristic for placing predictors of self-initiated cannabis cessation into a conceptual framework (Table 107.1).

[a]A literature search was conducted for data based on self-initiated teen cannabis use cessation papers published up to July 29, 2014 using the following databases: Google Scholar, PubMed, and OvidSp Medline. Different combinations of the following keywords were used in our search: cannabis cessation, marijuana cessation, adolescent(s), emerging adult(s), self-initiated, self-managed, non-treatment seeking. Our literature search yielded a total of 239 papers. After initial review of the paper title and abstract, duplicate papers and papers that were not specific to adolescents or emerging adults and non-treatment seeking cannabis cessation were removed. Only 6 papers remained relevant to the current review topic. These papers are signified by an asterisk (*) where they appear in the "Reference" section.

TABLE 107.1 Triadic Theory of Influence Matrix on Cannabis Cessation

Level of influence	Types of influence		
	Intrapersonal	**Cultural/attitudinal**	**Interpersonal**
Ultimate	*Definition:* Biological and/or personality attributes that influence the individual's self-efficacy to quit cannabis use	*Definition:* Characteristics of the individual's immediate environment that shapes attitudes towards cannabis cessation	*Definition:* Characteristics of the people that comprise the adolescent's most intimate social networks
	Constructs: Neurobiological (eg, positive and negative affect)	*Constructs:* Large social environment (eg, neighborhood/community (dis)approval of cannabis use and cessation), Neighborhood safety (ie, possibilities for victimization)	*Constructs:* Familial interactions
Distal	*Definition:* Psycho-physiological factors that motivate an individual to discontinue cannabis use	*Definition:* General values and behaviors an adolescent has that influences attitudes towards cannabis cessation	*Definition:* Social interactions between adolescents and their social networks (ie, role models) that encourage cannabis cessation
	Constructs: Self-image, deviancy, decision-making[a] (eg, avoidance and confidence)	*Constructs:* Acculturation, emerging adulthood (eg, vocational, education, family[b]), attitudes toward consequences of cannabis-related behavior, pro-drug use myths	*Constructs:* Peer social norms, peer (dis)approval of cannabis use and cessation
Proximal	*Definition:* Belief about ability to quit cannabis use	*Definition:* Subjective beliefs and evaluations about the costs and benefits of using or not using cannabis	*Definition:* Norms associated with cannabis cessation and pressure to use or quit
	Constructs: Self-efficacy to quit using cannabis	*Constructs:* Expected costs and benefits of using or quitting cannabis	*Constructs:* Desire to conform with others (eg, peers and role models)

This table lists the identified predictors associated with cannabis cessation within the framework of the triadic theory of influence.
Notes:
[a] *Considered as a coping style, facets of decision making are placed into the intrapersonal stream of TTI. Considered as a process of evaluation, other facets of decision-making might be placed into the cultural/attitudinal stream.*
[b] *Resolution of EA when one begins to establish a family would be considered a cultural/attitudinal variable according to TTI, whereas the quality of family attachments created would be considered an interpersonal variable.*

INTRAPERSONAL PREDICTORS

Intrapersonal predictors are biological and/or personality attributes influencing the individual's self-efficacy to avoid or use cannabis (Little et al., 2013, Petraitis et al., 1995). Intrapersonal factors include an individual's neurobiological systems (ultimate), self-image, and deviancy (distal), and establishment of cognitive functions (distal). The quality of development of neurobiological systems and cognitive function during childhood and adolescence can be shaped to help maximize social and emotional competence, which are influential in the self-regulation processes to help one quit cannabis use (Riggs & Greenberg, 2009). Also, it is plausible that subjective ability to refuse cannabis offers may be related to maintenance of self-initiated quitting, as a proximal intrapersonal factor (Petraitis et al., 1995), though no empirical studies exists on proximal factors and self-initiated quitting among adolescents.

Neurobiological

Youth susceptible to the positive reinforcing effects of cannabis may find it more difficult to quit using on their own. The potentially key active ingredient in cannabis, Δ^9-Tetrahydrocannabinol (THC), binds to cannabinoid receptors in the central nervous system, which leads to an altered psychological state of heightened perception or a "high" (APA, 2013). Positive affect associated with cannabis include feeling relaxed and calm, more sensation, reduced stress, mood enhancement (eg, evokes positive feelings and diminishes negative feelings), heightened sense of humor, and gaining insight/personal development (Iversen, 2003; Sussman et al., 1996). The positive affect(s) elicited during cannabis use may be a fortifying factor in cannabis (psychological) dependency. For those who initially obtain positive effects from use, subsequent adverse effects of cannabis use, such as anxiety, dysphoria, social withdrawal, impaired memory, paranoia, and possibly laziness or sleepiness (Sussman et al., 1996) may be a contributing factor in the decision to quit (Iversen, 2003). That is, subjective negative costs associated with regular use may lead to quitting.

Negative reinforcing effects (eg, withdrawal symptoms) may also apply to maintenance of cannabis dependency. Not surprisingly, frequency of cannabis use is a strong predictor of whether or not one will self-initiate cannabis cessation (Sussman & Dent, 2004; Chen & Kandel, 1998; Little et al., 2013; Kandel & Raveis, 1989; Rooke et al., 2011; Van den Bree & Pickworth, 2005). Less

frequent cannabis use leads to higher likelihood of successful quitting. Dependent users are relatively more likely to enter a formalized program and possibly seek out self-help groups (eg, Marijuana Anonymous; MA—Marijuana Anonymous, 1989), and are often sent to rehabilitation programs by an authority figure (eg, judge, teacher, family member; Rooke et al., 2011). After a half century of debate over the topic (Budney, Novy, & Hughes, 1999; Sussman et al., 1996) among clinicians and researchers, the *Diagnostic and Statistical Manual for Mental Disorders, Fifth Edition* (DSM-5) now acknowledges the existence of cannabis use withdrawal symptoms generally among heavy and prolonged users (eg, irritability, anxiety, sleep problems, decreased appetite, restlessness, depressed mood, and pain, such as headache or abdominal, and shakiness; APA, 2013). At least 50% of those adolescents and adults in treatment for cannabis dependence report such symptoms, which last up to 2 weeks (APA, 2013). Thus, at high levels of use, withdrawal symptoms may exist and cannabis users may wish to avoid them, making quitting more difficult.

Finally, cannabis may not be the drug of choice among some youth; that is, there may be individual difference moderators of whether or not youth will continue using cannabis. In a study by Chen and Kandel (1998), those who were depressed or expressed more depressive symptoms as an adolescent were more likely to self-recover from cannabis use later in life. Although the reasons for cannabis cessation among depressed individuals in the study was unclear, the authors posit cannabis may not provide the same psychoactive benefits, or "high," sought by their nondepressed counterparts, and were more likely to progress from cannabis to other illicit drugs (Chen & Kandel, 1998). However, other studies have found contradictory results—depressed individuals were more likely to continue using cannabis and were more likely to relapse after quitting (Rooke et al., 2011), possibly experiencing self-medicating benefits of use. Clearly, more work is needed on how aspects of depressed mood might be predictive of self-initiated cessation.

Self-Image and Deviant Behavior

Individuals who are concerned with their self-image are more likely to stop using cannabis (Sussman & Dent, 2004). This may be associated with the belief that engaging in cannabis-related behaviors may be linked with negatively perceived social labels. Self-image can be both a construct of individual perception and one's interaction with his or her social environment. In a study assessing factors predicting cannabis cessation, two of the top six social images identified by high risk youth were negative (eg, "silly," "spaced out"); however, it is important to note that adolescents continue to identify with perceived positive social images associated with cannabis use (Weiner et al., 1999).

Deviance is also correlated with self-initiated cannabis cessation in youth. Individuals who have a history of drug-related problems and legal issues are less likely to quit on their own (Sussman & Dent, 2004; Van den Bree & Pickworth, 2005). Those who show a pattern of delinquency may find themselves in a continuous cycle of drug exposure; for example, selling drugs, buying drugs, and using drugs. Conversely, those who are aware of and concerned about the consequences of cannabis use (eg, "getting in trouble," "jail," and "probation") are more likely to discontinue using on their own (Sussman & Dent, 2004; Van den Bree & Pickworth, 2005; Weiner et al., 1999). For these individuals, the consequences associated with cannabis use may outweigh the benefits.

Cognitive Processing

Those who have better executive cognitive processing (eg, attentional processes, planning) are less likely to continue cannabis use. Cognitive processing can be explicit (more conscious) or implicit (more automatic) (Sussman, Sun, Rohrbach, & Spruijt-Metz, 2012). Cessation is dependent on a deliberate or explicit effort to remove oneself from the stimuli that may trigger cannabis use (Rooke et al., 2011). For individuals who choose to self-manage quitting, mindful action to stop using are first steps to cannabis use reduction and cessation.

Decision-making is an explicit, deliberate, executive cognitive process that influences the decision to stop using cannabis. Individuals who exhibit higher avoidant and/or confident decision-making skills are more likely to quit on their own (Little et al., 2013). Though seemingly contrary decision-making styles, being decision-making avoidant and decision-making confident serve similar roles in cannabis cessation. Individuals who are decision-making avoidant tend to avoid making high-risk decisions, and are more risk adverse than their nonavoidant counterparts (Franken & Muris, 2005; Maner et al., 2007). Prolonged cannabis use may be seen as being of high risk, and may comprise unforeseeable consequences the decision-making avoidant individual may want to avoid.

Decision-making confidence is defined as exhibiting confidence in the decisions one makes (Mann, Harmoni, & Power, 1989). Individuals who perceive themselves as decision-making confident tend to be independent and decisive of their resolutions, and are happy with the choices they make (Paese & Sniezek, 1991). Individuals with a confident decision-making attitude may be less likely to seek or need the help of formal treatment programs because they believe they have the inherent competency to quit.

CULTURAL/ATTITUDINAL

Cultural/attitudinal predictors of self-initiated cannabis cessation include such variables as the large social environment (ultimate), acculturation (distal), emerging adulthood (distal), perception of pro-drug use myths (distal), and attitudes towards negative consequences of cannabis use in the short-term and long-term (distal). Inputs from ultimate (eg, large social environment) and distal (eg, acculturation) variables are influential in proximal cultural/attitudinal factors associated with quitting, which are the subjective beliefs and evaluations about the costs and benefits of using or not using a particular drug (Petraitis et al., 1995).

Larger Social Environment

A safe neighborhood social environment may facilitate a shorter duration of cannabis use. For example, individuals who report being relatively less victimized are more likely to quit (Sussman & Dent, 1999, 2004). One may speculate that those who are victimized seek the help of cannabis to cope with experiences of victimization (and this may occur in locations where cannabis is relatively widely distributed), whereas those who feel less victimized may be surrounded by a more protective and more encouraging social context that discourages cannabis use.

Social environmental-level variables may interact with intrapersonal-level variables to impact self-initiated cessation. For example, decision-making avoidant individuals tend to let others make decisions for them or go along with the decisions of the people they are surrounded by; thus, depending on the larger social context within which their family members, peers, and neighbors reside, self-initiated quitting may be greater or might be less likely. If persons live in a neighborhood that strongly disapproves of drug use or generally does not use drugs, then they are more likely to disapprove of and stop using cannabis (Rooke et al., 2011).

Acculturation

One's social environment is shaped in part by how acculturated one is within his or her community, which can serve as a protective or risk factor for cannabis use. Acculturation occurs when an individual becomes integrated to the mainstream values of the society he or she has entered. That is, there is a multi-dimensional process of blending one's own ethnic heritage with that of a new culture where the values of one's "home" country are joined with or replaced with new-country values and beliefs (Schwartz, Unger, Zamboanga, & Szapocznik, 2010). If the home country tends to be less favorable towards regular cannabis use than in the new country, then the less acculturated one is, the more likely one is to quit (Little et al., 2013). For example, there may be inherent factors present in some non-American cultures that prevent prolonged cannabis use (Lac et al., 2011). As a more specific example, Latino adolescents are more likely to self-initiate cannabis cessation than Whites in southern California (Sussman & Dent, 2004), possibly reflecting differences in culture. Values associated with family cohesion in the Hispanic culture may foster family and neighborhood bonding and increased parental and neighbor monitoring, which may divert the individual away from continued use of cannabis (Lac et al., 2011). Conversely, American culture may be more accepting of drug use and may create environments in which drug exposure is more common, especially since cannabis became legal in two states in the United States (Washington and Colorado; Donlan, 2013) and medical use of cannabis is widespread. For these reasons, the more acculturated one becomes to American culture, the more likely one's beliefs and values towards cannabis may adapt to tolerate its use.

Emerging Adulthood

Emerging adulthood (EA) is defined as a distinct time period between the ages of 18 and 25 where individuals transition from adolescence to young adulthood. During EA, individuals have the freedom to explore values and beliefs in their lives distinct from previously identified authority figures in childhood and are not confined to the limitations associated with adulthood. EA is characterized by five areas of development: (1) establishing one's own identity, (2) a time of feeling in-between, (3) a time of being self-focused, (4) a time of believing there are endless possibilities, and (5) a time where lifestyle is one of instability (Arnett, 2000). Perhaps the most important aspect of cannabis cessation associated with EA is how strongly an individual feels the current period of his life is a time of experimentation. Those who treat EA as a period to maximally experiment with individual lifestyles (eg, adhere to such expressions as "You Only Live Once" or "YOLO") are relatively less likely to quit (Little et al., 2013; Sussman & Arnett, 2014).

There is a strong association between older age and self-initiated cannabis cessation; many adolescent cannabis users tend to stop use around their mid-to-late twenties as societal roles of adulthood become relevant (Chen & Kandel, 1998; Kandel & Raveis, 1989). Those that hold traditional values consistent with mainstream societal norms are more likely to self-initiate cessation. For example, these individuals are motivated to quit to avoid testing positive on a drug test at work, and believe that using cannabis may pose vocational problems or prevent them from obtaining a job (Sussman & Dent, 1999, 2004;

Weiner et al., 1999). Individuals who are goal oriented (eg, have higher job aspirations or are actively seeking employment, who seek higher education or hold a degree higher than a high school education) are also more likely to discontinue use (Chen & Kandel, 1998; Sussman & Dent, 1999, 2004; Weiner et al., 1999). The duties established in adult life may act as other stimuli that detract from persistent cannabis use.

Perhaps the most influential EA event that determines whether one will self-recover from cannabis use is having a child or children. The construction of one's own nuclear family creates a socially defined "adult" role in which perceived acceptability of substance use may become rejected. This is particularly true for females who are about to embark on motherhood. When a female becomes pregnant, she usually quits using for fear of hurting the fetus (Chen & Kandel, 1998). Also, males who have children or are becoming a parent for the first time are also more likely to quit than males without children (Little et al., 2013; Kandel & Raveis, 1989; Sussman & Dent, 1999, 2004). Having a family (ie, marriage and becoming a parent) can be considered adaptive life strategies that may motivate individuals to quit and reinforce the quitting process (Rooke et al., 2011).

Attitudes and Pro-Drug Use Myths

Attitudes regarding the harmfulness of using cannabis are predictive of self-initiated cannabis cessation. Some studies have found that adolescents who believe cannabis is harmful to one's health are more likely to quit (Copersino et al., 2006; Sussman & Dent, 2004). In a study by Kandel and Raveis (1989), adolescents who later stopped using cannabis reported feeling healthier than young people who continued using cannabis. Additionally, individuals who have the attitude that it is wrong to use drugs (generally), and that it is wrong to use cannabis (specifically), showed shorter durations of cannabis use (Sussman & Dent, 2004). These attitudes may decrease the frequency of one's use and decrease the likelihood of socializing with people who approve of cannabis use because of the perceived consequences associated with the behavior.

Pro-drug use myths are inaccurate perceptions or expectancies related to drug use that individuals perceive to be true (Sussman & Ames, 2008). Those who hold more pro-drug use myths tend to believe their peers engage in similar cannabis-use behaviors, and expect the relative benefits of engaging in the behavior to outweigh the consequences of the behavior; for example, that smoking cannabis is relaxing (Kandel & Raveis, 1989). Those who endorse fewer pro-drug use myths—more accurate perception about the effects and consequences of drug and cannabis use—are more likely to stop using (Little et al., 2013; Sussman & Dent, 1999, 2004).

INTERPERSONAL PREDICTORS

Interpersonal predictors of cannabis use describe the microsocial environmental context through which individuals traverse. Self-initiated cessation may be influenced by perceptions or behaviors of the people who compose the adolescent's most intimate social support system (Petraitis et al., 1995). Salient adolescent interpersonal factors include peer social norms (distal), peer approval/disapproval of cannabis use (distal), and familial interactions (ultimate), which in turn affect proximal motivation to comply with significant others, or beliefs that significant others discourage cannabis use; that is, one may quit using cannabis to either fit in or please important people within one's social community.

Peer Influence

Peer norms and disapproval of cannabis are strong predictors of an individual's decision to quit using cannabis (Chen & Kandel, 1998; Kandel & Raveis, 1989; Little et al., 2013; Sussman & Dent, 1999, 2004; Van den Bree & Pickworth, 2005). If one perceives that one's friends believe there are negative consequences associated with cannabis use and disprove of cannabis-related behavior, then one is more likely to quit. Additionally, users who have friends who smoke cannabis less frequently, also tend to quit more so than those who have friends that smoke frequently (Sussman & Dent, 2004). These outcomes are consistent with other drug use cessation literature and social network analyses that emphasize the importance of peer influences on drug use behavior (Valente et al., 2007).

Youths who use cannabis solely as a social activity are more likely to quit than individuals who use cannabis alone (nonsocial use; Kandel & Raveis, 1989). Furthermore, those who perceive cannabis as a recreational activity are more likely to quit than individuals who experience and focus on the positive affect of cannabis use, for example, cannabis use is pleasurable, helps them feel more relaxed, and/or enhances sex (Kandel & Raveis, 1989). This finding is consistent with addiction literature that suggests once an individual is unable to choose the setting in which the drug use will take place (using perhaps more of necessity than recreationally), there has been a loss of control and a sense of compulsivity associated with the drug use (Sussman, Lisha, & Griffiths, 2011) (Table 107.2).

TABLE 107.2 Summary of Findings From Empirical Articles Included in the Review

Author name and year	*N*; age; follow-up	Definition of cannabis cessation	Analyses	Predictors of cessation
Chen and Kandel (1998)	*N* = 706; 15–16 year olds; 20 year follow-up	Having not use marijuana for the past year	Multivariate analyses of life event histories based on discrete-time methods.	Less marijuana use; older age; female; higher education; older age at initiation; more perceived harm of marijuana; used to cope; used socially; less delinquency; more depressive symptoms; became married; became parent
Kandel & Raveis (1989)	*N* = 1222; 15–16 year olds; 13 year follow-up	Having not use marijuana for the past year	Multivariate logistic regression	Less marijuana use; less illicit drug use; used marijuana for social reasons; excellent health self-rating; became married; lower household income
Little et al. (2013)	*N* = 522; 14–20 year olds; 1 year follow-up	Having not use marijuana for the past 30 days	Hierarchical logistic regression	Less baseline marijuana use; less peer substance use; less pro-drug use myths; believing that this was a time of life for experimentation (emerging adulthood)
Sussman and Dent (1999)	*N* = 566; 14–19 year olds; 1 year follow-up	Having not use marijuana for the past 30 days	Logistic regression	Older; less approval for drug use; negative drug use attitudes; less victimization
Sussman and Dent (2004)	*N* = 339; 14–19 year olds; 5 year follow-up	Having not use marijuana for the past 30 days	Logistic regression	Less baseline marijuana use; female gender; young adult; became married; less peer substance use
Van Den Bree & Pickworth (2005)	*N* = 13,718; 11–21 year olds; 1-year follow-up	Having not use marijuana for the past year	Multivariate logistic regression	Less delinquency; less peer substance use; more time spent doing activities with mother

This table provides a summary of the six empirical articles included in the review of the literature, organized around the Theory of Triadic Influence.

Family Influence

Compared to peer influences, parental and sibling influences play a minimal (or indirect) role in cannabis cessation. Contradictory studies show that living with both parents may be integral in self-initiated cessation (Sussman et al., 2012) or have no effect on cessation (Little et al., 2013). Yet, another study by Van den Bree and Pickworth (2005) show that parental bonding, especially for females, may play a contributing role in cessation. That is, girls who engage in activities with their mother are more likely to discontinue cannabis use (Van den Bree & Pickworth, 2005). Spousal interaction may also become influential in the cessation process as engaging in similar activities (ie, cannabis use) reinforces cannabis behavior (Chen & Kandel, 1998).

STRATEGIES NOTED TO HAVE BEEN USED FOR SELF-INITIATED CESSATION

Individuals who self-initiate cannabis cessation appear to employ several strategies for quitting. Most often, individuals divert their attention to other activities and remove themselves from social contexts that may encourage prolonged cannabis use (Boyd et al., 2005; Rooke et al., 2011). *Distraction* (ie, diversion, reinforcement of alternative behavior) and *stimulus removal* (ie, of cannabis and associated stimuli and contexts) are both strategies that help individuals quit, and have been found to be equally effective (Rooke et al., 2011). That is, individuals, on their own, will tend to keep busy doing activities generally incompatible with cannabis use, while, at the same time avoiding cannabis-related cues. Some distraction methods include exercising, seeking social support, and keeping busy (Hughes et al., 2008). Setting life goals, such as obtaining higher education, getting a job, and raising a family are all distraction methods that can deter cannabis use. Stimulus removal includes avoiding people who use cannabis, staying away from situations and environments where cannabis may be present, and throwing away all cannabis and cannabis related items from one's home (Rooke et al., 2011;Weiner et al., 1999). Early on in cessation, some persons may avoid other cues (eg, reading news items) related to cannabis use as well (Fig. 107.1).

IMPLICATIONS FOR FUTURE INTERVENTIONS

Identification of antecedents and strategies associated with self-initiated cannabis cessation has implications for future (formal and nonformal) intervention

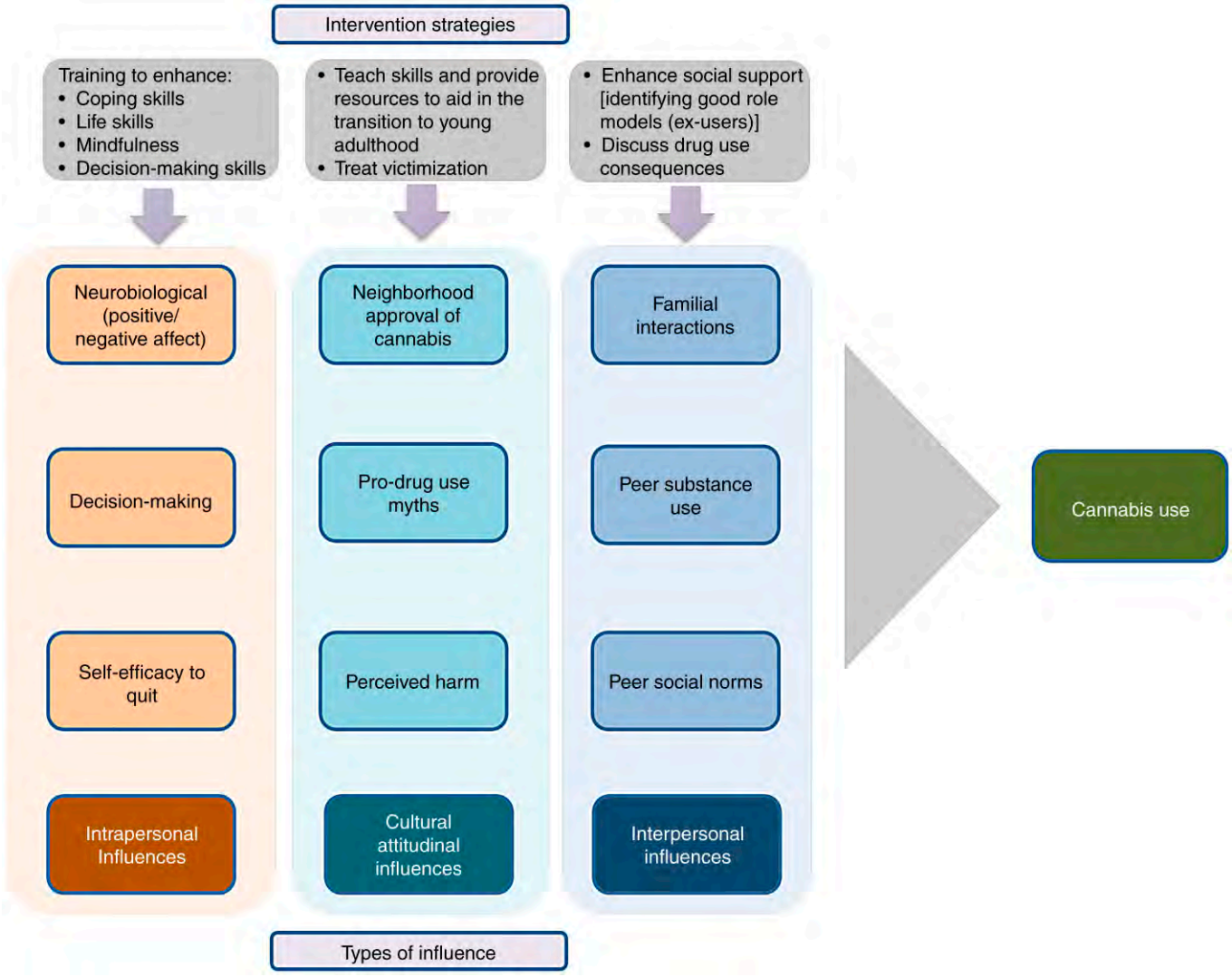


FIGURE 107.1 **Intervention strategies for self-initiated cannabis cessation.** This figure demonstrates possible intervention strategies for self-initiated cannabis cessation using the three streams of the Triadic Theory of Influence as a guide.

programs, and suggests successful programming that targets different streams of the TTI.

Intrapersonal Stream-Derived Interventions

Though much of the brain is developed during the childhood years (under the age of 5), neurobiological functions associated with emotional and social competence continue to be developed during the adolescent years (eg, understanding social cues, facial expressions, and stress adaptation). Adolescence is a critical time period for emotional and social processing that influences the ability to self-regulate one's behavior (Riggs & Greenberg, 2009). Knowing which of these neurobiological systems are most prominent in self-initiated cessation can provide an understanding of general tools necessary to quit. Introducing skills to cope with or reduce stress, such as coping skills, life skills, and mindfulness during childhood and throughout adolescence, may help individuals face stressful situations, and may aid in the quitting process (Sussman et al., 2012). Also, learning decision-making skills that may make one more risk-adverse or confident can have implications for current cannabis users – to lead them to avoid cannabis use situations and initiate distraction strategies, respectively.

Cultural/Attitudinal Stream-Derived Interventions

Cultural/larger social environments can be restructured to foster cannabis intolerant settings. Awareness of the inherent risks associated with acculturation implies the need to recognize the protective factors

for cannabis use associated with certain cultures (eg, family cohesion, parental attachment; Lac et al., 2011). Reinforcement of these cultural values can serve as preventive measures for prolonged cannabis use. Perhaps becoming more involved with one's ethnic culture may act as a distraction mechanism. Furthermore, prevention and/or treatment of victimization may be important to help individuals decide to quit using cannabis. Importantly, helping individuals define life goals and plan for the future, especially as one enters EA, can play a critical part in self-cessation. Introducing the concept of responsibility and learning skills and resources that will help individuals take on such responsibilities may assist in successful transition to young adulthood.

Interpersonal Stream-Derived Interventions

Social support for cannabis cessation, including promoting significant others to be good role models, and identifying good alternative role models, are important factors to be considered in informal future cannabis cessation. Generating discussions among adolescents and their peers about the consequences of cannabis use may discourage continued use, if carefully guided by a facilitator (Sussman & Ames, 2008). Teaching adolescents to be positive role models for their friends and children can cultivate a sustainable environment of cannabis disapproval, if established at a young age. Finally, future interventions should create an open dialogue between ex-users and adolescents about personal experiences associated with the drug. High-risk adolescents for drug use have reported they believe ex-users to be the most credible sources for offering advice on how to quit (Weiner et al., 1999). Interventions that introduce ex-users as positive role models can prompt adolescents to quit once they have seen others succeed.

CONCLUSIONS

Relatively little is known about the prolonged success and failures of self-initiated cannabis cessation. Most studies have been cross-sectional (Little et al., 2013; Sussman & Dent, 1999) or only of short duration (eg, no longer than five years; Kandel & Raveis, 1989; Sussman & Dent, 2004; Van den Bree & Pickworth, 2005), and only one study has looked at adolescent antecedents associated with cannabis cessation through young adulthood and adulthood (Chen & Kandel, 1998). Additional research is needed to determine whether self-initiated cessation is sustainable. For young persons who use cannabis regularly, and who seek the psychosocial benefits of cannabis use, quit attempts may be short lived (Table 107.3). Individuals who have psychological problems (eg, depression, anxiety, externalizing difficulties), and have problems coping with stressful situations are also more likely to relapse as they search for other outlets to cope with their problems (Copersino et al., 2006). Seeking out mental health resources may indirectly result in self-initiated cannabis use cessation. Research has suggested that prevalence of relapse in individuals who choose to quit on their own is much higher, compared to those who have participated in formal treatment (Rooke et al., 2011). Although most adolescents believe that quitting on one's own is the most effective means of achieving cannabis cessation, followed by clinical treatment (ie, "inpatient stay") and jail (Weiner et al., 1999), the susceptibility of nontreatment seeking adolescents to relapse suggest additional assistance may be necessary. On the other hand, while in-depth formalized treatments (eg, inpatient stays) may facilitate more sustained quitting, there is an increased likelihood young persons will become stigmatized through becoming officially labeled as a "cannabis addict." We speculate that a stepped-care approach may be most useful to adolescents, in which treatment begins with as informal an approach as possible.

In the past decade, self-reported cannabis use in adolescents has been on the rise (Johnston, O'Malley, Bachman, & Schulenberg, 2013), and recent legislative measures have placed cannabis in the forefront of political agendas (Donlan, 2013). A concern in most clinic-based programs is the ability to prevent relapse in clinical patients who must face the realities of life once they leave the clinical setting. However, with the study of self-initiated cessation, we at least begin to understand how young persons may manage, and have managed, these obstacles in the real world. This has implications for translational research practices, and may someday provide an evidence-based approach to generate more applicable settings and relatively informal means to assist with cannabis cessation in adolescents and emerging adults.

MINI-DICTIONARY

Acculturation A multidimensional process of blending one's ethnic heritage with that of a new culture where values of one's "home" country are joined with or replaced with new-country values.
Cultural/attitudinal predictor Cultural and attitudinal factors that influence one's subjective beliefs and values about cannabis use.
Decision-making avoidant A decision-making style where one prefers to avoid making high-risk decisions.
Decision-making confident A decision-making style where one exhibits confidence in the decisions one makes.
Emerging adulthood (EA) A developmental period that occurs generally between the ages of 18 and 25

TABLE 107.3 Summary of the Association of Psychosocial Predictors of Cannabis Cessation

Factors	Association	No association	Undecided
Intrapersonal			
Neurobiological disposition			
Positive affect	◦		
Negative affect	◦		
Self-image	◦		
Deviant behaviors	◦		
Cognitive processing	◦		
Decision-making	◦		
Perceived stress			◦
Coping skills			◦
Depression	◦		
Assertiveness		◦	
Sensation seeking		◦	
Social self-control		◦	
Delinquency	◦		
Cultural/attitudinal			
Socioeconomic status	◦		
Social environment			
Neighborhood approval or disapproval of cannabis use	◦		
Neighborhood safety	◦		
Acculturation	◦		
Emerging adulthood	◦		
Cannabis-related consequences attitudes	◦		
Morality of drug use			◦
Religiosity	◦		
Pro-drug use myths	◦		
Perceived harm	◦		
Interpersonal			
Peer social norms	◦		
Peer approval or disapproval of cannabis cessation	◦		
Familial interactions	◦		
Living with both parents		◦	
Parental status	◦		
Being married	◦		
Activities with mother	◦		
Closeness to parents	◦		
Peer substance use	◦		
Family conflict		◦	
Family's substance use		◦	
Marijuana as a social activity	◦		

Note: If discrepancy exists in the literature, the effect of the psychosocial predictor on cannabis cessation was evaluated based on whether more than two articles found the same effect.
This table provides a summary of the psychosocial predictors of cannabis cessation from the six empirical articles included in the review of the literature, organized around the theory of triadic influence.

where individuals transition from adolescence to young adulthood. EA is characterized by five areas of development: (1) establishing one's own identity, (2) a time of feeling in-between, (3) a time of being self-focused, (4) a time of believing there are endless possibilities, and (5) a time where lifestyle is one of instability.

"High." An altered psychological state of heightened perception that occurs as a result of cannabis use. This occurs (in part) when Δ^9-tetrahydrocannabinol (THC), an active ingredient found in cannabis, binds to cannabinoid receptors in the central nervous system.

Intrapersonal predictor Biological/personality factors that influence one's self-efficacy to avoid or use drugs.

Interpersonal predictor Social interaction factors that influence one's desires to conform to the social norms of the individual's immediate social circle.

Pro-drug use myths Inaccurate perceptions or expectancies related to drug use that individuals perceive to be true.

Self-initiated Cessation Self-initiated cessation refers to quitting without seeking formal treatment for cannabis cessation; that is, managing cessation in the real-world setting.

Theory of triadic influence (TTI) A theoretical framework developed by Petraitis, Flay, and Miller (Petraitis et al., 1995) that placed antecedents associated with experimental substance use into a theoretical framework.

References

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Washington, DC: APA.

Arnett, J. J. (2000). Emerging adulthood: a theory of development from the late teens through the twenties. *American Psychologist*, *55*(5), 469–480.

Boyd, S. J., Tashkin, D. P., Huestis, M. A., Heishman, S. J., Dermand, J. C., Simmons, M. S., & Gorelick, D. A. (2005). Strategies for quitting among non-treatment-seeking marijuana smokers. *American Journal on Addictions*, *14*(1), 35–42.

Budney, A. J., Novy, P. L., & Hughes, J. R. (1999). Marijuana withdrawal among adults seeking treatment for marijuana dependence. *Addiction*, *94*(9), 1311–1322.

*Chen, K., & Kandel, D. B. (1998). Predictors of cessation of marijuana use: an event history analysis. *Drug and Alcohol Dependence*, *50*(2), 109–121.

Copersino, M. L., Boyd, S. J., Tashkin, D. P., Huestis, M. A., Heishman, S. J., Dermand, J. C., Gorelick, D. A., et al. (2006). Quitting among non-treatment-seeking marijuana users: reasons and changes in other substance use. *American Journal on Addictions*, *15*(4), 297–302.

Davis, M. L., Powers, M. B., Handelsman, P., Medina, J. L., Zvolensky, M., & Smits, J. A. J. (2014). Behavioral therapies for treatment-seeking cannabis users a meta-analysis of randomized controlled trials. *Evaluation and the Health Professions*, *38*(1), 94–114.

Donlan, T. G. (2013). Should Pot Be Legal? *Barron's*, Available from: http://www.barrons.com/.

Flay, B. R., Snyder, F., & Petraitis, J. (2009). The theory of triadic influence. In R. J. DiClemente, R. A. Crosby, & M. Kegler (Eds.), *Emerging theories in health promotion practice and research* (pp. 451–510). San Francisco, CA: Jossey-Bass.

Franken, I. H., & Muris, P. (2005). Individual differences in decision-making. *Personality and Individual Differences*, *39*(5), 991–998.

Hughes, J. R., Peters, E. N., Callas, P. W., Budney, A. J., & Livingston, A. E. (2008). Attempts to stop or reduce marijuana use in non-treatment seekers. *Drug and Alcohol Dependence*, *97*(1–2), 180–184.

Iversen, L. (2003). Cannabis and the brain. *Brain*, *126*(6), 1252–1270.

Johnston, L. D., O'Malley, P. M., Bachman, J. G., & Schulenberg, J. E. (2013). *Monitoring the future national results on drug use: 2012 overview, key findings on adolescent drug use*. Ann Arbor, MI: Institute for Social Research, The University of Michigan.

*Kandel, D. B., & Raveis, V. H. (1989). Cessation of illicit drug use in young adulthood. *Archives of General Psychiatry*, *46*(2), 109.

Lac, A., Unger, J. B., Basáñez, T., Ritt-Olson, A., Soto, D. W., & Baezconde-Garbanati, L. (2011). Marijuana use among Latino adolescents: gender differences in protective familial factors. *Substance Use and Misuse*, *46*(5), 644–655.

*Little, M. a., Spruijt-Metz, D., Pokhrel, P., Sun, P., Rohrbach, L. A., & Sussman, S. (2013). Predicting self-initiated marijuana use cessation among youth at continuation high schools. *Frontiers in Psychiatry*, *4*, 69.

Maner, J. K., Richey, J. A., Cromer, K., Mallott, M., Lejuez, C. W., Joiner, T. E., & Schmidt, N. B. (2007). Dispositional anxiety and risk-avoidant decision-making. *Personality and Individual Differences*, *42*(4), 665–675.

Mann, L., Harmoni, R., & Power, C. (1989). Adolescent decision-making: the development of competence. *Journal of Adolescence*, *12*(3), 265–278.

MA—Marijuana Anonymous (1989). Marijuana Anonymous web page. Available from https://www.marijuana-anonymous.org/.

Paese, P. W., & Sniezek, J. A. (1991). Influences on the appropriateness of confidence in judgment: practice, effort, information, and decision-making. *Organizational Behavior and Human Decision Processes*, *48*(1), 100–130.

Petraitis, J., Flay, B. R., & Miller, T. Q. (1995). Reviewing theories of adolescent substance use: organizing pieces in the puzzle. *Psychological bulletin*, *117*(1), 67.

Riggs, N. R., & Greenberg, M. T. (2009). Neurocognition as a moderator and mediator in adolescent substance misuse prevention. *American Journal of Drug and Alcohol Abuse*, *35*(4), 209–213.

Rooke, S. E., Norberg, M. M., & Copeland, J. (2011). Successful and unsuccessful cannabis quitters: comparing group characteristics and quitting strategies. *Substance Abuse Treatment, Prevention and Policy*, *6*(30), 1–9.

Schwartz, S. J., Unger, J. B., Zamboanga, B. L., & Szapocznik, J. (2010). Rethinking the concept of acculturation: Implications for theory and research. *American Psychologist*, *65*(4), 237–251.

Sussman, S., & Ames, S. L. (2008). *Drug abuse: Concepts, prevention, and cessation*. New York, NY: Cambridge University Press.

Sussman, S., & Arnett, J. J. (2014). Emerging adulthood: developmental period facilitative of the addictions. *Evaluation & the health professions*, 0163278714521812.

*Sussman, S., & Dent, C. W. (1999). Brief report one-year prospective prediction of marijuana use cessation among youth at continuation high schools. *Addictive Behaviors*, *24*(3), 411–417.

*Sussman, S., & Dent, C. W. (2004). Five-year prospective prediction of marijuana use cessation of youth at continuation high schools. *Addictive Behaviors*, *29*(6), 1237–1243.

Sussman, S., Stacy, A. W., Dent, C. W., & Simon, T. R. (1996). Marijuana use: current issues and new research directions. *Journal of Drug Issues*, *26*(4), 695–733.

Sussman, S., Lisha, N., & Griffiths, M. (2011). Prevalence of the addictions: a problem of the majority or the minority? *Evaluation and the Health Professions*, *34*(1), 3–56.

Sussman, S., Sun, P., Rohrbach, L. A., & Spruijt-Metz, D. (2012). One-year outcomes of a drug abuse prevention program for older teens and emerging adults: evaluating a motivational interviewing booster component. *Health Psychology*, *31*(4), 476–485.

Valente, T. W., Ritt-Olson, A., Stacy, A., Unger, J. B., Okamoto, J., & Sussman, S. (2007). Peer acceleration: effects of a social network tailored substance abuse prevention program among high-risk adolescents. *Addiction*, *102*(11), 1804–1815.

*Van den Bree, M. B., & Pickworth, W. B. (2005). Risk factors predicting changes in marijuana involvement in teenagers. *Archives of General Psychiatry*, *62*(3), 311–319.

Weiner, M. D., Sussman, S., McCuller, W. J., & Lichtman, K. (1999). Factors in marijuana cessation among high-risk youth. *Journal of Drug Education*, *29*(4), 337–358.

CHAPTER

108

Treating Cannabis-Dependent Adolescents with Family Therapy: The case of Multidimensional Family Therapy

H. Rigter

Youth Interventions Foundation, Curium, Department of Child and Adolescent Psychiatry, Leiden University Medical Center, Leiden, The Netherlands

SUMMARY POINTS

- In adolescents, cannabis dependence often is part of multiple problem behavior. Treatment should focus on all problem behaviors to yield lasting effects on cannabis abuse and other behaviors.
- Cannabis dependent adolescents need treatment when there is a risk the condition will persist into adulthood. Risk factors for persistence are early start in life of cannabis use, and the combination of cannabis abuse with other problem behaviors.
- There is a range of cannabis treatments. The best treatment option for cannabis dependent adolescents consists in family/systems therapies, most notably Multidimensional Family Therapy (MDFT).
- An important feature of MDFT is its exceptional ability to interest adolescents and their parents to accept and follow the treatment. Key here are so-called motivational interventions, a rich arsenal of ways to enhance treatment motivation.
- MDFT is effective in populations with different ethnic backgrounds, and across countries (United States, Western Europe), despite huge variations in referral practices, treatment settings, and treatment policies.
- Implementation of a treatment program is hard work, requiring interventions at the levels of the therapist, the team, the treatment center, the funding agency, and local/regional and national policy makers. Each country has its own set of implementation challenges.

KEY FACTS OF CANNABIS DEPENDENCE

- Personal and social troubles of adolescents more strongly predict that they will develop cannabis dependence, than do the dose and potency of the cannabis preparation.
- At least half of cannabis dependent youth exhibit other problem behaviors as well, such as truancy, or criminality. Mental comorbidity is common.
- In many young adults, cannabis dependence dissipates without any treatment being given. However, in adolescents starting to take cannabis early in life, and showing other problem behaviors as well, cannabis dependence often persists, unless treatment is being offered.
- Cannabis dependence in youth is sensitive to treatment.
- Treatment works best if it takes into account the multiplicity of problem behaviors, rather than focusing on just one condition, such as cannabis dependence.

LIST OF ABBREVIATIONS

BSFT	Brief Strategic Family Therapy
CBT	Cognitive behavioral therapy
DSM	Diagnostic Statistical Manual, version IV
EMCDDA	European Monitoring Centre for Drugs and Drug Abuse
FFT	Functional family therapy

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00126-5
Copyright © 2017 Elsevier Inc. All rights reserved.

INCANT	The International Cannabis Need of Treatment trial
MDFT	Multidimensional family therapy
MST	Multisystemic therapy
NREPP	National Registry of Evidence-based programs and practices
SAMSHA	Substance Abuse and Mental Health Services Administration, US Ministry of Health

DO CANNABIS DEPENDENT YOUTH NEED TREATMENT?

This chapter focuses on adolescents, aged 12 through 18 years. Many youth experiment with taking cannabis, often occasionally but sometimes frequently. Across European Union member states and allied countries, at least 2% of youth consume cannabis daily or nearly every day (European Monitoring Centre for Drugs and Drug Abuse (EMCDDA), 2014a).

Frequent use of the drug does not necessarily lead to a cannabis use disorder, such as cannabis dependence. There is no simple dose-response relationship here. Personal and social troubles are stronger predictors of upcoming cannabis dependence than the number of joints smoked and the potency of the cannabis preparation are (van der Pol et al., 2013b).

Cannabis dependence may be transient. Over the years, many adults stop using cannabis or diminish their level of consumption on their own accord, without treatment. In a Dutch study, seven out of ten young cannabis dependent adults reduced their level of consumption of the drug in 3 years' time such that the diagnosis "cannabis dependence" no longer applied. Most of them had not sought any professional help (van der Pol, 2014).

In adolescents, however, cannabis dependence is quite persistent if starting at a young age and when combined with other problem behavior (Chassin, 2008; Hussong, Curran, Moffitt, Caspi, & Carrig, 2005). If untreated, cannabis dependence may affect education and life satisfaction levels of the users (Fergusson & Boden, 2008). When cannabis dependence might get persistent, treatment is to be recommended (van der Pol, 2014).

Frequent use of cannabis and cannabis dependence are associated with concurrent problem behavior, such as aggression, delinquency and truancy, and with mental comorbidity (Hussong et al., 2005; Phan et al., 2011; van der Pol et al., 2013a). Many young people with cannabis dependence are best described as youth with multiple problem behavior. Therefore, treatment needs to be multifocal, that is, it should not only address cannabis use, but other problems as well. The therapist may help the adolescent in reducing drug consumption, but if other problems as the ones mentioned are ignored, the youth will soon relapse into abusing drugs. For a treatment to be lastingly successful, it should target not just cannabis, but all major problem behaviors (Rigter et al., 2014).

So, cannabis dependent youth may need treatment. However, they rarely seek treatment on their own initiative. Treatment seeking for cannabis dependence is on the increase for adults, but less so for adolescents (European Monitoring Centre for Drugs & Drug Abuse, 2014a). In the five-cities European cannabis treatment trial INCANT—to be discussed subsequently—few adolescents sought treatment themselves. Quite a few youth in Brussels and Paris were referred to treatment by parents, one important reason in Paris being interim school reports warning their child might fail to pass to the next class at the end of the year (which, in Paris, would mean the young person would have to be expelled from school). One way to look at referral is by distinguishing externally coerced from noncoerced (self-determined) treatment seeking. Coercion is not only executed by Justice authorities, but also by treatment and care agencies or sometimes schools when the youth is sanctioned for not accepting treatment, such as "being kicked out." Coerced referral was common in The Hague (88%), Geneva (73%) and Berlin (54%), and uncommon in Brussels and Paris (Phan et al., 2011). Clearly, treatment and referral systems vary widely between countries. A positive outcome noticed in just one country cannot be taken as evidence for universal effectiveness of the intervention program concerned.

WHICH TREATMENTS ARE EFFECTIVE IN YOUTH WITH A CANNABIS USE DISORDER?

Adolescents with problem behavior can be treated individually or in groups of peers. Another treatment option is systems therapies, comprising family therapies as discussed in this chapter. Family therapies include sessions with the parents and with the family (parents plus youth), respectively, while other sessions—depending on the type of program—target the individual adolescent.

To reach youth who shy away from face to face treatment, treatment centers are experimenting with interventions to be offered on the computer or through internet. These interventions may be based on algorithms giving the youth access to information and to relevant tips to reduce substance use. Other interventions are similar, but allow for limited internet guidance by a therapist. According to a recent meta-analysis (Tait, Spijkerman, & Riper, 2013), such computer and internet assisted interventions are helpful in reducing cannabis use. However, the size of the effect is small—smaller than for treatments with face-to-face contact between user and therapist.

The cannabis users participating in the research studies analyzed were of all ages, but mostly adolescents and young adults (Tait et al., 2013). They were regular users of cannabis, often without clearly established cannabis dependence.

As for face to face treatment, interventions such as motivational interviewing, contingency management and cognitive behavioral therapy (CBT) should be mentioned. Elements of these approaches have been incorporated in family therapies. In adults, CBT appears to diminish cannabis and other substance abuse. This conclusion is tentative as an important part of the evidence relies on the outcomes of randomized trials in which CBT was compared with placement of persons on a waiting list (Davis et al., 2015), which is not top of the bill research methodology.

Also in adolescents, CBT may decrease cannabis and other substance use, but the evidence here is even weaker. Going from meta-analysis to meta-analysis, the balance for adolescent substance abuse CBT swings from effective to noneffective. Taken together, the verdict tends to be "effective" (Bender, Tripoli, Sartechi, & Vaughn, 2011; Waldron & Turner, 2008).

Family therapies are evidence based treatments for substance abusing adolescents (Baldwin, Christian, Berkeljon, & Shadish, 2012; Bender et al., 2011). In meta-analyses, these therapies as a group—data pooled for analysis purposes—are at least as effective as CBT in diminishing substance use. In trials directly comparing family therapy, such as Multidimensional Family Therapy (MDFT), with CBT, family therapy scored better in particular subgroups (the most impaired youth) and on several key measures. One example is a trial comparing MDFT with CBT. Both treatments helped adolescents to reduce their substance use as measured at 6-months follow-up, but the effect of MDFT grew stronger afterwards while the effect of CBT became weaker (Henderson, Dakof, Greenbaum, & Liddle, 2010).

Family therapies include MDFT, Multisystemic Therapy (MST), Functional Family Therapy (FFT) and Brief Strategic Family Therapy (BSFT). Analyses of pooled data indicate that these treatment programs are beneficial for adolescents in several ways. Cannabis consumption and other substance use decline. Delinquency, measured by the number of criminal offenses committed, decreases (Baldwin et al., 2012; Von Sydow, Retzlaff, Beher, Haun, & Schweitzer, 2013).

MDFT, MST, FFT, and BSFT have never been compared with each other in randomized controlled trials. The presently available meta-analysis data do not allow us to tell which family therapy works best (Baldwin et al., 2012). The EMCDDA decided to evaluate outcome data of cannabis treatment programs for adolescents using strict effectiveness criteria. This expert center concluded that only one treatment program met all criteria set, that is, MDFT (European Monitoring Centre for Drugs & Drug Abuse, 2013). A systematic literature review by the EMCDDA confirmed MDFT's value (European Monitoring Centre for Drugs & Drug Abuse, 2014b). The US Ministry of Health acknowledges just one family therapy as being helpful in combating adolescent substance abuse and other problem behavior, while being implementable in daily clinical practice. That family therapy was MDFT, http://www.nrepp.samhsa.gov/CERSeries.aspx.

The remainder of this chapter will focus on MDFT.

MULTIDIMENSIONAL FAMILY THERAPY IN A NUTSHELL

MDFT has been developed by Howard Liddle, presently at the University of Miami Medical Center, and his colleagues. It is a comprehensive, family-centered and developmentally-oriented treatment program for adolescents presenting with single or multiple problem behavior combinations of substance abuse, truancy and delinquency (Liddle & Rigter, 2013; Rigter et al., 2014). Mental and behavioral comorbidity is common among these youth.

Underlying theory in MDFT is the notion that an adolescent's problem behavior is multidimensionally determined, that is, is being shaped by factors from all major domains in the life of the youth. The first domain is the youth themselves: their personality, their experiences, their stage of development, their genes. The second one pertains to the parents, and the third one to the family: parents and young people in mutual interaction, possibly extending to other family members. The fourth domain consists of systems—social groups and structures—outside the family. Friends and peers of the youth are important. School, work, and leisure time activities do matter, as well. Police, Justice, and probation officers are also part of the fourth domain, especially when the young person has been arrested or convicted. In line with the distinction in domains, an MDFT therapist holds four types of sessions, that is, with the young person alone; with the parent(s) alone; with the family (youth and parents); and with representatives of other systems (friends, school mentor, etc.) present.

Aim of MDFT is to reduce substance abuse, to decrease antisocial behavior, and to prevent criminal recidivism, by achieving improvement in all four domains. The means to get these goals realized include the interventions listed in Table 108.1.

MDFT is carried out by certified MDFT therapists, who work in teams, one being the supervisor of the team. Training is practice-oriented, with the trainer regularly visiting the treatment center, and holding detailed consultation calls, and with the trainee sending in video-

TABLE 108.1 Main Interventions in MDFT

- Enhancing the motivation of the youth and parent(s) to accept and complete treatment
- Teaching the youth how to avoid and handle risky, problem behavior eliciting situations and how to be more selective in the choice of friends and leisure time activities
- Relapse prevention: helping the youth to resist substance abuse and crime provoking stimuli
- Improving family functioning and communication between family members, and resolving and preventing conflicts between parent(s) and their child
- Strengthening parental skills
- Outreaching interventions: the therapist contacts other systems as mentioned and wins support for arrangements (eg, adapted school program; or diverting the youth away from detention to community) that would benefit the youth.

tapes of treatment sessions in order to receive feedback and advice (Rigter et al., 2014).

MDFT is based on a manual, but is to some extent flexible. The therapist is expected not to just follow rules, but to take his or her own responsibility as well. On average, there are two sessions per week, for 4–6 months. The sessions take place at the therapist's office, or the family's home, or any other convenient place. Being an MDFT therapist means being the spider in the web for all treatment and guidance issues relevant to the particular young person and their family.

THE EFFECTS OF MULTIDIMENSIONAL FAMILY THERAPY

MDFT, delivered in outpatient settings, has been examined extensively in the United States. Eight randomized trials have been completed and another three are underway (Liddle & Rigter, 2013; Rigter et al., 2014). In these trials the researchers followed a methodologically strong approach by not comparing MDFT with an inactive control condition, but rather with active comparison treatments, namely:

- Youth: individual counseling,
- Youth: group counseling,
- Parents/families: group counseling,
- Youth: individual CBT,
- Youth: residential treatment.

An independent replication study has been carried out in Europe (2006–10). The study was named INCANT, the International Cannabis Need of Treatment trial (2006–10). It was set up on the initiative of government members of five Western European countries (Rigter et al., 2010). Subjects were 450 adolescents and their parents visiting treatment sites in Brussels, Berlin, Paris, The Hague, and Geneva (60–120 cases per site). The sites were quite different (Table 108.2), yet treatment results were similar.

INCANT was a randomized controlled trial with an open-label, parallel group design. The study compared 6 months of MDFT with individual psychotherapy, including CBT, at and across the sites mentioned. Assessments were at baseline and at 3, 6, 9, and 12 months after randomization. Fig. 108.1 shows the trial flow diagram. Note the low percentage of cases that were lost to 12-months follow-up (no more than about 10%); the data are strong because most study participants were willing to remain in the study. Of note, too, was the choice of the researchers to not exclude cases from the trial if not absolutely needed. The trial population reflected the diversity of adolescents and families seen in every day's clinical practice, which lends credence to the validity of the study outcomes. 85% of the INCANT adolescents were male. They all had a cannabis disorder, mostly

TABLE 108.2 Characteristics of the Treatment Sites in the International Cannabis Need of Treatment Trial

Site	Treatment sector	Location	Public or private
Belgium	Addiction care; mental health care	Brussels University affiliated outpatient clinic	Public MDFT funded by the federal government
France	Addiction care	Paris and suburbs Part of national addiction treatment infrastructure	Public MDFT funded by the federal government
Germany	Addiction care and youth care	Berlin Part of regional (state level) addiction treatment infrastructure	Public MDFT funded by federal and local governments
The Netherlands	Addiction care Forensic youth care	The Hague Part of national and regional addiction and forensic treatment infrastructures	Public Reimbursed by health insurance funds
Switzerland	Addiction care	Geneva Part of regional addiction and forensic treatment infrastructures	Public–private mix Mostly reimbursed by health insurance and social funds

Adapted from Rigter et al. (2013), courtesy of Drug and Alcohol Dependence

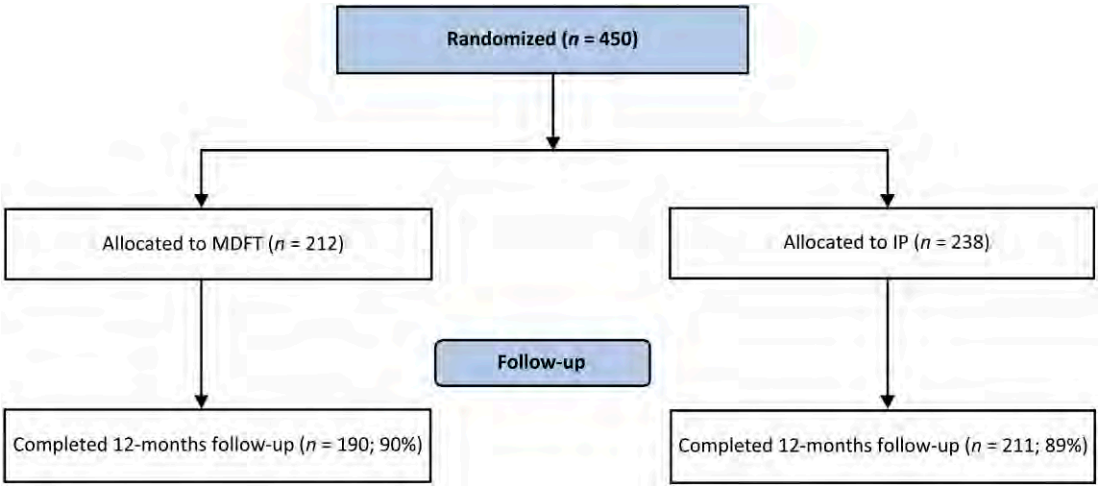


FIGURE 108.1 The International Cannabis Need of Treatment trial flow diagram. Across five West European sites, 450 adolescents plus one or both of their parents were included in the INCANT study, randomized across two treatment conditions and followed up for 3, 6, 9, and (shown here) 12 months. MDFT, Multidimensional Family Therapy; IP, individual psychotherapy (comparison treatment); *n*, the number of families. *Adapted from Rigter et al. (2011b). Copyright holder H. Rigter, the present author.*

(80%) cannabis dependence. One-third was delinquent (Phan et al., 2011).

INCANT trial outcomes included:

- The ability of European therapists to properly carry out MDFT,
- Treatment retention of adolescents and their parents,
- Treatment satisfaction,
- Reduction of cannabis-related problems,
- Reduction of criminality-related behavioral symptoms.

MDFT-trained European therapists scored well on measures of treatment integrity, also called "adherence," even somewhat better than American colleagues in US MDFT trials (Rowe et al., 2013). In INCANT, like in the US trials, MDFT succeeded in engaging and retaining 90% of the adolescents and their parents in treatment, while the corresponding percentage for the comparison treatment, with one exception, was two to three times lower (Rowe et al., 2013). Adolescents and parents were pleased with MDFT, more so than with the comparison treatment. Adolescents and parents agreed when rating treatment satisfaction. High treatment satisfaction scores predicted better treatment outcomes (Phan, 2014).

Following treatment, the diagnosis "cannabis dependence" became less prevalent. In MDFT, six out ten originally cannabis dependent youth were no longer classified as such 1 year after randomization. The comparison treatment was less effective in this regard (Fig. 108.2; Rigter et al., 2013). In addition, both treatments reduced rates of cannabis consumption. In adolescents who were the most severe users of cannabis at baseline—the "heavy cases"—MDFT outperformed individual psychotherapy, the comparison treatment, in decreasing cannabis use rates (Rigter et al., 2013).

As for so-called secondary outcomes, MDFT more than comparison treatment diminished symptoms of externalizing mental disorders, which are commonly seen as risk factor for developing criminal behavior (Schaub et al., 2014). The five European sites differed markedly in terms of addiction care, youth care, school policies and juvenile Justice procedures (Table 108.2; Rigter et al., 2011b). MDFT was effective in all settings and under all circumstances (Rigter et al., 2011a, 2013). One-third of the INCANT adolescents was part of an ethnic minority group. MDFT was effective regardless of ethnic background and also regardless of referral being coerced or not (Rigter et al., 2013; Schaub et al., 2014).

IMPLEMENTATION OF MDFT IN EUROPE

When the word of MDFT spread, therapists in the Netherlands wished to be trained in this treatment program. To this end, MDFT Academy was established in 2008. The Academy is now part of the Youth Interventions Foundation. In other words, a move was made from research to implementation. We shall address a few implementation issues here.

Accreditation

Increasingly, treatment programs need the green light of accreditation authorities and professional societies to

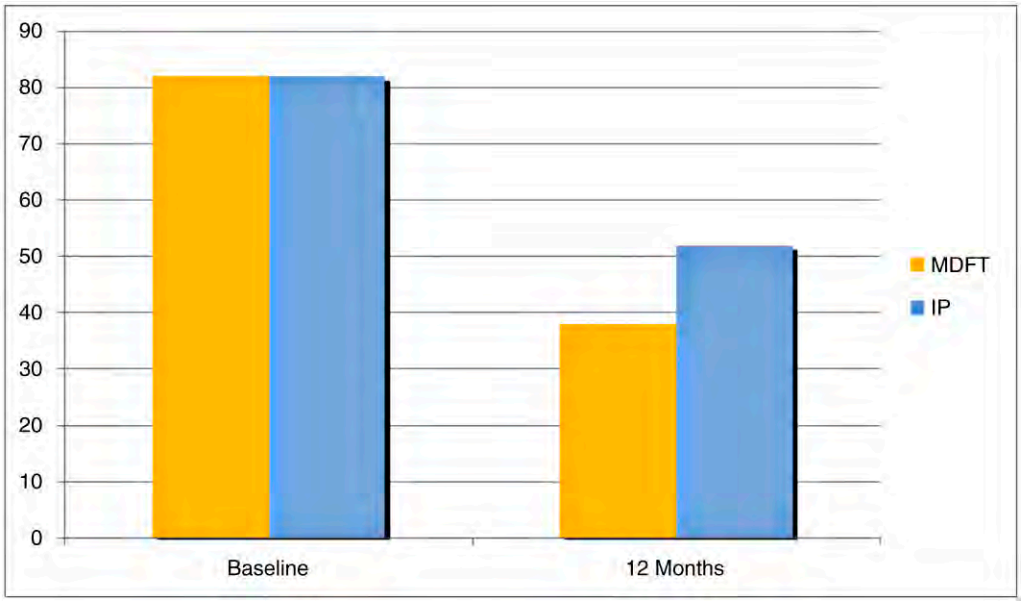


FIGURE 108.2 Multidimensional family therapy exceeds active comparison treatment in decreasing the rate of cannabis dependence. Proportion (percentage) of adolescents presenting with cannabis dependence diagnosis across treatment sites at baseline and 12-month follow-up. Results from the International Cannabis Need of Treatment trial trial. Proportion: percentage; MDFT, Multidimensional Family Therapy; IP, individual psychotherapy. *Adapted from Rigter et al. (2013), courtesy of Drug and Alcohol Dependence.*

get accepted and reimbursed as evidence based therapies. MDFT has been acknowledged and recommended by a number of US bodies, including the Substance Abuse and Mental Health Services Administration of the federal Ministry of Health (SAMSHA) (NREPP, 2014), and the National Institute of Justice (Crime Solutions, 2012). SAMSHA not only confirmed that MDFT is effective, but also selected MDFT as the only family therapy among five top treatment programs that "can be implemented with many different populations by providers of mental health and substance abuse services" (NREPP, 2014).

Accreditation of a treatment program in the United States does not suffice for European policy makers to follow suit. It needs to be proven that a treatment such as MDFT is effective and implementable in European context. INCANT provided the evidence. MDFT has now been accepted at the European Union level (EMCDDA, 2014b) and in several Western European countries. The highest hurdle to take was the Accreditation Committee of Behavioral Interventions, set up by the Dutch Ministry of Safety and Justice. This Committee uses ten accreditation criteria, requiring massive documentation. The treatment program needs to be effective and should be based on a convincing theory. Its target group—the persons to be treated—should be clearly delineated. Risk factors and protective factors should be spelled out and should be reflected in the treatment interventions to be undertaken. The treatment program should be phased, with motivating clients to accept and follow treatment at the beginning and offering aftercare at the end. Having a treatment quality assurance monitor is deemed essential. The Committee accredited MDFT in 2013. The accreditation status helped treatment centers to get MDFT reimbursed by funding agencies, such as health and social insurance companies.

Therapists

For a therapist, MDFT means breaking with old routines. From one session with the young person/family once every 2 or 3 weeks, to two or three sessions a week. From sessions exclusively held at the office of the therapist to sessions also in the family's home. From focus on just one problem behavior, such as cannabis abuse, to addressing all major problem behaviors and also family dysfunction. From being just one of the professionals helping the adolescent and the family to being the family's spider in the web. From working 9–5 to being accessible for the family also outside office hours.

One would expect that therapists would resist such changes in routines. Rather, therapists—more than treatment centers and policy makers—were the driving force in getting MDFT implemented.

Managers of Treatment Centers

Therapists may wish to change their ways, but they depend on the managers of their units. Implementation of a treatment program is bound to fail if managers are ignored or neglected. In MDFT, practitioners regularly visit the managers and convene meetings of managers to discuss issues that are relevant for their work.

Requirements for MDFT Teams

As said, MDFT therapists are part of a team. The team consists of 3 to 6 therapists, one being the supervisor. The team meets once every week or 2 weeks to discuss cases using session planning documentation, intervision, and videotaped recordings of treatment sessions. In addition, the supervisor carries out live supervision, that is, watching a session proceed by looking through a mirror screen.

Training

Candidate therapists and supervisors have a college or university education. They are psychologists, social workers, psychiatrists, and others, having at least a couple of years' experience in delivering psychosocial help and treatment to youth and families. The candidates are keen to learn, pragmatic, caring, nonjudgmental, energetic and skilled in communication. The supervisor must have leadership qualities. Training is given by certified trainers, recruited from the ranks of MDFT supervisors. At present in Europe, there are eight Dutch, two Belgian, three German, one French and one Swiss trainers.

Training to become a MDFT therapist or supervisor takes place in two 1-year steps. There is limited classroom (theoretical) training, namely 8 days in Year 1, followed by written exams. Training emphasis is on guiding therapists and supervisors on "the work floor," that is, in their daily clinical work. MDFT candidates videotape treatment and (supervisors) supervision sessions. The recordings are rated for treatment adherence and for treatment and supervision competence by the trainers and by independent evaluators, with ample feedback being given. The trainer regularly visits the team (site visits) for case review and live supervision. In addition, there are biweekly consultation calls between the trainer and the team.

At the end of Year 1, candidates (therapists and supervisors) passing certification requirements receive the MDFT Basic Level certificate. Training continues in Year 2, in a similar way with booster training sessions by top experts being added. At the end of Year 2, candidates meeting the requirements will be handed the MDFT Master Level certificate, which is valid indefinitely (Table 108.3).

Licensing

MDFT Academy will grant a free license to practice MDFT to teams having at least three Master Level certified members. Once every 3 years, the license is to be renewed, following an audit showing that the team is still doing fine.

Number of MDFT Teams in Europe

Between 2008 and 2014, close to 40 MDFT teams have been trained in the Netherlands or are still in training. Other countries are gearing up. Belgium has two teams, Finland nine, France four (with five more to come), Germany four, and Switzerland one. In 2015, five teams will be trained in Estonia. In the United States, approximately 60 MDFT teams are operational.

Innovation

MDFT is flexible. It allows for practice adaptations rendering this treatment program suitable for use in new target groups and in new treatment settings.

From the start, MDFT was an outpatient treatment program. It still is, but nowadays MDFT is also practiced in residential settings, such as Juvenile Detention Units in the Netherlands. Family oriented work (by all prison personnel) and MDFT are started right after the young person has been detained (Mos, Jong, Eltink, & Rigter, 2011). Sessions are held inside the Unit, during visiting hours of the parents, and during furlough when the young person is allowed to go home during weekends, initially under guidance, and then unguided. Sessions are frequent at the beginning of detention, infrequent in the middle part of detention, and increase in frequency a few months before the young person is being released. When the young person is free, MDFT continues for a while in an outpatient setting. Detention may take years. Therefore, detained/released young people may remain in MDFT treatment until the age of 23.

In the Netherlands, MDFT has also been adapted for use in residential youth care settings, targeting youth being placed out of home for any nonpenal reason (Rigter, Erftemeyer, & Mos, 2011a). In these settings, MDFT is applied to shorten residential stay, and to provide continuity of care for young people leaving the institution. One other application is to prevent residential placement at the last moment. Think of troubled families

TABLE 108.3 Requirements for Therapists Certified to Carry Out MDFT

- MDFT psychotherapists are *social workers, psychologists, or psychiatrists* with experience in treating youth/families
- The therapist is the key figure for the family for all problems (*spider in the web*)
- The therapist works in a *team* (3–6 therapists, one being the team's supervisor)
- *Caseload* at any given time: eight families per therapist
- The therapist is prepared to *leave the office* (eg, for sessions at the family's home), and to be contacted outside office hours
- He or she is interested in combining treatment and outreaching *case management*

with a young person to be placed out of home, with a juvenile judge's blessing, being offered the last-minute option of MDFT to avoid getting separated. In our initial experience in the residential youth care institute in The Hague in 2012, 81% of the families (youth and parents) concerned opted for placement-avoiding MDFT. MDFT healed families. None of the youth returned to be taken in after all. Preventing residential placement would save costs, adding to earlier findings that MDFT is a cost saving treatment program (French et al., 2003; Zavala et al., 2005).

Finally, MDFT has been adapted for use in youth with mild mental retardation. The effects of this application will be monitored in the next few years.

MINI-DICTIONARY

Accreditation Strict assessment of a treatment program by an authority, resulting in formally acknowledgment that the program is useful.

Cannabis dependence In DSM-IV, the most severe of the two cannabis use disorders, the other one being cannabis abuse. To classify for the diagnosis cannabis dependence, one must meet at least 3 out of 7 criteria relating to tolerance, withdrawal, inability to stop consumption, interference with daily activities and disruption of social life.

Certification Providing trainees with a diploma as testimony for having met the training requirements.

Domain Important and treatment relevant social realm in the life of adolescents. Domains include parents, family and systems (social structures) outside the family, such as friends, school, work, leisure time activities.

Evidence based Proof that a treatment program is consistently achieving its intended outcomes. There are "grades" of evidence. In this chapter, the term applies to the highest grade of evidence, with the judgment being based on practice-relevant data from at least several randomized controlled trials, involving active comparison treatments, with at least one trial having been conducted by researchers independent of the developers of the treatment program.

Family therapy A form of systems therapy. "Systems" include the family (the youth, his or her parents, other family members), but also other social structures (see Domain). Family therapy usually involves sessions with the parents and with the family (= youth plus parents). Some family therapies, such as MDFT, also have sessions with the youth alone.

Implementation Transferring insights from research to daily clinical and managerial practice through a diverse set of interventions at a variety of policy levels.

System A social circle, network. For instance, family, friends, school.

Treatment integrity Also called treatment adherence. The extent in which a therapist delivers the treatment properly, that is, according to preset specifications. This is assessed in MDFT by having treatment session tapes rated by independent staff. Also by having a trainer formally evaluate treatment competence, session planning, case assessment and the drawing up of the treatment plan.

Treatment program The word "program" signifies that the therapy is not just a single intervention, but an array of interventions, varying across stages of treatment and per domain and treatment goal.

Treatment retention The extent in which a youth and his or her parents accept the treatment offered and remain in treatment for a specified time or until a particular treatment goal has been achieved.

References

Baldwin, S. A., Christian, S., Berkeljon, A., & Shadish, W. R. (2012). The effects of family therapies for adolescent delinquency and substance abuse: a meta-analysis. *Journal of Marital and Family Therapy*, *38*, 281–304.

Bender, K., Tripoli, S. J., Sartechi, C., & Vaughn, M. G. (2011). A meta-analysis of interventions to reduce adolescent cannabis use. *Research on Social Work Practice*, *21*, 153–164.

Chassin, L. (2008). Juvenile justice and substance use. *Future of Children*, *18*, 165–183.

Crime Solutions: National Institute of Justice, Office of Justice Programs. (2012). *Multidimensional Family Therapy*. Washington, DC: NIJ. Available from http://crimesolutions.gov/ProgramDetails.aspx?ID=267

Davis, M. L., Powers, M. B., Handelsman, P., Medina, J. L., Zvolensky, M., & Smits, J. A. (2015). Behavioral therapies for treatment-seeking cannabis users: a meta-analysis of randomized controlled trials. *Evaluation and the Health Professions*, *38*(1), 94–114.

European Monitoring Centre for Drugs and Drug Abuse. (2013). *Treatment options for cannabis users*. Lisbon, Portugal: EMCDDA. Available from http://www.emcdda.europa.eu/best-practice/treatment/cannabis-users

European Monitoring Centre for Drugs and Drug Abuse. (2014a). *European Drug Report. Trends and developments*. Lisbon, Portugal: EMCDDA.

European Monitoring Centre for Drugs and Drug Abuse. (2014b). *Multidimensional family therapy for adolescent drug users: a systematic review*. Lisbon, Portugal: EMCDDA, EMCDDA Papers.

Fergusson, D. M., & Boden, J. M. (2008). Cannabis use and later life outcomes. *Addiction*, *103*, 969–976.

French, M. T., Roebuck, C., Dennis, M. L., Godly, S. H., Liddle, H. A., & Tims, F. M. (2003). Outpatient marijuana treatment for adolescents: economic evaluation of a multisite field experiment. *Evaluation Review*, *27*, 421–459.

Henderson, C. E., Dakof, G. A., Greenbaum, P. E., & Liddle, H. A. (2010). Effectiveness of Multidimensional Family Therapy with higher-severity substance abusing adolescents: report from two randomised controlled trials. *Journal of Consulting and Clinical Psychology*, *78*, 885–897.

Hussong, A. M., Curran, P. J., Moffitt, T. E., Caspi, A., & Carrig, M. (2005). Substance abuse hinders desistance in young adults' antisocial behavior. *Developmental Psychopathology*, *16*, 1029–1046.

Liddle, H., & Rigter, H. (2013). How developmental research and contextual theory drive clinical work with adolescents. *Harvard Review of Psychiatry*, *21*, 200–204.

Mos, K., Jong, J., Eltink, E., & Rigter, H. (2011). *Wegwijzer voor toepassing van MDFT in justitiële jeugdinrichtingen en aansluitende ambulante zorg (Guideline for using MDFT in juvenile detention units and in outpatient aftercare)*. Leiden: MDFT Academie, in Dutch.

NREPP (SAMSHA's National Registry of Evidence-based programs and practices). (2014). *Multidimensional Family Therapy: an Informational Resource*. Rockville, MD: SAMHSA, Comparative Effectiveness Research Series. Available from http://www.nrepp.samhsa.gov/CERSeries.aspx

Phan, O. (2014). *Treatment satisfaction outcomes of the European INCANT MDFT trial.* , Presentation at the conference Linking systemic practice and systemic research Heidelberg, March 6–8, 2014.

Phan, O., Henderson, C. E., Angelidis, T., Weil, P., van Toorn, M., Rigter, R., Soria, C., & Rigter, H. (2011). European youth care sites serve different populations of adolescents with cannabis use disorder.

Baseline and referral data from the INCANT trial. *BMC Psychiatry*, *11*, 110. Available from http://www.biomedcentral.com/1471-244X/11/110

Rigter, H., Pelc, I., Tossmann, P., Phan, O., Grichting, E., Hendriks, V., & Rowe, C. (2010). INCANT: a transnational randomized trial of multidimensional family therapy versus treatment as usual for adolescents with cannabis use disorder. *BMC Psychiatry*, *10*, 28, Available from http://www.biomedcentral.com/1471-244X/10/28.

Rigter, H., Erftemeyer, L., & Mos, K. (2011a). *Wegwijzer voor toepassing van MDFT in jeugdzorgplus en residentiële jeugdzorg (Guideline for using MDFT in residential youth care*. Leiden: MDFT Academie.

Rigter, H., Pelc, I., Phan, O., Tossmann, P., Hendriks, V., Rowe, C., & Schaub, M. (2011b). *Report on the INCANT Study. Multidimensional Family Therapy in Europe as a treatment for adolescents with cannabis use disorder and other problem behaviours*. Rotterdam, the Netherlands: Erasmus MC, Department of Public Health, www.incant.eu.

Rigter, H., Henderson, C. E., Pelc, I., Tossmann, P., Phan, O., Hendriks, V., Schaub, M., & Rowe, C. L. (2013). Multidimensional Family Therapy lowers the rate of cannabis dependence in adolescents: a randomised controlled trial in Western European outpatient settings. *Drug and Alcohol Dependence*, *130*, 85–93.

Rigter, H., Rowe, C., Gantner, A., Mos, K., Nielsen, P., Phan, O., Spapen, P., & Liddle, H. (2014). From research to practice: the international implementation of Multidimensional Family Therapy. In N. el-Guebaly, M. Galanter, & M. Carrá (Eds.), *Textbook of addiction treatment: International perspectives*. Berlin/Heidelberg: Springer Verlag, Chapter 43.

Rowe, C., Rigter, H., Henderson, C., Gantner, A., Mos, K., Nielsen, P., & Phan, O. (2013). Implementation fidelity of Multidimensional Family Therapy in an international trial. *Journal of Substance Abuse Treatment*, *44*, 391–399.

Schaub, M. P., Henderson, C. E., Pelc, I., Tossmann, P., Phan, O., Hendriks, V., Rowe, C., & Rigter, H. (2014). Multidimensional family therapy decreases the rate of externalising behavioural disorder symptoms in cannabis abusing adolescents: outcomes of the INCANT trial. *BMC Psychiatry*, *14*, 26. doi: 10.1186/1471-244X-14-26.

Tait, R. J., Spijkerman, R., & Riper, H. (2013). Internet and computer based interventions for cannabis use: a meta-analysis. *Drug and Alcohol Dependence*, *133*(1). Available from http://dx.doi.org/10.1016/j.drugalcdep.2013.05.012

van der Pol, P. (2014). *The dynamics of cannabis use and dependence*. Amsterdam, the Netherlands: University of Amsterdam, PhD thesis.

van der Pol, P., Liebregts, N., de Graaf, R., Korf, D. J., ten Have, M., van den Brink, W., & van der Laar, M. (2013a). Mental health differences between frequent cannabis users with and without dependence and the general population. *Addiction*, *108*, 1459–1469.

Van der Pol, P., Liebregts, N., de Graaf, R., Korf, D. J., van den Brink, W., & van der Laar, M. (2013b). Predicting the transition from frequent cannabis use to cannabis dependence: A three-year prospective study. *Drug and Alcohol Dependence*, *133*, 352–359.

Von Sydow, K., Retzlaff, R., Beher, S., Haun, M. W., & Schweitzer, J. (2013). The efficacy of systemic therapy for externalizing disorders of childhood and adolescence: a meta-content analysis of 46 randomized trials. *Family Process*, *52*, 576–618.

Waldron, H. B., & Turner, C. W. (2008). Evidence-based psychosocial treatments for adolescent substance abuse. *Journal of Child and Clinical Psychology*, *37*, 238–261.

Zavala, S. K., French, M. T., Henderson, C. E., Alberga, L., Rowe, C., & Liddle, H. A. (2005). Guidelines and challenges for estimating the economic costs and benefits of adolescent substance abuse treatments. *Journal of Substance Abuse Treatment*, *29*, 191–205.

CHAPTER

109

Cognitive Behavioral Therapy in Cannabis Use Disorder

F.M. Guven[*,**], *U.M. Camsari*[†], *O. Senormanci*[‡], *G. Oguz*[*,§]

*Turkish Association for Cognitive and Behavioural Therapies, Istanbul, Turkey
**Department of Psychiatry, Alcohol & Substance Use Disorders Treatment Center, Lara Anatolia Hospital, Antalya, Turkey
[†]Department of Psychiatry and Psychology, Mayo Clinic Health System, and Mayo Clinic College of Medicine, Rochester, MN, United States
[‡]Department of Psychiatry, Bülent Ecevit University School of Medicine, Zonguldak, Turkey
[§]Department of Psychology, Canik Basari University, Samsun, Turkey

SUMMARY POINTS

- Cannabis Use Disorder is a treatable psychiatric disorder; however, diagnosis and treatment can be a challenge.
- Cognitive Behavior Therapy (CBT) has no adverse effects, and can be combined with any other treatments of cannabis addiction.
- CBT is very effective in achieving abstinence and maintaining sobriety.
- Using CBT as a relapse prevention tool is known to increase the likelihood of maintaining sobriety and decrease the possibility of relapse.
- As much as its power, CBT seems to be underutilized in CUD and qualified therapists are scarce.

KEY FACTS OF CUD

- Because of the myths that cannabis is not harmful, even the users have problems they don't want to relate it with cannabis.
- Either because of that reason or because they are afraid of being stigmatized, patients have difficulty getting help from health care institutions.
- Treatment of CUD could be used as a prevention strategy from other substance use disorders.
- Lapse is inevitable in addiction of cannabinoids. But the lapse process can be used as an opportunity to hinder relapses.
- According to cognitive behavioral approach, because the use of cannabis is a learned behavior, it can be reverted.

KEY FACTS OF CBT

- The Cognitive Behavioral Model (CBM) doesn't see using cannabis as a personal trait, but as a behavior learned later in life.
- Learning which way to follow for not using cannabis, what may occur when the patient starts using cannabis again, and what are the harmful effects of cannabis may help patient quit using it more permanently.
- It is important for a patient to gain self-efficacy, learn how to deal with crisis, improving cognitive skills. It works better than stimulus control.
- It is scientifically proved that CBT techniques are effective not only in stopping using cannabis, but also in preventing relapses.
- Behavioral techniques aim at improving new behavior and attitudes that are incompatible with using cannabis. Once the behavior is changed, it can be reinforced by rewarding.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00127-7
Copyright © 2017 Elsevier Inc. All rights reserved.

- Cognitive techniques help the gained behaviors to become permanent. Otherwise, the learned behavior will be extinguished in time. If the patient is confronted with the stimulus that gives pleasure, addiction behavior may be reminded again.
- In high risk situations, the addicted person may think that everything is alright, but then the probability of using cannabis may rise again.

LIST OF ABBREVIATIONS

AVE	Abstinence-violation effects
BTs	Behavioral therapies
CBM	Cognitive Behavioral Model
CBT	Cognitive Behavioral Therapy
CNS	Central nervous system
CUD	Cannabis use disorder
HCV	Hepatitis C virus
SUDs	Substance use disorders
RP	Relapse prevention
THC	Tetrahydrocannabinol

INTRODUCTION

The word used for "cannabis" or "marijuana" is "esrar" in Turkish, has it is originated from Arabic meaning "mysteries, secrets" (Turkish Language Association, 2014). Cannabis is known to intervene with the normal synaptic effects of endogenous cannabinoids (endocannabinoid) along the reward circuitry which in turn disrupt memory and learning functions (Wolf, 2006).

Cannabis users tend to minimize, ignore, or completely deny the functional problems they face and which are associated with cannabis, and tend to continue using it. One of the most important reasons that the cannabis users are ambivalent to get help is the widely held opinion that the cannabis plant is not harmful as much as the other illegal addictive substances, which apparently can be regarded as a cognitive distortion. Every one person out of nine who tries cannabis may develop dependence, and also one in six people who try it in their teens have probably a higher risk of developing dependence (Wagner & Anthony, 2002; Hall & Degenhardt, 2009; UNODC, 2014). In addition, it has been suggested that cannabinoid use might be an independent factor in the development of mental disorders (Caspi et al., 2005). In the last decade, the demand to cannabis addiction treatment has significantly increased, especially in Europe, America, and Oceania (European Drug Report, 2013). Furthermore, it has been known that cannabis use increases the likelihood of codependence to both licit and illicit substances (Budney, Vandrey, & Fearer, 2011).

From a cognitive perspective, holding a belief that socialization could only be possible by using cannabis will cause addiction by decreasing self-efficacy (Witkiewitz & Marlatt, 2007). Unfortunately, living in an environment where the usage of cannabis is encouraged and confirmed, users tend to take risks in terms of health and having maladaptive cannabis related beliefs such as "Cannabis is not harmful for me, I'm impervious to it" that might be encouraging the use of cannabis, especially for teenagers and young adults (Connor et al., 2013). There may be different motivating factors to use cannabis: some of reported motives include enjoyment, feeling high, conformity, experimentation, social enhancement, getting rid of boredom, relaxation, and emotional regulation (Lee, Neighbors, & Woods, 2007; Zvolensky et al., 2009; Bonn-Miller, Vujanovic, & Zvolensky, 2008b). Conversely, cannabis use has been shown to be related with aggression, impulse control problems (Macdonald, Erickson, Wells, Hathaway, & Pakula, 2008), accidental problems (Asbridge, Hayden, & Cartwright, 2012), crime (Pedersen & Skardhamar, 2010), and sexually risky behaviors (Hendershot, Magnan, & Bryan, 2010; Andrea, Carroll, & Petry, 2013).

Cognitive Behavioral Therapy (CBT) is a well-defined and structured psychosocial treatment for cannabis-related disorders (Marijuana Treatment Project Research Group, 2004; Work Group on Substance Use Disorders, 2010; Sampl, Kadden, Hamilton, & Webb, 2002). CBT programs for Cannabis Use Disorder (CUD) have been suggested to be equally effective as other evidence-based psychosocial treatments in cannabis cessation (Hendriks, van der Schee, & Blanken, 2012). Evidence-based controlled studies have shown it to be more effective in maintaining sobriety (Nordstrom & Levin, 2007; Davis et al., 2015).

Modification of misbeliefs about the drugs achieved by CBT is assumed to help maintaining the sobriety state (Beck, Wright, & Newman, 1993; Wright, Sudak, Turkington, & Thase, 2010). So, CBT is considered superior to other treatments for Relapse Prevention (RP) in the treatment of SUDs (Witkiewitz & Marlatt, 2007). RP is based on cognitive skill development to lapse (a single instance) management for intervene relapse (problematic levels of use) (Marlatt & Gordon, 1985).

While CUD is a recurrent mental disorder as other Substance Use Disorders (SUDs) (Moore & Budney, 2003) with the help of improved self-efficacy, abstinence could be maintained (Stephens, Roffman, & Simpson, 1994).

In a more recent study conducted by Hoch et al. (2012), the effect of CBT was monitored in the third and sixth months in adults with CUD. The outcomes have indicated that CBT could be an effective treatment modality for achieving abstinence following both short-term and long-term cannabis use.

Evidence from numerous large scale trials supports the efficacy of CBT in SUDs, and larger treatment effect sizes were found for treatment of cannabis, followed by treatments for cocaine, opioids, and, with the smallest effect sizes, polysubstance dependence (McHugh, Hearon, & Otto, 2010). In this chapter, we would like to provide theoretical knowledge and practical advice for clinicians about Cognitive Behavioral Therapy approach in CUD.

CANNABIS USE AS A LEARNED BEHAVIOR

The Cognitive Behavioral Model (CBM) is not considered an alternative to the Neurobiological Model; these two models are complementary. According to both models, decreased tetrahydrocannabinol (THC) level in central nervous system (CNS) would trigger craving for cannabis (Marks, 2005; Wolf, 2006). Presumed changes that occur in the neurological pathway of reward circuitry could be underlying reason why individuals attribute positive thoughts to cannabis.

Learning Models

In view of CBM, addictive behaviors such as alcohol and drug dependence are acquired through experience. There are three suggested learning models in behavioral change: learning by association ("Pavlovian" or "classical" conditioning), learning by consequences ("operant" conditioning) (Kadden, 2001), and learning by expectancies (social learning) (Bandura, 1971). In learning by association, stimuli that are initially neutral can become triggers for cannabis cravings and repeated associations between those stimuli and cannabis use. Associations are reinforced with compulsive use, and would cause cravings. Cannabis use is also thought be reinforced by psychological factors well. An immediate result of cannabis smoking is decreased social anxiety and relaxation which could also play a role in positive reinforcement. As cannabis use reduces anxiety and tension, the contingency of the future use is increased. This process is called "negative reinforcement;" avoidance from cannabis withdrawal leads to continue using cannabis. Learning by expectancies and contribution of environmental factors to cannabis addiction is not well understood (Connor, Gullo, Feeney, Kavanagh, & Young, 2014).

Development and Maintenance of Cannabis Addiction

1. Predisposing factors. Having cognitive distortions in general (Leahy, 2003) or cognitive distortions specifically about cannabis (regarded as nonaddictive, herbal medicine, etc.) (Table 109.1), living in an environment that approves consumption of marijuana (environmental normalization), untreated psychiatric disorders, ongoing abuse of other substances, presence of psychosocial stressors, poor coping skills, social isolation and lack of a good support system (Witkiewitz & Marlatt, 2007).
2. High risk situations (HRS) might be environmental (cue objects, triggering events and places, or family members one uses with regularly) or individual (thoughts, emotions, or attitudes) (Witkiewitz & Marlatt, 2007). Being exposed to environments

TABLE 109.1 Cognitive Distortions About Cannabis Examples Are Given

Cognitive distortions[a]	Example
Control fallacy	"I am not addicted to cannabis, if I really want I can quit"
Selective abstraction (tunnel effect)	"Calm down there is no harm in it" "I won't become an addict" "No matter how much I smoke it doesn't bother me" "Only the weak become addicted" "If I don't smoke cannabis I can't relax"
Polarized thinking	"Cannabis is the only friend I have"
Minimization (negative effects of cannabis)	"Cannabis is not harmful to me" "No bad comes from use only once"
Magnification (positive effects of cannabis)	"I can't be happy/enjoy myself without cannabis"
Personalization	"My neighbor was rude to me because I am an addict"
Excuses	"I can't help myself, I just need to smoke cannabis" "I am a victim of my pothead friends, it is their fault"
Ignoring the evidence	"You can't quit once you became addicted "
Stigmatism	"I am a pothead" "I am a failure"
What if ?…	What happens if I don't quit? "I will be homeless"
Discounting the positive	"I can quit smoking for three months but anyone can do that"
Should statements	"I should have been a better parent now...."
Overgeneralization and selective abstraction	"Nothing is more pleasurable than smoking cannabis"

[a] *Leahy, R. (2003).*

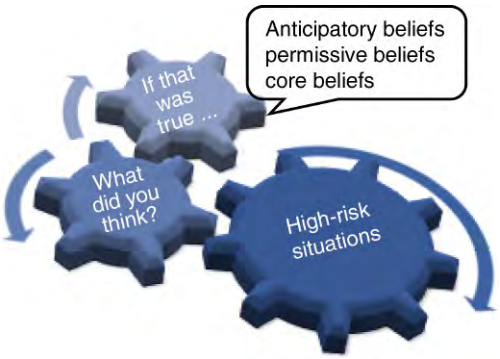


FIGURE 109.1 **High risk situations lead to change in internal states of cannabis addicted patients.** To learn the patient's beliefs, the therapist asks open ended questions "What did you think at that time?," complete the sentence "If that was true exactly…" (Vertical descendent method) (Leahy, 2003).

where cannabis is consumed, people who consume cannabis and any environmental cues could initiate the craving process. It is suggested that anticipatory beliefs will emerge when cannabis is being consumed, for example there might be cognitive distortions such as "smoking marijuana will make me look sexy"; therefore anticipatory thoughts might lead to permissive and core beliefs (Beck et al., 1993; Rangé & Mathias, 2012). An example for a permissive cognition would be "this actor smokes marijuana in every scene, and all girls are interested in him." High risk situations lead to change in internal state of cannabis addicted patients (Fig. 109.1).

3. Compulsive use usually reinforces existing precipitating, predisposing and perpetuating factors which lead to the emergence of automatic thoughts that intensify craving. Craving for cannabis follows a decision-making process about how to obtain the substance, and it is suggested that drug-seeking behaviors are controlled by automatic thought processes (Bowen, Chawla, & Marlatt, 2011).

APPROACHES IN CUD

CUD is a treatable chronic mental disorder. Comprehensive psychiatric interview and obtaining a detailed substance use history is crucial for early diagnosis.

Evaluation and Diagnosis

The interview about the history of addiction and diagnosis in CUD can be challenging for variety of reasons. Individuals might not be willing to admit their use, either because cannabis is illegal in most countries in the world, or because of the common cannabis-related belief is that is not a harmful substance (Pérez, Ariza, Sánchez-Martínez, & Nebot, 2010). As part of the evaluation it is important to focus on comorbid psychiatric or substance use disorders to determine the context in which cannabis may be used. It is common that cannabis is consumed as a self-medication for anxiety disorders, coping with stressful situations, or frequently for socialization (Zvolensky et al., 2009). Motivational Enhancement Therapy (MET) and Behavioral Therapies (BPs) facilitate establishing a rapport, and maintaining the therapeutic alliance (Sampl & Kadden, 2001; Nordstrom & Levin, 2007). Loss of functionality, spending large amounts of time and financial means in order to obtain or use cannabis, continued use despite the harmful consequences, and loss of control are some of the diagnostic symptoms (American Psychiatric Association, 2013). In cases of polysubstance abuse, withdrawal syndrome can overlap.

Comorbidity

It is known that cannabis abuse is a common comorbidity of depressive and anxiety disorders (Boden, Babson, Vujanovic, Short, & Bonn-Miller, 2013). Cannabis use and its association with psychotic syndromes have long been of academic interest (Griffin & Botvin, 2010). Codependence to other substances is common (Budney et al., 2011) (Bonn-Miller, Zvolensky, Bernstein, & Stickle, 2008a; Chadwick, Miller, & Hurd, 2013).

COGNITIVE-BEHAVIORAL APPROACH FOR CUD TREATMENT

CBT is designed to help patients to acquire certain behavioral skills to achieve and maintain abstinence. (Marks, 2005). CBT technique will focus on maladaptive thoughts which can be modified and, in turn, associated behaviors and emotions could be modified (Beck et al., 1993). Being an evidence-based approach, the CBT model has some unique advantages in addiction treatment since addictive behavior can also be empirically modeled as a learning model.

By definition, addictive behavior is resistant to change and recurrence is common. Stage of change, as defined by the Transtheoretical Change Model by Prochaska, DiClemente, and Norcross (1992), needs to be identified at the time of the initial evaluation. (Fig. 109.2).

During the remission phase, incidental use is usually considered as within the concept of "lapse," whereas going back to regular use is considered "relapse." As in other SUDs, CBT strategies are utilized during all three stages of CUD treatment, which are: preparation, achieving abstinence, and maintenance of sobriety

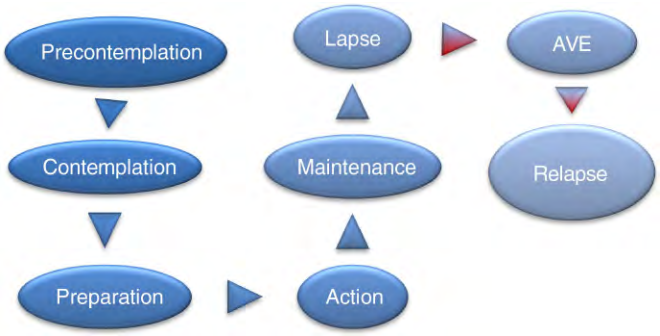


FIGURE 109.2 **Cognitive independence model.** The *red arrows* show that dealing with lapse and abstinence violation effects are two stages of change that will lead to be free from cannabis.

(Kadden, 2001). Main CBT principles to treat CUD are summarized in Table 109.2.

CBT METHODS FOR CUD

Establishing a therapeutic alliance is a priority. During an initial interview by using "guided discovery," a CBT method, the pattern of use and personal high risk situations (emotions, thoughts, attitudes, events, places) are determined. The suitable treatment method (group or personal therapy), intervals and duration of interview, targets of treatment can be assessed.

General principles of CBT will be applied to CUD, as it is applied to any other substance dependence. CBT can be used only by itself or along with medical treatment.

Seeing the Big Picture

Identifying cognitive distortions can help seeing the big picture. Patients usually have a variety of cognitive distortions about cannabis (Table 109.1) CBT techniques can be effectively used to modify cognitive distortions (Perkins, Conklin, & Levine, 2007). The therapist can start off by motivating the patient by sharing successful self-experiences, and positive outcomes in cannabis dependence therapy, as a technique used for improving self-efficacy.

The main initial target of the treatment is to achieve abstinence, and to make it as easy as possible for the patient. Using metaphors in therapy can be useful, as it would reduce the duration of sessions (Stott, Mansell, Salkovskis, Levander, & Cartwright-Hatton, 2012). While using metaphors, similar situations are used as examples to show the patient the connections between thought and behavior.

Cognitive Restructuring

In order to simplify the concept of cognitive restructuring, it might be useful to consider humans as living computers that consist of hardware and software. It is possible to terminate, uninstall, or even update a malfunctioning application that slows down the system. Updated applications can be considered similar to thought modification which can be used to modify cognitive distortions such as "there is no life without marijuana" can be modified with "marijuana is not a basic necessity."

Some of the methods (cognitive, behavioral, and emotional) that are used in cognitive restructuring are listed below (Marks, 2005):

Cognitive Methods

1. Assessing or interrupting negative automatic thoughts. Becoming aware of thoughts that involve in cannabis use.

TABLE 109.2 Main CBT Principles and Clinical Features of CUD Are Relatively Shown

Main principles of CBT	Clinical features of CUD
CBT is time limited and well-structured.	Difficulty of purposeful act, sense of time, memory and learning defects occur due to cannabis abuse.
It is individualized and reformulated based on patient's needs.	CUD has not only a heterogeneous clinic table but also a wide of range comorbidity.
It encourages the patient to ask questions of himself/herself.	Causes tendency of blaming himself/herself or significant others, finding excuses and wangling.
Patient shares the responsibility of treatment with the therapist.	Patient spends too much time to find and use cannabis, avoids and tries to delay responsibilities.
Can be put into practice with motivation enhancement techniques.	Patients usually don't accept change unless they face a critical problem.
"Quitting day" will be decided by patient and therapist together.	Cannabis cessation needs professional help, especially, in case of intensive and long term use.
Total abstinence is not a realistic target: increasing sobriety duration is the target.	Stimulus control is not always possible for user.

2. Understanding effective and self-beneficial explanations. Self-blaming is a sign of bad progress. It can be automatic, and can remain over time.
3. Self-imagine: learning to listen to the voice of a new person who don't use marijuana. Listening to the sound that would help you most. "Who do you want to be?"
4. Distraction: when exposed to high risk situations, distraction method helps immediately; but its effect is temporary.
5. Modifying maladaptive cognitive processes: maladaptive cognitions are modified with functional ones. For example, positive beliefs about marijuana can be modified by neutral or negative ones.
6. Focusing: choosing and managing what needs to be focused.
7. Imaginary: new behaviors can be imagined using past experience.

Behavioral Methods

1. Acceptance: quitting cannabis is a radical behavioral change.
2. Developing a negative attitude to cannabis: for example, the therapist can facilitate this by associating it with his/her respiratory system problems due to covered sensitization.
3. Becoming aware of the financial burden of the addictive behavior (Carroll et al., 2006).
4. Targeting the situations, habits, and environmental cues associated with cannabis use: alcohol, bong, friends using cannabis, and places where cannabis is used habitually.
5. Stimulus control: removing cannabis and triggering factors from sight.
6. Making an agreement: listing personal reasons for quitting cannabis use: individual and environmental disadvantages associated with the use (Litt, Kadden, & Petry, 2013).
7. Rewarding the behaviors: saving the money that patient would have spent on cannabis weekly or monthly, and using it for leisure (Stephens et al., 2002).

Emotional Methods

1. Relaxation exercises
2. Mindfulness-based relapse prevention
   a. Using mindfulness on a daily basis
   b. Using mindfulness activities for HRS (Bowen et al., 2011).

In CBT-oriented cannabis treatment programs, all methods should be individualized and tailored into patient's needs.

COGNITIVE BEHAVIORAL APPROACHES TO STAGES OF CUD

Regardless of the situation question needs to be asked: "What is the meaning of cannabis in you life?" should always be asked.

Preparation for Cannabis Cessation

Ambivalent patients may be using the substance as a way of dealing with the stressful situations, a coping strategy with unpleasant feelings, a way of spending time, or a break from stressful workloads. The ones brought by their family or referred for consultation by another doctor may not be ready for the treatment process. Psycho-education can be utilized as an effective technique with these patients. The cannabis users not aware of the negative effects of cannabis may think that using cannabis doesn't cause as much harm as smoking; but, for instance, if pregnant women are informed about the harmful effects of using cannabis in pregnancy, they may be kept away from using cannabis (Work Group on Substance Use Disorders, 2010). Educating the patients on withdrawal symptoms and reassuring them that these symptoms are temporary may also make a difference (Weinstein et al., 2010).

The Patients Who Are Ready for Change

As a first step, the patient's pattern of use should be questioned in detail. After that, patient and therapist can decide on a date together. Following detoxification, the next phase should be the Relapse Prevention (RP) process.

Maintaining Sobriety

In order to maintain positive behavioral change, the patient should be constantly motivated to stay in the RP stage. For the patients who are reluctant to ask for help, the therapist can use behavioral rehearsals (Wright et al., 2010). Social support has critical importance to recognize early signs of relapse (Bowen et al., 2011).

RP, developed by Marlatt and Gordon (1985) is widely used as a cognitive behavioral strategy. Relapse is not considered a stage itself but it's an unwanted return to an earlier stage from the maintenance or action stages. In the event of relapse, RP strategies and coping skills are likely to be compromised.

Intervention Strategies in "Lapse" Situations (Witkiewitz & Marlatt, 2007)

1. If behavioral techniques utilized during remission are compromised, the immediate result is usually "lapse," which is considered as an incidental use of substance without resuming regular dependence pattern. "Lapse" should be interpreted as an alarm signal.

2. "Lapse" as "mistake" is an opportunity for learning, therefore, focus on treatment can be resumed.
3. It may be difficult to remotivate the patient following a lapse. Focus should be given to take away the blame, if any, and reemphasizing the initial personal reasons for abstinence.
4. Possible reasons for lapse: HRS and triggering reasons for lapse should be questioned in detail, including the situation that it occurred, emotions and thoughts involved which can help further strengthening the RP strategies.
5. Preparing for immediate aftermath: note cards can be prepared for a crisis situation, including people to reach out, places to go. If possible, the clinician can allow the patient to contact him/her in the case of a lapse situation.

It is important to be aware of abstinence violence effects (AVE): cognitive restructuring period is a difficult process, and the general approach of the therapist should be gentle and considerate in a situation of a lapse. This kind of approach helps the patient focus on the crisis instead of regretting and blaming self.

Relapse Prevention Strategies (Witkiewitz & Marlatt, 2007)

1. Psychoeducation: teaching on relapse/lapse concepts, identifying early alarm signals, treatment plan in general (formulation).
2. It is asked from the patient to list HRS and managing techniques with them. The therapist should show how to do it in the session (role play).
3. Acquiring cognitive skills: setting boundaries in interpersonal relationships is important. Patients should also acquire communication skill of opposing insistences.
4. Realizing that emotions themselves and controlling reactions resulting from emotions should be considered differently.
5. Understanding cannabis craving: dealing with craving and exercising how to regain control of it.
6. Learning cognitive distortions and setbacks: helping the patient to modify cognitive perspectives for preventing relapse.
7. The ongoing medical treatment for other comorbid conditions and special situations should be taken into the consideration. Clinicians should also consider potential risks of co-dependence such as benzodiazepines and/or alcohol use.

CASE EXAMPLES

Establishing Therapeutic Alliance

A 32-year-old male photographer was referred to our clinic from the emergency room presenting with "Cannabis Related Problems." It was reported that while he was smoking cannabis he had a fall in his bedroom. The patient was brought to the hospital by his wife.

Dr Guven: Hi there, I hope you are doing better, I understand that you fell. I just wanted to let you know that urine toxicology came positive for cannabis. Is it possible that you were intoxicated with cannabis yesterday?

Patient: Yes, maybe.

Dr Guven: Would you feel comfortable talking about this?

Patient: Yes, we can do that.

Dr Guven: First off, I would like to ask you some questions about your past and current cannabis if that is okay with you. When was the first time you used cannabis?

Patient: About 10 years ago, I started sharing a joint with a friend. It eventually became a daily habit, I have been using regularly for about 5 years. I smoke a couple of joints a week.

Dr Guven: Okay, so how much exactly do you smoke daily?

Patient: Over the last few months I have smoked at least a joint almost every day. Some days two, other days up to four joints.

Dr Guven: It looks like you smoke more on some days than others. Any particular reason for that?

Patient: I don't know (smiles). If I don't smoke in the evening I can't sleep.

Dr Guven: So you have been using cannabis for sleeping? Is there anything else that marijuana helps you with?

Patient: I believe marijuana makes me creative.

Dr Guven: I understand that you think when you smoke marijuana you are more creative. Have you ever had a period when you remained abstinent for a while?

Patient: Two years ago I was preparing for my first photo exhibition. At that time I didn't use for three months.

Dr Guven: You said that cannabis makes you creative. I find it quite interesting that you didn't smoke cannabis while preparing for the exhibition.

Patient: I get handshakes when I smoke, so I try to be abstinent when I am preparing for exhibitions.

Dr Guven: I see, I would like to know you better in order to find out why this is happening.

Patient: How?

At the beginning their therapeutic alliance therapist (Dr Guven) suggested that they could work together to understand some of incomprehensible situations.

The session ended with a plan that focused on monitoring cravings until next follow-up (Table 109.3).

Unsuccessful Outcome

A 24 year old male university student who is studying Town Planning, living by himself in a metropolitan city. Initially using marijuana with friends at the weekends, he escalated to daily use over the past few months. As his use has increased, he has lost his interest in academics. Despite the significant increase in his use he reported less pleasure from smoking. He has noticed loss of excitement in relationship with his girlfriend and a decrease in socialization, if not associated with cannabis use. He was spending most of his money on cannabis, and he wasn't going out as often. He started to show problems with

TABLE 109.3 Cannabis Craving Diary Example

When?	What happened?	What did I think?	What did I feel?	Craving level (%)	What did I do?
05/05/14 Evening	I saw a bong on the shelf	I must not smoke marijuana anymore	Sad	80	Looking for some marijuana, automatically

his self-care. As his girlfriend started to worry about him, she decided to talk to his family about worsening functioning.

He was encouraged to enter our outpatient addiction treatment and was treated with the diagnoses of CUD and Psychosis NOS. However, he discontinued treatment against medical advice, within the third month of treatment. He continued thinking that he was not "addicted to" marijuana during this period. He dropped out from college. Within a year, he had a lapse when he was out. He thought that there was "nothing wrong" with taking just couple of puffs of at times. At that time he tried using synthetic cannabis. As his use escalated, he was brought to the psychiatric clinic by his family, but refused to enter treatment. With increasing pressure from the family and girlfriend, he decided to move in with his grandmother, who was then living in the suburbs.

After one year passed, he presented himself to our addiction center with request for help. Apparently he has been addicted to intravenous heroin, and was told that he contracted HCV. He reported that he started using heroin during a period when he was abstinent from cannabis, then he started using cannabis once a day to quit heroin, and ended up increasing both heroin and cannabis use. He apparently was able to remain sober from all substances for about 4 months; however, about 2 months later, he relapsed on marijuana and resumed his regular heavy use during an intense craving episode. He has succeeded in remaining abstinent from heroin since then, despite his strong cravings. He reported that he had increased cannabis cravings when he spent time in his bedroom where he used to use cannabis most of the time. Despite lack of full compliance with treatment recommendations, he shared how much he enjoyed applying some the techniques that he learned here in sessions (Table 109.4).

Successful Outcome

A 32-year old, high-school graduate single woman working as an accountant referred to our addiction services by the order of the traffic court, after she had a car accident and was found to have cannabis in her possession. She started using cannabis when she was in boarding school. At that time she was living away from her parents who lived in the suburbs. She was abstinent intermittently when her finances were not good. Eventually her use progressed to regular use, 3–4 times per week.

Dr Guven: My understanding is you have been sent here by the court with regards to your cannabis use. I am sure you are motivated to take care of the legal issues. Is there anything else that also could motivate you to quit using?

Patient: I guess, my parents would be very happy if I quit. I have caused enough trouble and worry for them.

Dr Guven: So you are saying that there may be more reasons to quit other than the legal trouble. That sounds wonderful.

Patient: I have been performing low in the past couple of months and was suspended from my job. I couldn't pay some of my bills, and asked help from my family. My parents refused to help me financially. I have had couple of no-shows lately, as well, because I would not wake up early following the nights that I smoke cannabis too much. My boss is also aware of my use, he suggested that he would be supportive if I seek professional help. My position at work is still vacant, and they are interested in having me back if I can get sober.

Dr Guven: That sounds like a good reason to quit. Anything else that motivates you?

Patient: I just don't want to lose everything. I don't want to be like my brother. His wife left him and his kid is now under legal protection because of his addiction problem. (tearful)

Dr Guven: It looks like you might be depressed about all this. Did you know that cannabis use commonly cause depression?

Patient: I guess it might be right. I am a little worried about losing some of my friends if I quit.

Dr Guven: Now, we will try to write down your reasons for quitting and the potential obstacles that could get in the way (Table 109.5). Perceived obstacles are usually maladaptive thoughts. If we work together, we can modify these thoughts.

TABLE 109.4 The Patients' Reasons and the excuses for not Using Cannabis Are Given

I don't want to use it, because…	I want to use it, because
It doesn't relieve me any more when I use it	It gives me pleasure
It makes me numb and I don't want to see anybody around	Helps me forget my loneliness
I need to see that I can give it up	It doesn't harm anybody
I want to say "no" when somebody offers me to use it	I am not addicted. I can use it whenever I want
I want to see what happens if I stop using it	My problems are not because of cannabis
When I am not awake I am losing my money and having economic problems	I can't sleep if I don't use it

TABLE 109.5 Reasons for Quitting Cannabis Use and Thoughts That Make Quitting Difficult Are Listed

Reasons	Obstacles
I may get arrested	Nobody can quit using cannabis
My family would be happy	I don't want to quit
I may lose my job	It makes me forget my debts
My coughing scares me	I can't sleep If I don't smoke

FIGURE 109.3 **Cannabis craving scale.**

I do think that some of this process has already started during this conversation. You remarked two different times about how much cannabis is making your life difficult and, in the beginning you gave it 30 points, which was increased to 60 points in the end (Fig. 109.3). As an assignment, you will list the events or situations in which your craving is triggered.

She completed 12 sessions carried out every other week. Before each session she gave urine toxicology tests part of the treatment contract. She has been sober for more than a year, and has founded the local chapter of "Marijuana Anonymous" in her hometown.

MINI-DICTIONARY

Attribution An individual's explanation why a result occurred.
Classical conditioning An originally neutral stimulus becomes a conditioned stimulus when paired with an unconditioned stimulus or with a conditioned stimulus.
Cognitive restructuring The general term applied to the process of changing the patient's thought patterns.
Contingency management This behavioral technique attempts to change those environmental contingencies that may influence substance abuse behavior. The goal is to increase certain behaviors that are incompatible with use.
Functional analysis A process used in cognitive behavioral therapy that probes the situations surrounding the patient's cannabis addicted.
Operant learning Refers to the process by which behaviors that are reinforced increase in frequency.
Social learning It occurs when individuals observe the behaviors in their own environment and at the end of a latent period, they finally obtain these behaviors by trial through some expectations.
Therapeutic alliance The development of a positive relationship between the therapist and patient.

Acknowledgments

First and foremost, I would like to thank The Head of Turkish Association of CBT, Prof Dr Mehmet Zihni SUNGUR and all other teachers who contributed to our knowledge. For translation of the chapter from Turkish to English I would like to thank Psychological Counselor Esra ALICI, Gülçin ŞENYUVA, Burcu SEVİM, and Sevgi KARADUMAN.

References

American Psychiatric Association. (2013). Cannabis-related disorders. In *Diagnostic and statistical manual of mental disorders* (5th ed., pp. 509–519). Arlington, VA: American Psychiatric Association.
Andrea, L. F., Carroll, K. M., & Petry, N. M. (2013). Marijuana use is associated with risky sexual behaviors in treatment-seeking poly-substance abusers. *American Journal of Drug and Alcohol Abuse*, *39*(4), 266–271.
Asbridge, M., Hayden, J. A., & Cartwright, J. L. (2012). Acute cannabis consumption and motor vehicle collision risk: systematic review of observational studies and meta-analysis. *British Medical Journal*, *344*(2), e536.
Bandura, A. (1971). *Social learning theory*. New York, NY: General Learning Press, 1–46.
Beck, A. T., Wright, F. D., & Newman, C. F. (1993). *Cognitive therapy of substance*. New York, NY: The Guilford Press.
Boden, M. T., Babson, K. A., Vujanovic, A. A., Short, N. A., & Bonn-Miller, M. O. (2013). Posttraumatic stress disorder and cannabis use characteristics among military veterans with cannabis dependence. *The American Journal on Addictions*, *22*(3), 277–284.
Bonn-Miller, M. O., Zvolensky, M. J., Bernstein, A., & Stickle, T. R. (2008a). Marijuana coping motives interact with marijuana use frequency to predict anxious arousal, panic related catastrophic thinking, and worry among current marijuana users. *Depression and Anxiety*, *25*(10), 862–873.
Bonn-Miller, M. O., Vujanovic, A. A., & Zvolensky, M. J. (2008b). Emotional dysregulation: association with coping-oriented marijuana use motives among current marijuana users. *Substance Use & Misuse*, *43*(11), 1653–1665.
Bowen, S., Chawla, N., & Marlatt, G. A. (2011). *Mindfulness-based relapse prevention for addictive behaviors*. New York, NY: The Guilford Press.
Budney, A. J., Vandrey, R. G., & Fearer, S. (2011). Cannabis. In P. Ruiz, & E. C. Strain (Eds.), *Lowinson and Ruiz's substance abuse: A comprehensive textbook* (5th ed., pp. 226–227). Philadelphia, PA: Lippincott Williams & Wilkins.
Carroll, K. M., Easton, C. J., Nich, C., Hunkele, K. A., Neavins, T. M., Sinha, R., Ford, H. L., et al. (2006). The use of contingency management and motivational/skills-building therapy to treat young adults with marijuana dependence. *Journal of Consulting and Clinical Psychology*, *74*(5), 955–966.
Caspi, A., Moffitt, T. E., Cannon, M., McClay, J., Murray, R., Harrington, H., Taylor, A., Arseneault, L., Williams, B., Braithwaite, A., Poulton, R., & Craig, I. W. (2005). Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-O-methyltransferase gene: longitudinal evidence of a gene X environment interaction. *Biological Psychiatry*, *57*(10), 1117–1127.
Chadwick, B., Miller, M. L., & Hurd, Y. L. (2013). Cannabis use during adolescent development: susceptibility to psychiatric illness. *Frontiers in Psychiatry*, *4*(129), 1–8.
Connor, J. P., Gullo, M. J., Chan, G., Young, R. M., Hall, W. D., & Feeney, G. F. X. (2013). Polysubstance use in cannabis users referred for treatment: drug use profiles, psychiatric comorbidity and cannabis-related beliefs. *Frontiers in Psychiatry*, *4*, 79.
Connor, J. P., Gullo, M. J., Feeney, G. F. X., Kavanagh, D. J., & Young, R. M. (2014). The relationship between cannabis outcome expectancies and cannabis refusal self-efficacy in a treatment population. *Addiction*, *109*(1), 111–119.
Davis, M. L., Power, M. B., Handelsman, P., Medina, J. L., Zvolensky, M., & Smits, J. A. (2015). Behavioral therapies for treatment-seeking cannabis users: a meta-analysis of randomized controlled trials. *Evaluation and the Health Professions*, *38*(1), 94–114.
European Monitoring Centre for Drugs and Drug Addiction. (2013). *European Drug Report 2013: Trends and developments*. Lisbon, Portugal: EMCDDA.

Griffin, K. W., & Botvin, G. J. (2010). Evidence-based interventions for preventing substance use disorders in adolescents. *Child and Adolescent Psychiatric Clinics of North America*, *19*(3), 505–526.

Hall, W., & Degenhardt, L. (2009). Adverse health effects of non-medical cannabis use. *Lancet*, *374*(9698), 1383–1391.

Hendershot, C. S., Magnan, R. E., & Bryan, A. D. (2010). Associations of marijuana use and sex-related marijuana expectancies with HIV/STD risk behavior in high-risk adolescents. *Psychology of Addictive Behaviors*, *24*(3), 404–414.

Hendriks, V., van der Schee, E., & Blanken, P. (2012). Matching adolescents with a cannabis use disorder to multidimensional family therapy or cognitive behavioral therapy: treatment effect moderators in a randomized controlled trial. *Drug and Alcohol Dependence*, *125*(1–2), 119–126.

Hoch, E., Noack, R., Henker, J., Pixa, A., Höfler, M., Behrendt, S., Bühringer, G., & Wittchen, H. U. (2012). Efficacy of a targeted cognitive-behavioral treatment program for cannabis use disorders (CANDIS *). *European Neuropsychopharmacology*, *22*(4), 267–280.

Kadden, R. M. (2001). *Cognitive-behavior therapy for substance dependence: Coping skills training*. Peoria and Bloomington, IL: Behavioral Health Recovery Management project, http://www.bhrm.org/guidelines/CBT-Kadden.pdf

Leahy, R. L. (2003). *Cognitive therapy techniques: A practitioner's guide*. New York, NY: The Guilford Publications.

Lee, C. M., Neighbors, C., & Woods, B. A. (2007). Marijuana motives: Young adults' reasons for using marijuana. *Addictive Behaviors*, *32*(7), 1384–1394.

Litt, M. D., Kadden, R. M., & Petry, N. M. (2013). Behavioral treatment for marijuana dependence: randomized trial of contingency management and self-efficacy enhancement. *Addictive Behaviors*, *38*(3), 1764–1775.

Macdonald, S., Erickson, P., Wells, S., Hathaway, A., & Pakula, B. (2008). Predicting violence among cocaine, cannabis, and alcohol treatment clients. *Addictive Behaviors*, *33*(1), 201–205.

Marijuana Treatment Project Research Group. (2004). Brief treatments for cannabis dependence: findings from a randomized multisite trial. *Journal of Consulting and Clinical Psychology*, *72*(3), 455–466.

Marks, D. F. (2005). *Overcoming your smoking habit: A self-help guide using Cognitive Behavioral Techniques*. London, UK: Robinson Publishing.

Marlatt, G. A., & Gordon, J. R. (1985). *Relapse prevention*. New York, NY: Guilford Press.

McHugh, R. K., Hearon, B. A., & Otto, M. W. (2010). Cognitive behavioral therapy for substance use disorders. *The Psychiatric Clinics of North America*, *33*(3), 511–525.

Moore, B. A., & Budney, A. J. (2003). Relapse in outpatient treatment for marijuana dependence. *Journal of Substance Abuse Treatment*, *25*(2), 85–89.

Nordstrom, B. R., & Levin, F. R. (2007). Treatment of cannabis use disorders: a review of the literature. *The American Journal on Addictions*, *16*(5), 331–342 http://www.ncbi.nlm.nih.gov/pubmed/17882603

Pedersen, W., & Skardhamar, T. (2010). Cannabis and crime: findings from a longitudinal study. *Addiction*, *105*(1), 109–118.

Pérez, A., Ariza, C., Sánchez-Martínez, F., & Nebot, M. (2010). Cannabis consumption initiation among adolescents: a longitudinal study. *Addictive Behaviors*, *35*(2), 129–134.

Perkins, K. A., Conklin, C. A., & Levine, M. D. (2007). *Cognitive Behavioral Therapy for smoking cessation: A practical guidebook to the most effective reatments*. New York, NY: Routledge.

Prochaska, J. O., DiClemente, C. C., & Norcross, J. C. (1992). In search of how people, change. Applications to addictive, behaviors. *The American Psychologist*, *47*, 1102–1114.

Rangé, B. P., & Mathias, A. C. B. (2012). Cognitive-behavior therapy for substance abuse. In I. R. De Oliveira (Ed.), *Standard and innovative strategies in cognitive behavior therapy* (pp. 157–170). Rijeka, Croatia: InTech, http://www.intechopen.com/books/standard-and-innovative-strategies-in-cognitive-behavior-therapy/cognitive-behavioral-therapy-for-alcohol-abuse

Sampl, S., & Kadden, R. (2001). *Motivational enhancement therapy and cognitive behavioral therapy for adolescent cannabis users: 5 sessions*. Rockville, MD: SAMHSA, http://www.drugsandalcohol.ie/17831/1/Motivational_Enhancement_Therapy_and_CBT_for_adolescent_cannabis_users.pdf

Sampl, S., Kadden, R., Hamilton, N., & Webb, C. (2002). *Cannabis Youth Treatment Series. Substance abuse (Vol. 4)*. Rockville, MD: Center for Substance Abuse, SAMHSA, http://integratedrecovery.org/wp-content/uploads/2010/08/CYT-Vol.1.pdf

Stephens, R. S., Babor, T. F., Kadden, R., Miller, M., McRee, B., Vendetti, J., Steinberg, K., et al. (2002). The Marijuana Treatment Project: rationale, design and participant characteristics. *Addiction*, *97*(Suppl. 1), 109–124.

Stephens, R. S., Roffman, R. A., & Simpson, E. E. (1994). Treating adult marijuana dependence: a test of the relapse prevention model. *Journal of Consulting and Clinical Psychology*, *62*(1), 92–99.

Stott, R., Mansell, W., Salkovskis, P., Levander, A., & Cartwright-Hatton, S. (2012). *Oxford guide to metaphors in CBT* (2nd ed.). Croydon, UK: Oxford University Press.

Turkish Language Association (2014). http://www.tdk.gov.tr

United Nations Office on Drugs and Crime. (2014). *Cannabis: a short review*. Vienna, Austria: UNODC, https://www.unodc.org/documents/drug-prevention-and-treatment/cannabis_review.pdf

Wagner, F. A., & Anthony, J. C. (2002). From first drug use to drug dependence: developmental periods of risk for dependence upon marijuana, cocaine, and alcohol. *Neuropsychopharmacology*, *26*(4), 479–488.

Witkiewitz, K., & Marlatt, G. A. (2007). *Therapist's guide to evidence-based relapse prevention*. Amsterdam, the Netherlands: Academic Press.

Wright, J. H., Sudak, D. M., Turkington, D., & Thase, M. E. (2010). *High-yield Cognitive Behavior Therapy for brief sessions*. Arlington, VA: American Psychiatric Publishing Inc.

Weinstein, A., Miller, H., Tal, E., Avi, I. B., Herman, I., Bar-Hamburger, R., & Bloch, M. (2010). Treatment of cannabis withdrawal syndrome using Cognitive Behavioral Therapy and relapse prevention for cannabis dependence. *Journal of Groups in Addiction & Recovery*, *5*(3–4), 240–265.

Wolf, M. E. (2006). Addiction. In G. J. Siegel, R. W. Albers, S. T. Brady, & D. L. Price (Eds.), *Basic neurochemistry: molecular, cellular and medical aspects* (7th ed., Part VIII, Chapter 56, pp. 911–926). New York, NY: Elsevier Academic.

Work Group on Substance Use Disorders. (2010). *Practice guideline for the treatment of patients with substance use disorders* (2nd ed.). Arlington, VA: American Psychiatric Association, http://psychiatryonline.org/pb/assets/raw/sitewide/practice_guidelines/guidelines/substanceuse.pdf

Zvolensky, M. J., Marshall, E. C., Johnson, K., Hogan, J., Bernstein, A., & Bonn-Miller, M. O. (2009). Relations between anxiety sensitivity, distress tolerance, and fear reactivity to bodily sensations to coping and conformity marijuana use motives among young adult marijuana users. *Experimental and clinical psychopharmacology*, *17*(1), 31–42.

CHAPTER

110

The Cannabis Withdrawal Syndrome—Symptoms and Time Course

M. Hesse, B. Thylstrup

Center for Alcohol and Drug Research, Aarhus University, Copenhagen Department, Copenhagen, Denmark

SUMMARY POINTS

- The literature on cannabis withdrawal phenomena and symptoms shows a fairly consistent picture.
- With the publication of the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) in 2013, a cannabis withdrawal syndrome is now officially recognized with defined criteria.
- The cannabis withdrawal syndrome begins within few days after cessation or reduction of use, symptoms peak after approximately 10 days, and are generally gone after around 30 days.
- The time course of the cannabis withdrawal syndrome may vary due to a wide range of underlying biological and psychological mechanisms.
- Individual symptoms vary in terms of their onset, with some symptoms beginning immediately after cessation or reduction (eg, insomnia and nervousness), and others typically developing later (eg, irritability and vivid, unpleasant dreams).
- The problem of demand characteristics which can be explained as the totality of cues that convey an experimental hypothesis to the subject may
- Study findings speak against the significance of demand characteristics in determining the course of the symptoms of cannabis withdrawal.
- The withdrawal symptoms listed in the DSM-5 are not the only symptoms that have been studied in clinical and experimental research, and other symptoms have been listed as symptoms that also may occur.
- The building evidence underlines the need for specific interventions that target symptoms related to cannabis withdrawal.

KEY FACTS

- Withdrawal or discontinuation syndromes occur after the discontinuation or reduction of use of a particular substance or a group of substances.
- Evidence is growing that the cannabis withdrawal syndrome is common, of clinical significance, and has a clear time course.
- Implications of withdrawal symptoms include the risk of relapse and effects on the wellbeing of patients.
- The symptoms are listed in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) and contains: (A) cessation of heavy and prolonged cannabis use; (B) three or more of the following within several days after cessation: (1) irritability, (2) nervousness, (3) sleep difficulty, (4) decreased appetite, (5) restlessness, (6) depressed mood, (7) physical symptoms and discomfort.
- The withdrawal symptoms listed in the DSM-5 are not the only symptoms that have been studied, and other symptoms may occur, including increased appetite (about as common as decreased appetite), and vivid unpleasant dreams.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00128-9
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

CUD Cannabis use disorder
DSM-5 Diagnostic and Statistical Manual of Mental Disorders-fifth edition
THC Tetrahydrocannabinol

INTRODUCTION

The Phenomenon of Cannabis Withdrawal Symptoms

Withdrawal or discontinuation syndromes occur after the discontinuation or reduction of use of a particular substance or a group of substances. The cannabis withdrawal phenomenon has received growing interest in recent years. Cannabis withdrawal does not cause significant medical or psychiatric problems comparable to opioid, alcohol, or benzodiazepine withdrawal (Tompkins, Smith, Mintzer, Campbell, & Strain, 2014). However, studies have found that cannabis withdrawal symptoms increases the risk of relapse and affect the wellbeing of patients, calling for targeted clinical interventions (Budney & Hughes, 2006; Budney, Hughes, Moore, & Vandrey, 2004; Kubarych et al., 2014).

With the publication of the Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 in 2013, a cannabis withdrawal syndrome is now officially recognized with defined criteria (APA, 2013) as a result of several earlier studies and discussions. Carroll, Rounsaville, and Bryant (1994) found that withdrawal was rarely endorsed by cannabis users, and Guttman scaling showed that only the most severely affected cannabis dependent patients reported cannabis withdrawal. However, the absence of specific criteria may have meant that the study sample did not interpret symptoms that occurred after cannabis cessation as withdrawal symptoms. Accordingly, later research has produced a different picture (see, for example, Budney & Hughes, 2006; Budney et al., 2004; Vandrey, Budney, Kamon, & Stanger, 2005). The cannabis withdrawal syndrome appears to be comparable to the tobacco withdrawal syndrome in severity, is associated with severity of cannabis problems at a later stage (Chung, Martin, Cornelius, & Clark, 2008; Cornelius, Chung, Martin, Wood, & Clark, 2008), and most symptoms increase immediately after cessation of use and decline over the next weeks (Allsop, Norberg, Copeland, Fu, & Budney, 2011; Budney, Moore, Vandrey, & Hughes, 2003; Milin, Manion, Dare, & Walker, 2008); see however Preuss, Watzke, Zimmermann, Wong, & Schmidt (2010).

SYMPTOMS OF CANNABIS WITHDRAWAL IN THE DSM-5

The symptoms of cannabis withdrawal that are listed in the DSM-5 contains: (A) cessation of heavy and prolonged cannabis use; (B) three or more of the following within several days after cessation: (1) irritability, (2) nervousness, (3) sleep difficulty, (4) decreased appetite, (5) restlessness, (6) depressed mood, (7) physical symptoms, and discomfort (APA, 2013). Some of the studies that have supported the inclusion of these symptoms involve the following.

Irritability

Most studies support the relevance of irritability and anger as common and significant symptoms of cannabis withdrawal (for example, Allsop et al., 2011; Arendt, Rosenberg, Foldager, Sher, & Munk-Jorgensen, 2007; Budney, Hughes, Moore, & Novy, 2001; Budney et al., 2003; Chung et al., 2008; Copersino et al., 2006; Haney, Ward, Comer, Foltin, & Fischman, 1999; Kouri & Pope, 2000; Levin et al., 2010; Mennes, Ben Abdallah, & Cottler, 2009). Irritability and anger has a later onset, and a longer duration than most other withdrawal symptoms (Budney et al., 2003; Hesse & Thylstrup, 2013). Additionally, more severe expressions of aggression, such as physical attacks, may occur later than milder symptoms of irritability (Levin et al., 2010).

Nervousness

Nervousness is a commonly reported symptom (Arendt et al., 2007; Budney et al., 2003; Chung et al., 2008; Copersino et al., 2006; Cornelius et al., 2008; Haney et al., 1999; Kouri & Pope, 2000; Levin et al., 2010; Mennes et al., 2009). Prospective research has found that nervousness associated with cannabis withdrawal is a source of significant distress (Allsop et al., 2011) that gradually declines in severity after cannabis cessation (Budney et al., 2003; Milin et al., 2008). However, not all studies have supported the significance of nervousness as a symptom of cannabis withdrawal (eg, Budney et al., 2001).

Sleep Difficulty

Sleep difficulty or insomnia is a frequently reported withdrawal symptom (Allsop et al., 2011; Arendt et al., 2007; Budney et al., 2001, 2003; Chung et al., 2008; Cornelius et al., 2008; Levin et al., 2010; Mennes et al., 2009) that decreases with time since cessation (Budney et al., 2001, 2003; Milin et al., 2008). However, studies have found some uncertainty regarding the time

course: prospective studies using self-report measures suggest that sleep problems decline fairly linearly after cessation of cannabis use (Budney et al., 2003; Milin et al., 2008), whereas a study using polysomnogram data indicated that sleep quality declined with no reversal (Bolla et al., 2010).

Decreased Appetite or Weight Loss

Decreased appetite or weight loss has been robustly reported across studies (Allsop et al., 2011; Budney et al., 2001, 2003; Copersino et al., 2006; Haney et al., 1999; Kouri & Pope, 2000; Levin et al., 2010; Mennes et al., 2009), with quick onset, followed by a relatively quick reversal (Budney et al., 2003).

Restlessness

Restlessness has generally been supported as a common and significant symptom of cannabis withdrawal (Allsop et al., 2011; Arendt et al., 2007; Budney et al., 2003; Chung et al., 2008; Cornelius et al., 2008; Levin et al., 2010; Mennes et al., 2009; Milin et al., 2008), with a slightly later onset than most symptoms (Budney et al., 2003).

Depressed Mood

Depressed mood is commonly reported (Arendt et al., 2007; Chung et al., 2008; Copersino et al., 2006; Cornelius et al., 2008; Kouri & Pope, 2000; Levin et al., 2010; Mennes et al., 2009). Some prospective studies have failed to show that it follows a clear withdrawal pattern (Budney et al., 2003), but other research supports its inclusion as a valid withdrawal symptom (Allsop et al., 2011).

Physical Symptoms

Physical symptoms including stomach pain, shakiness/tremors, sweating, fever, chills or headache have been reported at variable rates, and generally at lower rates compared with other symptoms (Chung et al., 2008; Cornelius et al., 2008; and for example Arendt et al., 2007; Haney et al., 1999; Kouri & Pope, 2000; Levin et al., 2010; Mennes et al., 2009), but some research has supported most of these symptoms as indications of withdrawal (Allsop et al., 2011); see however Budney et al. (2001).

Other Symptoms Related to Cessation of Cannabis Use

The withdrawal symptoms listed in the DSM-5 are not the only symptoms that have been studied in clinical and experimental research, and other symptoms have been listed as symptoms that may also occur (APA, 2013). A frequently reported symptom is increased appetite, about as common as decreased appetite (Copersino et al., 2006; Levin et al., 2010; Mennes et al., 2009), although Allsop et al. (2011) have reported that it was not valid as a withdrawal criterion.

One of the most commonly reported symptoms is vivid unpleasant dreams, which appears to be as common as the symptoms listed for the DSM-5 (Allsop et al., 2011; Arendt et al., 2007; Budney et al., 2001, 2003; Cornelius et al., 2008; Hesse & Thylstrup, 2013); see however Mennes et al. (2009). The time course of these dreams differs from other symptoms with a later onset and a longer duration (Budney et al., 2003; Levin et al., 2010), and the criterion "vivid unpleasant dreams" is not easily subsumed under any of the symptoms in the DSM-5. However, strange and unpleasant dreams are listed in the DSM-5 criteria for amphetamine and cocaine withdrawal.

VARIATIONS IN TIME COURSE OF WITHDRAWAL SYMPTOMS

The consistent findings that the time course of individual cannabis withdrawal symptoms varies may be caused by a wide range of underlying mechanisms. Some symptoms may occur as soon as cannabis use ceases and blood-levels of tetrahydrocannabinol (THC) and other components in cannabis begin to drop, whereas other symptoms may not occur until THC is nearly completely eliminated from the blood stream. For instance, a study by Kouri and Pope (2000) showed that self-reported increased anxiety, increased physical tension, and appetite loss began immediately after cessation of cannabis use, whereas irritability, low mood, and other physical symptoms, accelerated somewhat more slowly as THC levels dropped further (Kouri & Pope, 2000).

Similarly, the study by Hesse and Thylstrup (2013) found that nervousness increased immediately after cessation, whereas irritability showed an initial increase followed by a decrease (Hesse & Thylstrup, 2013). It is likely that medications affect the time course of cannabis withdrawal symptoms, although the study by Hesse and Thylstrup found that antipsychotics and antidepressants did not change the results of experienced severity of withdrawal symptoms across time (Hesse & Thylstrup, 2013). Other mechanisms that also may affect the time course and severity are the psychological factors, such as expectations to certain sensations after cessation of drug use, or other life stressors related to cessation of drug use or entering treatment. It is possible that these factors create a temporary increase in experienced stress over the first few days after

cessation of cannabis use, for example, when patients entering treatment become increasingly aware of both physical and psychological symptoms, or when they decide to make a change in lifestyle. This may be the case especially for patients entering residential treatment, and patients who experience multiple changes in their everyday life at the same time.

The Question of Demand Characteristics

Overall, the literature on cannabis withdrawal phenomenon and symptoms shows a fairly consistent picture that is largely in line with the cannabis withdrawal criteria in the DSM-5. However, a potential limitation is the problem of demand characteristics that can be explained as the totality of cues that convey an experimental hypothesis to the subject (Orne, 1962). In this case, giving patients cues that a given instrument is designed to measure withdrawal from cannabis may prompt them to respond in ways that they perceive matches what they believe about sensations related to withdrawal symptoms in general. If patients believe that withdrawal symptoms refer to a temporary cluster of specific symptoms that begin after cessation of a specific drug use, they may be more prone to report these symptoms after cessation of use, and stop reporting these symptoms after a while. Such cues may range from writing "cannabis withdrawal" in the heading of the study questionnaire, to asking on the same form, how many days it has been since the subject last used cannabis, or to having a cannabis craving scale on the same questionnaire. Some studies have addressed some aspects of demand characteristics, for example, by using filler items (Allsop et al., 2011), alternative headings, such as "Behavior Checklist" (Budney et al., 2001), or by asking participants to fill in other measures simultaneously with marijuana craving questionnaires (Budney et al., 2003). However, these studies have clearly stated the study focus on marijuana cessation during the recruitment phase, and have asked patients to specifically choose a quitting date for marijuana use.

One of the ways to reduce the impact of demand characteristics is to study cannabis withdrawal symptoms in polydrug use. Up until recently, very limited data exist on the cannabis withdrawal symptoms in patients with concurrent substance use disorders, other than cannabis use and tobacco use (Budney, Vandrey, Hughes, Thostenson, & Bursac, 2008; Vandrey, Budney, Moore, & Hughes, 2005). Arguably, there are good reasons for omitting studies of patient samples with polysubstance use when establishing the validity and time course of the cannabis withdrawal syndrome per se. For example, other drugs may influence mood, physical discomfort, sleep, appetite, and overall wellbeing that may mask or exacerbate specific symptoms that are associated with the cannabis withdrawal. However, polysubstance use is quite common in substance users (Carter et al., 2013), and polydrug dependent patients make up a large proportion of patients in substance abuse treatment services (Onyeka et al., 2012; Savant et al., 2013). If this patient group experiences clinically significant cannabis withdrawal symptoms, psychoeducation, behavioral, and pharmacological interventions that target the cannabis withdrawal symptoms interventions should be made available (Budney, Higgins, & Wong, 1996; Kidorf, Neufeld, King, Clark, & Brooner, 2007). Vorspan et al. (2010) conducted a small cross-sectional study that indicated that sleep disturbances, but not other cannabis withdrawal symptoms, were exacerbated in patients with comorbid opioid dependence (Vorspan et al., 2010). Hesse and Thylstrup (2013) conducted the first study to assess the DSM-5 cannabis withdrawal syndrome as described in the coming manual and the time course of the recommended withdrawal symptoms, based on 90 polydrug users' self-reports from residential illicit drug abuse treatment institutions in Denmark (Hesse & Thylstrup, 2013). To assess whether the DSM-5 symptoms would follow a curvilinear time course from cessation of cannabis use, characterized by an increase in severity immediately after cessation of use followed by a decrease in severity, the study used a scale that consisted of all the criteria listed in the DSM-5 for cannabis withdrawal. To reduce demand characteristics, the patients were not instructed to complete the scale questionnaire on the day that they last used cannabis, but were asked to describe how they felt right now. Additionally, the questionnaire included withdrawal symptoms currently listed for other substances; cocaine, amphetamine, opioids, tranquilizers, and alcohol. Study findings supported the existence of a cannabis withdrawal syndrome independent of other drug use that followed a clear time course with a peak in overall severity 10 days after last use, and a gradual decline over the next weeks (see Fig. 110.1).

The figure is divided into two, so that assessments conducted in week 1 are in the left panel, and assessments conducted in week 2 are in the right hand panel. Within each panel, severity of self-reported symptoms is shown as a function of days since last use of cannabis, with locally weighted regression estimation. As can be seen, the symptom severity increased from day 1 to day 10, and dropped gradually after this. For assessments from week 2, no patients reported using cannabis less than 7 days ago, so only an almost linear decline is seen, following roughly the same values as in the left-hand panel. Thus within this group, withdrawal symptoms follow a time course that is determined by days since last cannabis use, rather than time of assessment.

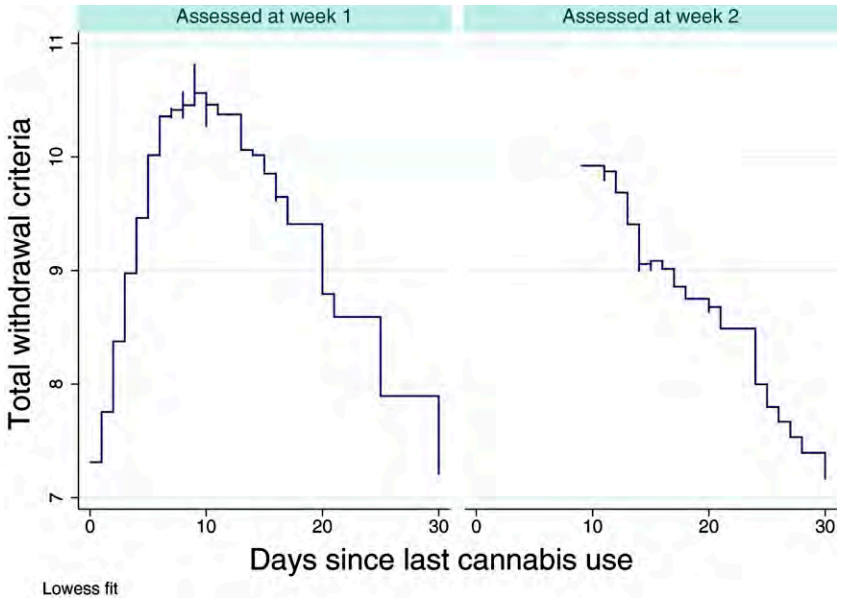


FIGURE 110.1 **Symptom severity for overall cannabis withdrawal syndrome.**

In sum, much of the research conducted supports the evidence and the inclusion of the cannabis withdrawal syndrome in the DSM-5. Although study findings also show diversities concerning which specific withdrawal symptoms that may be more prevalent than others, the cannabis withdrawal syndrome is partly independent of other drug use, and follows a clear time-course with a peak in overall severity 10 days after last use, followed by a gradual decline over the next 15–20 days.

CLINICAL IMPLICATIONS

Psychosocial Treatment

The building evidence on cannabis withdrawal syndrome underlines the need for specific interventions that target symptoms related to cannabis withdrawal, especially within the first month after cessation or reduction of use. This need that is stressed by the fact that adults seeking treatment for marijuana abuse or dependence average more than 10 years of near-daily use, and more than six serious attempts at quitting (Budney, 2006; Stephens, Babor, Kadden, Miller, & Marijuana Treatment Project Research, 2002). However, despite the accumulating evidence of the need for targeted interventions, psychosocial intervention studies on treatment of cannabis withdrawal symptoms are lacking. These studies are important for several reasons. For one, psychoeducation may better the patients' and family members' understanding of dependence and discomforts related to cessation or reduction of cannabis use, countering possible discomforts, such as, irritability, restlessness, or insomnia by giving advice on good sleep hygiene, relaxation, and avoidance of caffeine (Winstock, Ford, & Witton, 2010). Some studies have investigated patient motives for cannabis use, including positive and negative expectancies to cessation of use (Bonn-Miller, Zvolensky, & Bernstein, 2007; Zvolensky et al., 2007), while other studies have investigated specific interventions that address patient motives for use (Banes, Stephens, Blevins, Walker, & Roffman, 2014; Bonn-Miller et al., 2007; Zvolensky et al., 2007). One of the findings from these studies is that cognitive-behavioral therapy may work as relapse prevention by identifying situations and internal states that trigger use, and teach the patients relevant coping skills (Banes et al., 2014; Bonn-Miller et al., 2007; Zvolensky et al., 2007).

Because withdrawal symptoms may put patients at risk of relapse, treatment requires specific clinical attention on the specific cannabis withdrawal symptoms, besides the withdrawal syndrome itself. Besides information on relevant demographic factors, such as education, employment, marital status (Vendetti, McRee, Miller, Christiansen, & Herrell, 2002), screening for cannabis, tobacco and alcohol use, and other polydrug use, treatment should also consider screening for broader lifestyle questions that may be linked to the specific cannabis withdrawal symptoms, such as questions about sleep and eating problems (Chesney, Matsos, Couturier, & Johnson, 2014). As is the case for other drug use, cannabis dependence and withdrawal symptoms coexist with other mental disorders (Di Forti, Morrison, Butt, & Murray, 2007), for example, schizophrenia (Evins, Green, Kane, & Murray, 2012; Koskinen, Lohonen, Koponen, Isohanni, & Miettunen, 2010), anxiety (Crippa

et al., 2009), depression, bipolar disorder (Lev-Ran, Le Foll, McKenzie, George, & Rehm, 2013a), and personality disorders (Lev-Ran, Le Foll, McKenzie, George, & Rehm, 2013b). Clinical assessment may therefore include screening of relevant mental health problems, including age of onset, family history, externalizing and internalizing disorders, and temperament (Babor, Webb, Burleson, & Kaminer, 2002), as well as physical problems, such as deficits in memory and executive functions (Fisk & Montgomery, 2008; Meier et al., 2012; Solowij & Battisti, 2008), respiratory symptoms (Lee & Hancox, 2011; Tessmer et al., 2012), cardiovascular problems (Cottencin et al., 2010; Thomas, Kloner, & Rezkalla, 2014).

Pharmacological Treatment

Despite the strong focus on how pharmacological treatment can target the cannabis withdrawal syndrome, limited evidence is available concerning the effect of pharmacological treatment per se (Budney, Vandrey, Hughes, Moore, & Bahrenburg, 2007; Hart, 2005). However, there are currently ongoing trials that study the efficacy of this type of treatment. An interesting option is to either maintain or taper patients on THC-based medications (Haney et al., 2004; Milman et al., 2014). In a recent study, Allsop et al. (2014) evaluated the safety and efficacy of the cannabis extract nabiximols (Sativex), originally developed as a multiple sclerosis treatment, in a randomized clinical trial (Allsop et al., 2014). The study found that nabiximols attenuated cannabis withdrawal symptoms, and improved patient retention in a treatment-seeking cohort, but that placebo was as effective as nabiximols in promoting long-term reductions in cannabis use following medication cessation (Allsop et al., 2014). Other studies have looked at medications that traditionally have been used for somatic and mental health problems. For example, a study by Weinstein et al. (2014) on the use of escitalopram, (used in depression, panic disorder, anxiety disorder, and obsessive compulsive disorder), found that the medical treatment did not provide additional benefits compared to placebo (Weinstein et al., 2014). Also, a study by Vandrey, Smith, McCann, Budney, and Curran (2011) found that medications directed at particular symptoms, such as sleep disturbances, might reduce overall discomfort , and Mariani et al. (2014) are at the time of writing evaluating quetiapine, a short-acting atypical antipsychotic approved for the treatment of schizophrenia, bipolar disorder, and, along with an antidepressant, for the treatment of cannabis dependence (Mariani et al., 2014). Finally, a case study found that baclofen, an agonist for the $GABA_B$ receptors that until now was primarily used in the treatment of spasticity, may be of clinical utility for reduction of cannabis withdrawal symptoms, and the subjective effects of cannabis (Imbert, Labrune, Lancon, & Simon, 2014).

The diversity in studies on medication strategies, and their study findings, reflects the complexity in cannabis withdrawal syndrome that is also found in the related symptoms. The complexity indicates that different medications may be needed in targeting the various aspects of withdrawal symptoms. A review by Balter, Cooper, & Haney (2014) on recent medication development for the treatment of cannabis use disorder (CUD) describes that laboratory studies found that medication strategies, such as the cannabinoid receptor agonist, nabilone, and the adrenergic agonist, lofexidine, alone and in combination with dronabinol (synthetic THC), decreased cannabis withdrawal symptoms, whereas antidepressant, anxiolytic, and antipsychotic drugs targeting monoamines (norepinephrine, dopamine, and serotonin) in general failed to decrease withdrawal symptoms or laboratory measures of relapse. In clinical trials, dronabinol and multiple antidepressants (fluoxetine, venlafaxine and buspirone) seemed to fall short in decreasing cannabis use, whereas controlled clinical trials with gabapentin and *N*-acetylcysteine supported further research concerning medical use, as did open label and laboratory studies on lithium and oxytocin (Balter et al., 2014).

CONCLUSIONS

The abuse of cannabis may lead to specific cannabis withdrawal symptoms after cessation or reduction of use. Research literature on the cannabis withdrawal phenomena and withdrawal symptoms shows a fairly consistent picture that is largely in line with the cannabis withdrawal criteria in the DSM-5. The need for specific treatment interventions that target the cannabis withdrawal syndrome calls for further research on efficacious behavioral or pharmacological interventions of the withdrawal symptoms. Future research needs to take other substance-related problems, including the medical treatment of comorbid mental and physical health problems, into consideration when establishing the effect of psychosocial and pharmacological treatment.

MINI DICTIONARY

Demand characteristics The experimental artifact where participants form an interpretation of the experiment's purpose and subconsciously change their behavior to fit that interpretation.
Discontinuation The cessation or reduction of a pattern of substance use.
DSM-5 The Diagnostic and Statistical Manual, 5th edition.
Polydrug use The use of multiple substances by the same individual in a given time frame.
Residential treatment A drug and/or alcohol addiction treatment program, where patients reside at the residential treatment facility during treatment, which may be short-term (30 days or less) or long-ter (more than 30 days).

Symptom Any single complaint or problem that is caused by and shows a more serious and general problem.
Syndrome A group of signs and symptoms that occur together and characterize a particular abnormality or condition.
Time course The variation of a quantity over time.
Withdrawal A withdrawal syndrome, also called a discontinuation syndrome is a set of symptoms occurring in discontinuation or dosage reduction of some types of substances.

References

Allsop, D. J., Copeland, J., Lintzeris, N., Dunlop, A. J., Montebello, M., Sadler, C., …, McGregor, I. S. (2014). Nabiximols as an agonist replacement therapy during cannabis withdrawal: a randomized clinical trial. *JAMA Psychiatry*, *71*(3), 281–291.

Allsop, D. J., Norberg, M. M., Copeland, J., Fu, S., & Budney, A. J. (2011). The cannabis withdrawal scale development: patterns and predictors of cannabis withdrawal and distress. *Drug and Alcohol Dependence*, *119*(1-2), 123–129.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th edition). Arlington, VA: American Psychiatric Association.

Arendt, M., Rosenberg, R., Foldager, L., Sher, L., & Munk-Jorgensen, P. (2007). Withdrawal symptoms do not predict relapse among subjects treated for cannabis dependence. *American Journal of Addictions*, *16*(6), 461–467.

Babor, T. F., Webb, C., Burleson, J. A., & Kaminer, Y. (2002). Subtypes for classifying adolescents with marijuana use disorders: construct validity and clinical implications. *Addiction*, *97*(Suppl. 1), 58–69.

Balter, R. E., Cooper, Z. D., & Haney, M. (2014). Novel pharmacologic approaches to treating cannabis use disorder. *Current Addiction Reports*, *1*(2), 137–143.

Banes, K. E., Stephens, R. S., Blevins, C. E., Walker, D. D., & Roffman, R. A. (2014). Changing motives for use: outcomes from a cognitive-behavioral intervention for marijuana-dependent adults. *Drug and Alcohol Dependence*, *139*, 41–46.

Bolla, K. I., Lesage, S. R., Gamaldo, C. E., Neubauer, D. N., Wang, N. Y., Funderburk, F. R., …, Cadet, J. L. (2010). Polysomnogram changes in marijuana users who report sleep disturbances during prior abstinence. *Sleep Medicine*, *11*(9), 882–889.

Bonn-Miller, M. O., Zvolensky, M. J., & Bernstein, A. (2007). Marijuana use motives: concurrent relations to frequency of past 30-day use and anxiety sensitivity among young adult marijuana smokers. *Addictive Behaviors*, *32*(1), 49–62.

Budney, A. J. (2006). Are specific dependence criteria necessary for different substances: how can research on cannabis inform this issue? *Addiction*, *101*(Suppl. 1), 125–133.

Budney, A. J., Higgins, S. T., & Wong, C. J. (1996). Marijuana use and treatment outcome in cocaine-dependent patients. *Experimental and Clinical Psychopharmacology*, *4*, 396–403.

Budney, A. J., & Hughes, J. R. (2006). The cannabis withdrawal syndrome. *Current Opinion in Psychiatry*, *19*(3), 233–238.

Budney, A. J., Hughes, J. R., Moore, B. A., & Novy, P. L. (2001). Marijuana abstinence effects in marijuana smokers maintained in their home environment. *Archives of General Psychiatry*, *58*(10), 917–924.

Budney, A. J., Hughes, J. R., Moore, B. A., & Vandrey, R. (2004). Review of the validity and significance of cannabis withdrawal syndrome. *American Journal of Psychiatry*, *161*(11), 1967–1977.

Budney, A. J., Moore, B. A., Vandrey, R. G., & Hughes, J. R. (2003). The time course and significance of cannabis withdrawal. *Journal of Abnormal Psychology*, *112*(3), 393–402.

Budney, A. J., Vandrey, R. G., Hughes, J. R., Moore, B. A., & Bahrenburg, B. (2007). Oral delta-9-tetrahydrocannabinol suppresses cannabis withdrawal symptoms. *Drug and Alcohol Dependence*, *86*(1), 22–29.

Budney, A. J., Vandrey, R. G., Hughes, J. R., Thostenson, J. D., & Bursac, Z. (2008). Comparison of cannabis and tobacco withdrawal: severity and contribution to relapse. *Journal of Substance Abuse Treatment*, *35*(4), 362–368.

Carroll, K. M., Rounsaville, B. J., & Bryant, K. J. (1994). Should tolerance and withdrawal be required for substance dependence disorders? *Drug and Alcohol Dependence*, *36*(1), 15–22.

Carter, J. L., Strang, J., Frissa, S., Hayes, R. D., Team, S., Study, ELCoH., Hatch, S. L., & Hotopf, M. (2013). Comparisons of polydrug use at national and inner city levels in England: associations with demographic and socioeconomic factors. *Annals of Epidemiology*, *23*(10), 636–645.

Chesney, T., Matsos, L., Couturier, J., & Johnson, N. (2014). Cannabis withdrawal syndrome: An important diagnostic consideration in adolescents presenting with disordered eating. *International Journal of Eating Disorders*, *47*(2), 219–223.

Chung, T., Martin, C. S., Cornelius, J. R., & Clark, D. B. (2008). Cannabis withdrawal predicts severity of cannabis involvement at 1-year follow-up among treated adolescents. *Addiction*, *103*(5), 787–799.

Copersino, M. L., Boyd, S. J., Tashkin, D. P., Huestis, M. A., Heishman, S. J., Dermand, J. C., …, Gorelick, D.A. (2006). Cannabis withdrawal among non-treatment-seeking adult cannabis users. *American Journal of Addiction,*, *15*(1), 8–14.

Cornelius, J. R., Chung, T., Martin, C., Wood, D. S., & Clark, D. B. (2008). Cannabis withdrawal is common among treatment-seeking adolescents with cannabis dependence and major depression, and is associated with rapid relapse to dependence. *Addictive Behaviors*, *33*(11), 1500–1505.

Cottencin, O., Karila, L., Lambert, M., Arveiller, C., Benyamina, A., Boissonas, A., …, Reynaud, M. (2010). Cannabis arteritis: review of the literature. Journal of Addiction Medicine, *Journal of Addiction Medicine*, *4*(4), 191-196.

Crippa, J. A., Zuardi, A. W., Martin-Santos, R., Bhattacharyya, S., Atakan, Z., McGuire, P., & Fusar-Poli, P. (2009). Cannabis and anxiety: a critical review of the evidence. *Human Psychopharmacology: Clinical and Experimental*, *24*(7), 515–523.

Di Forti, M., Morrison, P. D., Butt, A., & Murray, R. M. (2007). Cannabis use and psychiatric and cognitive disorders: the chicken or the egg? *Current Opinion in Psychiatry*, *20*(3), 228–234.

Evins, A. E., Green, A. I., Kane, J. M., & Murray, R. M. (2012). The effect of marijuana use on the risk for schizophrenia. *Journal of Clinical Psychiatry*, *73*(11), 1463–1468.

Fisk, J. E., & Montgomery, C. (2008). Real-world memory and executive processes in cannabis users and non-users. *Journal of Psychopharmacology*, *22*(7), 727–736.

Haney, M., Hart, C. L., Vosburg, S. K., Nasser, J., Bennett, A., Zubaran, C., & Foltin, R. W. (2004). Marijuana withdrawal in humans: effects of oral THC or divalproex. *Neuropsychopharmacology*, *29*(1), 158–170.

Haney, M., Ward, A. S., Comer, S. D., Foltin, R. W., & Fischman, M. W. (1999). Abstinence symptoms following smoked marijuana in humans. *Psychopharmacology (Berlin)*, *141*(4), 395–404.

Hart, C. L. (2005). Increasing treatment options for cannabis dependence: a review of potential pharmacotherapies. *Drug and Alcohol Dependence*, *80*(2), 147–159.

Hesse, M., & Thylstrup, B. (2013). Time-course of the DSM-5 cannabis withdrawal symptoms in poly-substance abusers. *BMC Psychiatry*, *13*, 258.

Imbert, B., Labrune, N., Lancon, C., & Simon, N. (2014). Baclofen in the management of cannabis dependence syndrome. *Therapeutic Advances in Psychopharmacology*, *4*(1), 50–52.

Kidorf, M., Neufeld, K., King, V. L., Clark, M., & Brooner, R. K. (2007). A stepped care approach for reducing cannabis use in opioid-dependent outpatients. *Journal of Substance Abuse Treatment*, *32*(4), 341–347.

Koskinen, J., Lohonen, J., Koponen, H., Isohanni, M., & Miettunen, J. (2010). Rate of cannabis use disorders in clinical samples of patients with schizophrenia: a meta-analysis. *Schizophrenia Bulletin*, *36*(6), 1115–1130.

Kouri, E. M., & Pope, H. G. (2000). Abstinence symptoms during withdrawal from chronic marijuana use. *Experimental and Clinical Psychopharmacology*, *8*(4), 483–492.

Kubarych, T. S., Kendler, K. S., Aggen, S. H., Estabrook, R., Edwards, A.C., Clark, S. L., …, Gillespie, N. A. (2014). Comparing Factor, class, and mixture models of cannabis initiation and DSM cannabis use disorder criteria, including craving, in the Brisbane Longitudinal Twin Study. *Twin Research and Human Genetics*, 17(2), 89–98.

Lee, M. H., & Hancox, R. J. (2011). Effects of smoking cannabis on lung function. *Expert Review of Respiratory Medicine*, *5*(4), 537–546.

Lev-Ran, S., Le Foll, B., McKenzie, K., George, T. P., & Rehm, J. (2013a). Bipolar disorder and co-occurring cannabis use disorders: characteristics, co-morbidities and clinical correlates. *Psychiatry Research*, *209*(3), 459–465.

Lev-Ran, S., Le Foll, B., McKenzie, K., George, T. P., & Rehm, J. (2013b). Cannabis use and cannabis use disorders among individuals with mental illness. *Comprehensive Psychiatry*, *54*(6), 589–598.

Levin, K. H., Copersino, M. L., Heishman, S. J., Liu, F., Kelly, D. L., Boggs, D. L., & Gorelick, D. A. (2010). Cannabis withdrawal symptoms in non-treatment-seeking adult cannabis smokers. *Drug and Alcohol Dependence*, *111*(1-2), 120–127.

Mariani, J. J., Pavlicova, M., Mamczur, A. K., Bisaga, A., Nunes, E. V., & Levin, F. R. (2014). Open-label pilot study of quetiapine treatment for cannabis dependence. *American Journal of Drug and Alcohol Abuse*, *40*(4), 280–284.

Meier, M. H., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R. S., …, Moffitt, T. E. (2012). Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(40), E2657–E2664.

Mennes, C. E., Ben Abdallah, A., & Cottler, L. B. (2009). The reliability of self-reported cannabis abuse, dependence and withdrawal symptoms: multisite study of differences between general population and treatment groups. *Addictive Behaviors*, *34*(2), 223–226.

Milin, R., Manion, I., Dare, G., & Walker, S. (2008). Prospective assessment of cannabis withdrawal in adolescents with cannabis dependence: a pilot study. *Journal of the American Academy of Child and Adolescent Psychiatry*, *47*(2), 174–178.

Milman, G., Bergamaschi, M. M., Lee, D., Mendu, D. R., Barnes, A. J., Vandrey, R., & Huestis, M. A. (2014). Plasma cannabinoid concentrations during dronabinol pharmacotherapy for cannabis dependence. *Therapeutic Drug Monitoring*, *36*(2), 218–224.

Onyeka, I. N., Uosukainen, H., Korhonen, M. J., Beynon, C., Bell, J. S., Ronkainen, K., …, Kauhanen, J. (2012). Sociodemographic characteristics and drug abuse patterns of treatment-seeking illicit drug abusers in Finland, 1997-2008: the Huuti study. *Journal of Addictive Diseases*, *31*(4), 350–362.

Orne, M. T. (1962). On the social psychology of the psychological experiment: with particular reference to demand characteristics and their implications. *American Psychologist*, *17*, 776–783.

Preuss, U. W., Watzke, A. B., Zimmermann, J., Wong, J. W., & Schmidt, C. O. (2010). Cannabis withdrawal severity and short-term course among cannabis-dependent adolescent and young adult inpatients. *Drug and Alcohol Dependence*, *106*(2-3), 133–141.

Savant, J. D., Barry, D. T., Cutter, C. J., Joy, M. T., Dinh, A., Schottenfeld, R. S., & Fiellin, D. A. (2013). Prevalence of mood and substance use disorders among patients seeking primary care office-based buprenorphine/naloxone treatment. *Drug and Alcohol Dependence*, *127*(1-3), 243–247.

Solowij, N., & Battisti, R. (2008). The chronic effects of cannabis on memory in humans: a review. *Current Drug Abuse Reviews*, *1*(1), 81–98.

Stephens, R. S., Babor, T. F., Kadden, R., & Miller, M. Marijuana Treatment Project Research Group. (2002). The Marijuana Treatment Project: rationale, design and participant characteristics. *Addiction*, *97*(Suppl. 1), 109–124.

Tessmer, A., Berlin, N., Sussman, G., Leader, N., Chung, E. C., & Beezhold, D. (2012). Hypersensitivity reactions to marijuana. *Annals of Allergy, Asthma and Immunology*, *108*(4), 282–284.

Thomas, G., Kloner, R. A., & Rezkalla, S. (2014). Adverse cardiovascular, cerebrovascular, and peripheral vascular effects of marijuana inhalation: what cardiologists need to know. *American Journal of Cardiology*, *113*(1), 187–190.

Tompkins, D. A., Smith, M. T., Mintzer, M. Z., Campbell, C. M., & Strain, E. C. (2014). A double blind, within subject comparison of spontaneous opioid withdrawal from buprenorphine versus morphine. *Journal of Pharmacology and Experimental Therapeutics*, *348*(2), 217–226.

Vandrey, R., Budney, A. J., Kamon, J. L., & Stanger, C. (2005). Cannabis withdrawal in adolescent treatment seekers. *Drug and Alcohol Dependence*, *78*(2), 205–210.

Vandrey, R. G., Budney, A. J., Moore, B. A., & Hughes, J. R. (2005). A cross-study comparison of cannabis and tobacco withdrawal. *American Journal of Addiction*, *14*(1), 54–63.

Vandrey, R., Smith, M. T., McCann, U. D., Budney, A. J., & Curran, E. M. (2011). Sleep disturbance and the effects of extended-release zolpidem during cannabis withdrawal. *Drug and Alcohol Dependence*, *117*(1), 38–44.

Vendetti, J., McRee, B., Miller, M., Christiansen, K., & Herrell, J. Marijuana Treatment Project Research Group. (2002). Correlates of pretreatment drop-out among persons with marijuana dependence. *Addiction*, *97*(Suppl. 1), 125–134.

Vorspan, F., Guillem, E., Bloch, V., Bellais, L., Sicot, R., Noble, F., …, Gorelick, D. A. (2010). Self-reported sleep disturbances during cannabis withdrawal in cannabis-dependent outpatients with and without opioid dependence. *Sleep Medicine*, *11*(5), 499–500.

Weinstein, A. M., Miller, H., Bluvstein, I., Rapoport, E., Schreiber, S., Bar-Hamburger, R., & Bloch, M. (2014). Treatment of cannabis dependence using escitalopram in combination with cognitive-behavior therapy: a double-blind placebo-controlled study. *American Journal of Drug and Alcohol Abuse*, *40*(1), 16–22.

Winstock, A. R., Ford, C., & Witton, J. (2010). Assessment and management of cannabis use disorders in primary care. *British Medical Journal*, *340*, c1571.

Zvolensky, M. J., Vujanovic, A. A., Bernstein, A., Bonn-Miller, M. O., Marshall, E. C., & Leyro, T. M. (2007). Marijuana use motives: a confirmatory test and evaluation among young adult marijuana users. *Addictive Behaviors*, *32*(12), 3122–3130.

CHAPTER

111

School-Based Cannabis Prevention Programs

C. Ariza, F. Sánchez-Martínez, A. Pérez

Evaluation and Intervention Methods Service, Public Health Agency, Barcelona, Spain

SUMMARY POINTS

- Some transition periods in the natural history of cannabis use are good opportunities to intervene.
- There are a set of established evidence principles that a good school-based prevention needs to observe.
- The delivery methods are more important than the program's content for effectiveness.
- Cannabis, like other addictive substances, has its own social marketing that prevention programs need to deal with.
- Eight school-based drug prevention programs are described in this chapter.

KEY FACTS OF SCHOOL-BASED CANNABIS PREVENTION PROGRAMS

- School-based cannabis prevention programs are a set of cannabis preventive strategies, usually implemented in school settings.
- Based on the social influence model, these programs try to motivate young people to avoid using cannabis.
- Compared with other treatment resources, preventive programs are addressed to cannabis nonuser population, of school age.
- Little reductions in the incidence of new cannabis users obtained with these strategies have important impact in the individual and social burden of illness.
- The effect size of school-based cannabis prevention programs is low, but consistent, and can be maintained over time.

LIST OF ABBREVIATIONS

ADCA	Alcohol and other Drugs Council of Australia
ASSAD	Australian Secondary Students' Alcohol and Drug survey
CDC-YRBS	Centers for Disease Control and Prevention—Youth Risk Behavior Surveillance
CG	Control group
EDDRA	Exchange on Drug Demand Reduction Action
EG	Experimental group
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
ESPAD	European School survey Project on Alcohol and other Drugs
NREPP	National Registry of Evidence-Based Programs and Policies
RADAR	Register of Australian Drug and Alcohol Research
SAMHSA	Substance Abuse and Mental Health Services Administration

INFLUENCE OF THE NATURAL HISTORY OF CANNABIS CONSUMPTION ON PREVENTION

The natural history of cannabis consumption offers the principal stages that happen in the process that takes a person from occasional to regular user, and the exposure to the risk of becoming addicted. It is possible to identify relevant transition periods for prevention.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00129-0
Copyright © 2017 Elsevier Inc. All rights reserved.

TABLE 111.1 Comparative Data of Main Prevalences in the Progression of Cannabis Consumption (United States, Australia, and Europe, 2011[a])

	United States (%)	Australia (%)	Europe (%)
Tried cannabis the first time before 13 years	6.3–15.2	3.4	3.1–8.4[b]
Lifetime cannabis consumption	39.9	14.8	17.0
Current cannabis use (past month)	23.1	6.8	7.0

The table presents a comparative description of the main cannabis prevalence indicators in United States, Australia, and Europe in 2011.
[a] *United States: CDC (2012); Australia: White et al. (2012); Europe: Hibell et al. (2012).*
[b] *Range between European countries with extreme differences in figures about cannabis use (see Kokkevi, Gabhainn, & Spyropoulou, 2006).*

The first is the starting age for cannabis consumption. The prevalence of youngsters who tried cannabis before 13 years old is higher in US adolescents (6.3–15.2%) (CDC, 2012) than Europeans (3.1–8.4%) (Hibell et al., 2012) and Australians (White et al., 2012) (3.4%) (Table 111.1).

The best moment to start with school-based prevention programs is not clear. One study followed-up a representative sample of schoolchildren from secondary schools in Spain (n = 1056) from 7 to 10th grades (Guxens, Nebot, & Ariza, 2007). Participants completed a self-administered lifestyle questionnaire each year. Data about the progression on lifetime cannabis consumption is shown in Fig. 111.1.

The main relative increase in the prevalence occurs between the two first years of secondary school (13–14 years old). It is important to pay attention to this transition period, because early consumers have higher risk of persisting in long-term use and becoming addicted (Kokkevi et al., 2006) and exhibiting cannabis use-related problems and low academic results in later years (Ellickson, Tucker, Klein, & Saner, 2004). However, the quantitative increase occurred when students changed from 15 to 16 years old (Guxens et al., 2007), so this is the transition period when the majority of the target population come into contact with the drug. Thus, the most opportune age range to intervene is 14–15 years. Recent surveys showed that the average starting age for cannabis use was 14.7 years in a European country, Spain (Plan Nacional sobre Drogas, 2012), or 14.9 in a North American country, Canada (CAMH, 2013), However, cannabis consumption in the United States begins before 13 years, and cannabis preventive programs among American youngsters should probably start earlier than in the European or Australian context.

Two main indicators are important, "lifetime" and "current cannabis use." Whereas lifetime consumption can represent a milestone in the socialization process, the majority of authors accepted the progression to current use (30-day prevalence) as the first significant event to avoid (Thompson, 2001; Monshouwer, Smit, De Graaf, van Os, & Vollebergh, 2005). In 2011, 23.1% of United States (CDC, 2012), 6.8% of Australian (White et al., 2012), and 7% of European (Hibell et al., 2012) adolescents used cannabis the 30 days before the survey (Table 111.1). In a study prior to the design of school-based cannabis prevention program carried out in Spain, students' cannabis use was measured twice between the ages of 15 and 16 years. 23.7% of nonusers at baseline had started to consume 15 months later (23.0% boys and 24.2% girls). Among those who had reported lifetime cannabis

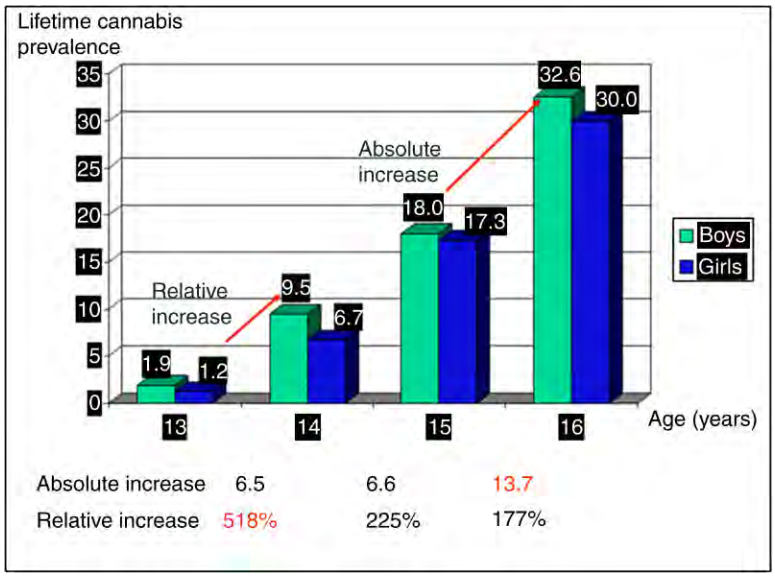


FIGURE 111.1 **Progression of lifetime cannabis consumption in a cohort from 13 to 16 year olds (Barcelona, 1998–2001).** The figure shows the turning points in the progression of lifetime cannabis use in a cohort of Barcelona teenagers.

use, 30.3% reported consumption during the previous month at the follow-up (Pérez, Ariza, Sánchez-Martínez, & Nebot, 2009). It seems that avoiding the progression to current use could be one of the main objectives for universal school-based cannabis prevention.

The third aspect with implications for prevention consists in associated factors that can predict the consumption. Factors associated with cannabis initiation among boys and girls are smoking, risky alcohol use, intention to consume cannabis, antisocial behavior, low academic performance (Thompson, 2001; Von Sydow, Lieb, Pfister, Hofler, & Wittchen, 2002; Ellickson et al., 2004; Guxens et al., 2007; Pérez et al., 2009). Cannabis use is more frequent in males than in females (Von Sydow et al., 2002), and the influence of socioeconomic status is controversial (Thompson, 2001). Other associated factors were: among boys, frequenting bars or discotheques, and not having organized activities in leisure time; among girls, having cannabis-using friends (Guxens et al., 2007; Pérez et al., 2009). This highlights the important influence of social norms, and especially peer influence in adolescent behavior and drug consumption. Preventive programs must cope with these influences, trying to consider these variables in the design of strategies. These data, on the other hand, underlines the role of behavioral and contextual variables.

MAIN CHARACTERISTICS OF SCHOOL-BASED CANNABIS PREVENTION PROGRAMS

School, community, and family are suitable settings for the implementation of cannabis prevention programs. Adolescents spend most of their time at school, being an appropriate place to implement prevention (National Crime Prevention Centre, 2009; Newton, Vogl, Teesson, & Andrews, 2011; Porath-Waller, Beasley, & Beirness, 2010). Faggiano et al. (2005) point out three reasons why schools are such a good place: prevention of substance use must focus on school-aged population because their beliefs and expectations are not still established; school is the best way of reaching a large number of young people; many countries can enforce educational policies.

Types of School-Based Cannabis Prevention Programs

School-based programs can be classified into; (1) *universal*, focused on the general student population, and (2) *selective* or *targeted*, focused on high-risk populations. In terms of components of the intervention, there are programs with only the school-based component (unimodal) and approaches that use family, peer, community, and school-based components (multimodal) (Nation et al., 2003; Norberg, Kezelman, & Lim-Howe, 2013).

Besides, school-based programs can be classified based on two criteria. The first criterion is according to the delivery method of the program. *Interactive programs* are those in which the students have the opportunity of interaction with peers and to experiment what they are learning (eg, role-play) (Faggiano et al., 2005; McGrath, Sumnall, McVeigh, & Bellis, 2006). *Noninteractive programs* are based on passive didactic methods, being the communication unidirectional from educator to students (Faggiano et al., 2005; Porath-Waller et al., 2010; Tobler, Lessard, Marshall, Ochshorn, & Roona, 1999).

The second criterion depends on the nature of the program content, which can be classified in three approaches: *skills-focused programs* (also known as social influence programs), *affective-focused programs* and *knowledge-focused* (Newton et al., 2011; Porath-Waller et al., 2010).

Skills-focused programs aim to improve students' abilities in generic, refusal and safety skills. This approach suggest that drug use originate direct or indirect in social influences from peers or media (McGrath et al., 2006; Botvin, 2000a) and aims to increase an awareness of social influences regarding drug use and to teach skills which allow adolescents to face pressure to consume drugs. It incorporates three components: (1) *information* given to youth must be appropriate and based on outcomes relevant to them (Newton et al., 2011); (2) *normative education* try to correct the misconception that the majority of population use drugs and to promote safer choices regarding drug consumption (Newton et al., 2011; McGrath et al., 2006; Botvin, 2000a). Correcting normative expectations is very important for resistance skills training programs to be successful (Botvin, 2000a); (3) *resistance skills training* provide adolescents with the abilities necessaries to identify and cope peer and media pressure to consume drugs, having a positive impact on cannabis use (Newton et al., 2011; McGrath et al., 2006; Botvin, 2000a). Botvin describes three resistance skills training: teaching students to recognize high-risk situations with peer pressure, increasing the awareness of media influences and teaching students abilities for refusing drugs' offers (Botvin, 2000a).

Affective-focused programs aim to modify inner qualities and to promote affective development increasing self-esteem, self-awareness, and self-acceptance (Faggiano et al., 2005; Porath-Waller et al., 2010; McGrath et al., 2006).

Knowledge-focused programs objective is to enhance students' knowledge of drugs, drug effects and their consequences in order to build negative attitudes toward drugs (Faggiano et al., 2005; McGrath et al., 2006).

Most authors agree on supporting that interactive methods for preventive interventions are more effective than noninteractive (Norberg et al., 2013; Porath-Waller

TABLE 111.2 Main Characteristics of School-Based Cannabis Prevention Programs

Types of school-based cannabis prevention programs	Population	*Universal:* general population
		Selective: high-risk population
	Components	*Unimodal:* only school-based component
		Multimodal: some of the family, peer, community, and school-based components
	Delivery methods	*Interactive*: students have the opportunity of interaction between peers
		Noninteractive: based on passive didactic methods, unidirectional communication from teacher to student
	Content	*Skills-focused program:* aim to enhance student's abilities in generic, refusal and safety skills
		Affective-focused program: aim to modify inner qualities and to promote affective development
		Knowledge-focused program: aim to improve students' knowledge of drugs and their effects
Individual components of school-based cannabis prevention programs	*Timing of delivery*: appropriate age of the participants for the intervention	
	Program facilitators: teachers, external educators, and peers are the most frequent program deliverers	
	Program intensity: dosage of the intervention (session length, number of sessions, spacing of sessions, and duration of the program)	
	Booster sessions: additional sessions which reinforce the progress made for the preliminary ones	

The table shows that school-based cannabis prevention programs can be classified regarding their target population, the components, the delivery methods used or their content.

et al., 2010; Tobler et al., 1999; McGrath et al., 2006). Also, here is strong evidence that skill-focused programs are the most effective form of school-level intervention for preventing youth drug use (Faggiano et al., 2005; Norberg et al., 2013; Porath-Waller et al., 2010; Tobler et al., 1999; Botvin, 2000a) (Table 111.2).

Individual Components of a School-Based Cannabis Prevention Program

The main components of a school-based prevention program are those:

Timing of Delivery

The optimal time of delivery is during early adolescence (Norberg et al., 2013; Porath-Waller et al., 2010). As mentioned before, the periods when preventive programs are more likely to work are: immediately before to initial experimentation, when most students are experiencing initial exposure, and when prevalence of use are high (McBride, 2003). However, literature regarding the optimal participant age for implementation is inconclusive (Porath-Waller et al., 2010; Gottfredson & Wilson, 2003).

Program Facilitators

Teachers, external educators and peers are the most frequent program deliverers. Some studies indicate that programs led by nonteachers are the most effective (Porath-Waller et al., 2010; McGrath et al., 2006). However, most of school-based programs are delivered by the teachers (Porath-Waller et al., 2010; Gottfredson & Wilson, 2003) because they are the nearest person to students. Insufficient motivation and lack of training, awareness or time are some of the main problems related to teachers' weak implementation. Therefore, teacher training and support from prevention experts is essential to give them the confidence necessary to implement properly the programs (Dusenbury & Falco, 1995; Elias, Gager, & Leon 1997).

Program Intensity

Participants must be exposed to sufficient dosage of intervention (Nation et al., 2003). Some authors reported that programs often offer fewer than 10 sessions and it could explain the decay of effects over time (Dusenbury & Falco, 1995).

Booster Sessions

Some studies report the benefit of including boosters in the program design for the maintenance of long-term reductions of drug use because they reinforce messages sometime after the intervention (Skara & Sussman, 2003; McBride, 2003).

The positive effects achieved by school-based preventive programs are often lower than expected, most of the

times due to an inadequate implementation (Sánchez-Martínez et al., 2010). Two dimensions have been defined to assess the implementation of a program: (1) *completeness* provides information on the amount of the intervention that has been applied, (2) *fidelity* measures the degree of implementation agreement to the initial design of the intervention (Sánchez-Martínez et al., 2010). The success of a preventive intervention is based largely on a correct implementation of the intervention.

BEST PRACTICES IN SCHOOL-BASED CANNABIS PREVENTION PROGRAMS

The purpose of this section is to select a group of programs that could be representative of the best practices that different researchers had been developing recently in the North American, Australian, and European contexts. This is not a systematic review. The information has been taken from review studies, but also from some intervention databases with the intention of providing an overview about what is doing now in this field, and also what appears to be working and can be considered as best practice for school-based cannabis prevention.

To select the interventions that we describe and compare in Tables 111.3 and 111.4 we take into account those of the three contexts we explored (North America, Australia, and Europe), and also reported the outcome evaluation and the quality rating obtained in each corresponding database.

North American Programs

The National Registry of Evidence-based Programs and Policies (NREPP) from the Substance Abuse and Mental Health Services Administration (SAMHSA) is the reference database. At the date of writing this text, 335 interventions are included in the registry, 117 of them about "substance abuse prevention." 86 of 117 were school-based programs.

There are three programs among the 86 school-based interventions that could be considered as best practices (Fig. 111.2): they feature the quality ratings obtained for design, implementation and materials and also for the level of effective evaluation. These are: LifeSkills Training (LST; Life Skills Training, 2014) (universal), promoted and evaluated in New York city and coordinated in the Cornell University; Project Alert (Alert Project, 2014) (universal, selective), designed at RAND corporation and evaluated in California, Oregon, and South Dakota, among other places; and Project Towards No Drug Use (TND; Towards No Drug Abuse, 2014) (universal, selective, indicated), promoted and evaluated from University of Southern California. All three programs focus on tobacco, alcohol and cannabis use, but none of them have specific content on cannabis.

Australian Programs

The Register of Australian Drug and Alcohol Research (RADAR) from the Alcohol and other Drugs Council of Australia (ADCA) is the source that we use in the selection of programs. This register contains 345 projects of which 146 are about school-based prevention and 33 of them including cannabis as a content, or their measurement variable in the objectives (Fig. 111.2). The two selected interventions have specific contents on cannabis. The first one, Climate Schools Model (CSM; Climate Schools Model, 2014) program, is a well-recognized intervention, even included in SAMHSA North American database. This program is classified as a universal prevention and integrated in a wider project that includes prevention intervention on alcohol and other illicit drugs and also Internet procedures (Newton, Teesson, Vogl, & Andrews, 2010). The second program is the Gatehouse prevention intervention (The Gatehouse Project, 2014), also universal prevention, that includes contents about health risk behaviors (cannabis use among them), but strategies for social and emotional support (Patton, Bond, Butler, & Glover, 2003) too.

European Programs

European programs are found on the Exchange on Drug Demand Reduction Action (EDDRA) database of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). This register contains 560 projects of which 470 are about school-based prevention and 251 include cannabis as a content or the measurement variable in the objectives (Fig. 111.2). From cannabis content, only eight obtained the classification "top level" quality. We excluded five projects for: (1) being addressed at 6–10 age group; (2) being implemented in special schools; and (3) concentrating their objectives in reducing or quitting cannabis. Consequently, we selected the other three for this section, which are included in Tables 111.3 and 111.4.

The first project is "Unplugged" (European Drug Addiction Prevention Trial, 2014), a universal program, targeted at adolescents from 12 to 14 years old. It is aimed to reduce the initiation, use and abuse of tobacco, alcohol and cannabis. The study was evaluated in a large European collaborative trial (EU-Dap) in seven European countries and coordinated in Italy. The second project is also a universal program, entitled "xkpts.com" (taking its name from the webpage resource it was based around; xkpts.com, 2014), and aimed at adolescents from 14 to 16 years old. The contents of this site were specifically about cannabis prevention and was

TABLE 111.3 Main Characteristics of the Eight Selected Best Practices for School-Based Cannabis Prevention Programs (United States, Australia, and Europe)

Program	Author(s)	Country of origin	Start year	Type of prevention	Target population	Specific versus multidrug prevention	Program leader	Number of sessions	Materials	Quality rating
Life Skills Training (LST)	Botvin, Baker, Dusenbury, Botvin, and Diaz (1995)	United States, New York City, Cornell University	1995	Universal	13–17 year olds	Substance use (alcohol, tobacco, cannabis, inhalants, polydrug)	Teachers	LST: 15 sessions—15 boosters (in 2 years)	Teacher's manual, student handouts, video material. Training workshop	4/4 (SAMHSA)
ALERT (revised)	Ellickson et al. (2004)	United States, RAND Corporation	1995	Universal	13–17 year olds	Substance use (alcohol, tobacco, cannabis)	Teachers	ALERT: 11 sessions—3 boosters	Teacher's manual, Peer teen manual, video material. Training workshop.	3.8/4 (SAMHSA)
Towards No-Drug Abuse (TND)	Sussman, Dent, Stacy, and Craig, (1998)	United States, California, University of Southern California	1994	Universal; selective	13–17 year olds	Alcohol and tobacco use; Cannabis and "hard drug" use	Teachers; Health educators	TND: 9 sessions (8 h)	Teacher's manual, student workbook, video material, board game. Training workshop.	3.1/4 (SAMHSA)
Climate Schools Model (CSM)	Newton et al. (2011)	Australia, University of New South Wales	2006	Universal	13–17 year olds	Alcohol and cannabis use	Teachers	CSM: 12 sessions (8 h)	Implementation guide for teachers and students, quizzes. Online course.	2.8/4 (SAMHSA)
The Gatehouse Project (GP)	Bond et al. (2004)	Australia,Victoria University of Melbourne	1997	Universal	13–17 year olds	Cannabis use	Teachers	GP: 20 sessions (15 h)	Team guidelines	—
Unplugged(EU-Dap Project)	Faggiano et al. (2005)	Europe, Italy, Turin, ASL TO3, Epidemiology Unit	2004	Universal	12–14 year olds	Substance use (alcohol, tobacco, cannabis, inhalants, cocaine, polydrug)	Teachers	Unplugged: 12 sessions (12 h)	Teacher's handbook, students workbook, cards. Training workshop	3/3 (EDDRA)
Xkpts.com	Ariza et al. (2013)	Europe, Spain, Barcelona Public Health Agency	2006	Universal	14–16 year olds	Cannabis use	Teachers	Xkpts.com: 4 sessions (10 h)	Teacher's guide, student workbook, video material, parents brochure. Training workshop	3/3 (EDDRA)
Pre-Venture	Conrod, Castellanos-Ryan, and Strang (2010)	Europe, UK, London, King's College London	2003	Selective; indicated	13–16 year olds	Substance use (alcohol, tobacco, cannabis, inhalants, cocaine, polydrug)	Health professionals	PreVenture: 2 sessions (3 h)	Health professional manual	3/3 (EDDRA)

SAMHSA, Substance Abuse and Mental Health Services Administration; EDDRA, Exchange on Drug Demand Reduction Action. The table describes the structure of each best practice selected for the article.

TABLE 111.4 Other Intervention and Evaluation Details of the Eight Selected Best Practices for School-Based Cannabis Prevention Programs (United States, Australia, and Europe)

Program	Theoretical model	Interactivity	Components	Family involvement	Evaluation design	Sample	Comparison group	Control group	Follow-up period	Outcome measure	Post-test effect	Effect size (Cohen "*d*"[a])
Life Skills Training (LST)	Social influence and competence enhancement model	Interactive	Unimodal	No family involvement	Blocked randomized trial	56 schools; 5954 students	Training provided by videotape	Treatment as usual	72 months	Monthly use (yes: 1, no: 0)	0.10 (E, high fidelity) versus 0.14 (C)[b]	0.09 (36)[c]; 0.09 (72)[c]
ALERT (revised)	Social influence model	Interactive	Multimodal	No family involvement	Cluster-randomized trial	55 schools; 4276 students	Treatment(Alert revised)	No treatment	18 months	Lifetime use; monthly use	13% (E) versus 17% (C)[b]; 9% versus 11%	0.15 (18)[c]; 0.08 (18)
Towards No-Drug Abuse (TND)	Motivation-skills-decision-making model	Interactive	Unimodal	No family involvement	Blocked randomized trial	21 schools; 1578 students	Classroom + School-as community condition	Treatment as usual	60 months	Monthly use	39.6% (E) versus 42.1% (C); 30.6% versus 34.5% (60)	0.07 (12); 0.04 (60)
Climate Schools Model (CSM)	Social influence model	Interactive	Unimodal	No family involvement	Cluster-randomized trial	10 schools; 764 students	—	Treatment as usual	6 months	Mean of times/ week in last 90 days	–0.06 (E) versus 0.20 (C)[b]	0.16 (6)
The Gatehouse Project	Attachment theory; social skills	Mixed: Noninteractive and interactive	Multimodal	No family involvement	Cluster-randomized trial	26 schools; 2678 students	—	Treatment as usual	24 months	Weekly new use in last 6 months	3.2% (E) versus 4.4% (C)	0.12 (24)
Unplugged (EU-Dap Project)	Comprehensive social influence approach	Interactive	Multimodal	Intensive skill training workshop	Randomized control trial	143 schools; 7079 students	Basic curriculum and peers; basic and parents	No treatment	18 months	Prevalence in last 30 days	3.3% (E) versus 5.3% (C)[b]	0.17 (18)[c]
Xkpts.com	ASE (attitudes-social influences-self-efficacy) model	Interactive	Multimodal	Handbook as basis for discussion	Quasi experimental pre-post	86 schools; 4848 students	Treatment as usual	—	15 months	New users in last month	8.3% (E) versus 11.7% (C)[b]	0.19 (15)[c]
Pre-Venture	Personality traits linked to risk for addictive and nonaddictive psychopatology	Noninteractive	Multimodal	No family involvement	Randomized control trial	732 high-risk students	—	Treatment as usual	24 months	Frequency of use in last 6 months	29.7% (E) versus 35.3% (C)	0.20 (24)[c]

E, experimental; C, comparison or control. The table explains how is the program of intervention and the evaluation for each one of the eight selected best practices.

[a] *Cohen's* d *was reported by authors or calculated from results.*

[b] *Statistically significant as* $p < 0.05$.

[c] *Statistically significant as 95% CI does not contain zero.*

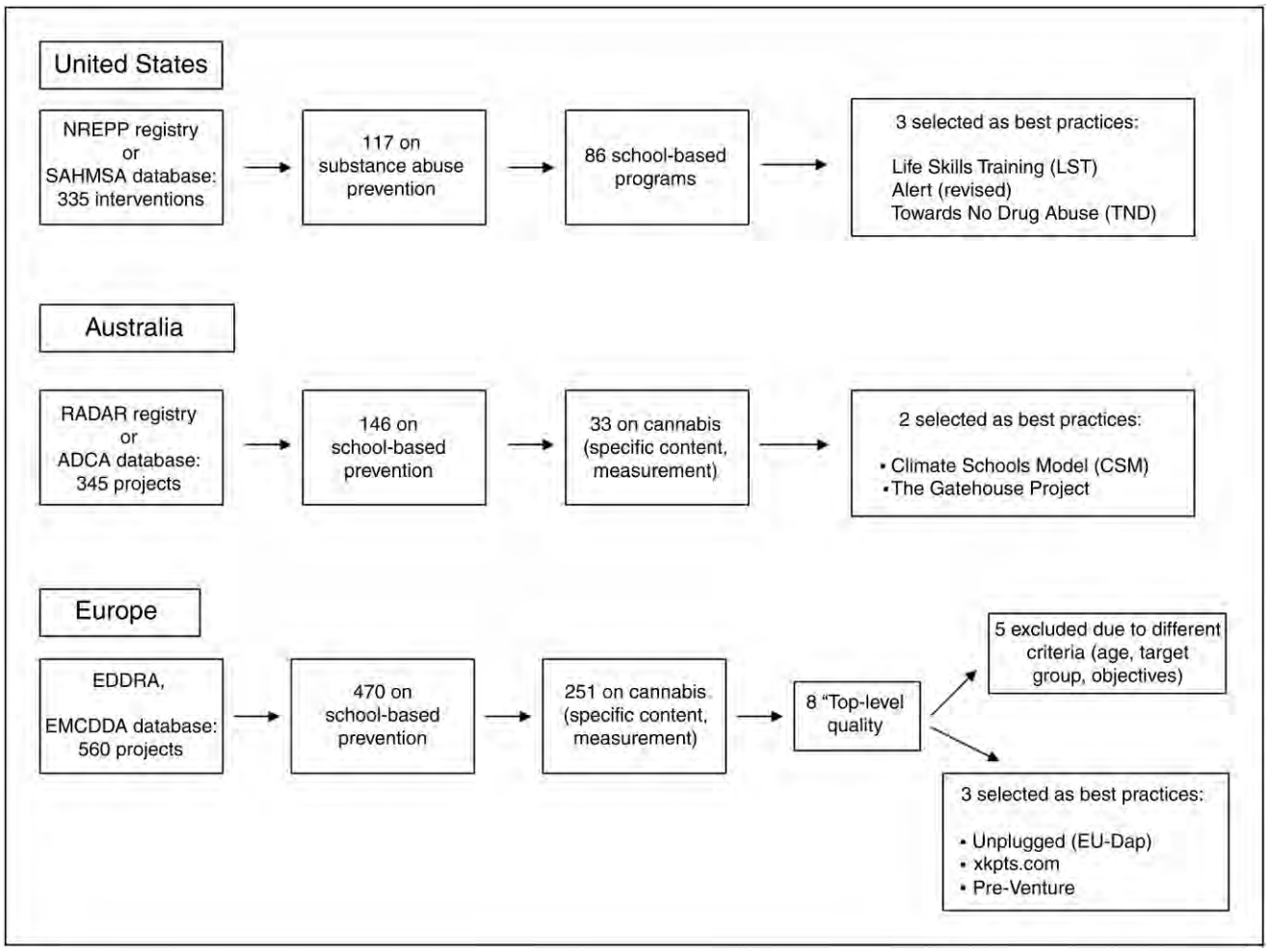


FIGURE 111.2 Flow-diagram of the identification process for best practices. The figure includes the flow-diagram with the detailed process for the selection of the eight best practices described in the article. *NREPP*, National Registry of Evidence-based Programs and Policies; *SAMHSA*, Substance Abuse and Mental Health Services Administration; *RADAR*, Register of Australian Drug and Alcohol Research; *ADCA*, Alcohol and Other Drugs Council of Australia; *EDDRA*, Exchange on Drug Demand Reduction Action; *EMCDDA*, European Monitoring Centre for Drugs and Drug Addiction.

promoted in Barcelona, Spain. The third is "Pre-Venture" (Pre-Venture, 2014), a selective program or personality-targeted cognitive behavioral intervention to reduce alcohol and drug misuse (with cannabis among these drugs). Implemented and coordinated in London, it is addressed to pupils aged 13–16.

Characteristics of the Selected Best Practices

The main characteristics of the selected best practices are shown in Table 111.3. The North American programs and the Gatehouse Australian Project are the older and started between 1994 and 1997. The three European projects and the Australian Climate Schools Model came later (2003–06).

Among the American projects, "Life Skills Training" (LST) was developed in New York City and the other two, project "Alert" and "Towards No Drug Abuse" (TND), in California. From Australian interventions, the "Climate Schools Model" (CSM) was created in New South Wales and the "Gatehouse Project" (GP) in Victoria. The first of three European programs, "Unplugged" (European Drug Addiction Prevention Trial, 2014) was tested in Austria, Belgium, Germany, Greece, Spain, Sweden and Italy and designed and coordinated in this last country. The program "xkpts.com" (xkpts.com, 2014) was designed and evaluated in Barcelona (Spain), and the project "Pre-Venture" (Pre-Venture, 2014) took place in London (United Kingdom).

The majority of the projects are of universal prevention, due to the fact that the classroom component is the element, which allows contact with the greatest number of population. However, two of the selected programs offered the possibilities to focus the intervention on high risk groups: (1) in the case of TND Project offering also actions to the remaining "normalized" population, and

(2) in the case of "Pre-venture," paying total attention to pupils with drug problematic behavior.

The majority of programs have a similar target population age range, of 13–17 year olds. Only "Unplugged" concentrated the intervention in the 12–14 age range.

Only two programs (CSM and "xkpts.com") include a specific session about cannabis in their content. GP worked on developing affective and emotional skills content in the classroom, which could be later applied to preventing students' cannabis use. The rest of the projects targeted substance use in general (tobacco, alcohol, and cannabis, and also other drugs, in combination).

All the universal prevention programs have the teacher as the main provider of the intervention. In the case of selective or indicated programs, health educators or health professionals were the main project leaders.

According to the number of sessions and length, program duration can be defined as short (ranging from 0 to 10 h) or long (more than 10 h) (Norberg et al., 2013). TND, CSM, "xkpts.com" and "Pre-Venture" are classified as short interventions, while LST, Project Alert, GP, and "Unplugged" are defined as long projects.

All the programs have a manual for teachers or the main provider of the intervention. In the case of five of these projects, a training workshop was provided for teachers. The remaining programs offered an online course and/or support. The majority of interventions also included a student workbook. In four cases (the three American programs and "xkpts.com") video material is offered among the resources.

The last column of Table 111.3 indicates the quality rating that the different databases classified their programs; thus, the reference punctuation is different according to the geographical procedure. North American programs were rated between 3.1 and 4 from SAMHSA score. The Australian RADAR database does not rate interventions; for this reason we use the SAMHSA rating for CSM. However, GP is not included in SAMHSA and therefore no rating is available. According to the EDDRA quality rating, the three European selected programs were classified as "top-level" score.

Other Intervention and Evaluation Details of the Selected Best Practices

The details that complement the intervention and effectiveness evaluation of each program are included in Table 111.4.

"LifeSkills Training" (LST) is based on both the social influence and competence enhancement models of prevention (http://www.lifeskillstraining.com/). This program helps young people to face the most important risk factors associated with alcohol, tobacco, and cannabis and, in an interactive format, develop the skills to understand and resist prodrug influences. Broad dissemination of LST started in 1995 and, since then, approximately 50,000 teachers, 10,000 schools, and 3 million students have participated in the program. LST has been extensively evaluated in different studies, several of which were conducted by its author, Gilbert Botvin and his coworkers (Botvin et al., 1995, 2000b). In one of them, carried out in 56 schools, an effect size of 0.09 in the monthly use of cannabis remained 6 years after the beginning of the study (Botvin et al., 1995).

Project "Alert" is based on the social influence model of prevention and designed to help young people to avoid using drugs (http://www.projectalert.com/). The project helped to develop an interactive scenario incorporating skills to resist cannabis and other social influences related to drugs. Its author, Phyllis Ellickson, and her coworkers participated in the majority of evaluation studies. In the second major evaluation, in 55 middle schools in South Dakota, was achieved an effect size of 0.15 in lifetime use of cannabis and from 0.08 in the monthly use at 18 months follow-up (Ellickson, McCaffrey, Ghosh-Dastidar, & Longshore, 2003). The dissemination of Project Alert began in 1995 and from then more than 50,000 teachers have been trained to deliver the intervention.

Project "Towards No Drug Abuse" (TND) is designed to help students to develop self-control and communication skills for resisting drug use, improving decision-making strategies and developing motivation to not use drugs (http://tnd.usc.edu). It was initially addressed at high-risk students and later adapted for all students attending traditional high schools. The project was designed by Steve Sussman and coworkers, who conducted some of the evaluations of the program between 1998 and 2009 (Sussman et al., 1998; Sun et al., 2006). In the last of these studies, a long-term outcome evaluation was carried out. Self-reported frequency of cannabis use in the past 30 days decreased by 22% among students in Project TND 12 months after the intervention, which was statistically significant compared to students in control group schools. The effect size at 60 months follow-up was 0.04 (Sun et al., 2006).

"Climate Schools Model" (CSM) is based on a social influence approach to prevention and uses cartoon storylines to engage student involvement (http://www.climateschools.com.au). Each lesson has two parts: an Internet-based activity (students log onto the CSM website and follow an episode of cartoon-based drama about real-life situations involving cannabis use); and a teacher-led activity that reinforce the information of cartoons and allows interactive communication between students. The intervention was developed in Australia in 2006 and also reviewed by NREPP of SAMHSA to assess quality of research. The program has been delivered to approximately 4000 students in Australia and also in the United Kingdom. The evaluation was conducted by

Newton et al. (2010). They showed that, in 10 Sydney metropolitan area schools, there was a decrease of intervention group frequency of cannabis use (mean difference, –0.06 times per week) compared to the control group (with a mean difference, 0.20 times per week) ($p < 0.02$), 6 months after the program. This significant difference between groups in cannabis use diminished 12 months following the intervention ($p = 0.11$). In terms of effect size, at 6 months follow-up Cohen's "*d*" was 0.16 (Newton et al., 2010).

The "Gatehouse Project" (GP) was developed in 1997 in Victoria, Australia, by George Patton and coworkers from the University of Melbourne (http://www.gatehouseproject.com). It includes a prevention program, derived from attachment theory and focused on three aspects of the social context: security, communication and participation. In addition, the curriculum in the early years of high school focused on skills for social functioning and emotional adjustment (Patton et al., 2003). The project was evaluated using a randomized controlled trial in 26 metropolitan Melbourne schools and the prevalence and incidence of cannabis use was examined 1 and 2 years after the program commenced. The intervention revealed a reduction of 0.8 in the incidence of weekly cannabis use in the intervention group (3.2%), related the control group (4.0%), which is not statistically significant. The reduction increased among nonsmokers at baseline. In terms of effect size these results supposed values of Cohen's "*d*" of 0.12 at 24 months (Bond et al., 2004).

"Unplugged" is a school-based drug prevention program based on the comprehensive social influence approach, that has been evaluated in the context of the European collaborative randomized controlled trial EU-Dap (http://www.eudap.net). It was conducted by Fabrizio Faggiano and coworkers in Turin (Italy), coordinating other six European countries between 2004 and 2007. The interactive program, with 12 classroom sessions, is delivered weekly by teachers and includes social skills, personal skills, knowledge, and normative education. The program is multimodal, with peers meetings, and also seminars for parents. The evaluation of the effectiveness of the prevention program, 18-month follow-up, showed a prevalence of any use of cannabis in the past 30 days of 3.3% in the intervention group versus 5.3% in control schools. That represented an absolute risk reduction in the multilevel model of the prevalence of cannabis use in past 30 days of 2.2% (Faggiano et al., 2010). That also supposes a Cohen's "*d*" of 0.17, the two values being statistically significant.

The program "xkpts.com" is a cannabis prevention intervention addressed to facing the progression from experimental use to current use (that is, any use in the past 30 days) (http://www.aspb.cat/quefem/escoles/canyes-petes.aspx). It is based on social influences model and skills training. The four-session program, developed over 10 h, spends two sessions on general issues regarding drug dependence and two specific sessions centered on group pressure to use cannabis and skills training to cope with the offer of cannabis. Parents are also involved and provided with a brochure about how to speak at home with their children about cannabis. The program also offers a webpage for students (http://www.sortimbcn.cat/xkpts/) with other different content related to that covered at school. Carles Ariza and coworkers conducted the effectiveness evaluation with a quasi-experimental pre-post controlled study. 4848 students from 86 Barcelona schools participated in the evaluation. Data showed an incidence of new users in past 30 days of 8.3% in the experimental group related to 11.7% in the control group, which was a statistically significant difference (Ariza et al., 2013). The effect size of this difference represents 0.19, according to Cohen's "*d*."

Finally, "Pre-Venture" is a personality targeted cognitive behavioral intervention to reduce alcohol and drug misuse (http://legacy.nreppadmin.net/ViewIntervention.aspx?id=264). Four personality dimensions have been linked to cannabis and other drug use: anxiety-sensitivity, hopelessness, impulsivity and sensation seeking. "Pre-Venture" began in Jan. 2004 and is currently being conducted in 25 mainstream schools in London boroughs. It was piloted by Patricia Conrod, in Canada (Conrod et al., 2010), and then developed at the National Addiction Centre to be developmentally and culturally appropriate for UK youth. The program offers psycho-educational manuals within interactive group sessions with students aged 13–16 years. Group sessions focuses on motivational factors for risky behaviors and provide students with coping skills to aid their decision making in situations involving anxiety and depression, thrill seeking, aggressive and risky behavior (eg, theft, vandalism and bullying), drugs and alcohol misuse. A randomized control trial was conducted with 732 high-risk students in London secondary schools. The intervention was associated with a decrease of frequency of cannabis use in the past 6 months from 5.6% at 24 months after the brief intervention (29.7% (EG) versus 35.3% (CG). This difference and a Cohen's "*d*" effect size of 0.20 were both statistically significant (Conrod et al., 2010).

MINI-DICTIONARY

Best practice Refer to interventions that are supposed to lead to desired outcomes.

Comparison group Group of people whose members have characteristics and demographics similar to those of the intervention group, but didn't receive the intervention.

Comprehensive intervention Array of interventions to address the main precursors or mediators of the target problem.

Comprehensive interventions include two dimensions: multiple interventions (about information, awareness, attitudes, social influences, skills…) and multiple settings (parents, peers, school, community…).

Effect size Effect of treatment for a study. Usually defined as the difference in means between the intervention and control groups, divided by the standard deviation of the control or both groups.

Interactive program Interventions which foster interpersonal skills and active engagement between students and teachers.

Outcome evaluation Systematic examination of the outcomes (changes, usually benefits), resulting from a set of activities implemented to achieve a stated goal, and a systematic examination of the extent to which those activities actually caused those outcomes to occur.

Selective prevention Strategies targeted to subsets of the total population that are deemed to be at risk for substance abuse by virtue of their membership in a particular population segment.

Skills-focused programs Preventive programs which content aim to increase an awareness of social influences regarding drug use and to teach skills which allow adolescents to face pressure to use drugs.

Unimodal/multimodal program Preventive programs can use an exclusive component (unimodal) (ie, school-based or family-based) or different components (multimodal) as family, peer, community, school-based…

Universal prevention Strategies addressed to the entire population (national, local community, school, neighborhood), with messages and programs aimed at preventing or delaying the abuse of alcohol, tobacco, and other drugs.

References

Alert Project. (2014). *National Registry of Evidence-based Programs and Policies*. Rockville, MD: SAMHSA, http://legacy.nreppadmin.net/ViewIntervention.aspx?id=62.

Ariza, C., Pérez, A., Sánchez-Martínez, F., Diéguez, M., Espelt, A., Pasarín, M. I., Suelves, J. M., De la Torre, R., & Nebot, M. (2013). Evaluation of the effectiveness of a school-based cannabis prevention program. *Drug and Alcohol Dependence*, *132*(1–2), 257–264.

Bond, L., Thomas, L., Coffey, C., Glover, S., Butler, H., Carlin, J. B., & Patton, G. (2004). Long-term impact of the Gatehouse Project on cannabis use of 16-year-olds in Australia. *Journal of School Health*, *74*(1), 23–29.

Botvin, G. J. (2000a). Preventing drug abuse in schools: social and competence enhancement approaches targeting individual-level etiologic factors. *Addictive Behaviors*, *25*(6), 887–897.

Botvin, G. J., Baker, E., Dusenbury, L., Botvin, E. M., & Diaz, T. (1995). Long-term followup results of a randomized drug abuse prevention trial in a white middle-class population. *Journal of the American Medical Association*, *273*, 1106–1112.

Botvin, G. J., Griffin, K. W., Diaz, T., Scheier, L. M., Williams, C., & Epstein, J. A. (2000b). Preventing illicit drug use in adolescents: long-term follow-up data from a randomized control trial of a school population. *Addictive Behavior*, *25*(5), 769–774.

Centre for Addiction and Mental Health (CAMH). (2013). At what age do students start to smoke cigarettes, drink alcohol and use cannabis? *CAMH Population Studies eBulletin*, *14*(1), 1–2. http://www.camh.ca/en/research/news_and_publications/Pages/research_population_ebulletins.aspx.

Centers for Disease Control and Prevention (CDC). (2012). Youth Risk Behavior Surveillance—United States, 2011. *Mortal and Mortality Weekly Reports*, *61*(4), 1–162.

Climate Schools Model (CSM). (2014). *National Registry of Evidence-based Programs and Policies*. Rockville, MD: SAMSHA, http://legacy.nreppadmin.net/ViewIntervention.aspx?id=306.

Conrod, P. J., Castellanos-Ryan, N., & Strang, J. (2010). Brief, personality-targeted coping skills interventions and survival as a non-drug user over a 2-year period during adolescence. *Archives of General Psychiatry*, *67*, 85–93.

Dusenbury, L., & Falco, M. (1995). Eleven components of effective drug abuse prevention curricula. *Journal of School Health*, *65*(10), 420–425.

Elias, M. J., Gager, P., & Leon, S. (1997). Spreading a warm blanket of prevention over all children: guidelines for selecting substance abuse and related prevention curricula for use in the schools. *Journal of Primary Prevention*, *18(1), 41–42*.

Ellickson, P. L., McCaffrey, D. F., Ghosh-Dastidar, B., & Longshore, D. L. (2003). New inroads in preventing adolescent drug use: results from a large-scale trial of Project ALERT in middle schools. *American Journal of Public Health*, *93*, 1830–1836.

Ellickson, P. L., Tucker, J. S., Klein, M. S., & Saner, H. (2004). Antecedents and outcomes of marijuana use initiation during adolescence. *Preventive Medicine*, *39*, 976–984.

European Drug Addiction Prevention Trial. (2014). *"Unplugged"(EU-Dap project)*. Lisbon, Portugal: EMCDDA, http://www.emcdda.europa.eu/html.cfm/index52035EN.html?project_id=IT&tab=overview.

Faggiano, F., Vigna-Taglianti, F. D., Versino, E., Zambon, A., Borraccino, A., & Lemma, P. (2005). School-based prevention for illicit drugs' use (Review). *Cochrane Database of Systematic Reviews*, *2*, CD003020.

Faggiano, F., Vigna-Taglianti, F. D., Burkhart, G., Bohrn, K., Cuomo, L., Gregori, D., Panella, M., Scatigna, M., Siliquini, R., Varona, L., Van der Kreeft, P., Vassara, M., Wiborg, G., & Galanti, M. R. (2010). The effectiveness of a school-based substance abuse prevention program: 18-month follow-up of the EU-Dap cluster randomized controlled trial. *Drug Alcohol Depend*, *108*, 56–64.

Gottfredson, D. C., & Wilson, D. B. (2003). Characteristics of effective school-based substance abuse prevention. *Prevention Science*, *4*(1), 27–38.

Guxens, M., Nebot, M., & Ariza, C. (2007). Age and sex differences in factors associated with the onset of cannabis use: a cohort study. *Drug and Alcohol Dependence*, *88*, 234–243.

Hibell, B., Guttormsson, U., Ahlström, S., Balakireva, O., Bjarnason, T., Kokkevi, A., & Kraus, L. ESPAD. (2012). *The 2011 ESPAD Report: Substance Use Among Students in 36 European Countries*. Stockholm, Sweden, and Lisbon, Portugal: The Swedish Council for Information on Alcohol and other Drugs (CAN), EMCDDA and Pompidou Group.

Kokkevi, A., Gabhainn, S. N., & Spyropoulou, M. Risk Behaviour Focus Group of the HBSC. (2006). Early initiation of cannabis use: a cross-national European perspective. *Journal of Adolescent Health*, *39*, 712–719.

"Life Skills Training" (LST). (2014). *National Registry of Evidence-based Programs and Policies*. Rockville, MD: SAMHSA, http://legacy.nreppadmin.net/ViewIntervention.aspx?id=109.

McBride, N. (2003). A systematic review of school drug education. *Health Education Research*, *18*(6), 729–742.

McGrath, Y., Sumnall, H., McVeigh, J., & Bellis, M. (2006). *Drug use prevention among young people: a review of reviews. Evidence briefing update*. London, UK: National Institute for Health and Clinical Excellence, http://www.nice.org.uk/niceMedia/docs/drug_use_prev_update_v9.pdf.

Monshouwer, K., Smit, F., DE Graaf, R., va Os, J., & Vollebergh, W. (2005). First cannabis use: does onset shift to younger ages? Findings from 1988 to 2003 from the Dutch National School Survey on Substance Use. *Addiction*, *100*, 963–970.

Nation, M., Crusto, C., Wandersman, A., Kumpfer, K. L., Seybolt, D., Morrissey-Kane, E., & Davino, K. (2003). What works in prevention: principles of effective prevention programs. *American Psychology*, *58*, 449–456.

National Crime Prevention Centre (NCPC). (2009). *School-based drug abuse prevention: promising and successful programs*. Ottawa, ON: Public Safety Canada, http://www.publicsafety.gc.ca/cnt/rsrcs/pblctns/sclbsd-drgbs/sclbsd-drgbs-eng.pdf.

Newton, N. C., Teesson, M., Vogl, L. E., & Andrews, G. (2010). Internet-based prevention for alcohol and cannabis use: final results of the climate schools course. *Addiction*, *105*, 749–759.

Newton, N. C., Vogl, L., Teesson, M., & Andrews, G. (2011). Developing the Climate Schools: Alcohol and Cannabis Module: a harm minimization, universal drug prevention program facilitated by the internet. *Substance Use and Misuse*, *46*, 1651–1663.

Norberg, M. M., Kezelman, S., & Lim-Howe, N. (2013). Primary prevention of cannabis use: a systematic review of randomized controlled trials. *PLoS One*, *8*(1), e53187.

Patton, G., Bond, L., Butler, H., & Glover, S. (2003). Changing schools, changing health? Design and implementation of the Gatehouse Project. *Journal of Adolescent Health*, *33*(4), 231–239.

Pérez, A., Ariza, C., Sánchez-Martínez, F., & Nebot, M. (2009). Cannabis consumption initiation among adolescents: a longitudinal study. *Addictive Behavior*, *35*, 129–134.

Plan Nacional sobre Drogas. (2012). *Encuesta Estatal sobre Uso de Drogas en Estudiantes de Secundaria (ESTUDES)*. Ministerio de Sanidad, Servicios Sociales e Igualdad: Madrid, Spain, http://www.pnsd.msc.es/Categoria2/observa/pdf/PresentESTUDES2012_2013.pdf.

Porath-Waller, A. J., Beasley, E., & Beirness, D. J. (2010). A meta-analytic review of school-based prevention for cannabis use. *Health Education and Behavior*, *37*, 709–723.

Pre-Venture. (2014). *Exchange on Drug Demand Reduction Action*. Vienna, Austria: EMCDDA, http://www.emcdda.europa.eu/html.cfm/index52035EN.html?project_id=10UK01&tab=overview.

Sánchez-Martínez, F., Ariza, C., Pérez, A., Diéguez, M., López, M. J., & Nebot, M. (2010). Process evaluation of the school-based cannabis use prevention program "xkpts.com" in adolescents from Barcelona in 2006. *Adicciones*, *22*(3), 2017–2026.

Skara, S., & Sussman (2003). A review of 25 long-term adolescent tobacco and other drug use prevention program evaluations. *Preventive Medicine*, *37*(5), 451–474.

Sun, W., Skara, S., Sun, P., Dent, C. W., & Sussman, S. (2006). Project Towards No Drug Abuse: long-term substance use outcomes evaluation. *Preventive Medicine*, *42*(3), 188–192.

Sussman, S., Dent, C. W., Stacy, A. W., & Craig, S. (1998). One-year outcomes of Project Towards No Drug Abuse. *Preventive Medicine*, *27*(4), 632–642Erratum in: *Preventive Medicine* (1998), *27*(5 Pt 1), 766.

The "Gatehouse Project" (GP) (2014). http://www.gatehouseproject.com

Thompson, K. M. (2001). Marijuana use among adolescents: trends, patterns and influences. *Minerva Pediatrica*, *53*, 313–323.

Tobler, N. S., Lessard, T., Marshall, D., Ochshorn, P., & Roona, M. (1999). Effectiveness of school-based drug prevention programs for marijuana use. *School Psychology International*, *20*, 105–137.

"Towards No Drug Abuse" (TND). (2014). *National Registry of Evidence-based Programs and Policies*. Rockville, MD: SAMSHA, http://legacy.nreppadmin.net/ViewIntervention.aspx?id=21.

Von Sydow, K., Lieb, R., Pfister, H., Hofler, M., & Wittchen, H. U. (2002). What predicts incident use of cannabis and progression to abuse and dependence? A 4-year prospective examination of risk factors in a community sample of adolescents and young adults. *Drug and Alcohol Dependence*, *68*, 49–64.

White, V., & Bariola, E. Centre for Behavioural Research in Cancer. (2012). *Australian secondary school students'use of tobacco, alcohol, and over-the-counter and illicit substances in 2011*. Melbourne, VIC: The Cancer Council Victoria, Report.

xkpts.com. (2014). *Exchange on Drug Demand Reduction Action*. Lisbon, Portugal: EMCDDA, http://www.emcdda.europa.eu/html.cfm/index52035EN.html?project_id=ES_06&tab=overview.

CHAPTER

112

The CapOpus Trial for Cannabis Use Disorders

C.R. Hjorthøj, M. Nordentoft

Mental Health Center Copenhagen, Copenhagen University Hospital, Copenhagen, Denmark

SUMMARY POINTS

- CapOpus was a randomized trial of 6 months' specialized psychosocial intervention compared to treatment as usual (TAU) for cannabis use disorder in people with schizophrenia and other psychoses.
- There was no effect on the primary outcome, that is, no reductions in frequency of cannabis use.
- People receiving CapOpus reduced the quantity of cannabis used, namely using less per day, albeit on the same number of days.
- People receiving CapOpus had more psychiatric treatment contacts, but admissions were shorter, probably indicating a more adequate level of treatment.
- CapOpus does not appear to be sufficient to implement as a treatment, but it may be useful in future combination treatment paradigms.

KEY FACTS ON THE CapOpus TREATMENT

- CapOpus was a randomized trial of cognitive behavior therapy and motivational interviewing for cannabis use disorder in people with schizophrenia and other psychoses.
- CapOpus specialized intervention did not reduce frequency of cannabis consumption.
- CapOpus reduced the amount of joints used to 27.3, compared to 48.2 per month in the comparison group.
- CapOpus led to fewer days admitted in psychiatric hospitals (IRR 0.72).
- CapOpus led to more psychiatric emergency room contacts and hospital admissions (IRR 3.47 and 2.24, respectively).

LIST OF ABBREVIATIONS

95% CI	95% Confidence interval
BACS	Brief assessment of cognition in schizophrenia
CBT	Cognitive behavior therapy
CSQ	Client satisfaction questionnaire
EQ-5D	Euroqol's five item quality of life instrument
ICD-10	International classification of diseases, 10th edition
IRR	Incidence rate ratio
MANSA	Manchester short assessment of quality of life
OR	Odds ratio
PANSS	Positive and negative syndrome scale for schizophrenia
SCAN	Schedules for clinical assessment in neuropsychiatry
SD	Standard deviation
TAU	Treatment as usual
THC	Δ^9-Tetrahydrocannabinol
TLFB	Timeline follow-back
WHO	World Health Organization

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00131-9
Copyright © 2017 Elsevier Inc. All rights reserved.

INTRODUCTION

As discussed in much detail elsewhere in this book, cannabis is the most commonly used illicit substance in the world, and it is especially prevalent in people with severe mental illness such as schizophrenia. Both current use and current misuse of cannabis is much more prevalent in people with schizophrenia than in the background population, the observation of which has implicated cannabis as a potentially etiological component in schizophrenia. The fact that cannabis can trigger cannabis-induced psychosis, which is probably phenotypically indistinguishable from schizophrenia, lends further credibility to this potential causal role.

Regardless of this potential role as a trigger for schizophrenia, a number of studies have observed that people with schizophrenia have a poorer prognosis on a range of outcomes if they use cannabis. More severe levels of psychotic symptoms, more hospitalizations, reduced adherence to antipsychotic medication, and a greater risk of homelessness, have all been linked to cannabis use in people with schizophrenia. Conversely, studies have also indicated that people with schizophrenia who use cannabis have fewer negative symptoms and better cognitive skills, compared to their noncannabis-using counterparts. However, randomized, blinded studies on the acute effects of Δ^9-tetrahydrocannabinol (THC) versus placebo given to people with schizophrenia indicate that negative symptoms are increased, and cognitive abilities decreased in the groups receiving THC. As such, it appears that cannabis is associated with worse outcomes on most if not all of the relevant studied outcomes, in people with schizophrenia. While this could be a case of confounding by indication (ie, the self-medication hypothesis), it raises the question whether, perhaps, the prognosis of this group of people with schizophrenia could be improved if they were to reduce or completely discontinue their use of cannabis.

Unfortunately, most trials conducted on cannabis use disorders in people with schizophrenia have been unsuccessful, and it appears that it may be easier to treat other types of substance use disorders in this population. For this reason, the CapOpus trial was initiated in 2007 under the leadership of Professor Merete Nordentoft from the Copenhagen University Hospital. The trial was a 6-month, parallel-group, observer-blinded superiority trial comparing CapOpus plus treatment as usual (TAU) with TAU alone, including patients—from Sep. 2007 to Dec. 2010—in Copenhagen, Denmark. Patients were referred from Danish early psychosis intervention services (Opus teams) (Petersen et al., 2005), Community Mental Health Centers (Nordentoft et al., 1996), Assertive Community Treatment teams (Vendsborg, Nordentoft, Hvenegaard, & Søgaard, 1999), and psychiatric wards. Patients were required to meet both the ICD-10 criteria for a schizophrenia spectrum psychosis (F2) and for a cannabis use disorder (F12); the participants included were aged 17–42 years, had to reside in the Copenhagen area, be able to participate in the trial without the aid of an interpreter, and be able and willing to give informed consent. Dependence to other substances was allowed, if participants themselves designated cannabis as their primary substance of abuse. The diagnosis of schizophrenia spectrum psychosis was usually established by the referring agent and, in cases of doubt, was established by the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) interview (Wing et al., 1990). At this point, participants were randomized to receive either CapOpus as an add-on to TAU, or TAU alone.

INTERVENTIONS

CapOpus

CapOpus was an add-on intervention to TAU (see section Treatment as Usual), which all patients received. Full details are given in the design paper (Hjorthøj et al., 2008) and full protocol (www.capopus.dk). The intervention was based on the EPPIC manual, and was similar to the Midas trial (Hinton, Elkins, & Edwards, 2002; Barrowclough et al., 2010). CapOpus lasted 6 months, with one or two weekly sessions. The intervention was centered around motivational interviewing (Miller & Rollnick, 2002; Miller, 1983) and cognitive behavior therapy (CBT). Emphasis was placed on analyzing advantages and disadvantages of continued use, and instructions in development of personal strategies, in relation to craving trigger situations. The sessions could take place in CapOpus offices, at the patient's home, or treatment facility.

An important part of the CapOpus treatment was to help the patient understand the mechanisms that complicate cessation of cannabis use. The overall target was harm reduction, that is, that harm from cannabis consumption is thought to be alleviated, even if consumption is not terminated, but only reduced.

Upon inclusion, the patient's case manager at the referring institution was offered education and supervision by one of the addiction consultants employed in the CapOpus trial.

To increase adherence to treatment, positive reinforcement was applied through contingency management in the form of complimentary food and social experiences, which may enhance outcomes of cognitive behavioral therapy or motivational enhancement (Denis, Lavie, Fatseas, & Auriacombe, 2006). Contingency management in the CapOpus trial had no connection to whether or not the use of cannabis is decreased. The uptake of this was minimal and was deemed to have influenced the consultant-participant alliance in a maximum of six participants.

The experimental CapOpus intervention was offered in addition to TAU. The interventions offered to the patients were registered, so that it was possible to estimate if the two intervention groups receive different forms of treatment as usual.

Caseload was intended to be 1:10, but was usually between 1:6 and 1:7.

Treatment as Usual

TAU consisted of the treatment available to patients had they not participated in the trial, provided by staff not employed by CapOpus. TAU was carried out in Opus, Community Mental Health Centers, or Assertive Community Treatment teams (Petersen et al., 2005; Nordentoft et al., 1996; Vendsborg et al., 1999). These facilities do not have explicit manuals regarding the treatment of substance use disorders. Caseload is approximately 1:10 in Opus and Assertive Community Treatment teams, and 1:20 to 1:30 in Community Mental Health Centers.

The primary outcome was the number of days with cannabis use in the past month, with recall assisted by Timeline Follow-Back (TLFB; Sobell & Sobell, 1992). This also allowed for quantification of number of "standard joints" last month (defined as 0.5 g of cannabis resin). TLFB-assisted self-report has been shown to validly estimate cannabis consumption in dual diagnosis patients (Hjorthøj, Hjorthøj, & Nordentoft, 2012; Hjorthøj, Fohlmann, Larsen, Arendt, & Nordentoft, 2012). Baseline information on other abuse diagnoses was collected using SCAN. Secondary outcomes were the Positive and Negative Syndrome Scale (PANSS; Kay, Fiszbein, & Opler, 1987), Global Assessment of Functioning (Endicott, Spitzer, Fleiss, & Cohen, 1976), WHO's Disability Assessment Schedule (WHO, 2000), EuroQol's Quality of Life Interview (EQ-5D; Brooks, 1996), Manchester Short Assessment of Quality of Life (MANSA) (Priebe, Huxley, Knight, & Evans, 1999), Brief Assessment of Cognition in Schizophrenia symbol coding (BACS; Keefe et al., 2004), Trail Making Tests A + B (Bowie & Harvey, 2006), Continuous Performance Test—Identical Pairs (Cornblatt, Lenzenweger, & Erlenmeyer-Kimling, 1989), Hopkins Verbal Learning Test (Brandt, 1991), Neuropsychological Assessment Battery mazes (Stern & White, 2003), and the Danish adaptation of the National Adult Reading Test (Nelson & O'Connell, 1978; Hjorthøj, Vesterager, & Nordentoft, 2012). The Client Satisfaction Questionnaire (CSQ) was administered posttreatment (Larsen, Attkisson, Hargreaves, & Nguyen, 1979). Outcome measures on healthcare utilization will be published separately.

Analyses were also conducted based on register-data, on which there was information for all participants in CapOpus. This was done using the nationwide Danish Psychiatric Central Research Register (Mors, Perto, & Mortensen, 2011). Information from this register was condensed into the following variables: time at risk of first psychiatric hospital admission; time at risk of first psychiatric emergency room visit; time at risk of either admission or emergency room visit; number of psychiatric hospital admissions; number of psychiatric emergency room visits; and number of days admitted to psychiatric hospitals.

Patients were randomized to CapOpus plus TAU (n = 52) or TAU alone (n = 51), with no indications of skewed distribution of cannabis consumption or other baseline characteristics between intervention groups. There was a follow-up rate of 66%.

EFFECT OF INTERVENTION ON CANNABIS CONSUMPTION

The effect of treatment on cannabis use is presented in Table 112.1 as incidence rate ratios (ratio-of-days) for the CapOpus group compared with the TAU group, showing no significant differences posttreatment or at follow-up. The absolute consumption of cannabis is also shown in Fig. 112.1. The ratio of cannabis-using days in CapOpus versus TAU was 0.76 [95% confidence interval (CI) 0.38–1.50] (p = 0.42) posttreatment, and 0.80 (95% CI 0.21 –3.10) (p = 0.75) at follow-up. CapOpus-participants smoked 20.9 (95% CI –1.0 to 42.9) fewer joints posttreatment per month than TAU participants (p = 0.06), whereas the difference was 13.3 (95% CI –8.5 to 35.1) (p = 0.23) at follow-up. There was no difference in rates of complete abstinence from cannabis in the two groups (OR = 1.31 (95% CI 0.47–3.64), p = 0.61 posttreatment, and OR = 0.64 (95% CI 0.25–1.68), p = 0.37, at the 10-month follow-up).

Did It Work Better on Some People?

Table 112.1 also shows the effect of treatment on different subpopulations within the randomized trial. It appeared that CapOpus was particularly efficient among those not abstinent at baseline, with a relative reduction of 26.1 (95% CI 3.5–48.7) standard joints per month (p = 0.02). CapOpus also appeared to be effective among the younger patients, defined as those who were 17–21 years old upon inclusion into the trial. Such CapOpus-participants reduced their number of cannabis-using days posttreatment by a third, and their number of monthly joints by half. Young TAU-participants increased their cannabis use to nearly daily consumption, and 40% more joints. Unemployed patients in CapOpus more than halved their number of posttreatment monthly joints, whereas unemployed patients in TAU did not alter their consumption (p = 0.03).

TABLE 112.1 Ratio of Number of Days (incidence rate ratios) of Days With Cannabis Use in the CapOpus Group Compared to TAU (95% Confidence Intervals)

	Baseline	Posttreatment		Follow-up		
Subgroups	**IRR (95% CI)**	**IRR (95% CI)**	**CapOpus versus TAU**	**IRR (95% CI)**	**CapOpus versus TAU**	***P* (group* time)**
All patients (*n* = 103)	0.94 (0.85–1.04)	0.76 (0.38–1.50)	*p* = 0.42	0.80 (0.21–3.10)	*0* = 0.75	0.25
Not abstinent at baseline (*n* = 91)	0.94 (0.85–1.04)	0.66 (0.32–1.37)	*p* = 0.26	0.64 (0.15–2.74)	*p* = 0.55	0.14
≤14 at baseline (*n* = 54)	1.00 (0.79–1.26)	1.52 (0.39–5.90)	*p* = 0.54	2.68 (0.18–39.93)	*p* = 0.48	0.71
15+ at baseline (*n* = 49)	0.98 (0.87–1.09)	0.65 (0.35–1.20)	*p* = 0.17	0.62 (0.19–2.08)	*p* = 0.44	0.14
Male (*n* = 78)	1.00 (0.88–1.12)	0.75 (0.32–1.73)	*p* = 0.50	0.57 (0.11–3.01)	*p* = 0.51	0.80
Female (*n* = 25)	0.78 (0.65–0.94)	0.74 (0.24–2.24)	*p* = 0.59	1.52 (0.17–13.90)	*p* = 0.71	0.04
17–21 years (*n* = 21)	1.07 (0.87–1.32)	0.39 (0.17–0.89)	*p* = 0.03	0.46 (0.10–2.23)	*p* = 0.34	0.002
22–30 years (*n* = 55)	0.96 (0.81–1.07)	0.91 (0.34–2.47)	*p* = 0.86	1.25 (0.17–9.08)	*p* = 0.83	0.29
31–42 years (*n* = 27)	0.83 (0.68–1.02)	1.10 (0.22–5.59)	*p* = 0.90	0.29 (0.01–7.51)	*p* = 0.46	0.01
Unemployed (*n* = 81)	0.99 (0.89–1.11)	0.61 (0.29–1.27)	*p* = 0.18	0.58 (0.14–2.49)	*p* = 0.46	0.04
Employed/student (*n* = 22)	0.76 (0.58–0.98)	1.91 (0.37–9.86)	*p* = 0.44	3.37 (0.13–89.69)	*p* = 0.47	0.42

TAU, Treatment as usual; IRR, incidence rate ratios.

Reproduced from Hjorthøj et al. (2013a). Psychiatric treatment following participation in the CapOpus randomized trial for patients with comorbid cannabis use disorder and psychosis. Schizophrenia Research, 151,*191–196, copyright 2013, with permission from Elsevier.*

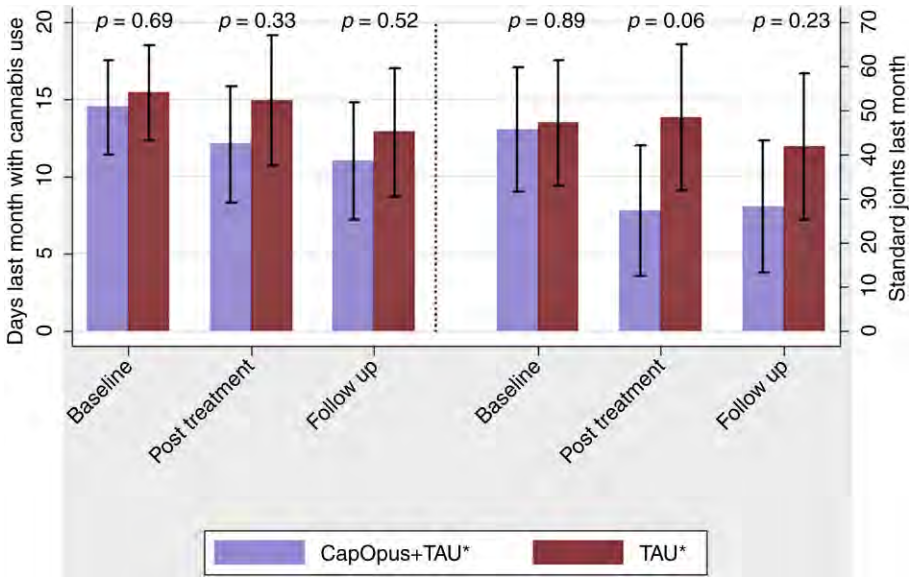


FIGURE 112.1 **Main results of the CapOpus trial as estimated marginal means.** *TAU*, Treatment as usual. *Source: Reproduced from Hjorthøj et al. (2013b). Specialized psychosocial treatment plus treatment as usual (TAU) versus TAU for patients with cannabis use disorder and psychosis: the CapOpus randomized trial,* Psychological Medicine, 43*(7), 1499–1510, with permission.*

CONTACT WITH TREATMENT FACILITIES

Table 112.2 shows the relative use of psychiatric services in CapOpus, compared to TAU. Participants in the CapOpus group had 3.47 (95% CI 2.64–4.57) times as many psychiatric emergency room contacts as those who had received TAU. The time to first emergency room contact was also much faster in the CapOpus group, as indicated by a hazard ratio of 2.02 (95% CI 1.22–3.34). Those who received CapOpus also had more psychiatric hospital admissions. However, those who received CapOpus were admitted fewer days to psychiatric inpatient wards (incidence rate ratio 0.72 (95% CI 0.68–0.75).

Time to first psychiatric emergency room contact and first psychiatric admission following inclusion into the trial are illustrated in Figs. 112.2 and 112.3, respectively.

TABLE 112.2 Effects of CapOpus on Level of Psychiatric Treatment

	TAU (n = 50)	CapOpus (n = 51)	
Cox proportional hazards regression analyses; hazard ratios and 95% confidence intervals			
Time to psychiatric emergency room contact	1 (ref.)	2.02 (1.22–3.34)	$p = 0.006$
Time to psychiatric hospital admission	1 (ref.)	1.48 (0.91–2.41)	$p = 0.12$
Time to any psychiatric contact	1 (ref.)	1.54 (0.96–2.46)	$p = 0.07$
Poisson regression analyses; incidence rate ratios and 95% confidence intervals			
Number of emergency room contacts	1 (ref.)	3.47 (2.64–4.57)	$p < 0.001$
Number of psychiatric hospital admissions	1 (ref.)	2.24 (1.65–3.03)	$p < 0.001$
Number of days admitted to psychiatric hospitals	1 (ref.)	0.72 (0.68–0.75)	$p < 0.001$

Reproduced from Hjorthøj et al. (2013b). Specialized psychosocial treatment plus treatment as usual (TAU) versus TAU for patients with cannabis use disorder and psychosis: the CapOpus randomized trial, Psychological Medicine, 43*(7), 1499–1510, with permission.*

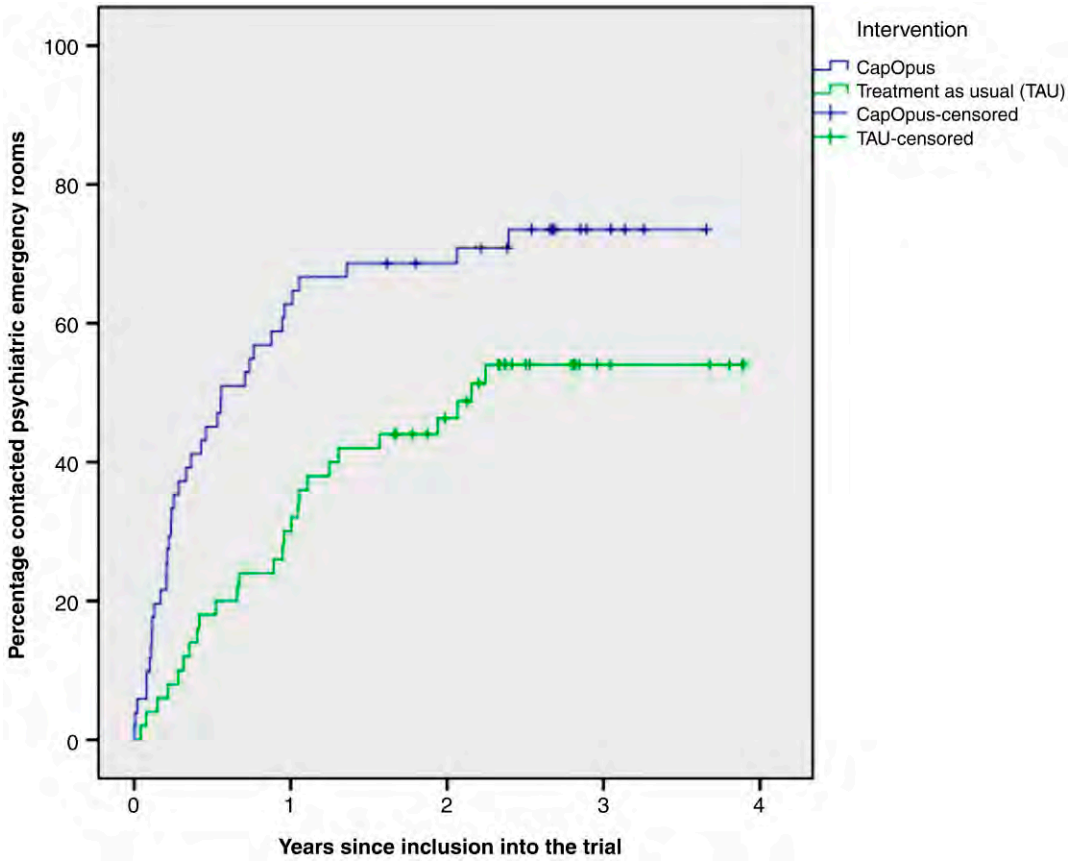


FIGURE 112.2 **Time to first psychiatric emergency room contact by intervention group.** Log rank: $p = 0.005$. *Source: Reproduced from Hjorthøj et al. (2013a). Psychiatric treatment following participation in the CapOpus randomized trial for patients with comorbid cannabis use disorder and psychosis.* Schizophrenia Research, 151, *191–196, copyright 2013, with permission from Elsevier.*

It is evident from these figures that the CapOpus group had a much faster contact with both types of psychiatric services in the period immediately following inclusion. This was at least partly explained by CapOpus consultants facilitating contact with other treatment facilities when needed.

CONCLUSIONS

The CapOpus intervention appeared to be somewhat effective in reducing the monthly number of joints used, but not in reducing the number of days on which this use took place. Those not abstinent at baseline,

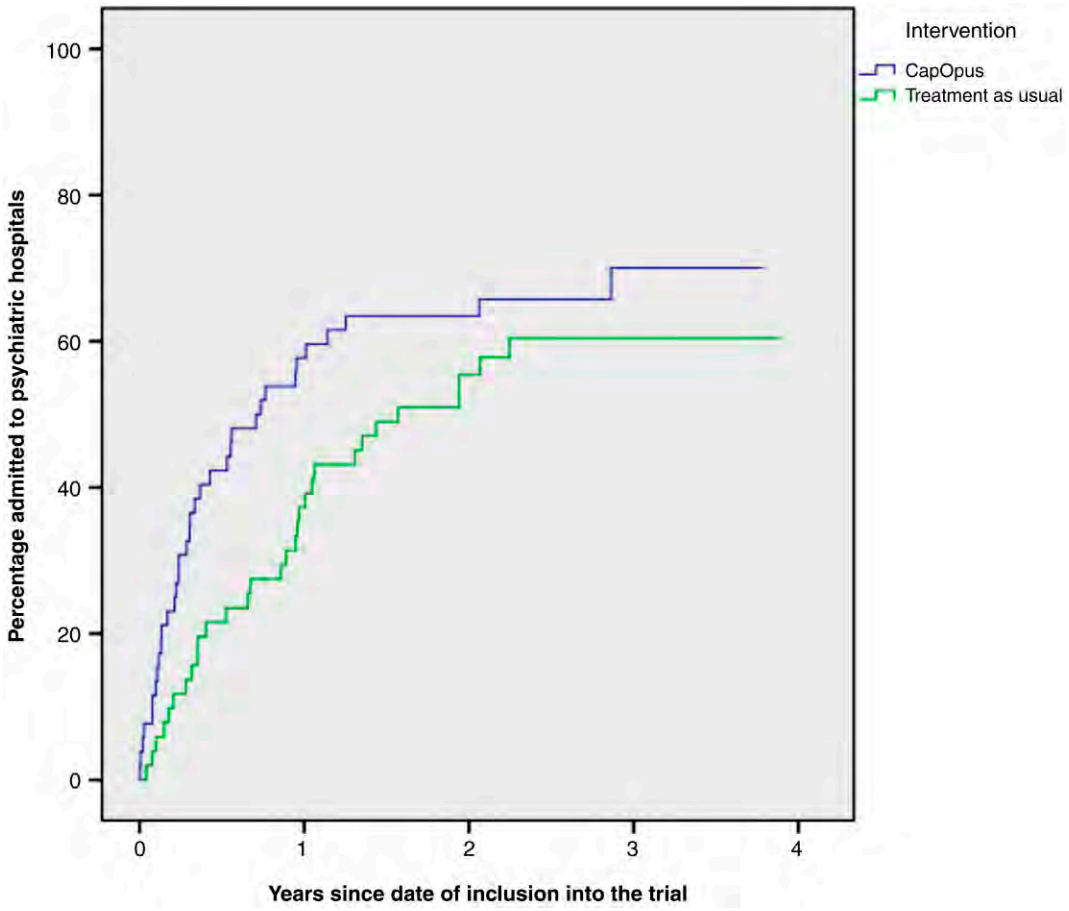


FIGURE 112.3 **Time to first psychiatric hospital contact by intervention group.** *Source: Reproduced from Hjorthøj et al. (2013a), Psychiatric treatment following participation in the CapOpus randomized trial for patients with comorbid cannabis use disorder and psychosis.* Schizophrenia Research, 151, *191–196, copyright 2013, with permission from Elsevier.*

younger participants, and unemployed participants appeared to have particularly positive effects of the CapOpus treatment. Although CapOpus increased the rates of psychiatric hospital admission and emergency room visits, those inpatient stays were of shorter duration when summed over the complete follow-up. This combination of results probably indicates that CapOpus patients were given more adequate treatment, and that those only receiving TAU had fewer inpatient contacts because they were undertreated, leading to prolonged stays in psychiatric wards, once they were finally admitted. This may have occurred by facilitation of referrals or admissions by the CapOpus consultants, or by the patients learning by the CapOpus consultants when to seek additional treatment on their own. The possibility that the increased treatment contact in CapOpus patients could be a detrimental consequence of CapOpus cannot be fully excluded, however. It may be a stressor for patients to start a new type of intervention with new staff, and stressors have been shown to exacerbate psychotic symptoms (Myin-Germeys & van Os, 2007; Norman & Malla, 1993). The odds of abstinence were similar in CapOpus and TAU, so it is unlikely that the increase in treatment contact was related to more withdrawal symptoms in the CapOpus group.

MINI-DICTIONARY

Negative symptoms Symptoms indicating a loss from normal function, such as affective flattening, apathy, and social withdrawal.

Odds ratio (OR), hazard ratio (HR) and incidence rate ratio (IRR) Different statistical terms that are typically all interpreted as indicating relative risk, that is, how much more likely it is that something occurs in one group compared with another.

Psychosis A mental state involving such elements as hallucinations and delusions.

p-value A statistical indicator of whether an association is likely the product of statistical chance rather than reflecting a true association. The number is interpreted as the percentage likelihood that the association could be due to chance. Usually, *p*-values below 0.05 (5%) are considered most likely to reflect true associations rather than statistical chance.

Randomized trial Study design in which participants are randomly assigned to the different interventions. This is considered the gold standard for testing interventions in medical research.

Schizophrenia A mental illness usually characterized by psychosis and negative symptoms.

Acknowledgments

I wish to acknowledge all of my coauthors from the original papers on the CapOpus trial: Professor Merete Nordentoft, Allan Fohlmann, Anne-Mette Larsen, Mette Madsen, Sonja Orlovska, Christian Gluud, and Mikkel Arendt.

References

Barrowclough, C., Haddock, G., Wykes, T., Beardmore, R., Conrod, P., Craig, T., et al. (2010). Integrated motivational interviewing and cognitive behavioural therapy for people with psychosis and comorbid substance misuse: randomised controlled trial. *British Medical Journal*, *341*, c6325.

Bowie, C. R., & Harvey, P. D. (2006). Administration and interpretation of the Trail Making Test. *Nature Protocols*, *1*, 2277–2281.

Brandt, J. (1991). The Hopkins verbal learning test: development of a new memory test with six equivalent forms. *Clinical Neuropsychologist*, *5*, 125–142.

Brooks, R. (1996). EuroQol: the current state of play. *Health Policy*, *37*, 53–72.

Cornblatt, B. A., Lenzenweger, M. F., & Erlenmeyer-Kimling, L. (1989). The continuous performance test, identical pairs version: II. Contrasting attentional profiles in schizophrenic and depressed patients. *Psychiatry Research*, *29*, 65–85.

Denis, C., Lavie, E., Fatseas, M., & Auriacombe, M. (2006). Psychotherapeutic interventions for cannabis abuse and/or dependence in outpatient settings. *Cochrane Database of Systematic Reviews*, *3*, CD005336.

Endicott, J., Spitzer, R. L., Fleiss, J. L., & Cohen, J. (1976). The global assessment scale. A procedure for measuring overall severity of psychiatric disturbance. *Archives of General Psychiatry*, *33*, 766–771.

Hinton, M., Elkins, K., & Edwards, J. (2002). *Cannabis and psychosis: An early psychosis treatment manual*. Melbourne, VIC: EPPIC.

Hjorthøj, C. R., Fohlmann, A., Larsen, A. M., Arendt, M., & Nordentoft, M. (2012a). Correlations and agreement between delta-9-tetrahydrocannabinol (THC) in blood plasma and timeline follow-back (TLFB)-assisted self-reported use of cannabis of patients with cannabis use disorder and psychotic illness attending the CapOpus randomized clinical trial. *Addiction*, *107*, 1123–1131.

Hjorthøj, C., Fohlmann, A., Larsen, A. -M., Madsen, M. T., Vesterager, L., Gluud, C., et al. (2008). Design paper: the CapOpus trial: a randomized, parallel-group, observer-blinded clinical trial of specialized addiction treatment versus treatment as usual for young patients with cannabis abuse and psychosis. *Trials*, *9*, 42.

Hjorthøj, C. R., Hjorthøj, A. R., & Nordentoft, M. (2012b). Validity of timeline follow-back for self-reported use of cannabis and other illicit substances—systematic review and meta-analysis. *Addictive Behaviors*, *37*, 225–233.

Hjorthøj, C. R., Orlovska, S., Fohlmann, A., & Nordentoft, M. (2013a). Psychiatric treatment following participation in the CapOpus randomized trial for patients with comorbid cannabis use disorder and psychosis. *Schizophrenia Research*, *151*, 191–196.

Hjorthøj, C. R., Fohlmann, A., Larsen, A. -M., Gluud, C., Arendt, M. C., & Nordentoft, M. (2013b). Specialized psychosocial treatment plus treatment as usual versus treatment as usual for patients with cannabis use disorder and psychosis—the CapOpus randomised trial. *Psychological Medicine*, *43*, 1499–1510.

Hjorthøj, C. R., Vesterager, L., & Nordentoft, M. (2013c). Test-retest reliability of the Danish Adult Reading Test in patients with comorbid psychosis and cannabis-use disorder. *Nordic Journal of Psychiatry*, *67*(3), 159–163.

Kay, S. R., Fiszbein, A., & Opler, L. A. (1987). The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophrenia Bulletin*, *13*, 261–276.

Keefe, R. S. E., Goldberg, T. E., Harvey, P. D., Gold, J. M., Poe, M. P., & Coughenour, L. (2004). The Brief Assessment of Cognition in Schizophrenia: reliability, sensitivity, and comparison with a standard neurocognitive battery. *Schizophrenia Research*, *68*, 283–297.

Larsen, D. L., Attkisson, C. C., Hargreaves, W. A., & Nguyen, T. D. (1979). Assessment of client/patient satisfaction: development of a general scale. *Evaluation and Program Planning*, *2*, 197–207.

Miller, W. R. (1983). Motivational Interviewing. *Behavior and Psychotherapeutics*, *11*, 91–111.

Miller, W. R., & Rollnick, S. (2002). *Motivational interviewing: Preparing people for change* (2nd ed.). New York, NY: Guilford Press.

Mors, O., Perto, G. P., & Mortensen, P. B. (2011). The Danish psychiatric central research register. *Scandinavian Journal of Public Health*, *39*, 54–57.

Myin-Germeys, I., & van Os, J. (2007). Stress-reactivity in psychosis: evidence for an affective pathway to psychosis. *Clinical Psychology Review*, *27*, 409–424.

Nelson, H. E., & O'Connell, A. (1978). Dementia: the estimation of premorbid intelligence levels using the New Adult Reading Test. *Cortex*, *14*, 234–244.

Nordentoft, M., Knudsen, H. C., Jessen-Petersen, B., Krasnik, A., Sælan, H., Treufeldt, P., et al. (1996). CCPP-Copenhagen Community Psychiatric Project. Implementation of community mental health centres in Copenhagen: effects of service utilization, social integration, quality of life and positive and negative symptoms. *Social Psychiatry and Psychiatric Epidemiology*, *31*, 336–344.

Norman, R. M., & Malla, A. K. (1993). Stressful life events and schizophrenia. I: a review of the research. *British Journal of Psychiatry*, *162*, 161–166.

Petersen, L., Jeppesen, P., Thorup, A., Abel, M. B., Ohlenschlaeger, J., Christensen, T. O., et al. (2005). A randomised multicentre trial of integrated versus standard treatment for patients with a first episode of psychotic illness. *British Medical Journal*, *331*, 602.

Priebe, S., Huxley, P., Knight, S., & Evans, S. (1999). Application and results of the Manchester Short Assessment of Quality of Life (Mansa). *International Journal of Social Psychiatry*, *45*, 7–12.

Sobell, L. C., & Sobell, M. B. (1992). Timeline Follow-Back: a Technique for Assessing Self-Reported Alcohol Consumption. In R. Z. Litten, & J. Allen (Eds.), *Measuring alcohol consumption: Psychosocial and biological methods* (pp. 41–72). Totowa, NJ: Humana Press.

Stern, R., & White, T. (2003). *Neuropsychological assessment battery*. Lutz, FL: Psychological Assessment Resources, Inc.

Vendsborg, P., Nordentoft, M., Hvenegaard, A., & Søgaard, J. (1999). *Opsøgende psykoseteam: Assertive Community Treatment*. Copenhagen: Institut for sundhedsvæsen.

WHO. (2000). *World Health Organization disability assessment schedule (WHODAS II)*. Geneva: WHO.

Wing, J. K., Babor, T., Brugha, T., Burke, J., Cooper, J. E., Giel, R., et al. (1990). SCAN: schedules for clinical assessment in neuropsychiatry. Archives of General Psychiatry, 47, 589–593.

CHAPTER

113

Treating Cannabis Use Disorders Through Technology-Assisted Interventions: the Telephone and Internet

P. Gates, J. Copeland

National Cannabis Prevention and Information Centre, University of New South Wales, Randwick, NSW, Australia

SUMMARY POINTS

- This chapter focuses on the use of technology-assisted interventions for managing cannabis use disorder.
- Treatment seeking for cannabis use disorder is particularly low compared to other illicit drugs despite meeting criteria for cannabis use disorder. Potential barriers include a fear of being stigmatized as a substance user and difficulty in accessing treatments even when they are available.
- Technology-assisted interventions may address these barriers by providing a low-cost treatment that is easily accessible and avoids potential stigma by minimizing personal contact with treatment providers.
- High-tech computer-based interventions are currently available to treatment seekers via the internet. These interventions have been shown to be comparable to face-to-face interventions in a handful of studies; however, little is known with regards to the mechanisms that work toward assisting with cannabis use reductions. Younger individuals and males seem to respond more positively to this treatment format, compared to older females. Dropout is a particular concern, as less than half of those who were involved with trials of these interventions were likely to complete them.
- Low-tech telephone counseling cannabis use interventions are not widely available to treatment seekers, with some exceptions. Two randomized controlled trials have assessed the efficacy of this form of delivery, although neither trial assessed the effectiveness of such an intervention in the long-term. Although not directly compared, the reported abstinence rates following this intervention type were comparable to face-to-face intervention. These interventions may be better received by older individuals and by females, compared to younger males. As with computer-based interventions, participant attrition has been a concern.

KEY FACTS OF COGNITIVE BEHAVIORAL THERAPY (CBT)

- CBT teaches management, coping and problem-solving skills to assist clients in recognizing and managing any actions that lead to an unwanted behavior.
- When applied to substance use, coping skills are paramount in order to avoid the unwanted behavior of failing to avoid substance use.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00132-0
Copyright © 2017 Elsevier Inc. All rights reserved.

- Substance use is perceived as a learned behavior following predictable outcomes and cognitive behavioral therapy aims to disrupt the connection between substance use and these outcomes.
- CBT is the most consistently evaluated form of substance use treatment and has been shown to be effective in helping adults reduce their use.

KEY FACTS OF MOTIVATIONAL ENHANCEMENT THERAPY

- Motivational enhancement therapy (MET) encourages talk about change but also provides feedback and education regarding the client's substance use.
- The client can "take stock" of their substance use as the therapist assists them in using information as a means of encouraging reductions to use.
- MET is commonly combined with cognitive behavioral therapy (CBT), where MET enhances and solidifies readiness to reduce use, and CBT is delivered when the client is ready to begin making this change.

KEY FACTS OF TECHNOLOGY-ASSISTED INTERVENTIONS IN TREATING CANNABIS USE DISORDER

- Technology-assisted interventions are being developed to treat cannabis use disorder in an attempt to enhance treatment accessibility, and avoiding potential stigma by minimizing personal contact with treatment providers.
- Telephone counseling is considered a "low-tech" intervention. When relating to management of cannabis use disorder, it refers to a treatment delivered by a trained counselor to the treatment seeker, via the telephone.
- Computer-based interventions are considered "high-tech" treatment. When relating to management of cannabis use disorder, this refers to a treatment delivered on a computer, giving a treatment seeker access to a predesigned intervention, with or without the assistance of a treatment provider and may be web based.

LIST OF ABBREVIATIONS

BI	Brief intervention
CBT	Cognitive behavioral therapy
CD	Computer delivered
F2F	Face-to-face
MET	Motivational enhancement therapy
MI	Motivational interviewing
PCT	Person centered therapy
UNODC	United Nations Office on Drugs and Crime

INTRODUCTION

Although most cannabis users do not feel the need to access drug treatment, it is due to the substantial prevalence of cannabis users (approximately 4% of the global population aged 15–64 years report recent use; UNODC, 2013), that cannabis treatment is important from a public health perspective (Copeland, Clement, & Swift, 2014). The literature on treating cannabis use disorders dates back to the 1970s, when the first national evaluation of substance use treatment in the United States found that most young people were entering treatment due to their cannabis use (Dennis, Babor, Roebuck, & Donaldson, 2002). Over the last 40 years, the efficacy of several face-to-face cannabis use interventions have been supported—most notably one to nine sessions of motivational enhancement therapy, combined with cognitive behavioral therapy [motivational enhancement therapy (MET) + cognitive behavioral therapy (CBT)] (Copeland et al., 2014; Denis, Lavie, Fatseas, & Auriacombe, 2006). Unfortunately, there remains to be a large "research to practice gap" hampering the large scale implementation and dissemination of MET + CBT treatments (Gotham, 2004). Attempts to address this gap have been hindered by a treatment system that is chiefly face-to-face, where the number of individuals reached is limited by the capacity and location of services (Bender, Tripodi, Sarteschi, & Vaughn, 2011; Dennis et al., 2002).

In an attempt to close this research-practice gap, some recent research has focused on technology-assisted interventions (Litvin, Abrantes, & Brown, 2013). That is, interventions which are supported by "high technology" (high-tech) such as computer-based programs, or applications and the internet, or by "low technology" (low-tech) such as interventions that use mobile and landline telephones to communicate. In particular, high-tech computer-based interventions have proven effective in large scale dissemination of treatments for illicit (Gainsbury & Blaszczynski, 2011; Moore, Fazzino, Garnet, Cutter, & Barry, 2011) and licit drug use (Rooke, Thorsteinsson, Karpin, Copeland, & Allsop, 2010). In addition, mobile-based interventions (Cohn, Hunter-Reel, Hagman, & Mitchell, 2011; Whittaker et al., 2009), interactive voice response interventions (Corkrey & Parkinson, 2002) and videoconferencing (often collectively referred to as telehealth or telemedicine) also show promise for licit drug treatment (Young, 2012; Benavides-Vaello, Strode, & Sheeran, 2013). Unlike these high-tech interventions that are rapidly increasing, comparatively low-tech interventions such as telephone counseling (telecounseling) remain largely overlooked, with two exceptions. First, telecounseling is a well-established treatment for tobacco smoking (Tzelepis et al., 2013; Stead, Perera, & Lancaster, 2006), and

thought to be cost-effective (Hollis et al., 2007). Second, the utility of the telephone is also beginning to be recognized as a promising modality for delivering continuing care (treatment that follows an initial episode of more intensive care) (Blodgett, Maisel, Fuh, Wilbourne, & Finney, 2014; Mckay, 2009).

The attractiveness of exploring technology-assisted interventions lies in their ability to transcend the limitations of traditional face-to-face treatments, particularly by being more accessible and low cost (improving treatment dissemination), and by reducing the work-load of service providers (improving the ease of treatment integration). In addition, as the treatment seeker does not need to physically enter treatment, the associated interactions with receptionists and dress codes are avoided, which may reduce any threats to personal space (Ranan & Blodgett, 1983; Hughes, Riggs, & Carpenter, 2001). This lack of face-to-face interaction may also reduce any experiences of stigma as treatment becomes more individualized (Livingston, Milne, Fang, & Amari, 2012), and anonymity can be maintained (thus reducing any potential threats to, eg, employment or child custody). The treatment itself can also be ended more easily by the treatment seeker (by logging off or hanging up the phone), which may result in greater candor and honesty (Reese, Conoley, & Brossart, 2006). Finally, the nature of the treatment also allows for greater operation hours, which may result in a greater ability to address in real time any experiences of craving or relapse to use (Borland, Balmford, Segan, Livingston, & Owen, 2003). Given these advantages of technology-assisted interventions, and an increasing need for cannabis-specific treatments, the findings from research on technology-assisted cannabis interventions are explored here.

DELIVERING A HIGH-TECH CANNABIS USE INTERVENTION

A recent systematic, metaanalytic literature review of computer-based cannabis use interventions has shown some initial support for their utility upon reviewing 10 studies; although the overall effect size was small [g = 0.16 (0.09–0.22); number-needed-to-treat ≈11] (Tait, Spijkerman, & Riper, 2013). As previous literature reviews on high-tech substance use interventions failed to find any cannabis-specific interventions (Gainsbury & Blaszczynski, 2011; Moore et al., 2011), Tait et al. (2013) expanded their inclusion criteria to identify any internet or computer delivered intervention with or without therapist guidance, conducted prior to Sep., 2012. All included studies were conducted in the past decade, with the initial study conducted in 2007 by Ondersma, Svikis, and Schuster (2007), who recruited a sample of new mothers.

In this review, only two interventions were classified as treatment for cannabis use (Tossmann, Jonas, Tensil, Lang, & Struber, 2011; Kay-Lambkin, Baker, Kelly, & Lewin, 2011), with the rest identified to be prevention programs. In addition, not included in this review, due to its nonrandomized design, was a computer-based counselor-guided cannabis use intervention (Budney et al., 2011), and a randomized controlled trial of a self-guided computer-based cannabis use intervention conducted after the review was published (Rooke, Copeland, Norberg, Hine, & Mccambridge, 2013). As such, a total of four separate trials of computer-based cannabis use treatment have been conducted, one of which was self-guided (Rooke et al., 2013). Some of the more pertinent information from these trials is described in Table 113.1.

In comparison to advice only, computer-based treatments (with and without guidance) were effective in reducing cannabis use frequency and quantity. Moreover, these treatments were comparative to similar interventions that were delivered face-to-face. Given the reduced costs involved with delivering such a cannabis intervention (minimal or no therapist guidance, compared to full therapist contact), the cost-effectiveness of computer-based interventions is likely.

With the research only just building, it is hard to determine the common mechanisms leading to positive outcomes across these treatments (Rooke et al., 2010). In particular, the ideal number of sessions to achieve positive treatment outcomes should be of particular interest in future research. Interestingly, only one trial found an association between treatment adherence (number of treatment sessions completed) and any reductions to cannabis use frequency at follow-up (Rooke et al., 2013). This is important, as few participants actually completed the treatments as they were intended. As such, it may be that initiating the computer-based treatment is more important in predicting treatment outcomes, as compared to actually completing it. If this is the case, the availability and ease of implementing these interventions is of particular advantage. Ideally, future research will address whether the number of intervention sessions or length of engagement to treatment predicts long-term outcomes.

Limitations of High-Tech Cannabis Use Interventions

The promising results from these trials are tempered by some limitations. First, a large number of participants were lost to follow-up, even when these interventions could be completed at the convenience of the participant and in their homes. Given that the reason for discontinuation is not known in most cases, it should not be assumed that the majority of dropouts felt that they could continue to reduce cannabis

TABLE 113.1 Summary of the Literature: Computer-Based Cannabis Use Interventions

Author/year	Target group	% male, mean age	Recruitment	Cannabis outcomes	Conditions I: intervention C: control	Treatment adherence[a]	N	Follow-up	% retained at follow-up	Outcomes[b]
Kay-Lambkin et al. (2011)	>15 years, comorbid depression	57%, 40 years	Advertisement health clinics	A: % with 50% reduction in past 30-day use; B: % abstinent; C: % harmful use	I: 1 BI + 9 CD-CBT/MI with F2F guidance C^1: 1 BI + 9 F2F-PCT C^2: 1 BI + 9 F2F-CBT/MI	5.3/9 sessions 29.9% completed	I: 97 C^1: 88 C^2: 87	3 months	59% (reported as total sample)	No sig. btw. group difference
Tossman et al. (2011)	No exclusion criteria, treatment seekers	71%, 25 years	Website	A: past 30-day frequency; B: past 30-day quantity	I: 50 days weekly online feedback + therapist C^1: advice only	58.3% declined participation and 21.2% discontinued	I: 863 C^1: 429	3 months	I: 11.6% C^1: 24.7%	A: $d = 0.20$, B: $d = 0.11$
Budney et al. (2011)	> 17 years, problematic cannabis use	53%, 33 years	Advertisement	A: past 30-day frequency; B: related problems; C: abstinence rates	I: 9 CD-MET + CBT + CM (3 sessions included F2F guidance) C^1: 9 F2F-MET + CBT + CM	6.9/9 sessions 62% completed	I: 16 C^1: 22	Completion of treatment at 6 weeks from baseline	I: 62% C^1: 41%	No sig. btw. group difference
Rooke et al. (2013)	> 17 years, treatment seekers	61%, 31 years	Website and advertisement	A: past 30-day frequency; B: past 3-day quantity; C: severity of dependence	I: 6 CD-CBT C^1: 6 advice only	3.5/6[c] sessions 28.4% completed	I: 119 C^1: 111	3 months	I: 54% C^1: 52%	A: $d = 0.33$, B: $d = 0.25$, C: $d = 0.33$

BI, Brief intervention delivered face-to-face; PCT, person centered therapy; CBT, cognitive behavioral therapy; MI, motivational interviewing; F2F, face-to-face; CD, computer delivered; MET, motivational enhancement therapy.

[a] *Treatment adherence is reported as the mean number of sessions completed and the percentage completing treatment as it was intended (all sessions).*

[b] *Based on intention to treat analyses.*

[c] *Treatment completion was related to past month smoking days.*

use without intervention, as compared to dropouts exiting treatment with the belief that it may not work for them. In addition, the intention-to-treat statistical approaches used to account for missing data can not completely correct for this problem. As such, future work should address the high treatment attrition rates before this form of intervention delivery can be truly considered efficacious. Second, computer-based interventions may be at particular risk of breaching participant anonymity, unless proper protection protocols are ensured. That is, some computer-based interventions may require email address registration—which could be made available or accessed by other individuals (Tossman et al., 2011), or require the use of a shared computer. Third, as mentioned, given that the research is in preliminary stages, relatively little is known about the specific mechanisms of change that result in positive treatment outcomes. Even regarding the broader literature associated with computer delivered interventions for alcohol and tobacco use, there have been no statistically significant differences in treatment outcome reported, when considering the role of normative feedback, availability of a discussion feature, inclusion of entertainment features, emphasis on relapse prevention, or in the number of treatment sessions that are completed (Rooke et al., 2010). As such, further research is required to refine and improve these high-tech interventions.

DELIVERING A LOW-TECH CANNABIS USE INTERVENTION

To date, two studies have investigated the feasibility of delivering a specific cannabis use intervention by telephone (Gates, Norberg, Copeland, & Digiusto, 2012b; Fernandes et al., 2010). As with high-tech interventions, these trials of low-tech telecounseling interventions were conducted relatively recently, and offer an initial investigation to a promising treatment delivery option.

In the first trial of a telephone-based cannabis intervention, 1744 adult participants were recruited from callers to a Brazilian cannabis-specific helpline, over 21 months. These participants were mostly male (74%), with a mean age of 25 years. The intervention was a single 20 min MET + CBT-based session with complimentary self-help materials (n = 873), while the control group received only the self-help materials (n = 871). In addition, participants were free to access external treatment, and over one third of participants reported doing so (38%). Follow-up data was collected at eight time points, over a 6-month period, and the first 262 individuals from each group to complete the 6-month follow-up assessment were selected for analysis. There was notable dropout from the trial, with less than half (47%) of participants completing the first follow-up assessment (conducted 1 day from the call), and less than one-third (30%) completing the 6-month follow-up. Unfortunately, the authors did not attempt any statistical control to address this rate of dropout, meaning that results reflect only the most motivated treatment completers.

The cannabis-use outcome reported was "abstinence," although the period of abstinence was not defined. Encouragingly, 73% of the intervention and 59% of the control group reported abstinence at 6 months, meaning that intervention participants were 160% times more likely to report abstinence at 6 months (95% CI = 120–200%). The intervention effect was greater for females, compared to males, and those that reported high motivation to reduce cannabis use at the baseline assessment. This rate of abstinence was particularly impressive, even among the control group who had spoken with helpline counselors prior to participation. Indeed, the reported rate of abstinence was comparable to an effective face-to-face intervention of eight sessions of MET + CBT that included contingency management, where 54% of participants reported abstinence at 6-month follow-up (Carroll et al., 2006).

In the second trial of a telephone-based cannabis intervention, 160 adult participants were recruited from an Australian cannabis-specific helpline over 14 months. These participants were mostly male (61.9%), with a mean age of 36 years. The intervention was four CBT + MET sessions, delivered over 4 weeks, with complimentary self-help materials (n = 79), and a wait-list control group protocol was implemented (n = 81). Follow-up data was collected posttreatment, and at 3 months from baseline, with the majority of participants being assessed (68.7%).

In this trial, there was no significant treatment effect on cannabis use frequency or quantity (which reduced in both groups; d = 0.6 and 0.4, respectively); however, intervention participants reported greater improvements to cannabis-related problems (d = 0.5) and cannabis dependence (d = 0.9), compared to control participants, at 3 months. In depth analysis showed that the intervention effect was due to increasing the participant's confidence to quit cannabis use that was found to be more important than session attendance in predicting positive outcomes. Importantly, this trial controlled for dropout effects, and addressed treatment attendance, including the use of medications and any external treatment. In addition, the promising treatment outcomes were comparable with a more comprehensive and intensive trial of 12 sessions of therapist delivered MI + CBT (improvement in the percentage of abstinent days reached 74% on average in the telephone trial, compared to 64% of days at 4 months in the face-to-face treatment) (The Marijuana Treatment Project Research Group, 2004).

Limitations of Low-Tech Cannabis Use Interventions

As with high-tech interventions, the promising results from these trials are tempered by some limitations. First, participant dropout was again a concern. Although this may be somewhat compensated for by an increased reach of technology-assisted interventions, future work will need to find a way to improve retention in the same way that face-to-face treatment motivates return visits (although treatment dropout is a problem common to any treatment type for substance use). Second, the short 3–6 month follow-up assessment periods of these technology-assisted interventions prevent any predictions of their longer term effectiveness. In broader substance use intervention research, even the treatments themselves continue for at least 3 months (Dutra et al., 2008), and longer term follow-up is the norm. Third, participants were not blind to their allocation into the telecounseling interventions or control groups, in these two trials. According to trial quality checks encouraged by the Cochrane Collaboration when conducting systematic reviews of interventions (The Cochrane Collaboration, 2011), this lack of blinding introduces a risk of bias in participant self-report. As such, future work that includes adequate blinding is recommended. Finally, although not assessed in the trials reported here, telephone services are known to suffer from infrastructure problems that warrant consideration. In fact, a 2002 review of 115 Australian helplines reported that for every two calls answered, one person would have failed to contact the service, despite trying an average of 3.95 attempts to make contact (Urbis Keys Young, 2002).

COMPARING TECHNOLOGY-ASSISTED CANNABIS USE INTERVENTIONS

The technology-assisted cannabis use interventions reviewed here show great promise. Currently, however, high-tech and low-tech cannabis use interventions are not widely disseminated throughout real-world treatment practices. This is reflective of the fact that the associated research is in its infancy, and that there is a subsequent absence of a "gold star" standard intervention. That said, technology-assisted interventions offer a promising solution to overcoming many cannabis treatment seeking barriers, such as the feeling that accessing face-to-face treatment will invoke stigma, or the barriers of treatment location and wait list problems (Gates, Copeland, Swift, & Martin, 2012a). So, which technology-assisted intervention is best and for whom?

In a study comparing two similar MET + CBT-based cannabis use interventions that were delivered via the telephone (Gates et al., 2012b) and the web (Rooke et al., 2013) (available at www.reduceyouruse.org.au), several notable differences relating to treatment effects, sample characteristics, treatment engagement, and relationships among these variables were noted (Rooke, Gates, Norberg, & Copeland, 2014). First, the overall treatment effect on cannabis use outcomes was greater for the telephone study, compared to the web-based study. Given that the treatment fundamentals were similar across platforms, this difference may have reflected the nonvisual human interaction present in the telephone study, compared to the self-guided web study. In addition, the participants were reportedly more motivated to quit cannabis in the telephone study (reporting lower study dropout, greater external treatment seeking, and greater self-efficacy)—which was found to be a key predictor of treatment outcome.

Second, participant age and gender may be important in regards to treatment seeking behaviors relating to technology-assisted interventions. That is, participants in the web study were younger than those in the telephone study, and treatment engagement and satisfaction was higher among males, compared to females in the web study. Notably, treatment outcomes were greater for females, compared to males in the initial investigation of a telephone-delivered cannabis intervention (Fernandes et al., 2010). Taking these findings into consideration, it may be important for treatment providers to be mindful of their client's age and gender, and motivations to quit, prior to suggesting a technology-assisted intervention for cannabis use disorder. That is, younger males may respond better to a computer-based intervention, with the possible exception of those with severe dependence or lower motivation to quit. Similarly, older females may respond better to a telephone-based intervention. Obviously, given the early stages of research into these interventions, further research on this issue is required before such recommendations should be firmly made.

CONCLUSIONS

Technology-assisted interventions for cannabis use disorder offer a promising means to address the increasing need for treatment (Copeland & Swift, 2009). While early research shows that these interventions are capable of achieving treatment outcomes that are similar to face-to-face delivered services, very little is known in regards to how this may generalize to a population of heavy cannabis users with a history of other illicit substance use, or any comorbid mental health conditions. In addition, little is known regarding how many sessions should be delivered, and what content is associated with greater treatment outcomes. Regardless, the advantages

of technology-assisted interventions with regards to increased treatment accessibility at a low cost, warrant future research to refine and improve these interventions, and discover how to best implement both high-tech and low-tech interventions.

MINI-DICTIONARY

Cannabis use disorder Refers to a psychiatric diagnosis of clinical importance, relating to mild to severe dependence on cannabis that is typically characterized by a lack of control over use, or use despite known harms.

Cognitive behavioral therapy (CBT) Refers to a style of counseling that assists clients to recognize and manage any actions leading to unwanted behavior. This is primarily done through teaching management, coping, and problem-solving skills.

Motivational enhancement therapy Refers to a variant of motivational interviewing that utilizes motivational interviewing techniques (such as expressing empathy and supporting self-efficacy) in the treatment of substance use. In addition to these techniques, motivational enhancement therapy includes feedback and education regarding the client's substance use, and comparisons with population norms.

Motivational interviewing Refers to a style of counseling originated in 1983 that involves a conversation about making change while attending to any ambivalence. This is done through the expression of empathy, the support of self-efficacy, and the highlighting of areas where the client's ideals are discrepant from their reality.

Technology-assisted interventions Refers to any intervention which utilizes communications technology, such as computers and/or telephones, to assist in delivering treatment. In some cases, the therapist is not required, as the treatment can be delivered completely through the use of technology, as seen in some computer-based interventions.

Treatment attrition Refers to the number of clients who are selected to participate in a given treatment but, for various reasons, either do not begin the treatment or leave prematurely, and do not complete the treatment as it was intended. Treatment attrition is a particular concern with regards to technology assisted interventions.

References

Benavides-Vaello, S., Strode, A., & Sheeran, B. C. (2013). Using technology in the delivery of mental health and substance abuse treatment in rural communities: a review. *Journal of Behavioral Health Services and Research*, *40*, 111–120.

Bender, K., Tripodi, S. J., Sarteschi, C., & Vaughn, M. G. (2011). A meta-analysis of interventions to reduce adolescent cannabis use. *Research on Social Work Practice*, *21*, 153–164.

Blodgett, J. C., Maisel, N. C., Fuh, I. L., Wilbourne, P. L., & Finney, J. W. (2014). How effective is continuing care for substance use disorders? A meta-analytic review. *Journal of Substance Abuse Treatment*, *46*, 87–97.

Borland, R., Balmford, J., Segan, C., Livingston, P., & Owen, N. (2003). The effectiveness of personalized smoking cessation strategies for callers to a Quitline service. *Addiction*, *98*, 837–846.

Budney, A. J., Fearer, S., Walker, D. D., Stanger, C., Thostenson, J., Grabinski, M., & Bickel, W. K. (2011). An initial trial of a computerized behavioral intervention for cannabis use disorder. *Drug and Alcohol Dependence*, *115*, 74–79.

Carroll, K. M., Easton, C. J., Nich, C., Hunkele, K. A., Neavins, T. M., Sinha, R., & Al., E. (2006). The use of contingency management and motivational/skills-building therapy to treat young adults with marijuana dependence. *Journal of Consulting and Clinical Psychology*, *74*, 955–966.

Cohn, A. M., Hunter-Reel, D., Hagman, B. T., & Mitchell, J. (2011). Promoting behavior change from alcohol use through mobile technology: the future of ecological momentary assessment. *Alcoholism: Clinical and Experimental Research*, *35*, 2209–2215.

Copeland, J., Clement, N., & Swift, W. (2014). Cannabis use, harms and the management of cannabis use disorder. *Neuropsychiatry*, *4*, 55–63.

Copeland, J., & Swift, W. (2009). Cannabis use disorder: epidemiology and management. *International Review of Psychiatry*, *21*, 96–103.

Corkrey, R., & Parkinson, L. (2002). Interactive voice response: review of studies 1989-2000. *Behavior Research Methods, Instruments and Computers*, *34*, 342–353.

Denis, C., Lavie, E., Fatseas, M., & Auriacombe, M. (2006). Psychotherapeutic interventions for cannabis abuse and/or dependence in outpatient settings. *Cochrane Database of Systematic Reviews*, CD005336.

Dennis, M., Babor, T. F., Roebuck, C., & Donaldson, J. (2002). Changing the focus: the case for recognizing and treating cannabis use disorders. *Addiction*, *97*, 4–15.

Dutra, L., Stathopoulou, G., Basden, S. L., Leyro, T. M., Powers, M. B., & Otto, M. W. (2008). A meta-analytic review of psychosocial interventions for substance use disorders. *American Journal of Psychiatry*, *165*, 179–187.

Fernandes, S., Ferigolo, M., Benchaya, M. C., Moreira, T. D. C., Pierozan, P. S., Mazoni, C. G., & Barros, H. M. T. (2010). Brief Motivational Intervention and telemedicine: a new perspective of treatment to marijuana users. *Addictive Behaviors*, *35*, 750–755.

Gainsbury, S., & Blaszczynski, A. (2011). A systematic review of Internet-based therapy for the treatment of addictions. *Clinical Psychology Review*, *31*, 490–498.

Gates, P., Copeland, J. a. N., Swift, W., & Martin, G. (2012a). Barriers and facilitators to cannabis treatment. *Drug and Alcohol Review*, *31*, 311–319.

Gates, P. J., Norberg, M. M., Copeland, J., & Digiusto, E. (2012b). Randomized controlled trial of a novel cannabis use intervention delivered by telephone. *Addiction*, *107*, 2149–2158.

Gotham, H. J. (2004). Diffusion of mental health and substance abuse treatments: development, dissemination, and implementation. *Clinical Psychology: Science and Practice*, *11*, 160–176.

Hollis, J. F., Mcafee, T. A., Fellows, J. L., Zbikowski, S. M., Stark, M., & Riedlinger, K. (2007). The effectiveness and cost effectiveness of telephone counselling and the nicotine patch in a state tobacco quitline. *Tobacco Control*, *16*, i53–i59.

Hughes, J. R., Riggs, R. L., & Carpenter, M. J. (2001). How helpful are drug abuse helplines? *Drug and Alcohol Dependence*, *62*, 191–194.

Kay-Lambkin, F. J., Baker, A. L., Kelly, B., & Lewin, T. J. (2011). Clinician-assisted computerised versus therapist-delivered treatment for depressive and addictive disorders: a randomised controlled trial. *Medical Journal of Australia*, *195*, S44–S50.

Litvin, E. B., Abrantes, A. M., & Brown, R. A. (2013). Computer and mobile technology-based interventions for substance use disorders: an organizing framework. *Addictive Behaviors*, *38*, 1747–1756.

Livingston, J. D., Milne, T., Fang, M. L., & Amari, E. (2012). The effectiveness of interventions for reducing stigma related to substance use disorders: a systematic review. *Addiction*, *107*, 39–50.

Mckay, J. R. (2009). Continuing care research: what we have learned and where we are going. *Journal of Substance Abuse Treatment*, *36*, 131–145.

Moore, B. A., Fazzino, T., Garnet, B., Cutter, C. J., & Barry, D. T. (2011). Computer-based interventions for drug use disorders: a systematic review. *Journal of Substance Abuse Treatment*, *40*, 215–223.

Ondersma, S. J., Svikis, D. S., & Schuster, C. R. (2007). Computer-based brief intervention: a randomized trial with postpartum women. *American Journal of Preventive Medicine*, *32*, 231–238.

Ranan, W., & Blodgett, A. (1983). Using telephone therapy for "unreachable" clients. *Social Casework*, *64*, 39–44.

Reese, R. J., Conoley, C. W., & Brossart, D. F. (2006). The Attractiveness of telephone counseling: an empirical investigation of client perceptions. *Journal of Counseling and Development*, *84*, 54–60.

Rooke, S., Copeland, J., Norberg, M., Hine, D., & Mccambridge, J. (2013). Effectiveness of a self-guided web-based cannabis treatment program: randomized controlled trial. *Journal of Medical Internet Research*, *15*, e26.

Rooke, S., Thorsteinsson, E., Karpin, A., Copeland, J., & Allsop, D. (2010). Computer-delivered interventions for alcohol and tobacco use: a meta-analysis. *Addiction*, *105*, 1381–1390.

Rooke, S. E., Gates, P. J., Norberg, M. M., & Copeland, J. (2014). Applying technology to the treatment of cannabis use disorder: comparing telephone versus Internet delivery using data from two completed trials. *Journal of Substance Abuse Treatment*, *46*, 78–84.

Stead, L. F., Perera, R., & Lancaster, T. (2006). Telephone counselling for smoking cessation. *Cochrane Database of Systematic Reviews*, *3*, CD002850.

Tait, R. J., Spijkerman, R., & Riper, H. (2013). Internet and computer-based interventions for cannabis use: a meta-analysis. *Drug and Alcohol Dependence*, *133*, 295–304.

The Cochrane Collaboration. (2011). The Cochrane Collaboration's tool for assessing risk of bias. In J. P. T. Higgins, & S. Green (Eds.), *Cochrane Handbook for Systematic Reviews of Interventions*. The Cochrane Collaboration, Version 5. Available from www.cochrane-handbook.org

The Marijuana Treatment Project Research Group. (2004). Brief treatments for cannabis dependence: Findings from a randomized clinical trial. *Journal of Consulting and Clinical Psychology*, *72*, 455–466.

Tossman, H. P., Jonas, B., Tensil, M. D., Lang, P., & Struber, E. (2011). A controlled trial of an internet-based intervention program for cannabis users. *Cyberpsychology, Behavior, and Social Networking*, *14*(11), 673–679.

Tzelepis, F., Paul, C. L., Walsh, R. A., Wiggers, J., Duncan, S. L., & Knight, J. (2013). Predictors of abstinence among smokers recruited actively to quitline support. *Addiction*, *108*, 181–185.

UNODC. (2013). *World Drug Report 2013*. Vienna, Austria: UNODC, United Nations publication, Sales No. E.13.XI.6.

Urbis Keys Young. (2002). *National review of tele counselling and web counselling services: final report*. Canberra: Australian Government Department of Health and Ageing.

Whittaker, R., Borland, R., Bullen, C., Lin, R. B., Mcrobbie, H., & Rodgers, A. (2009). Mobile phone-based interventions for smoking cessation. *Cochrane Database of Systematic Reviews*, CD006611.

Young, L. B. (2012). Telemedicine interventions for substance-use disorder: a literature review. Journal of Telemedicine and Telecare, 18, 47–53.

CHAPTER

114

Reducing Cannabis Use With a Real-Time Intervention Using Mobile Technology

M. Kells, L.A. Shrier***

***Division of Adolescent/Young Adult Medicine, Boston Children's Hospital, Boston, MA, United States**
****Division of Adolescent/Young Adult Medicine, Boston Children's Hospital, and Department of Pediatrics, Harvard Medical School, Boston, MA, United States**

SUMMARY POINTS

- Most young people who use marijuana frequently are not seeking treatment. Primary care settings can provide marijuana treatment to this population as part of routine care.
- Motivational interventions are effective for reducing substance use in adults, and may be effective for adolescents as well.
- Motivational enhancement therapy (MET) utilizes the principles of motivational interviewing (MI), as well as personalized feedback to enhance motivation for behavior change.
- Although marijuana may be used to cope with negative affect, chronic and frequent use may result in increased negative affect, leading to a cycle of dependence.
- Marijuana is frequently used in social settings. Social interaction is linked to higher dose and level of intoxication.
- Ecological momentary assessment (EMA) is a method of collecting real-time reports in the natural environment via mobile technology. Ecological momentary interventions (EMIs) use EMA data to provide intervention in real time.
- Designed for frequently-using youth not in treatment, the MOMENT intervention combines brief in-person MET in primary care with real-time self-assessment and responsive messaging in the natural environment using mobile technology.

KEY FACTS OF ECOLOGICAL MOMENTARY INTERVENTIONS

- EMIs deliver behavior-change treatment in real-life contexts, in real time.
- EMIs allow for practice of coping, avoidance, and other skills learned during in-person counseling in daily life, as factors influencing behavior are being experienced.
- EMIs include self-monitoring, a major component of cognitive behavioral therapy (CBT) and mindfulness training, often used in substance use reduction and relapse prevention.
- EMIs can target an individual's personal triggers for behavior, and provide treatment during that vulnerable time.
- EMIs are delivered using mobile technology, allowing for privacy and discretion, which may be particularly important for sensitive or stigmatizing behaviors.
- EMIs are feasible and acceptable to a range of populations, and appear effective for intervening on a variety of health behaviors, including substance use.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00133-2
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

CBT	Cognitive behavioral therapy
EMA	Ecological momentary assessment
EMI	Ecological momentary intervention
MET	Motivational enhancement therapy
MI	Motivational interviewing
SBIRT	Screening, brief intervention, and referral to treatment

FREQUENT MARIJUANA USE AMONG ADOLESCENTS AND YOUNG ADULTS

Marijuana continues to be the most common illicit drug of abuse by youth in the United States (Johnston, O'Malley, Bachman, & Schulenberg, 2012) and Europe (European Centre for Drugs and Drug Addiction, 2010). Frequent marijuana use among adolescents has been rising. The rate of US 12th graders who report daily marijuana use increased from 2.4% in 1993 to 6.5% in 2013 (Johnston et al., 2012). Declines in personal disapproval of use and perceived risk of regular use among youth, as well as the growing legalization of marijuana for both medical and recreational use, may herald further increases in marijuana use and abuse (Johnston et al., 2012).

Frequent marijuana use is associated with structural brain changes and negative effects in all domains of cognitive functioning (reviewed in Batalla et al., 2013). For those who initiate marijuana use during adolescence, these effects may persist despite cessation (Meier et al., 2012). Young people who use marijuana frequently are more likely to engage in polydrug use, have low academic intentions, and poorer academic outcomes (Ellickson, Tucker, Klein, & Saner, 2004b), less favorable developmental outcomes than their abstinent peers (Ellickson, Martino, & Collins, 2004a), and increased risk of developing a substance use disorder (Chen, Storr, & Anthony, 2009).

NEED FOR BRIEF TREATMENT IN PRIMARY CARE

Many young people recognize problems associated with their marijuana use, and try to quit (Copersino et al., 2010; Rhoads, Burke, Fredette, & Shrier, 2012), but often return to use (Hughes, Peters, Callas, Budney, & Livingston, 2008). Most individuals with marijuana use disorders are not in treatment programs. Adults cite lack of awareness of options, lack of perceived need for treatment, desire to continue use, and stigma as reasons for not seeking treatment (Gates, Copeland, Swift, & Martin, 2012). For adolescents, concerns regarding access, cost, and confidentiality (Sterling, Weisner, Hinman, & Parthasarathy, 2010), as well as limited specialized facilities and suboptimal care (Knudsen, 2009; SAMHSA, 2010) may also impede receipt of treatment. There is a need to develop engaging, effective, and accessible treatment options for the increasing population of youth with problematic marijuana use.

Primary care settings serving youth, in which providers are already identifying problematic substance use (Harris, Louis-Jacques, & Knight, 2014) and young people have already overcome barriers to accessing care, can offer time- and resource-efficient treatment. Screening, brief intervention, and referral to treatment (SBIRT) is recommended as part of routine health care for adolescents (Committee on Substance Abuse, 2011), as well as adults (Madras et al., 2009).

Because young persons may not adhere to lengthy treatment, a substance use intervention ideally would be effective after only 1 or 2 sessions. Brief interventions for drinking (Monti et al., 2007) and possibly for substance use in general (D'Amico, Miles, Stern, & Meredith, 2008) can be effective in youth. Research has begun to investigate the utility of brief interventions for marijuana-using young people in the primary care setting (Walton et al., 2013, 2014), with some focus on young persons not seeking treatment (de Gee, Verdurmen, Bransen, de Jonge, & Schippers, 2014; Martin & Copeland, 2008; Stein, Hagerty, Herman, Phipps, & Anderson, 2011; Walker & Roffman, 2006). The limited research thus far has been challenged by the need for parental involvement, mandated treatment, and multiple office visits.

MOMENTARY AND CONTEXTUAL FACTORS RELATED TO MARIJUANA USE

Several emotional, social, and environmental factors have been identified as antecedents and correlates of marijuana use (Bonn-Miller & Zvolensky, 2009; Shrier, Ross, & Blood, 2014b), and thus may be important targets for intervention. In particular, affective states have shown a strong link to marijuana desire and use behaviors in young people who are frequent users. As momentary positive affect increases, desire to use marijuana decreases and, as momentary negative affect increases, desire to use marijuana increases (Shrier, Walls, Kendall, & Blood, 2012). Further, young people report increased negative affect during the 24 h before marijuana use (Shrier et al., 2014b). Poorer mental health, including higher negative affect and anxiety, has also been linked to increased level of high from a marijuana use event (Shrier, Walls, Rhoads, & Blood, 2013b). Frequent users have reported overall anxiety reduction, compared to those who use less frequently (Metrik, Kahler, McGeary, Monti, & Rohsenow, 2011). Although anxiety reduction can occur in the immediate period following

use, long-term use may lead to overall increase in anxiety symptoms (Buckner et al., 2014), resulting in a negative cycle of use. In addition, young people have reported pleasure as a motivation for marijuana use (Shrier & Blood, 2014). In frequent users, marijuana use increases positive subjective-effect ratings, reduces tension, and produces a sense of euphoria (Hart et al., 2010). In this way, use may be tied to the desire to reduce unpleasant emotional states and enhance pleasurable states, consistent with an affect regulation model of marijuana use (Khantzian, 1997).

Setting is also associated with marijuana use behaviors. Young persons who use marijuana frequently report lowest desire for marijuana in the morning, and highest dose of marijuana per use event on weekend days (Shrier, Rhoads, Wall, & Blood, 2013a). Adolescents and young adults report that they desire marijuana more, and are more likely to use marijuana, when with friends versus alone (Shrier et al., 2012, 2013a), and individuals with social anxiety are more likely to use if they are with other users, when they are feeling more anxious (Buckner, Crosby, Wonderlich, & Schmidt, 2012b). Social environments also influence the amount of drug used and degree of high sought (Monti, Colby, & O'Leary, 2001). Social relationships that include using marijuana together and lack support for cessation have been cited as barriers to quitting (Rhoads et al., 2012).

ECOLOGICAL MOMENTARY ASSESSMENT AND INTERVENTION

Ecological momentary assessment (EMA), frequent, real-time data collection in the participant's natural settings, offers one approach to ascertaining marijuana-related social, environmental, and affective contexts as they vary throughout the day (Shrier et al., 2012). EMA reduces the likelihood of recall bias, enhances the ecological validity of results, and allows for multiple data collection opportunities over time, which has the potential to uncover pattern development (Heron & Smyth, 2010). Contextual and affective states immediately prior to marijuana use (Shrier et al., 2014b), as well as characteristics of use events (Shrier et al., 2013b), desire to use (Shrier et al., 2012), and reasons for use (Shrier & Blood, 2014) have been described through EMA.

Given that the factors influencing use are within the real-time situation, the ability to intervene at the moment of use or desire to use may be vital to affecting meaningful change in substance use behaviors. Ecological momentary intervention (EMI) delivers an intervention in real time in the natural setting, generally via mobile technology (eg, handheld computer, mobile phone). EMI may extend the effects of clinic-based interventions in which new skills are taught by allowing the opportunity to practice these skills in the context of real-life experience. EMI has been used to influence a number of health conditions and behaviors, including mental health disorders (Pramana, Parmanto, Kendall, & Silk, 2014), smoking cessation (Wetter et al., 2011), and alcohol dependence (Voogt, Kuntsche, Kleinian, Pelen, & Engels, 2014). In general, EMI appears feasible and acceptable, and may produce long-term benefits (Whittaker et al., 2012).

EMI offers particular advantages for changing addictive behaviors. Frequent electronic reporting on marijuana use and associated factors is a form of self-monitoring, which has been effective in changing health behavior (eg, to promote weight loss; Burke, Wang, & Sevick, 2011), although there is conflicting evidence about the effect of self-monitoring on substance use (Hufford, Shields, Shiffman, Paty, & Balabanis, 2002; Simpson, Kivlahan, Bush, & McFall, 2005). Cognitive behavioral therapy (CBT), which includes self-monitoring, may be effective in marijuana use treatment (Magill & Ray, 2009). In focusing on momentary experience, EMI may also enhance mindfulness (Kabat-Zinn, 1990), of which deficits may exist among individuals who abuse substances (Dakwar, Mariani, & Levin, 2011). Mindfulness training has been included in interventions for substance abuse treatment (de Dios et al., 2012) and relapse prevention (Witkiewitz, Marlatt, & Walker, 2005). EMIs can also be administered at times specific to an individual's needs (Heron & Smyth, 2010), for example, when experiencing personal emotional and social triggers for substance use (Shrier et al., 2014b). EMI administered on a mobile device offers privacy, which is of particular importance for managing stigma associated with substance abuse and its treatment (Luoma et al., 2007). Challenges of EMI research and intervention include upfront technological support and cost, as well as provider skepticism about new technology (Heron & Smyth, 2010).

THE MOMENTARY SELF-MONITORING AND FEEDBACK + MOTIVATIONAL ENHANCEMENT THERAPY (MOMENT) INTERVENTION

Based in the need for brief treatment for marijuana use in primary care, the importance of context in marijuana use, and the feasibility and potential effectiveness of EMIs for substance use disorders, we developed the MOmentary self monitoring and feedback + Motivational ENhancement Therapy (MOMENT) intervention to reduce marijuana use among frequently-using youth by intervening on the contextual influences of marijuana use in the natural environment. MOMENT has three components: (1) two 1-h MET sessions separated

by a week, (2) 2 weeks of momentary self-monitoring of affect, social contexts, and marijuana desire and use, and (3) feedback immediately following report of self-identified contextual factors that may lead to use.

Intervention Design

The brief MET component was adapted from interventions to reduce marijuana use among young people not seeking treatment (Walker & Roffman, 2006; Martin & Copeland, 2008; Walker et al., 2011). Brief face-to-face counseling creates a human connection that may enhance the EMI components of the intervention (Shrier et al., 2013a). MET includes the motivational interviewing (MI) concepts of expressing empathy, supporting self-efficacy, rolling with resistance, and developing discrepancy (Miller & Rollnick, 2012), plus personalized feedback on individualized assessment (Miller, Zweben, DiClemente, & Rychtarik, 1994). During the first MET session, the individual identifies personal emotional, social, and environmental triggers for marijuana use and selects his/her top 3, information that is used to individualize the feedback message component of the intervention (Table 114.1). Momentary self-monitoring of marijuana triggers, desire, use, and avoiding use is accomplished using a programmable mobile device (eg, handheld computer or mobile phone). When young persons indicate a "top 3" trigger context on a momentary report, they immediately receive a feedback message designed to support self-efficacy, and prompt consideration of their individualized coping strategies (Tevyaw & Monti, 2004), building on the second MET session. The young person will also receive messages after reports of desiring marijuana, having used marijuana, and having avoided use (Fig. 114.1).

Formative Research

In interviews with youth who use marijuana frequently and experts in MI, substance abuse treatment, and adolescent health, the approach of using counseling plus mobile technology to deliver an intervention to reduce marijuana use was explored (Shrier et al., 2013a). Both youth and clinicians gave the device human attributes such as caring and encouraging. Clinicians thought the device would be experienced as an extension of the counselor (eg, "virtual sponsor," "counselor in your pocket"), and that the momentary self-monitoring would be an intervention in itself. Young people and clinicians felt that the messages immediately following report of triggers for marijuana use might encourage reflection and alter behavior. Young people expressed the feeling that the timely messages would be well-received if they were personalized, and not too frequent or repetitive.

Pilot Study

In a pilot study, we tested the feasibility, acceptability and preliminary efficacy of the MOMENT intervention (Shrier, Rhoads, Burke, Walls, & Blood, 2014a). Of 27 young persons enrolled, 16 completed the intervention, and 14 provided complete follow-up data. The sample was primarily female (70%), had a median age of 19 years, and was ethnically and racially diverse. At baseline, marijuana use in the previous week was a median of 6 times. The study collected individual-, daily-, and momentary-level data at baseline, during the intervention, and 3 months postintervention (Tables 114.2 and 114.3).

During MET, young people most commonly selected as triggers for marijuana use negative affective states (stress, frustration, boredom, or anxiety; 86% of top-3

TABLE 114.1 Components of the MOMENT Intervention

Component	Description	Goals	Time
Motivational enhancement therapy	1:1 Motivational interview following a standardized manual	*Session 1:* Develop discrepancy between use and personal values and goals Identify top 3 triggers for use *Session 2:* Give personalized feedback. Evoke motivation for change Explore and enhance self-efficacy Develop a "game plan" for reducing use, including managing triggers	Two sessions 1 week apart 1 h each
Momentary self-monitoring	Report emotional states, social context, marijuana thoughts and behaviors, and other substance use	Enhance self-monitoring and recognize triggers for use	4–6/day × 2 weeks 2–4 min each
Immediate feedback	Message after reporting top-3 trigger, marijuana desire, use, avoiding use	Support self-efficacy Prompt use of coping strategies	<1 min each

Components of the MOMENT intervention include brief motivational enhancement therapy followed by 2 weeks of mobile self-monitoring of marijuana use and related factors, plus feedback messages immediately following report of self-identified triggers for use.

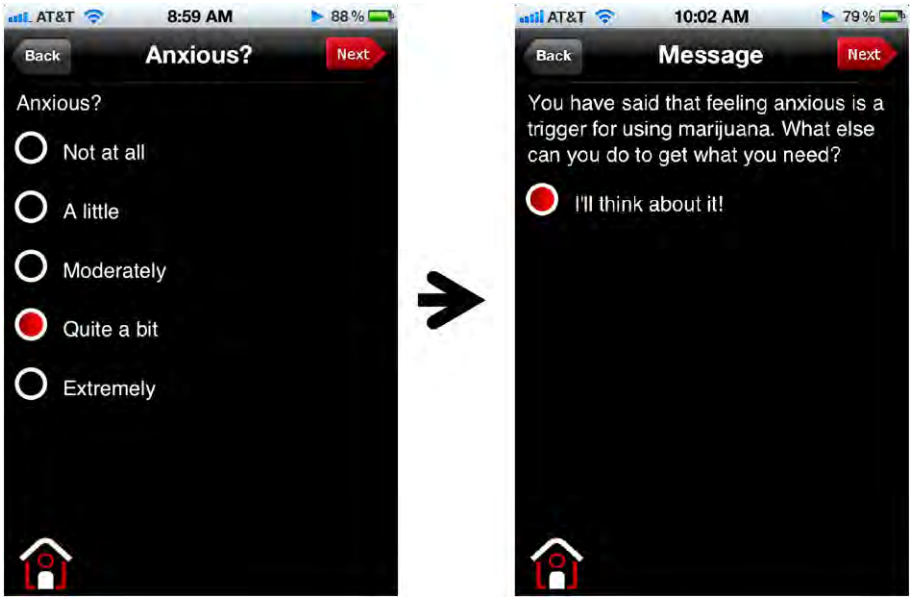


FIGURE 114.1 **Example of MOMENT momentary reporting and responsive messaging.** On the left is a momentary report of an individual's anxiety level. On the right is the responsive motivational message prompting the individual to think about strategies discussed during counseling to manage anxiety.

triggers), followed by being in comfortable or relaxed settings and states, being with friends (55%), being at own, friend's, or relative's home (45%), and hanging out resting/about to sleep (45%). The proportion of momentary reports of being in a personal top-3 trigger context did not change significantly over the study: 36% at baseline, 43% during intervention, and 38% at follow-up (p = 0.77). However, desire to use marijuana when in a top-3 trigger context and marijuana use after trigger exposure both declined from baseline to follow-up (Table 114.4 and Fig. 114.2). The pilot study was not powered to detect differences at a daily or individual level. However, nonsignificant trends for these outcomes were similar to those observed for the momentary-level outcomes (Table 114.4).

Participants indicated that they enjoyed the MET sessions, and found them useful. They reported ease with the technology, and low burden of participation. They felt that mobile messaging encouraged them to avoid use, and increased their awareness of triggers.

In sum, the pilot results suggest that the MOMENT intervention is acceptable to young persons with problematic marijuana use, and feasible to administer in primary care. The observed decrease in marijuana desire and use associated with exposure to triggering contexts, the target of MOMENT, may indicate an intervention effect, and warrants further study.

Randomized Controlled Trial

A randomized controlled trial is underway to further evaluate the feasibility and efficacy of the MOMENT intervention. Patients of primary care clinics affiliated with a large, urban, pediatric hospital in the northeastern

TABLE 114.2 MOMENT Pilot Study Data Collection

			Study phase		
Data level	**Data source**	**Variable example**	**Baseline**	**Intervention**	**3-Month follow-up**
Individual	Timeline follow-back calendar	30-day percent days abstinent	x		x
	Computer survey	Problems with use	x		x
Daily	Mobile device diary	No. of marijuana use episodes in 24 h	x	x	x
Momentary	Mobile device reports 4–6 times/day	Desire to use marijuana;	x	x	x
		marijuana use since last report	x	x	x

Daily diaries and momentary reports were collected for 1 week at baseline, 2 weeks during the intervention, and 1 week at 3-month follow-up. The timeline follow-back calendar (Sobell & Sobell, 1992) assessed marijuana use for the past 30 days.

TABLE 114.3 Individual-Level Mean and Standard Deviation for Momentary, Daily, and Individual Outcomes, by Phase of MOMENT Pilot Study

	Baseline	Intervention	Follow-up
Momentary outcomes, *N*[a]	518	677	377
Desire to use marijuana score (possible 0–9)	3.23 (1.75)	2.53 (1.98)	1.32 (1.50)
Use reported after context exposure, proportion of reports	0.28 (0.17)	0.26 (0.19)	0.19 (0.17)
Daily outcomes, *N*[a]	68	106	50
Use events per day	1.00 (0.61)	0.80 (0.66)	0.73 (0.72)
Readiness score (possible 0–9)	3.72 (2.21)	3.84 (2.26)	4.19 (3.16)
Importance score (possible 0–9)	4.03 (2.28)	4.67 (2.64)	4.18 (3.50)
Confidence score (possible 0–9)	5.76 (2.32)	5.85 (2.33)	4.76 (3.14)
Individual outcomes, *N*	27		15
Percent days abstinent (past 30 days)	37.9 (27.8)		47.3 (28.5)
POSIT score (possible 0–17)	5.67 (4.40)		3.93 (4.00)

POSIT, problem-oriented screening instrument for teenagers, substance use/abuse scale (Rahdert, 1991).
[a] *Twenty-two participants provided momentary and daily data for 1 week at baseline, 16 participants provided momentary and daily data for 2 weeks during the intervention, and 14 participants provided momentary and daily data for 1 week at follow-up.*
Reprinted from Shrier, L. A., Rhoads, A. M., Burke, P. J., Walls, C. E., & Blood, E.A. (2014a). Real-time, contextual intervention using mobile technology to reduce marijuana use among youth: a pilot study. Addictive Behaviors, *39(1), 173–180, copyright 2014, with permission from Elsevier.*

TABLE 114.4 Differences in Momentary, Daily, and Individual Outcomes Between Phases of the MOMENT Pilot Study

	Intervention versus baseline		Follow-up versus intervention		Follow-up versus baseline	
Momentary outcomes						
Desire to use marijuana	β (SE)	*p*	β (SE)	*p*	β (SE)	*p*
In top-3 trigger contexts	−0.22 (0.32)	0.48	−1.36 (0.37)	0.0002	−1.59 (0.33)	<0.0001
In other contexts	−0.53 (0.31)	0.08	−0.63 (0.27)	0.02	−1.16 (0.28)	<0.0001
Use reported after context exposure	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
In top-3 trigger contexts	0.85 (0.60, 1.20)	0.35	0.64 (0.35, 1.17)	0.14	0.54 (0.31, 0.95)	0.03
In other contexts	0.85 (0.58, 1.25)	0.41	0.83 (0.46, 1.49)	0.53	0.70 (0.42, 1.17)	0.17
Daily outcomes	RR (95% CI)	*p*	RR (95% CI)	*p*	RR (95% CI)	*p*
Use events per day	0.78 (0.60, 1.02)	0.07	0.93 (0.59, 1.46)	0.76	0.73 (0.49, 1.08)	0.11
	β (SE)	*p*	β (SE)	*p*	β (SE)	*p*
Readiness score	0.25 (0.24)	0.30	1.06 (0.99)	0.28	1.31 (1.09)	0.23
Importance score	0.42 (0.38)	0.27	0.03 (0.79)	0.97	0.45 (0.79)	0.57
Confidence score	0.36 (0.44)	0.40	0.32 (0.72)	0.65	0.69 (0.95)	0.47
Individual outcomes					*S*	*p*
Percent days abstinent (past 30 days)					27	0.13
POSIT score					−15.5	0.16

POSIT, Problem-oriented screening instrument for teenagers, substance use/abuse scale (Rahdert, 1991); SE, standard error; OR, odds ratio; CI, confidence interval; RR, rate ratio. Generalized estimating equations adjusted for age and gender were used for the momentary and daily outcomes. Wilcoxon signed rank tests were used for the individual outcomes.
Reprinted from Shrier, L. A., Rhoads, A. M., Burke, P. J., Walls, C. E., & Blood, E.A. (2014a). Real-time, contextual intervention using mobile technology to reduce marijuana use among youth: a pilot study. Addictive Behaviors, *39(1), 173–180, copyright 2014, with permission from Elsevier.*

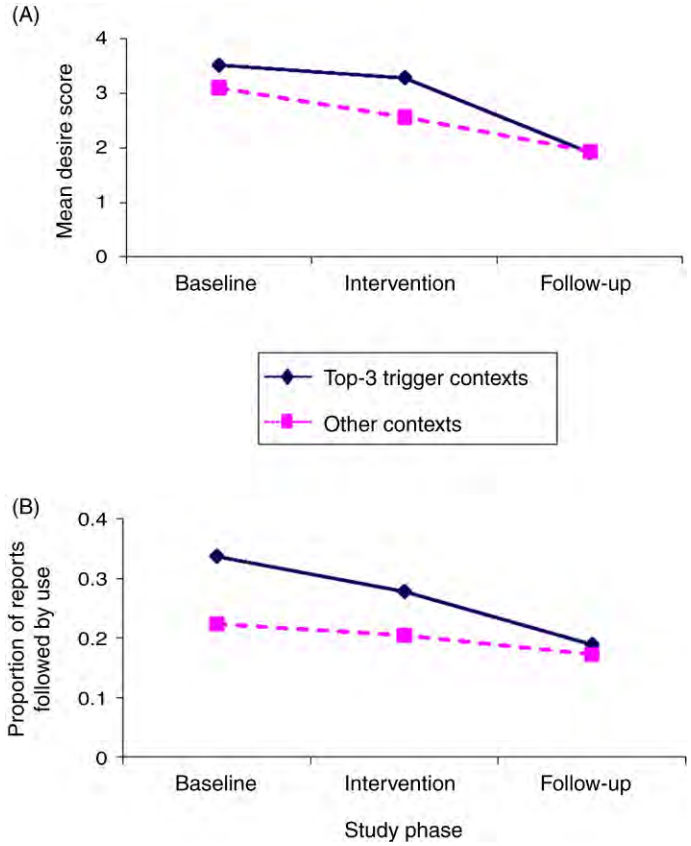


FIGURE 114.2 Mean momentary desire to use marijuana (A) and proportion of reports followed by marijuana use (B), by study phase and context type. In the MOMENT pilot study, desire to use marijuana tended to be higher in top-3 trigger contexts versus other contexts at baseline, but there was no difference in desire between context types at follow-up. Similarly, over the study marijuana use decreased after exposure to a top-3 trigger context more than after other contexts. *Source: Reprinted from Shrier, L. A., Rhoads, A. M., Burke, P. J., Walls, C. E., & Blood, E.A. (2014a). Real-time, contextual intervention using mobile technology to reduce marijuana use among youth: a pilot study.* Addictive Behaviors, *39(1), 173–180, copyright 2014, with permission from Elsevier.*

United States are being recruited if they are between 15 and 24 years of age, and use marijuana at least 3 times per week, on average. Research staff and clinical providers offer age-eligible patients who use marijuana the opportunity be screened and brochures in the clinics allow for self-referral.

Participants are randomly assigned to one of three arms: (1) MOMENT intervention including two MET sessions, momentary and daily self-monitoring via smartphone, and context- and behavior-dependent feedback, (2) no-feedback (MET and momentary self-monitoring), or (3) MET only. Regardless of arm, participants complete a computerized survey and timeline follow-back calendar, and collect momentary and daily data via smartphone for 1 week at baseline, and 3 months after intervention.

We will determine change in outcomes from baseline to 3 months postintervention for each arm, and then compare change in outcomes between arms. Analyses will examine individual-level outcomes (eg, 30-day percent days abstinent from marijuana), daily-level outcomes (eg, number of marijuana use events per day), and momentary-level outcomes (eg, marijuana desire when in a personal top-3 trigger context). Comparisons between MOMENT and each of the other arms will evaluate the value of adding feedback messages to the self-monitoring.

Field Notes From MOMENT MET Sessions

Most participants have reported social and negative affective states as triggers for use, consistent with previous research (Bucker, Crosby, Silgado, Wonderlich, & Schmidt, 2012; Shrier et al., 2014b). Additionally, many have believed that the high from marijuana helps them to focus on home- or school-related tasks (eg, household chores, completing a paper). However, several participants have recognized that their working memory is impaired after years of use, such that they have a hard time remembering and manipulating recent information, consistent with previous research (Vo, Schacht, Mintzer, & Fishman, 2014). Most participants have struggled to see a possible connection between identified negative outcomes (eg, inability to secure work, complete self-directed goals, perform in academic situations) and their frequent marijuana use. Some participants have

commented that marijuana use "can't be that bad" if local governments have decriminalized possession and begun to permit legal medicinal and/or recreational use for adults. Marijuana as a natural substance has also been a common theme among participants, and seems related to lower perceived harm of use.

Potential Challenges to Implementing the MOMENT Intervention

The use of mobile technology to deliver EMI can involve large upfront investments in time and monetary resources (Heron & Smyth, 2010). MOMENT includes only two manual-based counseling sessions, which limits the requirements for staff time and training. Nonetheless, staff time is needed to administer the MET, program the mobile device, and instruct the young persons. Clinic space is required for private sessions. Additional resources may be needed to provide MOMENT-compatible mobile devices for those adolescents who do not have one. MOMENT is designed for young people who are at high risk for adverse outcomes related to their frequent marijuana use, for whom it may be appropriate to allocate more resources (Shrier et al., 2014a).

The MOMENT intervention requires use of a mobile device, raising questions around usability, privacy, and confidentiality. Most young people in the United States and Europe have mobile phones, and are accustomed to using them to send and receive personal information (Nielsen, 2013; Sánchez-Martínez, & Otero, 2009). Our feasibility research has shown that young people are comfortable with the technological aspects of MOMENT, and are not concerned about using a mobile device to complete reports or to receive motivational messaging about marijuana (Black, de Moor, Kendall, & Shrier, 2014; Shrier et al., 2013a).

Young people with problematic substance use may have difficulty with adherence to brief treatment in primary care (D'Amico et al., 2008). Most attrition in the MOMENT pilot study was seen prior to the intervention phase, and may be related to misunderstanding the study burden or expectations. Of pilot participants who had the first MET session (n = 22), 73% (n = 16) completed the MOMENT intervention (Shrier et al., 2014a), similar to rates seen in other pilot studies of marijuana interventions for youth (de Dios et al., 2012; Walker & Roffman, 2006). The expectation for momentary reporting multiple times a day creates additional challenges with adherence. The momentary response rates in the MOMENT pilot study were slightly lower than EMA studies on substance use in adolescents and young adults, which typically range from approximately 60–80% (Black et al., 2014; Shrier et al., 2012), possibly reflecting difficulties associated with the effects of frequent marijuana use of our participants. Current research is seeking to identify strategies to enhance adherence to the momentary self-monitoring component of MOMENT.

Features of a young person's personality and history may need to be considered when administering the MOMENT intervention. For example, the intentional focus of the momentary assessments on negative affective states may exacerbate hypervigilance and sensitivity for those with a trauma history. Additionally, although face-to-face engagement during MET sessions may be beneficial to some, individuals with attachment disorders may lack the ability to make the connection to the counselor, limiting the utility of EMI as an extension of this relationship.

CONCLUSIONS

The MOMENT intervention aims to provide treatment for problematic marijuana use to the many affected adolescents and young adults who present to primary care but may not be actively seeking treatment. MOMENT offers brief motivational treatment in the medical home, where young people have already overcome concerns regarding access and confidentiality. Building on this established trust, MOMENT MET can quickly move into discussions and activities designed to elicit motivation to reduce marijuana use. The MET sessions are individualized in responding to the young person's thoughts and behaviors, standardized in following a detailed manual, and brief in limiting counseling time to only 2 h.

Recognizing that young people are engaging in their marijuana behaviors in variable social, emotional, and physical contexts outside of the clinical setting, MOMENT also uses mobile technology to extend the clinic-based counseling into the natural environment, via self-monitoring and responsive motivational messaging. The self-monitoring may bring the individual's attention to contextual factors related to their marijuana use as they arise, and thus may be tapping into processes that contribute to effectiveness in other treatments, such as CBT and mindfulness-based relapse prevention. Because they appear immediately following report of personal triggers for marijuana use, the messages are tailored to the individual's momentary experience. In this way, the automated messages may be experienced as personalized and relevant.

The MOMENT intervention appears feasible to administer in primary care, and acceptable to young people who use marijuana frequently and are not in treatment. Preliminary findings indicate that MOMENT may be associated with reductions in marijuana desire, and use related to real-life triggering contexts. Ongoing MOMENT research will continue to address issues of feasibility and efficacy, as well as begin to explore possible mechanisms for the observed changes in behavior.

In marrying the benefits of in-person motivational counseling with the advantages of mobile assessment and intervention in daily life, MOMENT offers a promising new approach to reducing marijuana use that may appeal to both the young people in need of treatment and the primary care clinicians who see them.

MINI-DICTIONARY

Ecological momentary assessment (EMA) Frequent, real-time data collection of feelings, thoughts, behaviors, and contexts in the natural environment.

Ecological momentary intervention (EMI) Behavioral intervention delivered via mobile technology in real time, in the natural environment.

Motivational enhancement therapy (MET) Client-driven behavior-change counseling technique based on Motivational Interviewing that provides personalized feedback to enhance behavior change.

Motivational interviewing Client-driven counseling technique that aims to increase motivation for and commitment to behavior change.

Screening, brief intervention, and referral to treatment (SBIRT) The evidence-based practice of identifying, managing, and preventing problem substance use and substance use disorders in the primary care setting.

References

Batalla, A., Bhattacharyya, S., Yucel, M., Fusar-Poli, P., Crippa, J. A., Nogue, S., …, Martin-Santos, R. (2013). Structural and functional imaging studies in chronic cannabis users: a systematic review of adolescent and adult findings. *PLoS One*, *8*, e55821.

Black, S. K., de Moor, C., Kendall, A. D., & Shrier, L. A. (2014). Feasibility of momentary sampling assessment of cannabis use in adolescents and young adults. *Journal of Child & Adolescent Substance Abuse*, *23*, 177–184.

Bonn-Miller, M. O., & Zvolensky, M. J. (2009). An evaluation of the nature of marijuana use and its motives among young adult active users. *American Journal on Addictions*, *18*, 409–416.

Buckner, J. D., Crosby, R. D., Silgado, J., Wonderlich, S. A., & Schmidt, N. B. (2012a). Immediate antecedents of marijuana use: an analysis from ecological momentary assessment. *Journal of Behavior Therapy and Experimental Psychiatry*, *43*, 647–655.

Buckner, J. D., Crosby, R. D., Wonderlich, S. A., & Schmidt, N. B. (2012b). Social anxiety and cannabis use: an analysis from ecological momentary assessment. *Journal of Anxiety Disorders*, *26*, 297–304.

Buckner, J. D., Zvolensky, M. J., Schmidt, N. B., Carroll, K. M., Schatschneider, C., & Crapanzano, K. (2014). Integrated cognitive behavioral therapy for cannabis use and anxiety disorders: rationale and development. *Addictive Behaviors*, *39*, 495–496.

Burke, L. E., Wang, J., & Sevick, M. A. (2011). Self-monitoring in weight loss: a systematic review of the literature. *Journal of the American Dietetic Association*, *111*, 92–102.

Chen, C. -Y., Sotrr, C. L., & Anthony, J. C. (2009). Early-onset drug use and risk for drug dependence problems. *Addictive Behaviors*, *34*, 319–322.

Committee on Substance Abuse. (2011). Substance use screening, brief intervention, and referral to treatment for pediatricians. *Pediatrics*, *128*, e1330–e1340.

Copersino, M. L., Boyd, S. J., Tashkin, D. P., Huestis, M. A., Heishman, S. J., Dermand, J. C., …, Gorelick, D. A. (2010). Sociodemographic characteristics of cannabis smokers and the experience of cannabis withdrawal. *American Journal of Drug and Alcohol Abuse*, *36*(6), 311–319.

D'Amico, E. J., Miles, J. N., Stern, S. A., & Meredith, L. S. (2008). Brief motivational interviewing for teens at risk of substance abuse consequences: a randomized pilot study in a primary care clinic. *Journal of Substance Abuse Treatment*, *35*, 53–61.

Dakwar, E., Mariani, J. P., & Levin, F. R. (2011). Mindfulness impairments in individuals seeking treatment for substance use disorders. *The American Journal of Drug and Alcohol Abuse*, *37*, 165–169.

de Dios, M. A., Herman, D. S., Britton, W. B., Hagerty, C. E., Anderson, B. J., & Stein, M. D. (2012). Motivational and mindfulness intervention for young adult female marijuana users. *Journal of Substance Abuse Treatment*, *42*, 56–64.

de Gee, E. A., Verdurmen, J. E., Bransen, E., de Jonge, J. M., & Schippers, G. M. (2014). A randomized controlled trial of a brief motivational enhancement for non-treatment-seeking adolescent cannabis users. *Journal of Substance Abuse Treatment*, *47*, 181–188.

Ellickson, P. L., Martino, S. C., & Collins, R. L. (2004a). Marijuana use from adolescence to young adulthood: multiple developmental trajectories and their associated outcomes. *Health Psychology*, *23*, 299–307.

Ellickson, P. L., Tucker, J. S., Klein, D. J., & Saner, H. (2004b). Antecedences and outcomes of marijuana use initiation during adolescence. *Preventive Medicine*, *39*, 976–984.

European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). (2010). *Annual Report 2010, the state of the drugs problem in Europe*. Luxembourg: Publications Office of the European Union.

Gates, P., Copeland, J., Swift, W., & Martin, G. (2012). Barriers and facilitators to cannabis treatment. *Drug and Alcohol Review*, *31*, 311–319.

Harris, S. K., Louis-Jacques, J., & Knight, J. R. (2014). Screening and brief intervention for alcohol and other abuse. *Adolescent Medicine State of the Art Reviews*, *1*, 126–156.

Hart, C. L., Ilan, A. B., Gevins, A., Gunderson, E. W., Role, K., Colley, J., & Foltin, R. W. (2010). Neurophysiological and cognitive effects of smoked marijuana in frequent users. *Pharmacology, Biochemistry and Behavior*, *96*, 333–341.

Heron, K., & Smyth, J. (2010). Ecological momentary interventions: incorporating mobile technology into psychosocial and health behavior treatments. *British Journal of Healthy Psychology*, *15*, 1–39.

Hufford, M. R., Shields, A. L., Shiffman, S., Paty, J., & Balabanis, M. (2002). Reactivity to ecological momentary assessment: an example using undergraduate problem drinkers. *Psychology of Addictive Behaviors*, *16*, 205–211.

Hughes, J. R., Peters, E. N., Callas, P. W., Budney, A. J., & Livingston, A. E. (2008). Attempts to stop or reduce marijuana use in non-treatment seekers. *Drug and Alcohol Dependence*, *97*(1–2), 180–184.

Johnston, L. D., O'Malley, P. M., Bachman, J. G., & Schulenberg, J. E. (2012). *Monitoring the future national survey results on drug use, 1975–2011. Vol. I: Secondary school students*. Ann Arbor, MI: Ann Arbor Institute for Social Research, The University of Michigan.

Kabat-Zinn, J. (1990). *Full catastrophe living: Using the wisdom of youth body and mind to face stress, pain, and illness*. New York, NY: Bantam Dell.

Khantzian, E. (1997). The self-medication hypothesis of substance use disorders: a reconsideration and recent applications. *Harvard Review of Psychiatry*, *4*, 231–244.

Knudsen, H. K. (2009). *Barriers to treating alcohol and drug problems among adolescents*. Greensboro, NC: Robert Wood Johnson Foundation's Substance Abuse Policy Research Program, http://saprp.org/knowledgeassets/knowledge_detail.cfm?KAID=20

Luoma, J. B., Twohig, M. P., Waltz, T., Hayes, S. C., Roget, N., Padilla, M., & Fischer, G. (2007). An investigation of stigma in individuals receiving treatment for substance abuse. *Addictive Behaviors*, *32*, 1331–1346.

Madras, B. K., Compton, W. M., Avula, D., Stegbauer, T., Stein, J. B., & Clark, H. W. (2009). Screening, brief interventions, referral to treatment (SBIRT) for illicit drug and alcohol use at multiple healthcare sites: comparison at intake and six months later. *Drug and Alcohol Dependence*, *99*, 280–295.

Magill, M., & Ray, L. A. (2009). Cognitive-behavioral treatment with adult alcohol and illicit drug users: a meta-analysis of randomized controlled trials. *Journal of Studies on Alcohol and Drugs*, *70*(4), 516–527.

Martin, G., & Copeland, J. (2008). The adolescent cannabis check-up: randomized trial of a brief intervention for young cannabis users. *Journal of Substance Abuse Treatment*, *34*, 407–414.

Meier, M. H., Caspi, A., Ambler, A., Harrington, H., Houts, R., Keefe, R. S., …, Moffitt, T. E. (2012). Persistent cannabis users show neuropsychological decline from childhood to midlife. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, E2657–E2664.

Metrik, J., Kahler, C. W., McGeary, J. E., Monti, P. M., & Rohsenow, D. J. (2011). Acute effects of marijuana smoking on negative and positive affect. *Journal of Cognitive Psychotherapy*, *25*, 31–46.

Miller, W. R., & Rollnick, S. (2012). *Motivational interviewing: Helping people change* (3rd ed.). New York, NY: Guildford Press.

Miller, W. R., Zweben, A., DiClemente, C. C., & Rychtarik, R. G. (1994). *Motivational enhancement therapy manual: A clinical research guide for therapists treating individuals with alcohol abuse and dependence*. Rockville, MD: NIAAA, Project MATCH Monograph Series. *Vol. 2*. DHHS Publication No. 94-3723.

Monti, P. M., Barnett, N. P., Colby, S. M., Gwaltney, C. J., Spirito, A., Rohsenow, D. J., & Woolard, R. (2007). Motivational interviewing versus feedback only in emergency care for young adult problem drinking. *Addiction*, *102*, 1234–1243.

Monti, P. M., Colby, S. M., & O'Leary, T. A. (Eds.). (2001). *Adolescents, alcohol and substance abuse: Reaching teens through brief interventions*. New York, NY: The Guilford Press.

Nielsen. (2013). *The mobile consumer: A global snapshot*. London, UK: The Nielsen Company, http://www.nielsen.com/content/dam/corporate/uk/en/documents/Mobile-Consumer-Report-2013.pdf

Pramana, G., Parmanto, B., Kendall, P. C., & Silk, J. S. (2014). The SmartCAT: an m-health platform for ecological momentary intervention in child anxiety treatment. *Telemedicine Journal and E-Health*, *20*, 419–427.

Rahdert, E. (1991). *The Adolescent Assessment/Referral System Manual*. Washington, DC: US Department of Health and Human Services, Alcohol, Drug Abuse, and Mental Health Administrations, DHHS Publication No. (ADM)91-1735.

Rhoads, A., Burke, P., Fredette, M. E., & Shrier, L. A. (2012). "We all here and we're all doing it": social and emotional contexts of frequent marijuana use in adolescents and young adults [abstract]. *Journal of Adolescent Health*, *50*, S41.

Sánchez-Martínez, M., & Otero, A. (2009). Factors associated with cell phone use in adolescents in the community of Madrid (Spain). *CyberPsychology and Behavior*, *12*(2), 131–137.

Shrier, L. A., & Blood, E. A. (2014). It depends on when you ask: motives for using marijuana assessed before versus after a marijuana use event. *Addictive Behaviors*, *39*, 1759–1765.

Shrier, L. A., Rhoads, A., Burke, P., Walls, C., & Blood, E. A. (2014a). Real-time, contextual intervention using mobile technology to reduce marijuana use among youth: a pilot study. *Addictive Behaviors*, *39*, 173–180.

Shrier, L. A., Rhoads, A. M., Fredette, M. E., & Burke, P. J. (2013a). "Counselor in Your Pocket": youth and provider perspective on a mobile motivational intervention for marijuana use. *Substance Use & Misuse*, *49*, 134–144.

Shrier, L. A., Ross, C. S., & Blood, E. A. (2014b). Momentary positive and negative affect predicting marijuana use events in youth. *Journal of Studies on Alcohol and Drugs*, *75*, 781–789.

Shrier, L. A., Walls, C., Rhoads, A., & Blood, E. A. (2013b). Individual and contextual predictors of severity of marijuana use events among young frequent users. *Addictive Behaviors*, *38*, 1448–1456.

Shrier, L. A., Walls, C. E., Kendall, A. D., & Blood, E. A. (2012). The context of desire to use marijuana: momentary assessment of young people who frequently use marijuana. *Psychology of Addictive Behaviors*, *26*, 821–829.

Simpson, T. L., Kivlahan, D., Bush, K. R., & McFall, M. E. (2009). Telephone self-monitoring among alcohol use disorder patients in early recovery: a randomized study of feasibility and measurement reactivity. *Drug and Alcohol Dependence*, *79*, 241–250.

Sobell, L. C., & Sobell, M. B. (1992). Timeline follow-back: a technique for assessing self-reported alcohol consumption. In R. Z. Litten, & J. P. Allen (Eds.), *Measuring alcohol consumption: Psychosocial and biochemical methods* (pp. 41–72). Totowa, NJ: Humana Press.

Stein, M. D., Hagerty, C. E., Herman, D. S., Phipps, M. G., & Anderson, B. J. (2011). A brief marijuana intervention for non-treatment-seeking young adult women. *Journal of Substance Abuse Treatment*, *40*, 189–198.

Sterling, S., Weisner, C., Hinman, A., & Parthasarathy, S. (2010). Access to treatment for adolescents with substance use and co-occurring disorders: challenges and opportunities. *Journal of the American Academy of Child and Adolescent Psychiatry*, *49*, 637–646.

Substance Abuse and Mental Health Services Administration. (2010). *Results from the 2009 National Survey on Drug Use and Health: Vol. I. Summary of National Findings*. Rockville, MD: SAMHSA, Office of Applied Studies, NSDUH Series H-38A, HHS Publication No. SMA 10-4856.

Tevyaw, T. O., & Monti, P. M. (2004). Motivational enhancement and other brief interventions for adolescent substance abuse: Foundations, applications, and evaluations. *Addiction*, *99*, 63–75.

Vo, H. T., Schacht, R., Mintzer, M., & Fishman, M. (2014). Working memory impairment in cannabis- and opioid-dependent adolescents. *Substance Abuse*, *35*(4), 387–390.

Voogt, C., Kuntshe, E., Kleinjan, M., Poelen, E., & Engels, R. (2014). Using ecological momentary assessment to test the effectiveness of a web-based brief alcohol intervention over time among heavy-drinking students: randomized controlled trial. *Journal of Medical Internet Research*, *16*, e5.

Walker, D. D., & Roffman, R. (2006). Motivational Enhancement Therapy for adolescent marijuana users: a preliminary randomized controlled trial. *Journal of Consulting and Clinical Psychology*, *74*, 628–632.

Walker, D. D., Stephens, R., Roffman, R., DeMarce, J., Lozano, B., Towe, S., & Berg, B. (2011). Randomized controlled trial of motivational enhancement therapy with nontreatment-seeking adolescent cannabis users: a further test of the teen marijuana check-up. *Psychology of Addictive Behaviors*, *25*, 474–484.

Walton, M. A., Bohnert, K., Resko, S., Barry, K. L., Chermack, S. T., Zucker, R. A., Zimmerman, M. A., Booth, B. M., & Blow, F. C. (2013). Computer and therapist based brief interventions among cannabis-using adolescents presenting to primary care: one year outcomes. *Drug and Alcohol Dependence*, *132*, 646–653.

Walton, M. A., Resko, S., Barry, K. L., Chermack, S. T., Zucker, R. A., Zimmerman, M. A., Booth, B. M., & Blow, F. C. (2014). A randomized controlled trial testing the efficacy of a brief cannabis universal prevention program among adolescents in primary care. *Addiction*, *109*, 786–797.

Wetter, D. W., McClure, J. B., Cofra-Woerpel, L., Costello, T. J., Reizel, L. R., Businelle, M. S., & Cinciripini, P. M. (2011). A randomized clinical trial of a palmtop computer-delivered treatment for smoking relapse prevention among women. *Psychology of Addictive Behaviors*, *25*, 365–371.

Whittaker, R., McRobbie, H., Bullen, C., Borland, R., Rodgers, A., & Gu, Y. (2012). Mobile phone-based interventions for smoking cessation. *Cochrane Database System Review*, *11:CD006611*.

Witkiewitz, K., Marlatt, G. A., & Walker, D. (2005). Mindfulness-based relapse prevention for alcohol and substance use disorders. Journal of Cognitive Psychotherapy, 19, 211–228.

Cannabis Neuropathology Resources and Recommended Reading

R. Rajendram, V.B. Patel**, V.R. Preedy**

***Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom**
****University of Westminster, School of Life Sciences, Department of Biomedical Science, London, United Kingdom**

KEY POINTS

- Cannabis (also commonly called marijuana) may have implications for various neuropathologies.
- Cannabis use has harmful effects in some individuals. On the other hand, it has been proposed that cannabis or its metabolites can confer therapeutic benefits.
- There is a vast amount of literature on cannabis in the public domain: literally hundreds of thousands of web pages.
- This chapter lists the most up-to-date resources on the regulatory bodies, journals, books, professional bodies, and websites that are relevant to an evidence-based approach to the investigation of cannabis.

INTRODUCTION

Addiction to cannabis (also commonly called marijuana) is a chronic relapsing neuropathological disorder which affects the neural circuits for memory, motivation, and executive decision making. It is associated with characteristic behaviors that focus on obtaining and using cannabis regardless of the consequences. Cannabis misuse is associated with specific cognitive disturbances, and imaging evidence of brain dysfunction (Ghosh & Basu, 2015). Significant biochemical and structural abnormalities have also been reported in postmortem studies of addicted individuals (Ghosh & Basu, 2015). Although cannabis has significant potential for addiction, it does have some reported therapeutic benefits. For example, cannabis has been shown to reduce some painful symptoms of multiple sclerosis (Whiting et al., 2015). Medicines that act via the endocannabinoid receptor pathway may have other novel therapeutic uses in the future.

Although cannabis has been in use since time immemorial, it is only relatively recently that the understanding of the neuropathology of addictions began to increase exponentially. Vast amounts of data on cannabis are now easily available to anyone with an interest. It is challenging, even for experienced scientists, to remain up-to-date. For those without experience and extensive knowledge of the literature, it is difficult to determine which sources are reliable. This is especially important as there are literally hundreds of thousands of pages on the web that have information on cannabis, from personal to scientific perspectives.

To assist colleagues who are interested in understanding more about this subject, we have therefore produced tables containing reliable, up-to-date resources on cannabis in this chapter. These resources have been recommended by the authors of the chapters of this book, all of whom are eminent researchers in the field. The experts who assisted with the compilation of these tables of resources are acknowledged further.

Tables 1–5 list the most up-to-date information on the regulatory bodies and organizations (Table 1), professional bodies (Table 2), journals (Table 3), books (Table 4), and websites (Table 5) that are relevant to cannabis.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00135-6
Copyright © 2017 Elsevier Inc. All rights reserved.

TABLE 1 Regulatory Bodies and Organizations

Alcohol and other Drugs Council of Australia (ADCA)
Department of Health and Human Services www.hhs.gov
European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) www.emcdda.europa.eu/publications/html.cfm/index34913EN.html
Food and Drug Administration www.fda.gov
The Substance Abuse and Mental Health Services Administration (SAMHSA) www.samhsa.gov
United Nations Office on Drugs and Crime (UNODC) www.unodc.org/wdr2016/
US National Library of Medicine and National Institutes of Health www.ncbi.nlm.nih.gov/pubmed
World Health Organization www.who.int/en

This table lists regulatory bodies and organizations relevant to cannabis.

TABLE 2 Professional Societies and Organizations

Drug and Alcohol Findings www.findings.org.uk
International Association of Forensic Toxicologists (TIAFT) www.tiaft.org
International Positive Psychology Association www.ippanetwork.org
Department of Immunology and Allergology, Faculty of Medicine and Health Sciences, University of Antwerp www.uantwerpen.be/en/rg/tpr
Schizophrenia Society of Canada www.schizophrenia.ca
Society of Forensic Toxicologists (SOFT) www.soft-tox.org
University Hospital Antwerp www.uza.be
University of Pennsylvania www.authentichappiness.sas.upenn.edu/

This table lists professional societies and other bodies involved with cannabis.

TABLE 3 Journals Publishing Articles on Cannabis

Drug and Alcohol Dependence
Addictive Behaviors
Addiction
PLoS One
International Journal of Drug Policy
American Journal on Addictions
Forensic Science International
Schizophrenia Research
Journal of Substance Abuse Treatment
Psychopharmacology
Psychology of Addictive Behaviors
Substance Use and Misuse
Journal of Adolescent Health
American Journal of Drug and Alcohol Abuse
Journal of Studies on Alcohol and Drugs
Drug Testing and Analysis
Psychological Medicine
Journal of Psychopharmacology
Drug and Alcohol Review
Journal of Psychoactive Drugs
Psychiatry Research
British Journal of Pharmacology
Current Pharmaceutical Design
European Addiction Research
Journal of Analytical Toxicology
Journal of Addiction Medicine
Journal of Substance Use
Journal of Addictive Diseases
BMC Psychiatry
Australian and New Zealand Journal of Psychiatry
Neuropsychopharmacology
Journal of Child and Adolescent Substance Abuse
Journal of Dual Diagnosis
Frontiers in Psychiatry
BMC Public Health
Substance Abuse
Journal of Drug Education
Pediatrics
American Journal of Psychiatry
Journal of Psychiatric Research

This table lists the journals publishing original research and review articles related to cannabis. The list was generated using SCOPUS (www.scopus.com/). The journals are listed in descending order of the total number of articles published on cannabis in the past 5 years.

TABLE 4 Relevant Books and Other Publications

Decuyper, I., Ryckebosch, H., Van Gasse, A. L., Sabato, V., Faber, M., Bridts, C. H., & Ebo, D. G. (2015). Cannabis allergy: what do we know anno 2015? *Archivum Immunologiae et Therapiae Experimentalis*, *63*(5), 327–332.

Ebo, D. G., Swerts, S., Sabato, V., Hagendorens, M. M., Bridts, C. H., Jorens, P. G., De Clerck, & L. S. (2013). New food allergies in a European non-Mediterranean region: is Cannabis sativa to blame? *International Archives of Allergy and Immunology*, *161*, 220–228.

Faber, M., Van Gasse, A. L., Sabato, V., Hagendorens, M. M., Bridts, C. H., De Clerck, L. S., & Ebo, D. G. (2015). Marihuana allergy: beyond the joint. *Journal of Investigational Allergology and Clinical Immunology*, *25*, 70–72.

Faggiano, F., & Molinar, R. (2014). Multidimensional family therapy for adolescent drug users. A systematic review. *EMCDDA—European Monitoring Centre for Drugs and Drug Addiction*. Lisbon, Portugal: EMCDDA.

Hall J. E. (2011). *Guyton and Hall textbook of medical physiology*. Philadelphia, PA, USA: Saunders.

Kahn, C. R., Weir, G. C., King, G. L., Jacobson, A. M., Moses, A. C., & Smith, R. J. (2008). *Joslin's diabetes mellitus*. Philadelphia, PA, USA: Lippincott Williams & Wilkins.

Swerts, S., Van Gasse, A. L., Leysen, J., Faber, M., Sabato, V., Bridts, C. H., Jorens, P. G., De Clerck, L. S., & Ebo, D. G (2014). Allergy to illicit drugs and narcotics. *Clinical and Experimental Allergy*, *44*, 307–318.

This table lists books and other publications on cannabis.

TABLE 5 Relevant Scientific Internet Resources

Complementary Medicine
http://umm.edu/health/medical/altmed

Crime Survey for England and Wales
www.gov.uk/government/publications/drug-misuse-findings-from-the-2013-to-2014-csew/drug-misuse-findings-from-the-201314-crime-survey-for-england-and-wales

DISEASES
http://diseases.jensenlab.org

GenMANIA
www.genemania.org

HighWire
http://highwire.stanford.edu

National Institutes of Health grants:
www.nih.gov

National Institute on Drug Abuse (NIDA) for teens
http://teens.drugabuse.gov/drug-facts/marijuana

National Highway Traffic Safety Administration (NHTSA)
www.nhtsa.gov

PubMed
www.ncbi.nlm.nih.gov/pubmed

RAND Corporation
www.rand.org/pubs/research_reports/RR864.html

Exchange on Drug Demand Reduction Action. (EMCDDA)
http://www.emcdda.europa.eu/themes/best-practice/examples

National Registry of Evidence-based Programs and Policies. SAMHSA.
http://www.nrepp.samhsa.gov/01_landing.aspx

Register of Australian Drug and Alcohol Research (RADAR)
www.radar.org.au

UpToDate
www.uptodate.com/home

This table lists some scientific internet resources on cannabis or related disease or conditions. Other sites for understanding or investigating diseases or pathologies are also listed (eg, information on genes or sources of secondary information).

Acknowledgments

We would like to thank the following authors for contributing to the development of this resource:
J. Allen, C. Ariza, S. Bachmann, D. Ebo, Z.M. Coskum, D. Herzig, C. Moore, H. Rigter, and P. Tibbo.

References

Ghosh, A., & Basu, D. (2015). Cannabis and psychopathology: the meandering journey of the last decade. *Indian Journal of Psychiatry*, *57*, 140–149.

Whiting, P. F., Wolff, R. F., Deshpande, S., Di Nisio, M., Duffy, S., Hernandez, A. V., Keurentjes, J. C., Lang, S., Misso, K., Ryder, S., Schmidlkofer, S., Westwood, M., & Kleijnen, J. (2015). Cannabinoids for medical use: a systematic review and meta-analysis. *Journal of the American Medical Association*, *313*, 2456–2473.

Page left intentionally blank

CHAPTER

e17

Short Instruments to Screen for "Problematic" Cannabis Use in General Population Surveys

*B. Annaheim**, S. Legleye***,†

***Institute for Biomedical Ethics (IBMB), University of Basel, Basel, Switzerland**
****Institut national d'études démographiques (INED), Paris, France**
†University of Paris-Saclay, Univ. Paris-Sud, UVSQ, CESP, Inserm, Versailles, France

SUMMARY POINTS

- A systematic review of the literature identified 20 self-administrable, brief, and simple instruments that assess a broad spectrum of cannabis-related problems.
- Eleven instruments which had not undergone validations outside the clinical context were subsequently removed from the preliminary list.
- The remaining nine instruments (ASSIST, CAST, CPQ-A, CRAFFT, CUDIT, CUPIT, DAST-10, PUM, SIP-AD) were subject to further in-depth analysis.
- Several instruments theoretically were very promising (eg, CUPIT, PUM). However, only few had proven their usefulness in empirical studies.
- CAST and CUDIT were finally selected as present-day's most appropriate instruments for use in general population surveys.

KEY FACTS OF GENERAL POPULATION CANNABIS SCREENING INSTRUMENTS

Epidemiological research among the general population can usually afford neither the time nor the cost of complex, lengthy or face-to face interviews. Thus, screening instruments should conform to the following criteria:

- be *simple* and easy to understand.
- be brief, that is, *short* and *quick* to administer.
- be *self-administrable* or at least do not require specific training to administer.
- be *in* the *public domain*, or at least inexpensive to obtain.
- *encompass a broad spectrum of cannabis-related problems*, and identify those individuals displaying initial signs of problems that have not (yet) reached pathological level.
- *validated in general population samples*, as results from clinical trials are not necessarily transferable to the general population.
- *validated for adolescents and young adults*, as tools designed for screening adults are not automatically suitable for screening adolescents too, and vice versa.
- *validated among cannabis users*, as tools designed and tested for other substance use are not necessarily also suitable for cannabis users.

LIST OF ABBREVIATIONS

ADIS	Adolescent drug involvement scale
ASSIST	Alcohol, smoking and substance involvement screening test
AUDIT	Alcohol use disorders identification test
CAGE-AID	Cut down-annoyed-guilty-eye opener—adapted to include drugs
CAST	Cannabis abuse screening test

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00116-2
Copyright © 2017 Elsevier Inc. All rights reserved.

CPQ-A-S	Cannabis problems questionnaire, adolescent, short version
CRAFFT	Car-relax-alone-family-forget-trouble
CUDIT	Cannabis use disorders identification test
CUPIT	Cannabis use problems identification test
DAP	Drug and alcohol problem—quick screen
DAST	Drug abuse screening test
MPS	Marijuana problem scale
POSIT	Problem oriented screening instrument for teenagers
PUM	Problematic use of marijuana
RAFFT	Relax-alone-friends-family member with alcohol/drug problem-trouble
SASI	Substance abuse screening instrument
SIP-AD	Short inventory of problems—alcohol and drugs
SMAST-AID	Short Michigan alcohol screening test—adapted to include drugs
SSI-SA/-AOD	Single screening instrument for substance abuse/alcohol and other drugs
TICS	Two-item conjoint screening
THC	Tetrahydrocannabinol

WHY AND HOW TO ESTIMATE THE PREVALENCE OF "PROBLEMATIC" CANNABIS USE IN THE GENERAL POPULATION

This chapter identifies and analyzes existing screening instruments that are adequate for estimating the prevalence of "problematic" cannabis use in social-epidemiological, general population, studies.

Although illegal, using cannabis is widespread (see Chapter 19 in this handbook). Nevertheless, a majority of users smoke cannabis in moderation, and in a socially integrated manner. They use it for purposes of leisure, relaxation, or pleasure. For a minority of users, however, their cannabis use is more like a coping strategy to deal with the challenges of everyday life and can be associated with health, social, legal, financial, and other problems (eg, Asbridge, Duff, Marsh, & Erickson, 2014; Beck & Legleye, 2008).

There is a public health interest in quantifying those people whose cannabis use is related to such problems, as it would make it possible to estimate the magnitude of the "cannabis problem" in the general population, and aid in the development of adequate prevention strategies and treatment measures. Although only a small proportion of cannabis users will ever require medical treatment, cannabis use is still important from a public health perspective due to the large number of users involved (eg, Asbridge et al., 2014; Beck & Legleye, 2008). Furthermore, even recreational use can be linked to a series of potential health and psychosocial hazards (Hall, 2014). The field of public health, therefore, must have at its disposal instruments that estimate the proportion of individuals who encounter problems related to their cannabis use.

Screening seems an appropriate method to estimate the proportion of such individuals within the population and to observe trends over time. Screening does not produce formal diagnoses of substance use disorders. It is conducted in the preclinical phase of a disease and refers to a preliminary assessment which is designed to identify individuals in a broad population whose substance use is potentially harmful or problematic. Screening the general population usually involves short questionnaires, which enable the classification of respondents as either "positive" or "negative," indicating substance use that is "problematic," "harmful," or "risky," while the latter indicates "nonproblematic" or "controlled" use (Babor et al., 2007).

In contrast to tobacco and alcohol, where the use of *Fagerström Test for Nicotine Dependence* (Fagerström, 1978) and AUDIT (Saunders, Aasland, Babor, de la Fuente, & Grant, 1993) or CAGE (Ewing, 1984) are common, none of the existing instruments that screen for cannabis-related problems has yet to emerge as an international standard (Asbridge et al., 2014; Piontek, Kraus, & Klempova, 2008).

In the absence of an agreed-upon and validated screening instrument for cannabis-related problems, some scholars suggest to simply measure frequency and quantity of use, as is often done in alcohol research (Newton et al., 2011; Rehm et al., 2013). Yet, such instruments are not specific enough, and can lead to a high rate of false positive cases (see also Asbridge et al., 2014). Though this might be reasonable in the clinical context, for general population surveys, a screening instrument needs to show high specificity. With cannabis, measuring quantity is even more challenging than frequency of use. Quantity can vary widely with the same frequency. Daily use, for example, can mean having only one puff, or smoking several "joints" (cannabis-cigarettes) in 1 day. Additionally, THC content of consumed cannabis or cannabis-tobacco ratios of a joint can differ dramatically (Temple, Brown, & Hine, 2011; Zeisser et al., 2012). Evidence also suggests that potency of cannabis is increasing in many countries (McLaren, Swift, Dillon, & Allsop, 2008), thereby increasing the risk of adverse consequences. Furthermore, specific frequencies and quantities may be associated with a range of consequences whose severity can vary across individuals. These concerns point to the need for more sensitive means of determining "problematic" cannabis use, as opposed to

simple quantity-frequency measures (see also Asbridge et al., 2014).

Another approach suggests using structured diagnostic interviews according to the International Classification of Diseases (ICD) or the Diagnostic and Statistical Manual of Mental Disorders (DSM). Again, while this approach might be suitable for the clinical context, it is less than satisfactory for social-epidemiological, general population surveys. First, since such tests are long, complex, and costly, they are often difficult to implement in large nationwide surveys. Self-administered surveys can be difficult for individuals with literacy problems (who are more at risk to present cannabis related problems) to complete. Others can also perceive self-administered surveys as boring, which can lead to a very high number of missing answers. Furthermore, face-to-face administration can require special training for interviewers. Second, in terms of public health, it makes more sense to assess a broader spectrum of cannabis-related problems. Thus, a strict focus on clinical diagnoses may exclude important information about users who do not fulfil the whole criteria for substance use disorders (Degenhardt, Coffey, Carlin, Swift, & Patton, 2008). On the contrary, a diagnosis of cannabis use disorder does not necessarily equate to *concrete* harm or problems (see Temple et al., 2011).

To this end, one can conclude that an early and rough estimation of the prevalence of "problematic" cannabis use through general population surveys is vital, and specific cannabis screening instruments are the most appropriate tools to employ.

General population screening must comply with a number of conditions. First, it must be short, quick, efficient, and as nonintrusive as possible (Pilowsky and Wu, 2013). General population screening instruments should be simple, easy to understand, and able to be self-administered, for a maximum degree of accuracy. As mentioned earlier, such instruments should encompass a broad spectrum of cannabis-related problems for the purposes of identifying individuals who display initial signs of problems, but have not necessarily reached a pathological level, yet. Optimally, such screening instruments should be suitable for adolescents, as well as adults, and in the public domain.

However, to this day, no consensus exists for a validated screening instrument that conforms to these criteria. A clear definition is also lacking regarding what constitutes *"problematic" cannabis use*. The European Monitoring Centre for Drugs and Drug Addictions (EMCDDA) has set up a working group to resolve the issue (EMCDDA, 2007). At present, and until a definition is agreed upon, the EMCDDA applies the term to simply refer to consumption patterns "*...leading to negative consequences on a social or health level, both for the individual user and for the larger community*" (Beck & Legleye, 2008, p. 31).

Although an agreed-upon and validated screening instrument for "problematic" cannabis use has yet to be established, many instruments already exist. While some were conceptualized for clinical screening and must therefore be administered by a trained professional, others are capable of being self-administered, making them ideally suited for general population studies where face-to-face interviews are not feasible. Certain instruments are cannabis-specific, while others cover all illegal drugs and, in some instances, alcohol as well. Several reviews of cannabis screening instruments have already been undertaken (Annaheim, 2013; Beck & Legleye, 2008; Bashford, 2010; Obradovic, 2013; Piontek et al., 2008; Stephens & Roffman, 2005; Mdege & Lang 2011). At present, however, none of these reviews includes all of the following criteria: up-to-date, cannabis-specific, focused on population-based (but not clinical) screening, exhaustive, and grounded on a systematic selection of screening instruments.

The present chapter contains a systematic review of the literature, and analyzes and selects the instrument best suited to screen for cannabis-related problems in general population surveys.

SELECTION OF ADEQUATE INSTRUMENTS FROM THE LITERATURE

To identify publications dealing with nonclinical cannabis screening instruments, we first searched in PubMed, and in Web of Science (these searches included publications in English, French, German and Spanish, dating from 1990 onward, ie, to July 2014). Then, we checked our findings by performing a Google Scholar search, restricted to recent publications (ie, from 2010 to July 2014) for *reviews* not previously discovered, with the EMCDDA Evaluation Instruments Bank and the US Alcohol and Drug Abuse Institute (ADAI) Library of the University of Washington.

All tests had to conform to the following criteria for eligibility and relevance (checklist): the tests had to be simple and easy to understand, brief (maximum 20 items/10 minimum), capable of being self-administered or not requiring specific training for administration. They also had to be in the public domain, measuring a broad concept of "problematic" cannabis use (not solely frequency of use, craving, or dependence, for example), as described in the preceding section. Tests that addressed drug use in general were excluded if the same test was available in its cannabis-specific version [eg, *Drug Use Disorders Identification Test* (DUDIT) was excluded in favor of *Cannabis Use Disorders Identification Test* (CUDIT)].

Consequently, 20 brief cannabis screening instruments were preselected for closer inspection concerning their empirical approval.

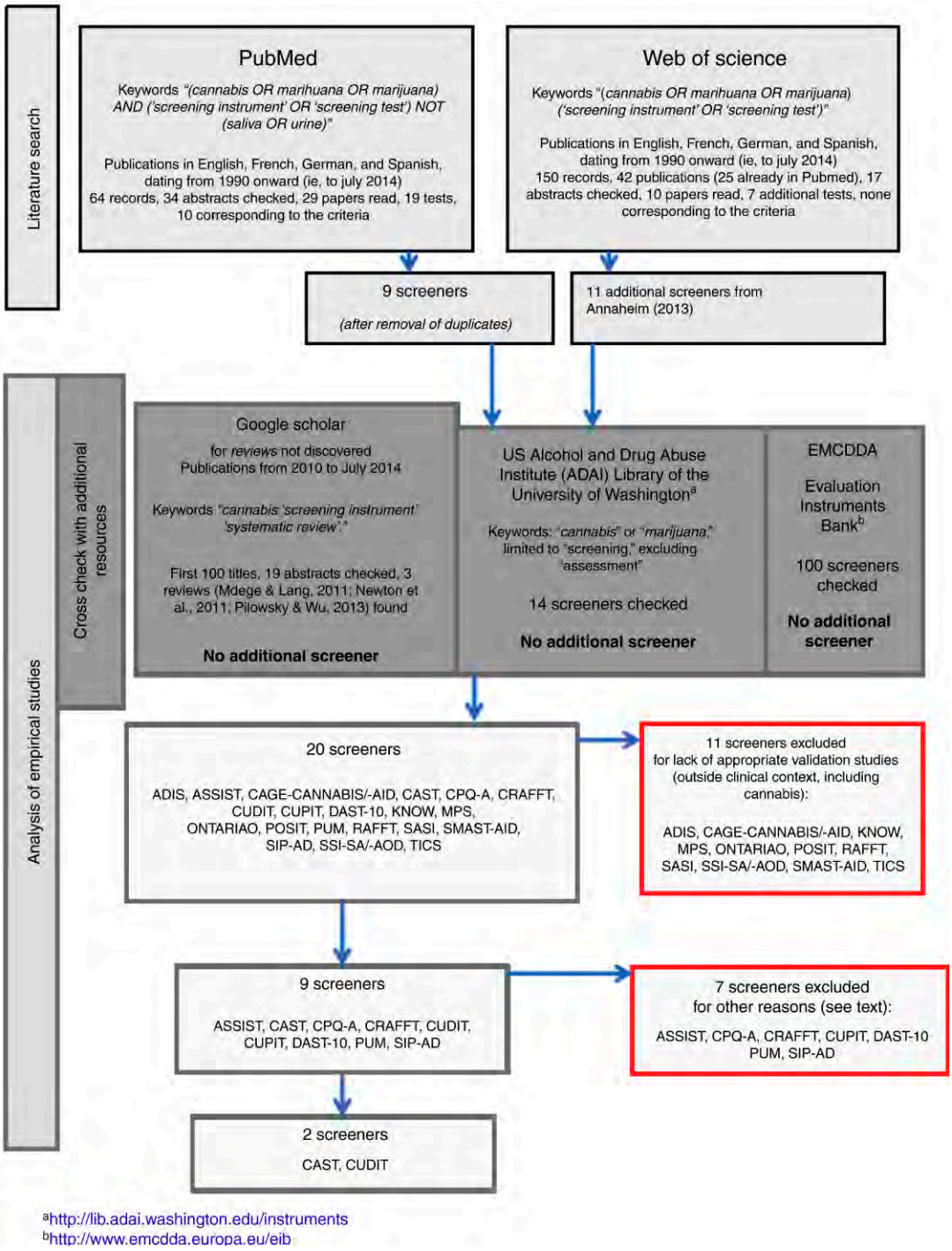


FIGURE e17.1 The process of selecting and analyzing screening instruments. *Source: Based on the PRISMA flow diagram [cf. Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. The PRISMA Group (2009)]. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement.* PLoS Medicine, *6(6), e1000097) and shows the way the most useful screening tests (screeners) were identified. For the tests' spelled-out names see List of Abbreviations.*

Thus, in a second step, 11 tests—although potentially useful—had to be excluded from the preselected list, because they had not yet undergone an evaluation in a general population sample. With lacking nonclinical empirical investigations, a concrete judgment about the suitability of those tests, for use in general population surveys, was not possible. Nine tests were finally selected. The detail about the literature search is provided in Fig. e17.1.

CHARACTERISTICS OF SELECTED SCREENING INSTRUMENTS

The remaining nine instruments (see Fig. e17.1) were subjected to a more detailed analysis (see Table e17.1).

ASSIST: The *Alcohol, Smoking and Substance Involvement Screening Test* was developed by a working group of the World Health Organization (WHO) to identify

TABLE e17.1 Brief, Self-Administrable (Cannabis) Screening Instruments

Name (acronym)	Basic reference/origin	Purpose (measured concept)/ dimensions/items	Validation studies outside clinical context	Gold standard, cut-off, sensitivity/ specificity
Alcohol, Smoking and Substance Involvement Screening Test (*ASSIST*)	WHO ASSIST Working Group (2002)	To detect psychoactive substance use and related (moderate and severe) problems, that is, risky, hazardous, harmful use (WHO ASSIST Working Group, 2002)	Davis, Thomas, Jesseman, and Mazan (2009): cannabis-specific validation in two Canadian adult general population samples (n = 13,909/1235; n = 1181/160 past 3 months users)	Any problem with cannabis in the last 12 months, cutoff 8, 77/67
		Theoretical dimensions (items): 1. Frequency of use (1, 2) 2. Abuse/dependence [craving (3), failed to do what expected (5), concerned others (6), loss of control (7)] 3. Problems [health, social, legal, financial (4)]	Thake and Davis (2011): cannabis-specific validation in Canadian adult general population sample (n = 16,674; n = 1179 past 3 months users)	Cannabis before driving, cutoff 8, 53/78; any other illicit drug use in the last 12 months, cutoff 8, 52/70; any harm because of use in the last 12 months, cutoff 8, 69/77
			Asbridge et al. (2014): cannabis-specific evaluation[a] in Canadian adult population sample (n = 13,082; n = 775 past 3 months users) and long-term, socially integrated regular cannabis users sample from four Canadian cities (n = 165)	None
			Barreto, de Oliveira Christoff, and Boerngen-Lacerda (2014): noncannabis-specific validation (different psychoactive substances) in Brazilian convenience student sample (n = 170)	None: ASSIST (interview format) as gold standard for self-administered ASSIST
Cannabis Abuse Screening Test (*CAST*)	French Monitoring Centre for Drugs and Drug Addictions OFDT (in collaboration with EMCDDA): based on a review by Reynaud (2002) and validated by Legleye, Karila, Beck, and Reynaud (2007)	Description of different aspects of harmful cannabis use and screening of "problematic cannabis use" (EMCDDA, 2007; Beck & Legleye, 2008)	Legleye et al. (2007)—lifetime; binary CAST: cannabis-specific validation in French convenience sample among scholars from schools and universities (n = 1728 aged 14–22)	POSIT, cutoff 4 (binary CAST), 93/81

		Items: morning use (1), smoking alone (2), memory problems (3), concerned others (4), tried to cut down (5), other problems (6)	PNSD (2009): cannabis-specific validation in Spanish student sample (n = 26,454; n = 3569 past 12 months users aged 14–18)	DSM-IV TR dependence, cutoff 5, 83/87
			Legleye, Piontek, and Kraus (2011): cannabis-specific validation in French nationwide random sample (n = 2566 past 12 months users aged 17–18)	DSM-IV TR (M-CIDI) dependence, cutoff 3, 84/72 DSM-IV-TR (M-CIDI) abuse, cutoff 3, 79/81
			Cuenca-Royo et al. (2012): cannabis-specific validation among Spanish volunteers (n = 245 past 12 months users aged 18–25)	DSM-IV TR (PRISM-IV), SUD, cutoff 9, 74/69; dependence, cut-off 12, 57/84
			Fernandez-Artamendi et al. (2012): cannabis-specific validation in Spanish convenience sample (n = 144 past month users aged 16–20)	DSM-IV TR, dependence, cut-off 3, 83/77
			Bastiani et al. (2013): cannabis-specific validation in Italian nationwide random sample of pupils (n = 5787 past 12 months users aged 15–19)	DSM-IV TR, ≥4 criteria, cut-off 7, 80/80
			Legleye, Piontek, Kraus, Morand, and Falissard (2013): cannabis-specific validation in French random nationwide adolescent sample (n = 2566 past 12 months users aged 17–18)	Latent class analysis of DSM-IV TR (M-CIDI) Moderate/severe, cut-off 3, 78/81 Severe, cut-off 7, 88/85
			Gyepesi et al. (2014): cannabis-specific validation in almost pure random sample among Hungarian high school and college students (n= 476 and 439 past 12 months users mean aged 19–24 years)	DSM-IV TR (M-CIDI) High school: CUD, cut-off 2, 62/97; dependence, cutoff 2, 86/95; college: CUD, cutoff 2, 68/97; dependence, cutoff 2, 78/94
			Legleye et al., 2015: cannabis-specific validation in French nationwide random sample (n = 550 past 12 months users aged 15–62)	DSM-IV TR (M-CIDI), CUD, cut-off 3, 80/75, dependence, cutoff 5, 81/79 Latent class analysis, moderate/severe, cut-off 3, 78/82; severe, cutoff 7, 97/86 or cutoff 8, 94/89
Cannabis Problems Questionnaire for adolescents, short version (*CPQ-A-S*)	Proudfoot, Vogl, Swift, Martin, and Copeland (2010): based on adolescent version (CPQ-A) (Martin, Copeland, Gilmour, Gates, & Swift, 2006) of CPQ (Copeland, Gilmour, Gates, & Swift, 2005)	Problems related to cannabis use (Proudfoot et al., 2010); [CPQ-A: intended to supplement DSM-IV diagnostic in exploration of cannabis-related problems (Martin et al., 2006)]	Proudfoot et al. (2010): cannabis-specific validation in Australian student sample (n = 1734; n = 126 past 3 months users), combined with a community sample (n = 99 past 3 months users aged 14–18)	None: strong predictor of cannabis heavy use in logistic modeling

(*Continued*)

TABLE e17.1 Brief, Self-Administrable (Cannabis) Screening Instruments (*cont.*)

Name (acronym)	Basic reference/origin	Purpose (measured concept)/ dimensions/items	Validation studies outside clinical context	Gold standard, cut-off, sensitivity/ specificity
		Items: loss of control (1), paranoia (2, 11), concerned others (4), financial problems (5), social reduction (3, 12), trouble with police (6), health problems (7–10)	Fernandez-Artamendi, Fernandez-Hermida, Muniz-Fernandez, Secades-Villa, and Garcia-Fernandez (2012): cannabis-specific validation in Spanish student sample (n = 748; n = 144 past month users)	DSM-IV TR dependence, cutoff 3, 83/78
Car-Relax-Alone-Forget-Family-Trouble (*CRAFFT*)	Knight et al. (1999): constructed by combining and modifying items from RAFFT, DAP, and POSIT	Harmful use of alcohol and drugs (Knight et al., 1999)	Cummins et al. (2003): validation for alcohol and other drug use, in American-Indian and Alaska-Native adolescents (13–19 years) from US public schools and outpatients clinics (n = 70)	Frequent cannabis use, cutoff 2, 100/75
		Items: driving while intoxicated (1), motives for use (2), smoking alone (3), memory problems (4), concerned others (5), gotten into trouble (6)	Bernard et al. (2005): validation for alcohol and other drug use of a French version, in Swiss mixed, substance using treatment (n = 83) and general population (n = 80) adolescent sample (14–19 years)	ADAD, cutoff 4, 74/65; MINI, cutoff 3, 94/61
			Karila et al. (2007): validation for alcohol and other drug use, in French school student sample (n = 1728)	POSIT, cutoff 2, 93/77
			Subramaniam, Cheok, Verma, Wong, and Chong (2010): validation for alcohol and other drug use, in Singapore military recruits (16–26 years) male sample (n = 22,248)	CIDI auto 2.1, cutoff 1, 71/74
			Pérez Gomez and Scoppeta Diaz-Grandos (2011): validation for alcohol and other drug use, in Colombian student (14–18 years) sample (n = 432)	None: item response analysis
			Skogen, Boe, Knudsen, and Hysing (2013): validation for alcohol and other drug use, in Norwegian general population adolescents (17–19 years) sample (n = 9680)	Self-report of "any problem with drug use," cutoff 1, 81/72

Cannabis Use Disorders Identification Test (*CUDIT*)	Adamson and Sellman (2003): adaptation of Alcohol Use Disorders Identification Test (AUDIT) (Saunders et al., 1993)	Originally: DSM-IV abuse and dependence (Adamson & Sellman, 2003), when used in general population: "problematic cannabis use" (Annaheim, Rehm, & Gmel, 2008; Annaheim, Scotto, & Gmel, 2010); CUDIT is based on AUDIT (see Saunders et al., 1993) that theoretically measures alcohol-related problems and the following three subconcepts: 1. original version (items): a. frequency/quantity (1–3) b. symptoms of dependence [loss of control (4), failed to do what expected (5), morning use (6)] c. negative consequences (guilt (7), memory problems (8), injuries (9), concerned others (10) 2. revised version CUDIT-R (Adamson et al., 2010): deleting 2 and replacing 3 items (3, 6, 7, 9, 10) with hazardous use, devoting a great deal of time, thoughts about quitting 3. revised version for general population (Annaheim et al., 2010): replacing 3 items (2, 7, 9) with motives for use, social reduction, difficulties at work/school	Annaheim et al. (2008): cannabis-specific validation in Swiss adolescents and young adults general population sample (n = 5025; n = 593 past 6 months users) Annaheim et al. (2010): cannabis-specific validation in Swiss adolescents and young adults general population sample (n = 5722; n = 558 past 6 months users) Thake and Davis (2011): cannabis-specific validation in Canadian adult general population sample (n = 16'674; n = 1179 past 3 months users) Bruno, Marshall, and Adamson (2013): cannabis-specific validation among adult, nontreatment seeking cannabis users in New Zealand (n = 368)	Different accepted indicators of problematic use: smoking before school/work, cutoff 8, 64/69; smoking before driving, cutoff 6, 58/59; depressive symptoms, cutoff 6, 50/59; coping motives, cut-off 6, 66/67; self-evaluation, cutoff 7, 71/70 None: item response analysis Cannabis before driving, cutoff 6, 51/83; any other illicit drug use in the last 12 months, cutoff 6, 53/76; any harm because of use in the last 12 months, cutoff 6, 63/83 DSM-IV TR dependence, cutoff 11, 78/79; DSM-5 mild, cutoff 9, 75/77; DSM-5 moderate, cutoff 11, 80/77; DSM-5 severe, cutoff 13, 86/81
Cannabis Use Problems Identification Test (*CUPIT*)	Bashford, Flett, and Copeland (2010)	Currently diagnosable and potentially problematic cannabis use Theoretically three sub-concepts according DSM-IV/ICD-10 (items): 1. risky use [frequency (1, 2)] 2. dependence/using behavior [times using (3), morning use (4), being stoned (5), loss of control (9), craving (6, 7, 8, 10)] 3. health/social problems [work/school problems (11), lack of energy (12), social reduction (13, 14), memory problems (15), loss of control (16)]	Bashford et al. (2010): cannabis-specific validation among volunteers in New Zealand, who had been using cannabis at least once during the last 12 months, from general population (n = 36), drug treatment clinics (n = 36), justice system (n = 55), and students/job seekers (n = 83)	DSM-IV TR/ICD-10 CUD: cutoff 20, 87/79

(*Continued*)

TABLE e17.1 Brief, Self-Administrable (Cannabis) Screening Instruments (*cont.*)

Name (acronym)	Basic reference/origin	Purpose (measured concept)/ dimensions/items	Validation studies outside clinical context	Gold standard, cut-off, sensitivity/ specificity
Drug Abuse Screening Test (*DAST-10*)	Skinner (1982): Adaption of Michigan Alcohol Screening Test (MAST) (Selzer, 1971)	Drug abuse or dependence; DAST-10 is the shortened version of the original DAST (28 items) and the DAST-20	Bedregal, Sobell, Sobell, and Simco (2006): noncannabis-specific validation among Spanish speaking individuals (Hispanics/ Latinos) without alcohol/drug problems (n = 127), drug and (n = 60) alcohol abusers (n = 35) in the United States	Drug abusers in treatment programs: cutoff 4, 90/97
		Items: (poly-) drug use in general (1, 2), loss of control (3), "blackouts" (4), guilt (5), concerned others (6), social reduction (7), illegal activities (8), withdrawal (9), medical problems (10)	McCabe, Boyd, Cranford, Morales, and Slayden (2006): noncannabis-specific validation among undergraduate students in the United States (n = 4500; n = 1735 past 12 months drug users)	None: correlation analyses
			Grekin et al. (2010): noncannabis-specific validation among 300 low-income (mostly African-American) women in the United States, recruited right after giving birth (in clinical setting)	Biological drug screening, sensitivity = 0.47
			Pérez Gálvez, García Fernández, de Vicente Manzanaro, Angustias Oliveras Valenzuela, and Lahoz Lafuente (2010): noncannabis-specific validation of Spanish version in 259 Spanish adults (n = 121 drug addicts/n = 138 healthy controls)	DSM-IV TR, cutoff 3, 96/95
			Evren, Ogel, Evren, and Bozkurt (2014): noncannabis-specific validation of Turkish version among 202 prisoners (n = 78 with drug problems/n = 124 without drug problems)	Addiction Profile Index-Short, cutoff 4, 88/74
			Yongseok (2014): noncannabis-specific validation of Korean version in general population adults (n = 1000; n = 503 "normal" residents/n = 497 at risk adults, ie, from prisons, on probation or working at bars)	Being arrested because of use, cutoff 2, 66/93

Problematic Use of Marijuana (Cannabis) [*PUM (PUC)*]	Okulicz-Kozaryn and Sieroslawski (2007)	ICD-10 harmful use, problems in interpersonal relationships/psychophysical functioning and abuse (Bashford, 2010) Items: skipped school (1), arguments with family/friends (2, 3), bought cannabis (4), memory problems (5), smoking alone (6), craving (7), financial problems (8)	Okulicz-Kozaryn and Sieroslawski (2007): cannabis-specific validation among general population cannabis using adolescents recruited through snow-ball sampling in Poland (n = 1277)	ICD-10 dependence, cutoff 2, 81/88
Short Inventory of Problems—Alcohol and Drugs (*SIP-AD*)	Blanchard, Morgenstern, Morgan, Lobouvie, and Bux (2003): shortened version of Inventory of Drug Use Consequences (Tonigan & Miller, 2002)—itself a modified form of the Drinker Inventory of Consequences (Miller, Tonigan, & Longabaugh, 1995)	Problems related to alcohol and substance use in general: Items: being unhappy (1), physical appearance changed (2, 9), failed to do what expected (3), personality changes (4, 13), risk taking (5), impulsiveness (6, 7), financial problems (8, 15), family/friendship was hurt (10, 11), activity reduction (12), damaged reputation (14)	Gillespie, Holt, and Blackwell (2007): noncannabis-specific validation among 421 university students in the United States (n = 287 past year alcohol/drug users) Hagman et al. (2009): noncannabis-specific validation among 469 male regular "clubbers"/club drug users who have sex with men from New York City	None: logistic modeling None: item response analysis

DSM-IV TR, Diagnostic and Statistical Manual of Mental Disorders, Text Revision; M-CIDI, Munich Composite International Diagnostic Interview; PRISM, Psychiatric Research Interview for Substance and Mental Disorders (PRISM-IV); SUD, substance use disorders (dependence or abuse); CUD, Cannabis use disorders (same as SUD in this context).
The table lists basic reference, origins, purpose, and available nonclinical validation studies for every screening test selected after a checklist (see Key Facts).
Reynaud, M. (2002). *Usage nocif de substances psychoactives*. Paris: La Documentation française.
PNSD (2009). *Consumo problemático de cannabis en estudiantes españoles de 14-18 años: validación de escalas*. Madrid: Ministerio de Sanidad y Consumo. http://www.pnsd.msc.es/Categoria2/publica/pdf/ConsProblematico_cannabis.pdf
Copeland, J., Gilmour, S., Gates, P., & Swift, W. (2005). The cannabis problems questionnaire: factor structure, reliability, and validity. *Drug and Alcohol Dependence*, *80*(3), 313–319.
Selzer, M. L. (1971). The Michigan alcoholism screening test: the quest for a new diagnostic instrument. *American Journal of Psychiatry*, *127*, 1653–1658.
Tonigan, J. S., & Miller, W. R. (2002). The inventory of drug use consequences (InDUC): test-retest stability and sensitivity to detect change. *Psychology of Addictive Behaviors*, *16*(2), 165–168.
Miller, W. R., Tonigan, J. S., & Longabaugh, R. (1995). *The drinker inventory of consequences (DrInC): An instrument for assessing adverse consequences of alcohol abuse*. Test manual. (*Vol. 4*, Project MATCH Monograph Series). Rockville, MD: National Institute on Alcohol Abuse and Alcoholism.
[a] *Not a validation in narrower sense of the word.*

TABLE e17.2 The Cannabis Abuse Screening Test (CAST)

Questions		Answers (scoring)
Over the past 12 months...		
1	Have you smoked cannabis before midday?	Never (0), rarely (1), from time to time (2), fairly often (3), very often (4)
2	Have you smoked cannabis when you were alone?	Never (0), rarely (1), from time to time (2), fairly often (3), very often (4)
3	Have you had memory problems when you smoked cannabis?	Never (0), rarely (1), from time to time (2), fairly often (3), very often (4)
4	Have friends or family members told you that you should reduce or stop your cannabis consumption?	Never (0), rarely (1), from time to time (2), fairly often (3), very often (4)
5	Have you tried to reduce or stop your cannabis use without succeeding?	Never (0), rarely (1), from time to time (2), fairly often (3), very often (4)
6	Have you had problems because of your cannabis use (argument, accident, poor results at school, etc.)?	Never (0), rarely (1), from time to time (2), fairly often (3), very often (4)

The table displays the CAST in its original, nondichotomized version (Legleye et al., 2011).

substance use and related problems in medical care settings, through face-to-face interaction with a health professional. The tool is composed of seven questions per substance that encompass lifetime and current use, symptoms of dependence, health, social, legal, and financial problems (WHO ASSIST Working Group, 2002). Only recently, its usefulness was also tested in survey research.

Davis et al. (2009) assessed the effectiveness of the cannabis portion of ASSIST to identify "problematic" users, comparing it to self-reported harms. They concluded that a greater balance of sensitivity with specificity could be obtained when using a higher threshold for "problematic" cannabis use than what was suggested in clinical applications of the instrument.

In a subsequent Canadian study, Thake and Davis (2011) confirmed the higher threshold for the nonclinical setting and declared ASSIST more successful in identifying harm than a simple frequency of use measure. At the same time, they found CUDIT (see "Cannabis Use Disorders Identification Test") of better performance for use in general population surveys than ASSIST.

Asbridge et al. (2014) compared ASSIST to a frequency (and quantity) measure. Their results underline a disproportionately high impact that the inclusion of a simple measure of frequency into ASSIST has on defining "problematic" cannabis use. Moreover, they confirmed the higher threshold for general population surveys.

Finally, a new self-report format of ASSIST was compared to the original interview-format in a small convenience sample of Brazilian university students (Barreto et al., 2014). The authors emphasized, however, the inability of extrapolation of their results to the general population or even university students in general.

To conclude, data on ASSIST's psychometric performance in the general population are available, today, and indicates the need for a higher threshold. Nevertheless, that data remain of a very limited nature (eg, no information on dimensionality of the scale). In addition, another short cannabis screening instrument (CUDIT) has shown better psychometric performance than ASSIST when tested in a large general population sample (Thake & Davis, 2011). Thus, based on the present state of knowledge, there is not enough evidence that would prove ASSIST's usefulness outside the clinical setting.

CAST: The *Cannabis Abuse Screening Test* is a six-item cannabis-specific tool aimed at describing some patterns of use and at screening "problematic" use according to Beck and Legleye (2008) and ICD-10 diagnosis. Its items encompass patterns of potential nonrecreational use, symptoms of dependence, social aspects, memory, and other problems. The test originally used five ordered categories for answers (never, rarely, from time to time, fairly often, very often). It was initially designed for the entire lifespan, and used a dichotomization of the answers instead of a full range (Legleye et al., 2007). Subsequently, the time frame was restricted to "the past 12 months" and the dichotomization abandoned (Table e17.2). It was validated using adolescents and young adults (mostly students). Studies were conducted in various countries, including nationwide representative samples using two gold standards: the DSM-IV diagnoses (Bastiani et al., 2013; Cuenca-Royo et al., 2012; Gyepesi et al., 2014; Legleye et al., 2011), or an empirical gold standard obtained by latent class analysis of the DSM-IV (Legleye et al., 2013). Its validity was also assessed in the general population (aged 15–64) in France (Legleye et al., 2015). The difference between binary or full-range scorings were found low in French adolescents (Legleye et al., 2011), as were the gender differences (Legleye et al., 2013), and the same thresholds were found for adolescents and adults.

CAST appears to be unidimensional, and shows good internal consistency and high discriminative power

between diagnoses. Its test-retest reliability was found to be very good (Molinaro, Siciliano, Curzio, Denoth, & Mariani, 2012). It was used for the European School Survey Project on Alcohol and Other Drugs (ESPAD) Study (Pabst, Kraus, & Piontek, 2012), applying a binary scoring. Its structural equivalence across 13 countries was found to be high (Bougeard, Eslami, & Qannari, 2014).

At this time, CAST has proved its psychometric and screening properties in various populations and countries, and with various statistical methods and gold standards. Existing crossnational surveys allow studying its crosscultural invariance. It is the most widely used tool for research among general population adolescents and adults. According to the criteria of our review, it is considered to be one of the most useful tools for use in general population surveys.

CPQ-A-S: The *Cannabis Problems Questionnaire* for adolescents is a 27-item tool which has been found to be a reliable and valid screen for cannabis problems in adolescents. Originally, it was a clinician-administered interview (Martin et al., 2006). More adequate for our purposes, however, is its shortened, 12-item version, CPQ-A-S, which has been constructed also for research in the community, and is intended to identify young people at risk of harm due to their cannabis use (see Proudfoot et al., 2010). CPQ-A-S puts a high emphasize on health aspects, with about half of its items assessing physical or mental health problems (eg, chest infection, cough, paranoia). In addition, it contains items on patterns of use, social, legal, and financial aspects.

When validated in a student and adolescent community sample (Proudfoot et al., 2010), CPQ-A-S has shown good reliability and validity and loaded on a single factor. However, the test has shown rather moderate internal consistency.

Further, CPQ-A-S has been validated in a student sample and compared to CAST (Fernandez-Artamendi et al., 2012). The researchers defined both instruments as reliable and valid to identify "problematic" cannabis use in young people. CPQ-A-S appeared to be a better predictor of cannabis users with psychopathological symptoms and concern about their cannabis use, while CAST had higher internal consistency, as well as higher specificity to detect cannabis dependence according to DSM-IV diagnoses.

At this time, CPQ only has been validated in adolescent samples and there is no short form of the tool that is conceptualized also for adults. Moreover, outside the clinical setting, specificity is at least as important as sensitivity and other instruments (CAST) have shown higher specificity while encompassing less items (ie, take less time to be administered). Therefore, based on the present state of knowledge, CPQ-A-S cannot be counted as one of the most useful tools for research among general population adolescents and adults.

CRAFFT: CRAFFT is a mnemonic, based on six questions that ask for cannabis-related problems from a lifetime perspective. It was constructed by combining and modifying items from other screening tests and consequently validated in the clinical context (Knight et al., 1999). Its items encompass patterns of use, harmful use, cognitive and social aspects, although no health aspects.

The tool has been used in French (Bernard et al., 2005; Karila et al., 2007), Spanish (Pérez Gomez & Scoppeta Diaz-Grandos, 2011), Norwegian (Skogen et al., 2013), and other language versions (Subramaniam et al., 2010). Thus, CRAFFT is a widely used screening instrument among adolescents that has generally been found of adequate psychometric performance (Dhalla, Zumbo, & Poole, 2011).

More specifically, however, among adolescents *outside* the clinical context, CRAFFT has shown insufficient internal consistency and relatively low specificity in several validation studies (see Bernard et al., 2005; Cummins et al., 2003; Karila et al., 2007; Knight et al., 1999; Pérez Gomez & Scoppeta Diaz-Grandos, 2011; Skogen et al., 2013; Subramaniam et al., 2010). Yet, to the knowledge of the authors, CRAFFT has never been used and validated among general population adults older than 26 years. Although there are numerous validation studies from outside the clinical context, there seem to be hardly any studies using a representative general population sample, other than (school) students or military recruits. Moreover, there is, to the knowledge of the authors, not a single evaluation of CRAFFT that focuses on cannabis use instead of analyzing several illegal substances, and often also alcohol, at the same time.

For all these reasons, CRAFFT cannot be one of the most adequate screening instruments for use in general population surveys.

CUDIT: The *Cannabis Use Disorders Identification Test* was constructed as a brief 10-item screen to identify individuals who had used cannabis in a problematic or harmful way during the preceding 6 months (Adamson & Sellman, 2003). It was directly adapted from the established AUDIT (Saunders et al., 1993) and simply created by merely replacing the word "alcohol" with the word "cannabis." CUDIT covers patterns of use, symptoms of dependence, and negative (social) consequences of cannabis use. Originally constructed (Adamson & Sellman, 2003) and revised (Adamson et al., 2010: CUDIT-R) for the clinical context, it was subsequently also used and validated for research in the general population (Table e17.3).

Annaheim et al. (2008) found CUDIT related to accepted concepts of "problematic" cannabis use (eg, coping motives for cannabis use, depressive symptoms), but with a modest internal consistency and a rather weak psychometric performance of some items. Thus,

TABLE e17.3 The Cannabis Use Disorders Identification Test (CUDIT)

	Questions	Answers (scoring)
Have you used any cannabis over the past 6 months? If YES, please answer the following questions about your cannabis use: Over the past 6 months...		
1	... how often have you used cannabis?	Never (0), monthly or less (1), 2–4 times a month (2), 2–3- times a week (3), 4 or more times a week (4)
2	Which of the following statements best fits your personal situation?	"I smoke cannabis for fun, because it's something special" (0), "I smoke cannabis out of habit, because it's part of my daily life" (4)
3	... how often were you "stoned" for 6 or more hours?	Never (0), less than monthly (1), monthly (2), weekly (3), daily or almost daily (4)
4	How often during the past 6 months did you find that you were not able to stop using cannabis once you had started?	Never (0), less than monthly (1), monthly (2), weekly (3), daily or almost daily (4)
5	How often during the past 6 months did you fail to do what was normally expected from you because of using cannabis?	Never (0), less than monthly (1), monthly (2), weekly (3), daily or almost daily(4)
6	How often during the past 6 months did you need to use cannabis in the morning to get yourself going after a heavy session of using cannabis?	Never (0), less than monthly (1), monthly (2), weekly (3), daily or almost daily (4)
7	How often during the past 6 months have you refrained from taking part in leisure time activities that you originally wanted to do, such as going out, sports, hobbies, etc., because of using cannabis?	Never (0), less than monthly (1), monthly (2), weekly (3), daily or almost daily (4)
8	How often during the past 6 months have you had a problem with your memory or concentration after using cannabis?	Never (0), less than monthly (1), monthly (2), weekly (3), daily or almost daily (4)
9	How often during the past 6 months have you had difficulties at work or school, because of using cannabis?	Never (0), less than monthly (1), monthly (2), weekly (3), daily or almost daily (4)
10	Has a relative, friend or a doctor or other health worker been concerned about your use of cannabis or suggested you cut down over the past 6 months?	No (0), yes (4)

The table displays a revised version of the CUDIT for use in general population surveys (Annaheim et al., 2010), based on the original CUDIT (Adamson & Sellman, 2003).

in a follow-up study, Annaheim et al. (2010) suggested revising CUDIT for the social-epidemiological research context, replacing items of unsatisfactory psychometric properties with new ones. Corresponding results indicated generally improved psychometric performance.

In a general population study, Thake and Davis (2011) found—original—CUDIT of good psychometric performance. It outperformed a simple frequency measure, as well as ASSIST, with regards to sensitivity and specificity, when compared to different external criteria as indicators of "problematic" cannabis use (self-evaluation of harm, driving under the influence, use of other illicit drugs).

Recently, another revised version of the tool—CUDIT-R (Adamson et al., 2010), originally amended for the clinical context—was also tested in a community sample of cannabis users (Bruno et al., 2013). CUDIT-R showed unidimensionality, and strongly correlated with measures of frequency of use, as well as cannabis use disorder according to DSM-5.

To conclude, CUDIT is a cannabis-specific brief screening test that measures a broad spectrum of problems related to cannabis use among adolescents and adults. When validated in different (large) general population samples in Switzerland, Canada, and New Zealand, it has shown good psychometric performance.

CUPIT: The *Cannabis Use Problems Identification Test* is a 16-item screen for the detection of currently and future "risky" or "problematic" use, respectively. CUPIT was constructed for the treatment setting but also for use in the community and as a research tool. It is intended to measure a DSM/ICD tridimensional concept of risky use, dependence and using behavior, as well as health and social problems (Bashford et al., 2010). More concretely, its items cover patterns of use, symptoms of dependence, as well as social aspects.

CUPIT was found to be of excellent test-retest reliability, high internal consistency, a high ability to discriminate between different diagnostic subgroups (nonproblematic, risky, problematic use), and of highly significant

longitudinal predictive validity (Bashford et al., 2010). CUPIT's potential to detect future "risky" or "problematic" use is probably its main advantages over other tests. None of the other tests, to the knowledge of the authors, has been tested for predictive validity in longitudinal studies, yet.

However, the only sample that was used to construct and validate CUPIT was of limited representativeness. It was rather small (n = 212), not random, and only consisted of 36 general population adolescents and adults (Bashford et al., 2010).

To this end, regardless of CUPIT's novel capability to classify both currently diagnosable and also potentially problematic cannabis use and its good psychometric properties when tested in a particular sample, evidence from validation studies is lacking currently. Such validation studies could prove this test to be one of the most adequate screening instruments for use in general population surveys.

DAST-10: The *Drug Abuse Screening Test* was designed after the *Michigan Alcohol Screening Test* to provide a 28-item instrument for clinical and nonclinical screening to detect drug abuse or dependence disorders. Shortened, 20- and 10-item versions of the test are also available (see Skinner, 1982). Because of its brevity, the latest version is most relevant for our purposes of review. DAST-10 covers items on illegal (poly-) drug use in general, symptoms of dependence, in addition to health, social, and legal problems.

Outside the clinical setting, the psychometric performance of DAST-10 was analyzed among a small subgroup of Spanish-speaking individuals in the United States. The test was found to be reliable and unidimensional (Bedregal et al., 2006). At about the same time, its psychometric performance was tested among drug using undergraduate students in the United States, and declared a promising instrument for detecting drug abuse among college students (McCabe et al., 2006).

A few years later, it was validated against drug-biomarkers among 300 low-income women, immediately after having given birth, and found of low sensitivity (Grekin et al., 2010). Further, a Spanish version was validated among drug addicts and healthy controls (Pérez Gálvez et al., 2010).

Recently, Evren et al. (2014) have undertaken a validation of a Turkish version of DAST-10 among prisoners. Finally, a Korean version was validated in a sample of "normal" and "at risk" residents (Yongseok, 2014).

Although, not clinical samples in the narrowest sense, those samples cannot be considered representative for the community, as not probabilistic and mostly composed of "at risk" individuals—that is, alcohol and illegal drug users in general, or otherwise especially vulnerable (eg, low-income, prisoners) individuals. Moreover, DAST-10 has never been tested concerning its performance specifically among cannabis users. Therefore, the available studies hardly allow one to draw conclusions about its possible performance concerning cannabis use among general population adolescents and adults and in the (Western) European context. For those reasons, DAST-10 does not conform to this review's preconditions for counting as one of the most adequate screeners.

PUM (PUC): The *Problematic Use of Marijuana (Cannabis)* screening tool is an eight-item scale that intends to measure ICD-10 harmful use, along with problems in interpersonal relationships and psycho-physical functioning. Its items cover patterns of use, symptoms of dependence, as well as cognitive, social, and financial aspects.

PUM was constructed and validated in Poland among adolescent cannabis users, recruited by snowball-sampling, where it has shown good psychometric performance (Okulicz-Kozaryn & Sieroslawski, 2007; Piontek et al., 2008).

Although a promising cannabis-specific screening instrument, since its construction in 2007 and validation in a nonprobabilistic adolescent sample, it has not been further validated (Okulicz-Kozaryn, K., personal communication, Oct. 14, 2014). For that reason, evidence is lacking that would allow one to select the PUM as one of the most adequate screening instruments for use in general population surveys among adolescents and adults.

SIP-AD: The *Short Inventory of Problems—Alcohol and Drugs* was constructed by Blanchard et al. (2003). Its shortened 15-item version covers health, social and financial aspects, as well as risk taking and negative personality changes. Although recently validated in a multitude of clinical settings (Allensworth-Davies, Cheng, Smith, Samet, & Saitz, 2012), there is scarce information on its performance in the general population.

Gillespie et al. (2007) provide some empirical validity for SIP-AD as a useful measure for assessing negative consequences associated with substance use in general. However, they call the representativeness of their sample into question, and state that inferences can neither be drawn to other student bodies, and to an even lesser degree, the general population. Further, they assume that the extension of SIP-AD to nonclinical samples might be unmerited in the test's present incarnation.

Hagman et al. (2009) refined a shortened, 10-item SIP-AD as a unidimensional construct, and found a high degree of reliability and validity. Yet, the results from their study sample—469 regular club drug-using males, who have sex with men—are hardly transferable to the general population.

Moreover, neither of the studies were cannabis-specific, but included a multitude of other illegal drugs, as well as alcohol. For those reasons, it cannot be decided how SIP-AD would actually perform for cannabis use in

general population samples of adolescents and adults of both sexes.

CONCLUSIONS

At the time of this review, two screening tests were found to be sufficiently studied in general population surveys to be considered reliable or promising for adolescent and (young) adult populations: CAST and CUDIT.

CAST is the shortest and the most studied. It was validated in many different contexts toward different gold-standards derived from DSM-IV, and compared to another screening test. The test has proven good psychometric and screening performances, and the study of its transcultural equivalence has begun. However, it has not been yet validated toward DSM-5, nor studied in longitudinal settings.

CUDIT is somewhat longer, and also shows appealing performances in general population samples of three countries and was validated toward DSM-5, although only in a small sample. It has been compared to another screening test but, to date, has not been studied in longitudinal surveys nor studied in cross-national surveys.

CAST and CUDIT cover different aspects of cannabis use and its consequences. These tests have not been compared with each other. Validation studies of these tests used different survey methodologies (self-administered paper or Internet, telephone, etc.), focusing on different population targets (adolescents, young adults, general population). Additionally, despite the use of DSM-derived gold standard, no study focused on the communalities of these tests and the gold standard: what do these tests measure that is common to DSM and what is not? In parallel, no thorough comparison of the CAST with agreed-upon concepts of problematic cannabis use was done. This is a general problem for all tests concerned by this review.

Further development of research may consider: (1) a study of the common structure of CAST and CUDIT and DSM-5, especially the description of what is common and what is not between them, regarding concepts and factorial structures; (2) a survey comparing the tests together; (3) a study of the added value of these tests, compared to frequency threshold and DSM diagnoses in cross-sectional studies; (4) the added value of these tests in predicting evolutions of cannabis use or psychosocial health damages, compared to frequency thresholds and DSM diagnoses; (5) the cultural invariance of these tests in crossnational surveys, especially the determination of specific thresholds for international comparisons. Of course, other compelling tests could also be studied that way.

MINI-DICTIONARY

General population survey In contrast to clinical investigations, a probability-based survey of people that are selected without any link with the topic of the study.

"Problematic" cannabis use There is no generally agreed-upon definition. Commonly, the expression is used to describe a broader concept of cannabis-use-related issues (including Cannabis use disorders as per the international classifications ICD or DSM) and refers to consumption patterns leading to negative consequences on a social or health level, for the individual cannabis user and the larger community.

Psychometric properties The characteristics of a test that is used for psychological, sociological or public health measurement and of its items (questions). Psychometric properties are examined with the intention to construct, develop and improve such tests.

Screening Research or clinical procedure that refers to a preliminary assessment and aims at identifying individuals in a broader population whose substance use is (potentially) harmful. Thus, screening is a method to estimate the prevalence of "problematic" cannabis use within the general population.

Screening instrument/test Brief questionnaires which enable the classification of respondents to a "positive" (problematic) and a "negative" (nonproblematic) group, depending on the answers they give. Screening tools do not produce formal diagnoses.

Acknowledgments

The work of the first author (Beatrice Annaheim) was financed by a grant from the *Swiss Academy of Medical Sciences* (SAMW) and kindly supported by the *Institute for Biomedical Ethics* (IBMB) at University of Basel, Switzerland, headed by Prof Bernice S. Elger. Stéphane Legleye wants to thank the EMCDDA for funding the first validation of CAST and François Beck for his collaboration in this adventure. Both authors would also like to thank Dorit Barlevy (IBMB) for proofreading of the manuscript.

References

Adamson, S. J., & Sellman, J. D. (2003). A prototype screening instrument for cannabis use disorder: the Cannabis Use Disorders Identification Test (CUDIT) in an alcohol-dependent clinical sample. *Drug and Alcohol Reviews*, *22*(3), 309–315.

Adamson, S. J., Kay-Lambkin, F. J., Baker, A. L., Lewin, T. J., Thornton, L., Kelly, B. J., & Sellman, J. D. (2010). An improved brief measure of cannabis misuse: the Cannabis Use Disorders Identification Test-Revised (CUDIT-R). *Drug and Alcohol Dependence*, *110*(1–2), 137–143.

Allensworth-Davies, D., Cheng, D. M., Smith, P. C., Samet, J. H., & Saitz, R. (2012). The Short Inventory of Problems-Modified for Drug Use (SIP-DU): validity in a primary care sample. *American Journal of Addiction*, *21*(3), 257–262.

Annaheim, B. (2013). Who is smoking pot for fun and who is not? An overview of instruments to screen for cannabis-related problems in general population surveys. *Addiction Research and Theory*, *21*(5), 410–428.

Annaheim, B., Rehm, J., & Gmel, G. (2008). How to screen for problematic cannabis use in population surveys: an evaluation of the Cannabis Use Disorders Identification Test (CUDIT) in a Swiss sample of adolescents and young adults. *European Addiction Research*, *14*(4), 190–197.

Annaheim, B., Scotto, T. J., & Gmel, G. (2010). Revising the Cannabis Use Disorders Identification Test (CUDIT) by means of Item Response Theory. *International Journal of Methods in Psychiatric Research*, *19*(3), 142–155.

Asbridge, M., Duff, C., Marsh, D. C., & Erickson, P. G. (2014). Problems with the identification of 'problematic' cannabis use: examining the issues of frequency, quantity, and drug use environment. *European Addiction Research*, *20*(5), 254–267.

Babor, T. F., McRee, B. G., Kassebaum, P. A., Grimaldi, P. L., Ahmed, K., & Bray, J. (2007). Screening, Brief Intervention, and Referral to Treatment (SBIRT): toward a public health approach to the management of substance abuse. *Substance Abuse*, *28*(3), 7–30.

Barreto, H. A., de Oliveira Christoff, A., & Boerngen-Lacerda, R. (2014). Development of a self-report format of ASSIST with university students. *Addictive Behaviors*, *39*(7), 1152–1158.

Bashford, J. (2010). *Screening and assessment for cannabis use disorders*. Randwick, NSW: National Cannabis and Information Centre, Guidelines background papers: http://ncpic.org.au/static/pdfs/background-papers/screening-and-assessment-for-cannabis-use-disorders.pdf

Bashford, J., Flett, R., & Copeland, J. (2010). The Cannabis Use Problems Identification Test (CUPIT): development, reliability, concurrent and predictive validity among adolescents and adults. *Addiction*, *105*(4), 615–625.

Bastiani, L., Siciliano, V., Curzio, O., Luppi, C., Gori, M., Grassi, M., & Molinaro, S. (2013). Optimal scaling of the CAST and of SDS Scale in a national sample of adolescents. *Addictive Behaviors*, *38*(4), 2060–2067.

Beck, F., & Legleye, S. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). (2008). Measuring cannabis related problems and dependence at the population level. In *A cannabis reader: Global issues and local experiences* (pp. 29–57). (Vol. 2). Lisbon: EMCDDA.

Bedregal, L. E., Sobell, L. C., Sobell, M. B., & Simco, E. (2006). Psychometric characteristics of a Spanish version of the DAST-10 and the RAGS. *Addictive Behaviors*, *31*(2), 309–319.

Bernard, M., Bolognini, M., Plancherel, B., Chinet, L., Laget, J., Stephan, P., & Halfon, O. (2005). French validity of two substance-se screening tests among adolescents: a comparison of the CRAFFT and DEP-ADO. *Journal of Substance Use*, *10*(6), 385–395.

Blanchard, K. A., Morgenstern, J., Morgan, T. J., Lobouvie, E. W., & Bux, D. A. (2003). Assessing consequences of substance use: psychometric properties of the inventory of drug use consequences. *Psychology of Addictive Behaviors*, *17*(4), 328–331.

Bougeard, S., Eslami, A., & Qannari, E. M. (2014). Multigroup analyses to study Cannabis Abuse Screening Test in thirteen European countries. *Oral presentation at the eighth international conference on partial least squares and related methods*. Paris: HEC, May 2014.

Bruno, R., Marshall, S., & Adamson, S. (2013). Screening for DSM-5 cannabis dependence using the Cannabis Use Identification Test-Revised (CUDIT-R). *Poster presented at the College on Problems of Drug Dependence (CPDD) 75th Annual Meeting*. San Diego, June 2013.

Cuenca-Royo, A. M., Sanchez-Niubo, A., Forero, C. G., Torrens, M., Suelves, J. M., & Domingo-Salvany, A. (2012). Psychometric properties of the CAST and SDS scales in young adult cannabis users. *Addictive Behaviors*, *37*(6), 709–715.

Cummins, L. H., Chan, K. K., Burns, K. M., Blume, A. W., Larimer, M., & Marlatt, G. A. (2003). Validity of the CRAFFT in American-Indian and Alaska-Native adolescents: screening for drug and alcohol risk. *Journal of Studies on Alcohol*, *64*(5), 727–732.

Davis, C. G., Thomas, G., Jesseman, R., & Mazan, R. (2009). Drawing the line on risky use of cannabis: assessing problematic use with the ASSIST. *Addiction Research and Theory*, *17*(3), 322–332.

Degenhardt, L., Coffey, C., Carlin, J. B., Swift, W., & Patton, G. C. (2008). Are diagnostic orphans at risk of developing cannabis abuse or dependence? Four-year follow-up of young adult cannabis users not meeting diagnostic criteria. *Drug and Alcohol Dependence*, *92*(1–3), 86–90.

Dhalla, S., Zumbo, B. D., & Poole, G. A. (2011). Review of the psychometric properties of the CRAFFT instrument: 1999–2010. *Current Drug Abuse Reviews*, *4*(1), 57–64.

EMCDDA (2007). *Drug Situation. Screening for problem or dependent cannabis use*. Drugnet Europe, April–June 2007, *Vol. 58*. http://www.emcdda.europa.eu/html.cfm/index31435EN.html

Evren, C., Ogel, K., Evren, B., & Bozkurt, M. (2014). Psychometric properties of the Turkish versions of the Drug Use Disorders Identification Test (DUDIT) and the Drug Abuse Screening Test (DAST-10) in the prison setting. *Journal of Psychoactive Drugs*, *46*(2), 140–146.

Ewing, J. A. (1984). Detecting alcoholism. The CAGE questionnaire. *Journal of the American Medical Association*, *252*(14), 1905–1907.

Fagerström, K. O. (1978). Measuring degree of physical dependence to tobacco smoking with reference to individualization of treatment. *Addictive Behaviors*, *3*(3–4), 235–341.

Fernandez-Artamendi, S., Fernandez-Hermida, J. R., Muniz-Fernandez, J., Secades-Villa, R., & Garcia-Fernandez, G. (2012). Screening of cannabis-related problems among youth: the CPQ-A-S and CAST questionnaires. *Substance Abuse Treatment Prevention and Policy*, *7*, 13.

Gillespie, W., Holt, J. L., & Blackwell, R. L. (2007). Measuring outcomes of alcohol, marijuana, and cocaine use among college students: a preliminary test of the Shortened Inventory of Problems-Alcohol and Drugs (SIP-AD). *Journal of Drug Issues*, *37*(3), 549–567.

Grekin, E. R., Svikis, D. S., Lam, P., Connors, V., Lebreton, J. M., Streiner, D. L., Smith, C., & Ondersma, S. J. (2010). Drug use during pregnancy: validating the Drug Abuse Screening Test against physiological measures. *Psychology of Addictive Behaviors*, *24*(4), 719–723.

Gyepesi, A., Urbán, R., Farkas, J., Kraus, L., Piontek, D., Paksi, B., Horváth, G., Magi, A., Eisinger, A., Pilling, J., Kökönyei, G., Kun, B., & Demetrovics, Z. (2014). Psychometric properties of the cannabis abuse screening test in Hungarian samples of adolescents and young adults. *European Addiction Research*, *20*(3), 119–128.

Hagman, B. T., Kuerbis, A. N., Morgenstern, J., Bux, D. A., Parsons, J. T., & Heidinger, B. E. (2009). An Item Response Theory (IRT) analysis of the Short Inventory of Problems-Alcohol and Drugs (SIP-AD) among non-treatment seeking men-who-have-sex-with-men: evidence for a shortened 10-item SIP-AD. *Addictive Behaviors*, *34*(11), 948–954.

Hall, W. (2014). What has research over the past two decades revealed about the adverse health effects of recreational cannabis use? *Addiction*, *110*(1), 19–35.

Karila, L., Legleye, S., Beck, F., Corruble, E., Falissard, B., & Reynaud, M. (2007). Validation of a questionnaire to screen for harmful use of alcohol and cannabis in the general population: CRAFFT-ADOSPA. *Presse Medicale*, *36*(4 Pt 1), 582–590.

Knight, J. R., Shrier, L. A., Bravender, T. D., Farrell, M., Vander Bilt, J., & Shaffer, H. J. (1999). A new brief screen for adolescent substance abuse. *Archives of Pediatrics and and Adolescent Medicine*, *153*, 591–596.

Legleye, S., Karila, L., Beck, F., & Reynaud, M. (2007). Validation of the CAST, a general population Cannabis Abuse Screening Test. *Journal of Substance Use*, *12*(4), 233–242.

Legleye, S., Piontek, D., & Kraus, L. (2011). Psychometric properties of the Cannabis Abuse Screening Test (CAST) in a French sample of adolescents. *Drug and Alcohol Dependence*, *113*(2–3), 229–235.

Legleye, S., Piontek, D., Kraus, L., Morand, E., & Falissard, B. (2013). A validation of the Cannabis Abuse Screening Test (CAST) using a latent class analysis of the DSM-IV among adolescents. *International Journal of Methods in Psychiatric Research*, *22*(1), 16–26.

Legleye, S., Guignard, R., Richard, J. -B., Kraus, L., Pabst, A., & Beck, F. (2015). Properties of the Cannabis Abuse Screening Test (CAST) in the general population. *International Journal of Methods in Psychiatric Research*, *24*(2), 170–183.

Martin, G., Copeland, J., Gilmour, S., Gates, P., & Swift, W. (2006). The Adolescent Cannabis Problems Questionnaire (CPQ-A): psychometric properties. *Addictive Behavior*, *31*(12), 2238–2248.

McCabe, S. E., Boyd, C. J., Cranford, J. A., Morales, M., & Slayden, J. (2006). A modified version of the Drug Abuse Screening Test among

undergraduate students. *Journal of Substance Abuse Treatment*, *31*(3), 297–303.

McLaren, J., Swift, W., Dillon, P., & Allsop, S. (2008). Cannabis potency and contamination: a review of the literature. *Addiction*, *103*(7), 1100–1109.

Mdege, N. D., & Lang, J. (2011). Screening instruments for detecting illicit drug use/abuse that could be useful in general hospital wards: a systematic review. *Addictive Behaviors*, *36*(12), 1111–1119.

Molinaro, S., Siciliano, V., Curzio, O., Denoth, F., & Mariani, F. (2012). Concordance and consistency of answers to the self-delivered ESPAD questionnaire on use of psychoactive substances. *International Journal of Methods in Psychiatric Research*, *21*(2), 158–168.

Newton, A. S., Gokiert, R., Mabood, N., Ata, N., Dong, K., Ali, S., Vandermeer, B., Tjosvold, L., Hartling, L., & Wild, T. C. (2011). Instruments to detect alcohol and other drug misuse in the emergency department: a systematic review. *Pediatrics*, *128*(1), e180–e192.

Obradovic, J. (2013). *Guide pratique des principaux outils de repérage de l'usage problématique de cannabis chez les adolescents*. Paris, France: OFDT, http://www.ofdt.fr/BDD/publications/docs/epfxiot9.pdf

Okulicz-Kozaryn, K., & Sieroslawski, J. (2007). Psychometric properties of the Problematic Marijuana Use (PUM) Test for adolescents. *Postepy Neurologii, Neurochirurgii, i Psychiatrii*, *16*, 105–111.

Pabst, A., Kraus, L., & Piontek, D. (2012). The Cannabis Abuse Screening Test (CAST): examining the prevalence of cannabis-related problems among adolescents in 13 European countries. In B. Hibell, U. Guttormsson, S. Ahlström, O. Balakireva, T. Bjarnason, A. Kokkevi, & L. Kraus (Eds.), *The 2011 ESPAD Report. Substance use among students in 36 European countries* (pp. 158–163). Stockholm, Sweden: The Swedish Council for Information on Alcohol and Other Drugs (CAN).

Pérez Gálvez, B., García Fernández, L., de Vicente Manzanaro, M. P., Angustias Oliveras Valenzuela, M., & Lahoz Lafuente, M. (2010). Validación española del Drug Abuse Screening Test (DAST-20 y DAST-10). *Revista Salud y Drogas*, *10*(1), 35–50.

Pérez Gomez, A., & Scoppeta Diaz-Grandos, O. (2011). El CRAFFT/CARLOS como Instrumento para la Identificación Temprana de Consumo de Alcohol y Otras SPA: una adaptación al español. *Revista Colombiana de Psicología*, *20*(2), 265–274.

Pilowsky, D. J., & Wu, L. T. (2013). Screening instruments for substance use and brief interventions targeting adolescents in primary care: a literature review. *Addictive Behaviors*, *38*(5), 2146–2153.

Piontek, D., Kraus, L., & Klempova, D. (2008). Short scales to assess cannabis-related problems: a review of psychometric properties. *Substance Abuse Treatment, Prevention and Policy*, *3*, 25.

Proudfoot, H., Vogl, L., Swift, W., Martin, G., & Copeland, J. (2010). Development of a short cannabis problems questionnaire for adolescents in the community. *Addictive Behaviors*, *35*(7), 734–737.

Rehm, J., Marmet, S., Anderson, P., Gual, A., Kraus, L., Nutt, D. J., Room, R., Samokhvalov, A. V., Scafato, E., Trapencieris, M., Wiers, R. W., & Gmel, G. (2013). Defining substance use disorders: do we really need more than heavy use? *Alcohol and Alcoholism*, *48*(6), 633–640.

Saunders, J. B., Aasland, O. G., Babor, T. F., de la Fuente, J. R., & Grant, M. (1993). *Addiction*, *88*(6), 791–804.

Skinner, H. A. (1982). The drug abuse screening test. *Addictive Behaviors*, *7*(4), 363–371.

Skogen, J. C., Boe, T., Knudsen, A. K., & Hysing, M. (2013). Psychometric properties and concurrent validity of the CRAFFT among Norwegian adolescents. Ung@hordaland, a population-based study. *Addictive Behavior*, *38*(10), 2500–2505.

Stephens, R. S., & Roffman, R. A. (2005). Assessment of cannabis use disorders. In D. M. Donovan, & G. A. Marlatt (Eds.), *Assessment of addictive behaviors* (pp. 248–273). New York, NY: The Guilford Press.

Subramaniam, M., Cheok, C., Verma, S., Wong, J., & Chong, S. A. (2010). Validity of a brief screening instrument-CRAFFT in a multiethnic Asian population. *Addictive Behaviors*, *35*(12), 1102–1104.

Temple, E. C., Brown, R. F., & Hine, D. W. (2011). The 'grass ceiling': limitations in the literature hinder our understanding of cannabis use and its consequences. *Addiction*, *106*(2), 238–244.

Thake, J., & Davis, C. G. (2011). Assessing problematic cannabis use. *Addiction Research and Theory*, *19*(5), 448–458.

WHO ASSIST Working Group. (2002). The Alcohol, Smoking and Substance Involvement Screening Test (ASSIST): development, reliability and feasibility. *Addiction*, *97*(9), 1183–1194.

Yongseok, K. (2014). Validating a Korean version of the Drug Abuse Screening Test-10 (DAST-10). *Journal of Social Service Research*, *40*(2), 232–241.

Zeisser, C., Thompson, K., Stockwell, T., Duff, C., Chow, C., Vallance, K., Ivsins, A., Michelow, W., Marsh, D., & Lucas, P. (2012). A 'standard joint'? The role of quantity in predicting cannabis-related problems. *Addiction Research and Theory*, *20*(1), 82–92.

CHAPTER

e18

Self-report of Cannabis Use

T. Van der Linden

Department drugs and toxicology, National Institute of Criminalistics, Brussels, Belgium

SUMMARY POINTS

- This chapter focuses on self-reporting of cannabis use.
- Self-reports are noninvasive, but can be biased.
- Several guidelines are available for conducting surveys on cannabis use through self-report.
- Cannabis is the most widely used illicit drug according to several self-reporting studies.
- Several factors influence the validity of self-report, such as social stigma, acceptance of the drug user identity, problems with cognitive function, the study setting or the fear for (legal) consequences.
- Concordance between self-report and biological measurements varies for different studies, partly depending on the characteristics of the respondents, and the study settings.

KEY FACTS AND TYPES OF SURVEYS

- A survey is a method of using questionnaires or interviews to gather information that can be generalized to a population.
- Collection can be done by: self-administered questionnaires (given in group settings, individual or by mail), personal interviews (conducted in home or other settings), telephone interviews, computer-assisted interviews, or combinations of the aforementioned.
- Census: every individual in the population is studied.
- Sample survey: a subgroup representative of the population is studied.
- Cross-sectional surveys: studying a group of people only at one point in time.
- Panel surveys: collecting data from the same individuals at two or more points in time.
- Cohort studies: combination of cross-sectional and panel design: studying different representative samples from a population at multiple points in time.

KEY FACTS OF EMCDDA

- European Monitoring Centre for Drugs and Drug Addiction.
- Established in 1993, and inaugurated in Lisbon in 1995.
- Exists to provide the European Community and EU Member States with a factual overview of European drug problems, and a solid evidence base to support the drug debate.
- Prevalent numbers on drug use in annual European Drug Reports.

KEY FACTS OF UNODC

- United Nations Office on Drugs and Crime.
- Global leader in fight against illicit drugs and international crime.
- Established in 1997, UNODC operates in all regions of the world.
- Provides field-based technical cooperation projects to enhance the capacity of member states to counteract illicit drugs, crime, and terrorism.
- Provides research and analytical work to increase knowledge and understanding of drugs and crime issues.
- Estimates based on the UNODC annual report questionnaire show that globally 3.8% of 15–64 year-olds have used cannabis in 2012.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00122-8
Copyright © 2017 Elsevier Inc. All rights reserved.

LIST OF ABBREVIATIONS

EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
EMQ	European model questionnaire
ESPAD	European School Survey Project on Alcohol and other Drugs
GDS	Global Drug Survey
HBSC	Health behavior in school-aged children
NDSHS	National Drug Strategy Household Survey
NSDUH	National Survey on Drug Use and Health
SAMHSA	Substance Abuse and Mental Health Services Administration
THC	Tetrahydrocannabinol, Δ^9-Tetrahydrocannabinol
THCCOOH	11-nor-9-carboxy-delta-9-tetrahydrocannabinol
UNODC	United Nations Office on Drugs and Crime
WHO	World Health Organization

INTRODUCTION

Gathering information on the prevalence of illicit drug use, such as cannabis use, can be done in several ways. Biological samples are taken and analyzed to get scientifically proven results. Self-reports are less invasive, but these results could be more biased. In this (part of the) chapter, methods of self-reporting questionnaires are briefly mentioned; prevalence for different populations based on self-report are given; points on the validity of self-report are discussed, and concordance between self-report and biological analyses is given (Table e18.1).

METHODS FOR SELF-REPORT

International guidelines are drawn to decide which questions, concepts, and epidemiological indicators (eg, prevalence, frequency, incidence) have to be included in a population survey on illicit drug use and which methodological aspects (eg, data collection method, sample size) are indispensable to use in periodical studies. (Tieberghien, De Moor, De Corte, & Mortelmans, 2009)

In Europe, most countries (eg, Austria, Cyprus, Finland, Hungary, Italy, Lithuania, and Spain) have established national surveys based solely on illicit drug use, mostly in combination with the use of other psychoactive substances, such as alcohol and tobacco. Some countries piggyback drug prevalence measurements to another survey (eg, health survey, crime survey). Both approaches have their (dis)advantages. For example, when conducting a single survey, researchers are free to create the most appropriate questionnaire, research design, and sampling. Piggybacking on the other hand requires a lower budget, but it also implies that the questionnaire and the research design have to be subordinated to the main questionnaire (Tieberghien et al., 2009).

Guidelines in Europe

Prevalence and patterns of drug use among different groups of population (eg, adults, young people) is one of five EMCDDA key indicators that assess and monitor the drugs situation among EU Member States. Data from general and school population drug surveys provide information on patterns of use, risk perceptions, social and health correlates, and consequences of use of psychoactive substances.

For surveys conducted among the adult population (Fig. e18.1) in households, the EMCDDA provides a list

TABLE e18.1 Summary of Concordance Between Self-Report and Biological Measurements in Several Studies

Study	Concordance between self-report and biological measurements
University students Zaldívar Basurto et al. (2009)	High sensitivity and specificity
Driving population (Van der Linden et al., 2014)	Low sensitivity, high specificity and accuracy
Treatment groups	1. High number of false negative self-report (Buchan et al., 2002) 2. High consistency between both methods (Neale & Robertson, 2003) 3. High correlation (Hjorthoj et al., 2012) 4. Low number of false negatives (Akinci et al., 2001)
Armed forces (Mayet et al., 2013)	Good sensitivity, high specificity
HIV-infected youth (Nichols et al., 2014)	Rather good concordance
Emergency department (Macdonald et al., 2014)	Very low number of false negatives
Pregnant women (El Marroun et al., 2011)	Low sensitivity, very high specificity

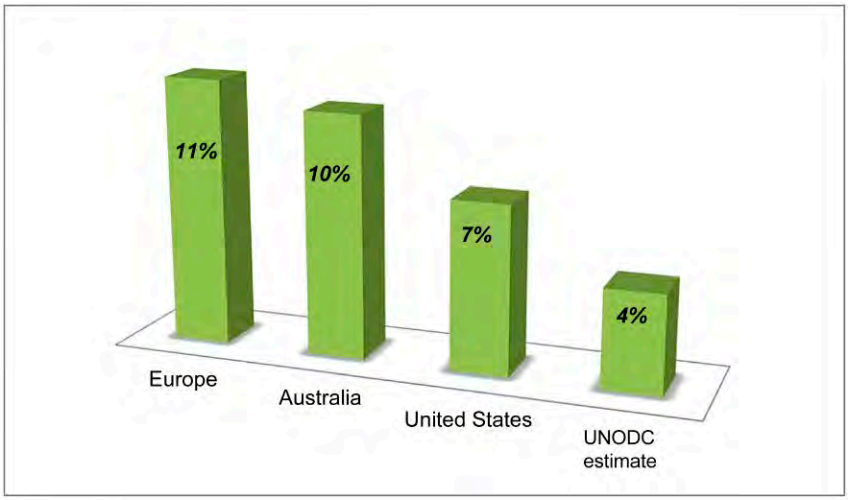


FIGURE e18.1 Prevalence of cannabis use through self-report of adult population. Europe, European Drug Report 2014 (EMCDDA, 2014); Australia, National Drug Strategy Household Survey 2013 (NDSHS, 2014); United States, National Survey on Drug Use and Health (SAMHSA, 2014); UNODC estimate, World Drug Report 2014 (UNODC, 2014).

of core items, called the European model questionnaire (EMQ) that can, for example, be included in questionnaires of national surveys. With the EMQ data on the period, prevalence of drugs can be collected, as well as information on age of first use, and frequency of use (EMCDDA, 2009).

Population surveys on drug use in Europe are less compatible with the recommendations of the WHO or UNODC (Tieberghien et al., 2009).

Methods for representative school surveys may be found in internationally coordinated school survey projects, and several national projects in Europe and the United States. School surveys collect mostly data in classrooms through anonymous questionnaires on the use of alcohol and a range of drugs (EMCDDA, 2009).

Every year, the EMCDDA reports prevalence numbers on drug use in Europe in their *European Drug Report: trends and developments*.

Surveys in the United States and Australia

In Australia, the National Drug Strategy Household Survey (NDSHS) is conducted every 3 years, and the Australian institute of Health and Welfare has been reporting on these surveys since 1998. The survey was commissioned and funded by the Australian Government Department of Health. The scope of the 2013 NDSHS (the 11th survey in the series) was the Australian residential population aged 12 years or older. The 2013 survey collected data from 23,855 people across Australia. The 2013 survey was conducted using a self-completion drop-and-collect questionnaire method (NDSHS, 2014).

The National Survey on Drug Use and Health (NSDUH) is an annual survey (since 1971) on the use of illicit drugs, alcohol and tobacco in the civilian, noninstitutionalized population of the United States aged 12 years and older, involving interviews with approximately 70,000 randomly selected individuals. The Substance Abuse and Mental Health Services Administration (SAMHSA) funds NSDUH, and is an agency of the US Public Health Service in the US Department of Health and Human Services (DHHS) (SAMHSA, 2014).

Global Drug Survey

Every year, the Global Drug Survey (GDS) is launched, an online questionnaire that approaches drug-use. This implicates that automatically drug users are appealed to respond to the questionnaire. The surveyed group is, therefore not representative for the whole population, but gives a unique insight in (the patterns of) drug-use (GDS, 2014).

Youth Surveys

ESPAD (European School Survey Project on Alcohol and other Drugs) is a collaborative effort of independent research teams in more than 40 European countries, and the largest cross-national research project on adolescent substance use in the world. The overall aim with the project is to collect repeatedly comparable data on substance use among 15–16-year-old students in as many European countries as possible (ESPAD, 2012).

HBSC (Health Behavior in School-aged Children) is a pioneer cross-national study gaining insight into young people's well-being (Fig. e18.2), health behaviors, and their social context. It collects data every 4 years on

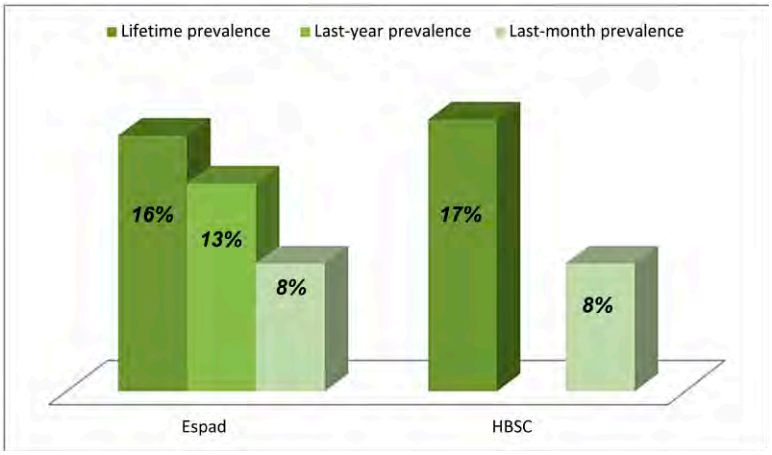


FIGURE e18.2 Prevalence of cannabis use through self-report of youth population. ESPAD: European School Survey Project on Alcohol and other Drugs 2011 (ESPAD, 2012); HBSC: Health Behavior in School-aged Children, 2009–2010 (HBSC, 2012).

11-, 13-, and 15-year-old boys and girls, including information on cannabis use. This research collaboration with the WHO Regional Office for Europe is conducted in 43 countries and regions across Europe and North America (HBSC, 2012).

PREVALENCE DATA BASED ON SELF-REPORT

European General Population

The 2014 European Drug Report published by the EMCDDA revealed that almost one-quarter of the adult population in the European Union, or over 80 million adults, are estimated to have used illicit drugs at some point in their lives. In most cases, they have used cannabis (73.6 million).

An estimated 11% of young Europeans (15–34) used cannabis in the last year, with 14% of these aged 15–24. Cannabis use is higher generally among males, and this difference is usually accentuated for more intensive or regular patterns of use. Just less than 1% of European adults are estimated to be intensive (daily or almost daily) users; over two-thirds of this group are aged between 15 and 34 years, and in this age group over three-quarters are male (EMCDDA, 2014).

General Population in Australia and the United States

Information provided through the 2013 National Drug Strategy household Survey showed that 10% of the Australian population aged 14 years and older recently used cannabis, while lifetime use was 35% (NDSHS, 2014).

Information provided by the 2012 National Survey on Drug Use and Health showed that almost 19 million inhabitants of the United States aged 12 or older (7%) used cannabis in the past month. Daily or almost daily use (used on 20 or more days in the past month) increased from 5.1 million persons in 2007 to 7.6 million persons in 2012. (SAMHSA, 2014)

Global Drug Survey

The GDS2014 conducted during November/December 2013 comprised almost 80,000 responses. 48% of the respondents declared to have smoked cannabis in the last 12 months, with Australia having the lowest percentage (35%), and Mexico the highest (78%) (GDS, 2014).

Youth

Information provided by the 2011 ESPAD revealed that lifetime cannabis use was reported by 16% of the students, with more reporting by boys (19 vs 14% for girls). Cannabis use in the past 12 months was reported by 13% of all students, with 15% among boys and 11% among girls. Use in the past 30 days was claimed by 8% of the boys and 5% of the girls (7% average) (ESPAD, 2012).

Information provided by the 2009/2010 HBSC showed that 17% of the interviewed 15 year olds have ever used cannabis (15% for girls, 20% for boys; highest in Canada, lowest in Macedonia). An average of 8% (6% for girls, 9% for boys) have used cannabis in the last 30 days (HBSC, 2012).

VALIDITY OF SELF-REPORT

Evaluation of drug use based on the subject's self-report is the most widely used practice for epidemiological research in addiction, as it has two very clear advantages, low cost and the possibility of collecting an

abundance of information from many people. However, the validity of estimations based on their use has been questioned frequently.

Points Affecting Validity

The variables that influence validity can be classified generally as (1) social context factors, (2) personal characteristics of the informer, and (3) demands of the task. Also, the design of the self-report on substance use and especially the conditions under which it is used are important factors (Zaldívar Basurto et al., 2009).

The illegal nature of drug abuse, privacy, face-saving, and possible criminal sanctions is all factors associated with social pressure, largely affecting the reliability of self-report outcomes.

Reasons for under- or overreporting of drug use are based mainly on social stigma. As social stigma of the use of a certain drug increases, underreporting of the drug use increases. It has been shown that underreporting is most frequent for heroin, which is the least socially accepted drug. When a person is addicted to a certain drug already for a longer period of time, reporting of drug use will be more truthful, due to the fact that higher acceptance of the drug user identity is expected. Response distortion to avoid social stigma is a serious risk in surveys of value-laden issues, such as illicit drug use. Survey respondents may be unwilling to report drug use to avoid adverse reactions from others, or to present themselves to the interviewer in a favorable way. Conversely, respondents with positive views of drug use may exaggerate their drug use to impress the interviewer or others, or to live up to a self-image that perceives drug us as positive. Many types of behavior viewed as socially desirable are overreported, while those viewed as less desirable are underreported. Several studies also indicate that the tendency to underreport varies across social groups that hold differing norms and values regarding the desirability of the behaviors or traits under investigations. Thus, even when validity studies indicate a bias toward underreporting a stigmatized behavior, the bias cannot be assumed to be constant across all respondents (Harrel, 1997).

Wrongness perceived in drug use and, therefore, the tendency to underreporting cannot be generalized to all populations (eg, a study showed how some teenagers who perceived use of alcohol to be socially desirable tended to increase their use report), so favorable/unfavorable attitudes about use in each specific sample must be known beforehand.

Not all under- or overreporting is deliberate, for example, it is an accepted phenomenon that, especially in the sometimes chaotic life of a drug user, cognitive function may limit accurate reporting. The cognitive complexity of accurately recalling drug use in the past may sometimes also result in errors (Harrel, 1997) They may be unable to report drug use accurately, particularly when questions involve detailed accounts of drug consumption at times in the past. They may not be able to remember the circumstances of use, when they used a drug, or even whether they ever used a particular drug. Heavier drug users are likely to find particular facts more difficult to recall and may experience memory impairment.

Factors other than social desirability also threaten the accuracy of self-reported drug use data. Respondents may fear legal consequences to reporting drug use, if they distrust survey assurances of confidentiality. The assumption is that the assurance of confidentiality and anonymity will offset any potential tendency of the respondent to distort the accuracy of responses, and a greater similarity between self-report and test results will be achieved (Harrel, 1997).

Also, the setting in which self-reports are provided can affect the willingness to report an illegal behavior. For example, arrestees may provide less accurate information on their drug use to intake personnel at a jail, than to a researcher on a self-administered questionnaire given with a guarantee of confidentiality. Reluctance to disclose drug use can occur even in an alternative setting, such as a clinic (which is expected to be less conducive to underreporting), if the respondent is interested in conveying a positive image to the interviewer (Harrel, 1997).

Concordance with Biological Measurements

University Students

Zaldívar Basurto et al. (2009) determined the validity of a self-report on recent drug use (cocaine and cannabis) in a sample of university students. The self-report sensitivity to recent use of cannabis was 91.8%, and specificity 89.6%. Agreement between the self-report on use of cannabis and the urine test is rather high, with a statistically significant kappa of 0.666, and a rather high percentage of agreement (0.89) (Zaldívar Basurto et al., 2009).

Driving Population

Van der Linden, Silverans, and Verstraete (2014) compared the number of Belgian drivers who self-reported cannabis use by questionnaires during a roadside survey to the result of toxicological analysis. The sensitivity of the questionnaire was low, while specificity and accuracy were high. Kappa statistics revealed a fair agreement between self-report and positive findings for tetrahydrocannabinol (THC) in oral fluid and blood, and moderate agreement with 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THCCOOH) in blood. Self-report largely underestimates driving under the influence of cannabis, particularly recent use. Data also indicated

that maybe some respondents gave a "socially desirable" answer, stating "I smoked cannabis >24 h ago," while in fact it was more recently (Van der Linden et al., 2014).

Drug Users in Treatment

Buchan, L Dennis, Tims, and Diamond (2002) evaluated the agreement between adolescent self-reported cannabis use in outpatient treatment and toxicological analysis. Over two thirds tested positive after stating not to have used in the week prior to examination and one-third even tested positive after stating soberness for 4 weeks. The findings suggest both the advantages of multiple sources of information (Buchan et al., 2002).

Neale and Robertson (2003) compared self-report and oral fluid testing for detecting drug use among individuals starting drug treatment. In 80% of the cases, self-report on cannabis use and oral fluid testing were consistent (Neale & Robertson, 2003).

Hjorthoj, Fohlmann, Larsen, Arendt, and Nordentoft (2012) found that timeline follow-back-assisted self-report correlates highly with plasma THC in patients with comorbid cannabis use disorder and psychosis (Hjorthoj et al., 2012).

Akinci, Tarter, and Kirisci (2001) evaluated the concordance between self-report and urine screen in a study with adolescent sons of fathers with and without a substance use disorder. The researchers observed a discordance rate of 13%, most of it was related to underreporting; however, a small proportion of subjects overreported their marijuana use. Almost one-third of the total sample denied use but screened positive in urine, and more than 10% stated to have used cannabis but screened negative in urine (Akinci et al., 2001).

Armed Forces

Mayet et al. (2013) evaluated the accuracy of self-report of past-month cannabis use in a representative sample of French military staff members, and evaluated the scale of the prevarication bias. The prevalence for self-reported use was 16.1%, and for positive testing 13.4%. The discriminant power of self-report was good, with an area under the receiver operating characteristics curve of 0.90. Specificity was 94.5%, but sensitivity was lower (85.7%). The lowest sensitivity values were observed in the higher categories of personnel and in the Navy, which could suggest some prevarication in these subpopulations who might believe they were more exposed to sanctions if detected (Mayet et al., 2013).

HIV-Infected Youth

Nichols et al. (2014) examined the relationship of self-report measures of substance use with plasma testing of a range of substances of abuse in two groups of youth (youth living with HIV (= YLWH) and uninfected youth). The first showed a percentage of positive self-report over the past 3 months for cannabis of 49% (20% daily use). Positive self-reported use for uninfected youth was 53% (11% daily use). Positive plasma toxicology assays for YLWH were 36%, while for the uninfected group this was 27%. Overall concordance between any self-reported use of cannabis and toxicology assay was 79% for YLWH and 87 for uninfected youth (kappa = 0.587, p = 0.432). The data support, particularly for daily users, self-report as a valid indicator of substance use in research studies of youth with or without HIV-infection (Nichols et al., 2014).

Emergency Department Patients

Macdonald et al. (2014) assessed the concordance of self-reports of cannabis, cocaine, and amphetamines with oral fluid on-site testing in an emergency department setting. Among those who admitted use of cannabis, 21% tested positive. Of those who reported no use 2% had a positive oral fluid test (Macdonald et al., 2014)

Pregnant Women

In the Netherlands, El Marroun et al. (2011) studied the agreement between self-report and urine analysis concerning maternal cannabis use. Sensitivity and specificity of self-report compared to urine analysis were 0.36 and 0.99. The researchers suggest a two-step approach involving self-report information followed by urine analysis. (El Marroun et al., 2011)

DISCUSSION AND CONCLUSION

Some remarks should be taken into account when investigating the concordance between biological analyses and self-reports of cannabis use. Depending on the biological matrix used, the measured analytes and the detection times are different. For instance, THCCOOH is measured in urine, and can sometimes be detected several weeks after consumption, while in oral fluid THC is the analyzed substance, with a much shorter detection window. This could result in false positive self-reports while, in fact, the respondents gave correct information that is not detectable anymore in the biological matrix.

Self-reporting stays the most widely used procedure for gathering information on prevalence numbers on drug use. Since cannabis is the most socially accepted drug, the validity of self-report tends to be the highest for this drug class, but is highly depended on several parameters, such as environment,

anonymity, etc. This review, for instance, has shown that when sanctions (eg, driving population, high ranks in the army) or social stigma are feared (pregnant women), validity tends to be lower. When reporting drug use is part of a treatment (eg, outpatient treatment, people with substance use disorder), validity tends to be higher. It offers a reliable approximation for measuring drug use, if conducted under optimum conditions.

Although the self-reported use of drugs has certain limitations or biases that cannot be obviated, it can offer a reliable approximation for measuring drug use, if conducted under optimum conditions. One of the biggest advantages it has is that it can provide certain information (history of use, determinants, and consequences of use, etc.) that cannot be found in any other type of measure.

MINI-DICTIONARY

Accuracy The proportion of correctly identified positive and negative results from all the test results.

$$Accuracy = \frac{TP+TN}{TP+TN+FP+FN}$$

FN False negative: number of cases with a negative self-report and a positive confirmation analysis.
FP False positive: number of cases with a positive self-report and a negative confirmation analysis.
Kappa Cohen's kappa measures the agreement between two raters who each classify N items into C mutually exclusive categories. The equation for κ is:

$$K = \frac{\Pr(a)-\Pr(e)}{1-\Pr(e)}$$

Where $\Pr(a)$ is the relative observed agreement among raters, and $\Pr(e)$ is the hypothetical probability of chance agreement, using the observed data to calculate the probabilities of each observer randomly saying each category. If the raters are in complete agreement then $\kappa = 1$. If there is no agreement among the raters other than what would be expected by chance (as defined by $\Pr(e)$), $\kappa = 0$.
Sensitivity The proportion of positive cases that are correctly identified by the test.

$$Sensitivity = \frac{TP}{TP+FN}$$

Specificity The proportion of negative cases that are correctly identified by the test.

$$Specificity = \frac{TN}{TN+FP}$$

TN True negative: number of cases with a negative self-report and a negative confirmation analysis.
TP True positive: number of cases with a positive self-report and a positive confirmation analysis.

References

Akinci, I. H., Tarter, R. E., & Kirisci, L. (2001). Concordance between verbal report and urine screen of recent marijuana use in adolescents. *Addictive Behaviors*, *26*(4), 613–619.

Buchan, B. J., L Dennis, M., Tims, F. M., & Diamond, G. S. (2002). Cannabis use: consistency and validity of self-report, on-site urine testing and laboratory testing. *Addiction*, *97*(Suppl 1), 98–108.

El Marroun, H., Tiemeier, H., Jaddoe, V. W., Hofman, A., Verhulst, F. C., van den Brink, W., & Huizink, A. C. (2011). Agreement between maternal cannabis use during pregnancy according to self-report and urinalysis in a population-based cohort: the Generation R Study. *European Addiction Research*, *17*(1), 37–43.

EMCDDA. (2009). *An overview of the general populations survey (GPS) key indicator*. Lisbon, Portugal: European Monitoring Centre for Drugs and Drug Addiction.

EMCDDA. (2014). Chapter 2: Drug use and drug-related problems. In *European Drug Report 2014: Trends and developments* (pp. 88). Lisbon, Portugal: European Monitoring Centre for Drugs and Drug Addiction.

ESPAD. (2012). *ESPAD 2011 Summary*. Stockholm, Sweden: The Swedish Council for Information on Alcohol and Other Drugs (CAN), and the Pompidou Group of the Council of Europe.

GDS. (2014). *The 2014 Global Drug Survey*. London, UK: Global Drug Survey.

Harrel, A. V. (1997). *The validity of self-reported drug use data: The accuracy of responses on confidential self-administered answered sheets*. Rockville, MD: National Institute on Drug Abuse (vol. 167, pp. 37–58). NIDA Research Monograph.

HBSC. (2012). *HBSC 2009/2010 survey*. St Andrews, UK: HBSC International Coordinating Centre.

Hjorthoj, C. R., Fohlmann, A., Larsen, A. M., Arendt, M., & Nordentoft, M. (2012). Correlations and agreement between delta-9-tetrahydrocannabinol (THC) in blood plasma and timeline follow-back (TLFB)-assisted self-reported use of cannabis of patients with cannabis use disorder and psychotic illness attending the CapOpus randomized clinical trial. *Addiction*, *107*(6), 1123–1131.

Macdonald, S., Cherpitel, C. J., Stockwell, T., Martin, G., Ishiguro, S., Vallance, K., & Brubacher, J. (2014). Concordance of self-reported drug use and saliva drug tests in a sample of emergency department patients. *Journal of substance use*, *19*(1–2), 147–151.

Mayet, A., Esvan, M., Marimoutou, C., Haus-Cheymol, R., Verret, C., Ollivier, L., Migliani, R., et al. (2013). The accuracy of self-reported data concerning recent cannabis use in the French armed forces. *European Journal of Public Health*, *23*(2), 328–332.

NDSHS. (2014). *National Drug Strategy Household Survey*. Canberra, ACT: AIWH.

Neale, J., & Robertson, M. (2003). Comparisons of self-report data and oral fluid testing in detecting drug use amongst new treatment clients. *Drug and Alcohol Dependence*, *71*(1), 57–64.

Nichols, S. L., Lowe, A., Zhang, X., Garvie, P. A., Thornton, S., Goldberger, B. A., Sleasman, J. W., et al. (2014). Concordance between self-reported substance use and toxicology among HIV-infected and uninfected at risk youth. *Drug and Alcohol Dependence*, *134*, 376–382.

SAMHSA. (2014). *Results from the 2013 National Survey on Drug Use and Health: Summary of National Findings*. Rockville, MD: Substance Abuse and Mental Health Services Administration (No. NSDUH Series H-48, HHS Publication No. (SMA) 14-4863).

Tieberghien, J., De Moor, S., De Corte, T., & Mortelmans, D. (2009). *Summary Report: Feasibility study of a recurring survey on drug use in the general population*. Brussels, Belgium: Belgian Science Policy Office (Report No. SP1960).

UNODC. (2014). *World Drug Report 2014*. Vienna, Austria: UNODC, Available from http://www.unodc.org/documents/data-and-analysis/WDR2014/World_Drug_Report_2014_web.pdf.

Van der Linden, T., Silverans, P., & Verstraete, A. G. (2014). Comparison between self-report of cannabis use and toxicological detection of THC/THCCOOH in blood and THC in oral fluid in drivers in a roadside survey. *Drug Testing and Analysis*, *6*(1–2), 137–142.

Zaldívar Basurto, F., García Montes, J. M., Flores Cubos, P., Sánchez Santed, F., López Ríos, F., & Molina Moreno, A. (2009). Validity of the self-report on drug use by university students: correspondence between self-reported use and use detected in urine. *Psicothema*, *21*(2), 213–219.

CHAPTER

e19

CANDIS Program: Modular Treatment of Cannabis Use Disorders

E. Hoch, H. Rohrbacher***

***Department of Psychiatry, Ludwig Maximilian University, Munich, Germany**
****Practice for Cognitive Behaviour Therapy, Munich, Germany**

SUMMARY POINTS

- Cannabis is the most commonly used illicit drug worldwide.
- Frequent cannabis use increases the risk of psychosocial, mental, and physical health problems, such as cannabis dependence.
- The criteria for cannabis dependence include withdrawal, tolerance, continued use despite cannabis-related problems, and time excess in obtaining and recovering from the drug.
- During the past two decades several psychotherapeutic interventions for primary cannabis use disorders have been developed and evaluated.
- Empirical evidence was found for the effectiveness of cognitive-behavioral therapy (CBT) in combination with motivational interviewing (MI), contingency management (CM) and relapse prevention (RP).
- CANDIS therapy is one of the first manualized cannabis-specific treatment programs in Europe and was developed at the Technische Universität Dresden (Dresden University of Technology), Germany.
- CANDIS program was tailored to the specific problems and needs of adolescents and adults with primary cannabis use disorders and combines motivational enhancement therapy, cognitive-behavioral therapy and problem solving.
- It consists of ten one-on-one sessions, spanning over a period of 8–12 weeks.
- It was shown to be effective in achieving abstinence as well as reducing cannabis use, addiction severity, disability days, and the overall level of psychopathology.
- Treatment effects could also be achieved after translating the intervention to clinical practice.

KEY FACTS OF RANDOMIZED CONTROLLED TRIAL

- A randomized controlled trial (RCT) is a study, in which participants are randomly (by chance) allocated to receive one of several interventions.
- One of the interventions is a control condition.
- Participants in the control condition receive either standard therapy, a placebo therapy or no intervention at all (eg, waiting-control group).
- Effectiveness of the experimental treatment is measured in comparison with the control condition.
- RCT are quantitative studies as outcomes are measured.
- RCT is considered as the gold standard for clinical trials, because it minimizes selection and allocation biases.

KEY FACTS OF MOTIVATIONAL INTERVIEWING

- Motivational interviewing (MI) is a directive, goal-oriented counseling style, aiming to increase the client's intrinsic motivation to change.

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00130-7
Copyright © 2017 Elsevier Inc. All rights reserved.

- MI evolved from experience in the treatment of problem drinkers, and was described and elaborated by Miller and Rollnick (1991).
- MI elicits behavior change by helping clients to explore and resolve ambivalence.
- The central principles of MI are expressing empathy, developing discrepancy, rolling with resistance, and supporting self-efficacy.
- MI affirms the client's freedom of choice and avoids confrontation and argument.

LIST OF ABBREVIATIONS

CBT Cognitive behavioral therapy
CM Contingency management
CU Cannabis use
CUD Cannabis use disorders
ITT Intention-to-treat
MI Motivational interviewing
RCT Randomized-controlled trial
RP Relapse prevention

INTRODUCTION

Cannabis is the most commonly used illicit drug worldwide, particularly among young people (UNODC, 2014). There are an estimated number of 3 million Europeans (15– 64 years) who use the drug on a daily or almost daily basis (EMCDDA, 2014). This pattern of regular or heavy cannabis use (CU) continued over years most clearly predicts increased risk of psychosocial, mental and physical health problems (Danovitch & Gorelik, 2012; Hall & Degenhardt, 2014; Hoch et al., 2015; Karila, Roux, & Rolland, 2014; Volkow, Baler, Compton, & Weiss, 2014). The most probable adverse health effects are cannabis use disorders (CUD). Cannabis dependence is a mental disorder with a distressing, chronic and relapsing nature. Core dependence criteria are withdrawal, tolerance, continued use despite cannabis-related problems and time excess in obtaining and recovering from the drug [ICD-10 (WHO, 1992), DSM-V; (APA, 2013)]. The lifetime risk of dependence among all cannabis users has been estimated at about 9%, it increases to one in six among those who initiate in adolescence (Anthony, 2006). Europe has seen the numbers of clients entering drug treatment for the first time for cannabis problems rise in the past 15 years. It is the most frequently mentioned drug among first-time clients and the secondary illegal drug of concern among all treatment seekers (EMCDDA, 2014). In many cases, cannabis is not the only reason for treatment seeking. Epidemiological studies consistently report that comorbidity between CUD and other mental disorders is high (Agosti, Nunes, & Levin, 2002; Grant & Pickering, 1998; Wittchen et al., 2007). In the general population 90% of individuals with CUD meet lifetime diagnostic criteria of at least one further mental disorder in comparison to 55% of individuals without CUD (Agosti et al., 2002). Associations between cannabis use, anxiety and panic disorders, bipolar disorders, symptoms of mania, anxiety disorders, suicidal ideations, major depression, schizoaffective disorders, psychotic symptoms, symptoms of schizophrenia, and use of other legal or illegal drugs were found (Hoch et al., 2015).

The knowledge of how to treat primary cannabis use disorders with or without comorbid conditions has been relatively limited for a long time. It is only during the past two decades that rigorously controlled studies of interventions for cannabis use disorders have appeared in the literature. One metaanalysis (Davis et al., 2015) examined the efficacy of psychotherapeutic interventions for primary cannabis use disorders. A comprehensive literature research produced 10 well-controlled randomized controlled trials (RCTs; n = 2027). Five studies were from the United States (Carroll et al., 2006; Kadden, Litt, Kabela-Cormier, & Petry, 2007; Marijuana Treatment Project Research-group, 2004; Stephens, Roffman, & Simpson, 1994; Stephens, Roffman, & Curtin, 2000), four studies were from Australia (Copeland, Swift, Roffman, & Stephens, 2001; Gates, Norberg, Copeland, & Digiusto, 2012; Martin & Copeland, 2008; Rooke, Copeland, Norberg, Hine, & McCambridge, 2013) and one study was from Europe (Hoch et al., 2012). The metaanalysis found evidence that cognitive-behavioral therapy (CBT) in combination with motivational interviewing (MI), contingency management (CM) and relapse prevention (RP) were effective in treating cannabis use disorders. The average patient receiving a behavioral intervention fared better than 66% of those in the control condition. In this chapter the European cannabis-specific treatment program "CANDIS" is described. It was developed and tested in the framework of a translational cannabis treatment research program (Hoch et al., 2012) at Technische Universität Dresden (Dresden University of Technology) in Germany. From 2004 until 2010, the research program was funded by the German Ministry of Research and Education and the German Ministry of Health.

WHAT IS THE CANDIS TREATMENT PROGRAM?

CANDIS (abbreviation of "Cannabis Disorder") is an empirically supported treatment program tailored to the specific problems and needs of adolescents (≥16 years) and adults with primary cannabis use disorders. It combines motivational enhancement therapy,

cognitive-behavioral therapy and problem solving (treatment modules are described in sections "Cognitive Behavioral Therapy" and "Problem Solving Training"). The treatment consists of two weekly 90-min sessions in-person therapy. A treatment manual (Hoch et al., 2011b) specifies all relevant aims, topics and actions to be covered within each session, including assessments and urine drug screenings. The manual also includes information on cannabis, cannabis-related health effects, cannabis use disorders, the basic therapy principles, case reports and how to address typical problems (eg, ambiguous motivation) during treatment.

Who Can be Treated With CANDIS Treatment Program?

CANDIS was developed for persons with primary cannabis use problems. Many people with CUD are ambivalent with regard to changing their cannabis use patterns or treatment seeking. To participate at the program an individual should at least (1) be willing to participate at a treatment session, and be able to participate regularly and to follow the therapy plan. Further inclusion criteria for CANDIS therapy are: (2) primary cannabis use problems, (3) minimum age of at least 16 years, (4) average intellectual performance capability, (5) stable social integration. The intervention can both be helpful for those who are still using cannabis or those who already quitted. *Exclusion criteria* are: age under 16 years, acute severe alcohol dependence (particularly with withdrawal symptoms) or dependence on other illegal drugs, current medication with psychotropic substances, severe intoxication by opiates and stimulants, severe psychiatric disorder (eg, psychosis) and acute suicidality, severe learning disorders or neurological defects. Young adolescent cannabis users often complain about problems with their parents or at school. For this group of patients family-therapeutic concepts (eg, multidimensional family therapy; Liddle, 2002) may be more helpful than CANDIS therapy because family members, teachers and other relevant contact persons can be involved in the therapeutic process.

What Type of Clinician Should Deliver CANDIS?

Psychologists, psychotherapists, medical doctors, and counselors with experience in the field of addiction treatment can deliver CANDIS program to their clients. A special CANDIS "hands-on" training on the nature of CUD, the treatment manual, content and structure of each therapy session, typical problems in the therapeutic process is recommended. Moreover, ongoing supervision is essential to maintain high levels of treatment fidelity.

What Are the Aims of CANDIS?

The CANDIS program has several targets for the therapeutic process. The patient should learn (1) to recognize his or her cannabis use problems, (2) to accept the therapy and participate regularly, (3) to aim for abstinence as primary treatment goal (if patients are not willing to achieve complete abstinence, a reduction in cannabis use may be an alternative aim for treatment), (4) to stop cannabis consumption, (5) to remain abstinent by using relapse preventive measures, and (6) to learn identifying and solving mental and social problems adequately and efficiently.

Treatment Modules

Motivational Enhancement Therapy

The principles and techniques of Motivational Enhancement Therapy (MET) (Miller & Rollnick, 1991) are applied in the CANDIS-program to resolve motivational issues that inhibit positive behavior change. MET is a client-centered and directive counseling style, aiming to increase intrinsic motivation through exploring and decreasing ambivalent thoughts and attitudes. The central principles of MI are (1) expressing empathy, (2) developing discrepancy, (3) rolling with resistance, and (4) supporting self-efficacy. Typical strategies comprise (1) asking open questions, (2) listening reflectively, (3) affirming, (4) eliciting change talk, (5) exploring the pros and cons of a behavior change, (5) envisioning a changed (or nonchanged) future, and (6) exploring goals and values. MI techniques play an important role in the early therapy sessions (session 1–3) and throughout the whole therapeutic process (session 1–10).

Cognitive Behavioral Therapy

Cognitive-behavioral strategies (CBTs) (Monti, Abrams, Kadden, & Cooney, 1989) build the core of the CANDIS-therapy and help patients to successfully change their problematic cannabis use patterns. They are based on the theory that learning processes play a crucial role in the development and maintenance of substance use disorders and that these maladaptive reactions can be replaced by alternative, more functional behaviors. Patients learn cognitive and behavioral skills that support them in understanding, reducing or stopping their cannabis use and maintaining their treatment goals. Central CBT-elements are as follows:

1. *Psychoeducation:* after getting feedback of the results of the diagnostic session patients receive detailed information on the short-term and long-term effects of the drug cannabis. A biopsychosocial model for the development of cannabis dependence is an important basis for the deduction of the therapy

rational. The patients learn that social factors (eg, peer pressure) as well as psychological learning (operant and classical conditioning, model learning) can initiate cannabis use and progression to harmful use patterns (eg, abuse and dependence). They get information on how THC affects the brain (endocannabinoid system).

2. *Understanding consumption patterns:* before initiating any consumption change the patients are guided to understand their own use patterns (session 3). They are instructed to fill out consumption protocols in order to attentively "self-observe" their (mostly automatic) behavior reactions. Through functional analysis the patients learn to identify their problematic behavior, its triggers as well as its consequences, which continue to maintain it.
3. *Preparation of a target day:* after deciding for a specific goal (abstinence or reduction) the therapist helps the patient in session 4 to prepare the consumption stop/target day. The patient has been informed that abstinence as opposed to reduction is the most promising goal from a physiological as well as psychological point of view. The patient chooses a specific day that lies between session 4 and session 5. He or she explores together with the therapist helpful alternative coping strategies as well as how to prevent high risk-situations. He is further encouraged to increase (new) pleasant activities.
4. *Coping with cravings and urges:* in session 5, the patients learn helpful strategies for coping with cravings and urges. They further learn to identify (and avoid) triggers that increase urges and cravings.
5. *Relapse prevention:* in session 6, the patients are prepared how to foresee and deal with high-risk situations that may threaten their goal to remain abstinent. They further develop a personal emergency plan and learn how to deal with a slip in order to prevent a relapse.
6. *Refusal/social competence training:* the aim of session 10 is to increase the awareness for social situations in which it could be difficult to refuse the offer to smoke cannabis. The patient and therapist practice helpful refusal skills (role-plays are conducted).

Problem Solving Training

Patients with cannabis use disorders often report having severe problems in several life areas (eg, difficulties with the job or university, family or other relational problems, financial or legal issues). These problems can be completely unrelated with the consumptions pattern (eg, illness of a family member), but often are directly (eg, partner does not accept the drug consumption) or indirectly (eg, lack of motivation, concentration problems, or poor work performance) associated with the cannabis use disorder. In most cases these problems hamper a lasting abstinence, as many patients lack problem-solving skills and try to neglect problems and adverse feelings (eg, anger, sadness, frustration, anxiety) through the use of cannabis. Many chronic users describe their situation as a vicious circle of unresolved problems and the heavy use of cannabis. In order to break that negative circle, the goal in session 7 and 8 is to acquire appropriate problem solving strategies (D'Zurilla & Goldfried, 1971). Problem solving training (PST) can be regarded as a form of self-management training, in which the individual learns how to discover the most effective way of responding toward a problem. The problem solving training of D'Zurilla and Goldfried (1971) comprises five stages: (1) general orientation, (2) problem definition and formulation, (3) generation of alternatives, (4) decision making, and (5) verification. Malouff, Thorsteinsson, and Schutte (2007) showed in their metaanalysis of 30 studies encompassing $n = 2895$ participants the efficacy of problem solving training. The intervention was effective in comparison to no treatment ($d = 1.37$), treatment as usual ($d = 0.54$), and attention placebo ($d = 0.54$). Significant moderators included whether the PST included problem-orientation training, and whether homework was assigned.

Therapy Sessions

CANDIS treatment program has a clear structure: each session has special topics to be covered (see Fig. e19.1). According to our experience such a clear structure helps to define targets for the therapeutic interventions and facilitates to learn new behavior. In an initial *informational meeting* the patient is carefully informed about the treatment program. Realistic and positive treatment expectations should be fostered as they help the patients to get the feeling of being in good hands and build up a confidential relationship. According to Rogers, Dorfman, Gordon, and Hobbs (2005) especially suitable interview techniques promoting a confident therapeutic relationship are devotion and receptiveness (active hearing), empathy and acceptance of the other person. Sometimes, it can be necessary to clarify with patients that they usually have to give more information about themselves than they might be familiar with from classical medical treatment situations. In a second meeting with the patient a *diagnostic interview (anamnesis)* is conducted, including screenings for cannabis use, cannabis use disorders, comorbid mental and physical disorders, motivation to change etc. (Hoch et al., 2011b). Drug screenings (eg, urine test) are recommended for diagnostic purposes at the beginning and during the course of treatment. In sessions 1, 2, and 3, enhancement of motivation for change cannabis use patterns and participation in CANDIS treatment are major targets. In *session 1*, psychoeducation about CUD, with an emphasis on how cannabis affects the brain, its

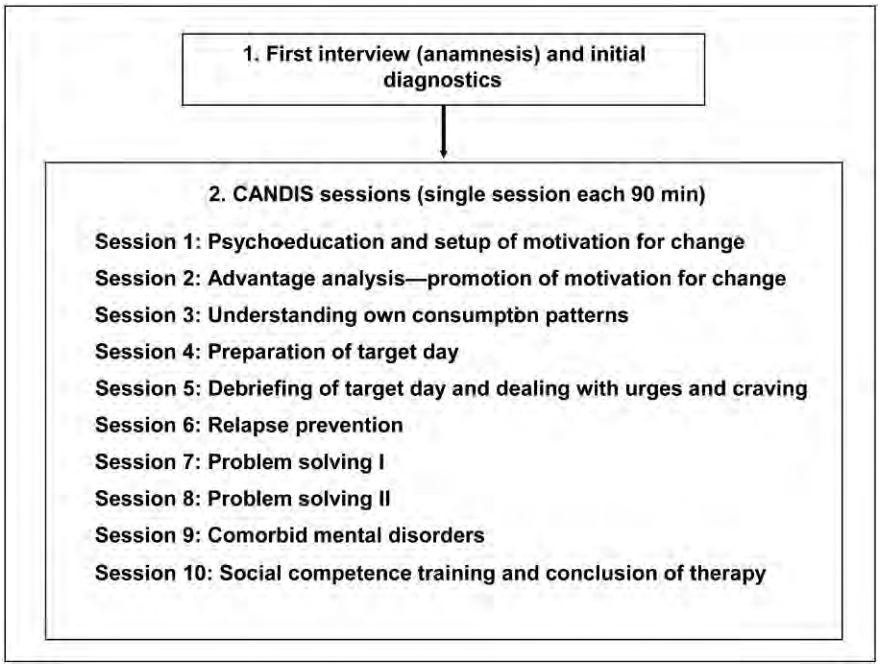


FIGURE e19.1 **CANDIS treatment sessions.**

positive and adverse health effects, and the endogenous cannabinoid system are given. An individual etiological model of CU emphasizing genetic, neurobiological, psychological and social risk factors for CU initiation and maintenance is derived. Principles of classical conditioning, operant conditioning, and model learning are introduced, in order to derive an individual learning history and change concept. Feedback of the results of the diagnostic session it provided to the patients. Motivational enhancement techniques are applied in this context to raise awareness of problems associated with cannabis. In *session 2*, a careful balancing of reasons for abstinence, as well as for cannabis consumption, is performed, in order to create motivational ambivalence. Motivational enhancement therapy techniques are used to resolve ambivalence, to find a treatment goal (eg, cannabis abstinence or reduction in cannabis use), and to promote behavior modification. Therapists worked toward helping patients to address barriers to change and to see the advantages of abstinence. If patients rejected abstinence as a therapy goal, they were supported in pursuing CU-reduction. *Session 3* consists of a discrimination training to help patients in recognizing situations that likely lead to CU. In a functional analysis, patients identified and examined their antecedents and consequences of CU. In *sessions 4–8*, several skills trainings are implemented, for example, quit day preparations, coping with withdrawal and craving, prevention of (re-)lapses, planning for emergencies, refusing cannabis offers, enhancing social support). Patients were also taught how to cope with negative thoughts, how to utilize contingency management (a system of self-reward using material goods and pleasant activities) and how to establish and reinforce positive activities. In *sessions 7 and 8*, the problem solving training is conducted. Patients learn to identify other life problems (eg, unemployment) and to use problem solving strategies (D'Zurilla & Goldfried, 1971). In *session 9*, we elaborate how comorbid mental disorders (anxiety, depression, substance use disorders) may contribute to continued CU and relapse while applying techniques from previous sessions to improve coping. If necessary, patients are given information on treatment providers for treatment after termination from the CANDIS treatment. In *session 10*, social competence training for the rejection of cannabis offers is introduced. Session 10 is also the conclusion of the CANDIS therapy.

A CASE REPORT

Mike is a 25 year old student who participated in the CANDIS treatment project. He had his first joint at the age of 15. Initially, he only smoked cannabis at the weekend, when he met his friends. After some time, his cannabis use became more regular. Mike enjoyed the pleasurable and relaxing effects of the drug after long days at school. Moreover, he was able to relax and sleep better at night. At the age of 18, Mike became a university student. His flatmate was also a regular cannabis user, and the two of them intensively played computer games and smoked bongs (5–10 bongs a day). From that point of time on, cannabis became central to his life. Often, Mike was not able to turn up at the university. He spent a lot of time in his bedroom, stayed in bed, and smoked pot. His desire to do new activities or meet people

was limited. He needed more cannabis to get the effect he wanted, and gave up playing soccer and going out with noncannabis using friends, which was something he had always enjoyed in the past. Mike became very moody, and felt depressed. He conducted several attempts to cut down his cannabis use, but craving and feelings of compulsion to use cannabis were too difficult to manage. Mike decided to take part in the CANDIS project. Within ten therapy sessions he stopped using cannabis, and managed to cope with craving and symptoms of withdrawal.

SCIENTIFIC EVALUATION

To develop and test the efficacy of modular treatment program for older adolescents and adults with cannabis use disorders, we conducted a large translational cannabis treatment research program (Hoch et al., 2011a, 2012, 2014). The first phase was a RCT conducted at Technische Universität Dresden (Dresden University of Technology) in order to develop and test a cannabis-specific intervention combining CBT, MET, and problem-solving training. In a second phase, the intervention was translated to 11 outpatient addiction treatment centers in Germany, where the treatment's effectiveness could be tested in the context of a multisite RCT.

CANDIS I (2004–07)

The aim of this first study phase was to examine the efficacy of modular CANDIS treatment program for older adolescents and adults with DSM-IV cannabis use disorders. A randomized controlled clinical trial of n = 122 patients (aged 16–44 years) with DSM-IV cannabis dependence as the main substance use diagnosis was conducted (Hoch et al., 2012). Patients were randomly assigned to either Active Treatment (AT, n = 90) or a Delayed Treatment Control group (DTC, n = 32) (Fig. e19.2).

Assessments were conducted at baseline, during each therapy session, at posttreatment and at follow-up assessments at 3 and 6 months. Ethical approval to conduct the study was obtained from the Human Research Ethics Committee of the Technische Universität Dresden (Dresden University of Technology). The study was registered in the clinical trials registry of the US National Institute of Health. Patients were recruited and treated (Jan. 26–Dec. 31, 2006) at an addiction outpatient clinic. Patients were referred via other outpatient drug treatment centers, the clinic homepage, newspaper advertisement, flyers, newspaper, radio, word-of-mouth and others sources. Treatment consisted of 10 sessions of therapy, detailed in a strictly enforced manual. Patients randomly assigned to the control group were informed

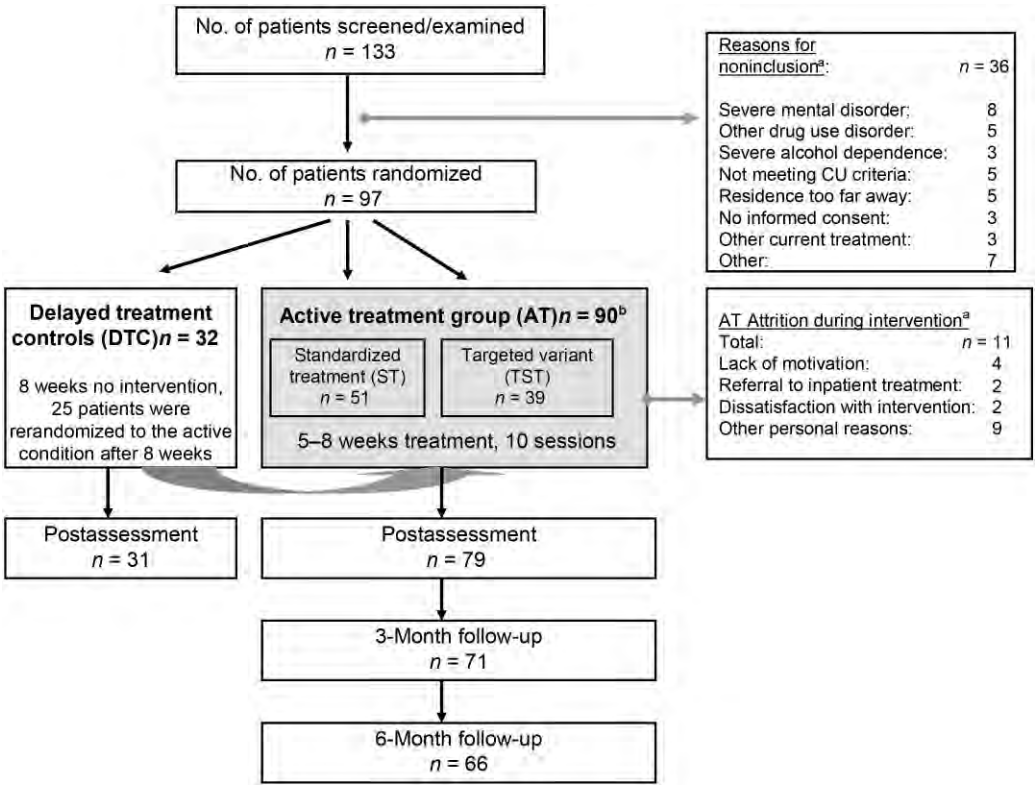


FIGURE e19.2 Patient flow through the study (from Hoch et al., 2012). [a] Multiple reasons possible; [b]Among the n = 90 subjects in the AT group were n = 25 rerandomized subjects from the DTC-condition.

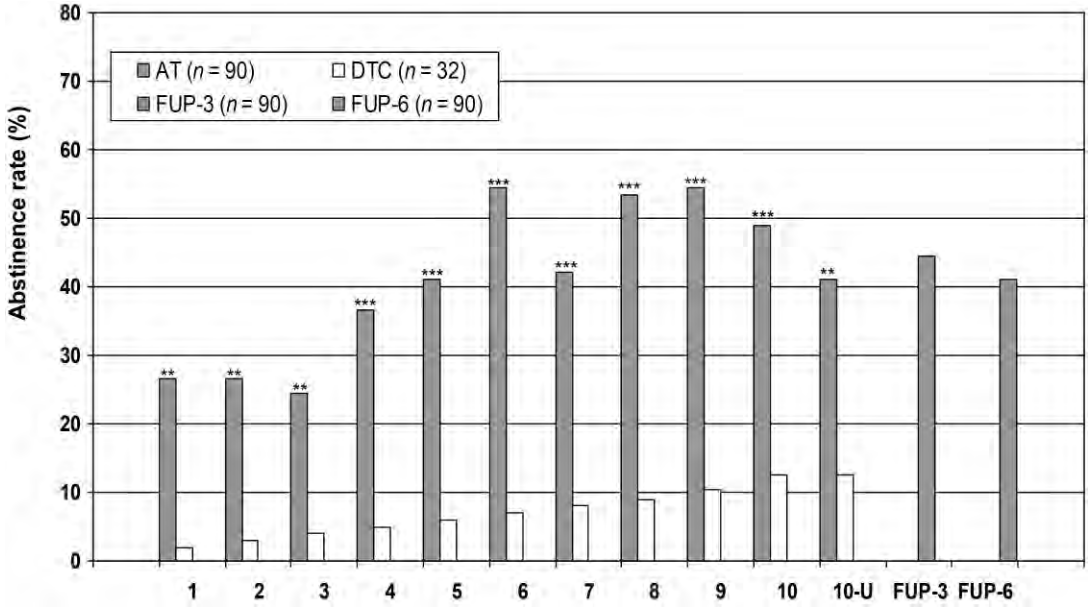


FIGURE e19.3 **Abstinence[a] rates (intention-to-treat-analysis, $n = 122$) (from Hoch et al., 2012).** [a]Abstinence is defined as complete abstinence in the past 7 days, no observations of abstinence at baseline; DTC abstinence rates were interpolated for session 1–9; ***$p < 0.001$ for DTC versus AT; ** $p < 0.01$ for DTC versus AT; session 10 findings are reported without and with (10-U) taking into account positive urine screenings.

that treatment will start in 8 weeks. Identical to AT patients, they were informed about the rationale and study procedures and completed the baseline assessment. They were instructed to reduce or even discontinue their cannabis use during the waiting period. Before starting the delayed treatment, patients completed a second assessment. Study therapists ($n = 10$) were clinical psychologists with training in behavior therapy. All study therapists attended a 1-week training on the treatment manual, which thoroughly addressed the nature of CU and CUD and trained all aspects of the manual using video and role plays. Therapists also received detailed instructions on how to solve frequent administration problems. Throughout the study therapists were supervised every 14 days. Each treatment session was videotaped. 50% were randomly selected and reviewed by supervisors. Feedback was provided to therapists on treatment adherence and therapist competency in a monthly feedback session. The treatment retention rate at the end of treatment was 88%. Abstinence was achieved in 49% of AT patients and in 13% of those in DTC ($p = 0.001$; intend-to-treat (ITT) analysis). Further, AT patients improved significantly ($p = 0.001$) in the frequency of cannabis use per week, addiction severity, number of disability days, and overall level of psychopathology. Program effects were maintained over a 3-month (abstinence rate: 51%) and 6-month follow-up (45%) period. The treatment program was effective in obtaining abstinence as well as reducing cannabis use and improved the associated social and mental health burden (Fig. e19.3).

CANDIS II (2007–10)

In a secondary project phase, the intervention was translated to routine practice. A multisite, randomized controlled trial of 279 treatment seekers with ICD-10 cannabis use disorders, aged 16–63, was conducted in 11 outpatient addiction treatment centers in Germany (Hoch et al., 2014). Patients were randomly assigned to an Active Treatment (AT, $n = 149$) or Delayed Treatment Control (DTC, $n = 130$). Treatment consisted of 10 sessions of fully manualized individual CANDIS. Assessments were conducted at baseline, during each therapy session, at posttreatment and at 3- and 6-month follow-ups. Ethical approval to conduct the study was obtained from the Human Research Ethics Committee of the Technische Universität Dresden (Dresden University of Technology). The study was registered in the clinical trials registry of the US National Institute of Health. Participating therapists recruited and treated patients from May 2, 2008 to Apr. 30, 2009. In order to avoid any systematic selection bias, every new patient to come to each counseling and treatment center who had regularly used cannabis in the previous year was informed about and invited to participate in the study. Patients who reported having been administered any other cannabis-specific treatment in the previous 3-month period were not approached. Patients were asked to provide written informed consent during the initial face-to-face interview, in which they were also asked to complete a prestudy questionnaire designed to determine their eligibility. Anonymized data of eligible patients was reported to the study

headquarter in Dresden, where they were randomized and assigned to one of the two treatment groups within 2 working days. Patients did not receive any payment for their participation. Those who were excluded from the study were treated as usual. Therapists participating in the study were given 2 days' training on the treatment manual, study procedures, assessment instruments, and data collection. They were supervised quarterly by the research staff. Each treatment session was videotaped. Random samples of these tapes (one out of every 30 sessions) were then sent from the study therapists to the study center in Dresden, where the research staff checked them for treatment fidelity using a standardized assessment form. Study therapists were blind to the number and sequence of the selected videotapes. They were given written feedback on treatment adherence and therapist competency. Adherence to the treatment manual was also evaluated after each therapy session, using a standardized questionnaire. Patients were at a mean age of 26.6 years (SD = 8.2, range: 16–63 years), predominantly males (86.7%) and not married (86%). On average, patients reported their first cannabis use at the age of 15.2 years, and their first regular use at 18.8 years of age. Of the total sample, 89.5% had used cannabis in the previous 4 weeks. They had consumed cannabis on mean of 18.8 of the 28 days in this 4-week period, and 42.7% were daily users. The majority of patients (87.1%) met ICD-10 diagnostic criteria for lifetime cannabis dependence (mean number of reported symptoms: 4.9). Prior cannabis quit attempts were reported by 85% of the sample (mean number of quit attempts: 7.9). The only observed baseline difference between groups was the number of cannabis dependence symptoms in the previous 4 weeks (AT vs DTC; $p = 0.005$). At post assessment 53.3% of AT patients reported abstinence (46.3% negative urine screenings) compared to 22% of DTC patients (17.7% negative drug screenings) ($p < 0.001$, intention-to-treat analysis). AT patients improved in the frequency of cannabis use, number of cannabis dependence criteria, severity of dependence, as well as number and severity of cannabis-related problems. Effect sizes were moderate to high. While abstinence rates in the AT group decreased over the 3-month (negative urine screenings: 32.4%) and 6-month (negative urine screenings: 35.7%) follow-up periods, the effects in secondary outcomes were maintained.

CONCLUSIONS

The intervention can successfully be translated to and applied in clinical practice. It has the potential to improve access to evidence-based care for chronic CUD patients.

MINI-DICTIONARY

CANDIS Cannabis disorders

CANDIS program CANDIS therapy is an empirically supported treatment program tailored to the specific problems and needs of adolescents and adults with cannabis use disorders. It combines motivational enhancement therapy, cognitive-behavioral therapy and problem solving.

Contingency management Contingency management is a behavioral treatment approach that is based on the principles of operant conditioning. Patients receive rewards to reinforce positive behaviors such as abstinence.

Cravings Cravings are a hallmark of substance use disorders, and describe the intense desire to use the drug.

Lapse A lapse (or slip) describes a brief return to addictive behaviors after a period of abstinence that can be successfully stopped by the individual, and does not lead to a regression of previous consumption patterns.

Motivational interviewing Motivational interviewing is a client-centered and directive counseling style, aiming to increase intrinsic motivation through exploring and decreasing ambivalent thoughts and attitudes.

Problem solving training Problem solving training is a form of self-management training in which the individual learns how to generate and choose the most effective way of responding toward a defined problem.

Relapse A relapse describes the complete return to addictive behaviors after a period of abstinence.

Relapse prevention Relapse prevention is an essential component of substance abuse treatment. RP techniques involve the identification and prevention of high risk situations as well as the development of individual emergency plans in order to maintain abstinent.

Tetrahydrocannabinol (THC) Tetrahydrocannabinol is the principal psychoactive compound of cannabis and acts on specific molecular targets, called cannabinoid receptors.

The biopsychosocial model The biopsychosocial model is a general model that attributes the development of mental disorders (eg, substance use disorders) to an interplay of certain social, psychological, and biological factors.

References

Agosti, V., Nunes, E., & Levin, F. (2002). Rates of psychiatric comorbidity among U.S. residents with lifetime cannabis dependence. *The American Journal of Drug and Alcohol Abuse*, *28*(4), 643–652.

Anthony, J. (2006). In R. A. Roffman, & R. S. Stephens (Eds.), *Cannabis dependence: Its nature, consequences and treatment* (pp. 58–105). Cambridge, UK: Cambridge University Press, 2006.

APA. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Arlington, VA: American Psychiatric Publishing.

Carroll, K. M., Easton, C. J., Nich, C., Hunkele, K. A., Navins, T. M., Sinha, R., & Rounsaville, B. J. (2006). The use of contingency management and motivational/skills-building therapy to treat young adults with marijuana dependence. *Journal of Consulting and Clinical Psychology*, *74*, 955–966.

Copeland, J., Swift, W., Roffman, R. A., & Stephens, R. S. (2001). A randomised controlled trial of brief cognitive behavioral interventions for cannabis use disorder. *Journal of Substance Abuse Treatment*, *21*, 55–64.

D'Zurilla, T. J., & Goldfried, M. R. (1971). Problem solving and behavior modification. *Journal of Abnormal Psychology*, *78*, 107–126.

Danovitch, I., & Gorelik, D. A. (2012). State of the art treatments for cannabis dependence. *Psychiatric Clinic North America*, *35*(2), 309–326.

Davis, M. L., Powers, M. B., Handelsman, P., Medina, J. L., Zvolensky, M., & Smits, J. A. (2015). Behavioral therapies for treatment-seeking cannabis users: a meta-analysis of randomized controlled trials. *Evaluation and the Health Professionals*, *38*(1), 94–114.

European Monitoring Center for Drugs and Drug Addiction. (2014). *Annual Report: The state of the drugs problem in Europe*. Lisbon, Portugal: EMCDDA.

Gates, P. J., Norberg, M. M., Copeland, J., & Digiusto, E. (2012). Randomized controlled trial of a novel cannabis use intervention delivered by telephone. *Addiction*, *107*, 2149–2158.

Grant, B., & Pickering, R. (1998). The relationship between cannabis use and DSM-IV cannabis abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. *Journal of Substance Abuse*, *10*(3), 255–264.

Hall, W. D., & Degenhardt, L. (2014). The adverse health effects of chronic cannabis use. *Drug Test Analysis*, *6*, 39–45.

Hoch, E., Bonnet, U., Thomasius, R., Ganzer, F., Havemann-Reinecke, U., & Preuss, U. (2015). Risks associated with the non-medical use of cannabis. *Deutsches Ärzteblatt*, *112*(16), 271–278.

Hoch, E., Bühringer, G., Henker, J., Rohrbacher, H., Noack, R., Dittmer, K., Pixa, A., Rühlmann, A., & Wittchen, H. -U. (2011a). Untersuchungskonzept für die CANDIS-Studie zur Behandlung von cannabisbezogenen Störungen: Ein Beispiel translationaler Forschung. [Design of the CANDIS*-study for the treatment of cannabis use disorders: an example of translational research]. *Sucht*, *57*, 183–192.

Hoch, E., Bühringer, G., Pixa, A., Dittmer, K., Henker, J., Seifert, A., & Wittchen, H. -U. (2014). CANDIS treatment program for cannabis use disorders: findings from a randomized multi-site translational trial. *Drug and Alcohol Dependence*, *134*, 185–193.

Hoch, E., Noack, R., Henker, J., Pixa, A., Höfler, M., Behrendt, S., Bühringer, G., & Wittchen, H. -U. (2012). Efficacy of a targeted cognitive-behavioral treatment program for cannabis use disorders (CANDIS). *European Neuropsychopharmacology*, *22*, 267–280.

Hoch, E., Zimmermann, P., Henker, J., Rohrbacher, H., Noack, R., Bühringer, G., & Wittchen, H. U. (2011b). *Modulare Therapie von Cannabisstörungen. Das CANDIS-Programm [Modular therapy of cannabis use disorders: The CANDIS-program]*. Göttingen, Germany: Hogrefe.

Kadden, R. M., Litt, M. D., Kabela-Cormier, E., & Petry, N. M. (2007). Abstinence rates following behavioral treatments for marijuana dependence. *Addictive Behavior*, *32*, 1220–1236.

Karila, L., Roux, P., & Rolland, B. (2014). Acute and long-term effects of cannabis use. A review. *Current Pharmaceutical Design*, *20*, 4112–4118.

Liddle, H. A. (2002). *Multidimensional Family Therapy for adolescent cannabis users. Cannabis Youth Treatment (CYT) Series* (Vol. 5). Rockville, MD: Center for Substance Abuse Treatment, SAMHSA.

Malouff, J. M., Thorsteinsson, E. B., & Schutte, N. S. (2007). The efficacy of problem solving therapy in reducing mental and physical health problems: a meta-analysis. *Clinical Psychological Review*, *27*, 46–57.

Marijuana Treatment Project Research-group. (2004). Brief treatments for cannabis dependence: findings from a randomised multi-site trial. *Journal of Consulting and Clinical Psychology*, *72*, 455–466.

Martin, G., & Copeland, J. (2008). The adolescent cannabis check-up: randomized trial of a brief intervention for young cannabis users. *Journal of Substance Abuse Treatment*, *34*(4), 407–414.

Miller, W. R., & Rollnick, S. (1991). *Motivational interviewing: Preparing people to change addictive behavior*. New York, NY: Guilford.

Monti, P., Abrams, D., Kadden, R., & Cooney, N. (1989). *Treating alcohol dependence*. New York, NY: Guilford Press.

Rogers, C., Dorfman, E., Gordon, T., & Hobbs, N. (2005). *Die klientenzentrierte Gesprächspsychotherapie (17. Auflage)*. Frankfurt, Germany: Fischer.

Rooke, S., Copeland, J., Norberg, M., Hine, D., & McCambridge, J. (2013). Effectiveness of a self-guided web-based cannabis treatment program: randomized controlled trial. *Journal of Medical Internet Research*, *15*(2), e26.

Stephens, R. S., Roffman, R. A., & Curtin, L. (2000). Comparison of extended versus brief treatments for marijuana use. *Journal of Consulting and Clinical Psychology*, *68*, 898–908.

Stephens, R. S., Roffman, R. A., & Simpson, E. E. (1994). Treating adult marijuana dependence: a test of the relapse prevention model. *Journal of Consulting and Clinical Psychology*, *62*, 92–99.

United Nations Office on Drugs and Crime. (2014). *World Drug Report 2014*. Vienna: United Nations publication.

Volkow, N. D., Baler, R. D., Compton, W. M., & Weiss, S. R. B. (2014). Adverse health effects of marijuana use. *New England Journal of Medicine*, *370*(23), 2219–2227.

Wittchen, H. U., Fröhlich, C., Behrendt, S., Günther, A., Rehm, J., Zimmermann, P., Lieb, R., & Perkonigg, A. (2007). Cannabis use and cannabis use disorders and their relationship to mental disorders: a 10-year prospective-longitudinal community study in adolescents. *Drug and Alcohol Dependence*, *88*(1), 60–70.

WHO. (1992). *ICD-10 Classifications of mental and behavioural disorder: Clinical descriptions and diagnostic guidelines*. Geneva: World Health Organisation.

CHAPTER

e20

Engaging Cannabis Users in Treatment

F. Kay-Lambkin, A. Healey**, A. Baker†, W. Swift*, L. Thornton*, A. Turner†*

NHMRC Centre for Research Excellence in Mental Health and Substance Use, National Drug and Alcohol Research Centre, University of New South Wales, Randwick, NSW, Australia

***School of Psychology, The University of Newcastle, University Drive, Callaghan, NSW, Australia**

†**Priority Research Centre for Translational Neuroscience and Mental Health, University of Newcastle, Callaghan, NSW, Australia**

SUMMARY POINTS

- This chapter focuses on issues of engaging cannabis users in treatment.
- Cannabis users access traditional substance use treatment services less often than do any other substance users (including alcohol), and report higher rates of dropout.
- Outcomes for cannabis use are significantly improved with longer psychological and behavioral interventions, providing an impetus for better efforts at engaging, and retaining cannabis users in treatment.
- The physiological effects of cannabis, along with the psychological and social consequences of the drug, play a key role in the ability and willingness of cannabis users to engage in treatment targeting their cannabis use directly.
- Generally, cannabis users perceive cannabis as a relatively benign drug, with few consequences for their functioning, wellbeing and long-term health. The reality is quite different.
- Cannabis users have reported difficulties in establishing a bond with their therapist; a key feature of therapeutic alliance that is associated with treatment engagement and retention.
- A range of strategies is suggested to overcome the difficulties with engaging cannabis users in treatment. These include the integration of non-face-to-face treatments (eg, Internet-delivered) that have demonstrated benefits for cannabis users particularly; targeting early treatments for cannabis users to those aspects of their lifestyle for which they are willing to seek treatment (eg, lifestyle, tobacco use); and establishing specialized cannabis clinics where trained therapists can be adequately supported and resourced to treat the complexities of cannabis use.

KEY FACTS OF ENGAGEMENT

- Encouraging people with substance use problems to enter and remain in treatment (treatment engagement) is a key concern and priority of substance use research and service provision.
- Definitions of "engagement" incorporate aspects of treatment attendance, treatment motivation, patient involvement, and the attitude and behaviors of both the client and therapist.
- It is widely acknowledged in the research and clinical literature that engaging clients is particularly difficult in drug and alcohol settings, whether in terms of accessing services, attending appointments, and developing and maintaining motivation for treatment.
- Studies measuring "engagement" are generally focused on exploring aspects related to improving treatment seeking, treatment retention, or the client-therapist relationship (therapeutic alliance).

Handbook of Cannabis and Related Pathologies. http://dx.doi.org/10.1016/B978-0-12-800756-3.00134-4
Copyright © 2017 Elsevier Inc. All rights reserved.

- Exactly which factors influence a person's decision to seek and remain in treatment are not well understood; however, client retention in treatment is consistently related to enhanced treatment outcomes. This is particularly true for cannabis users.
- One of the most influential and studied factors related to treatment engagement and attendance is therapeutic alliance, particularly in the first few weeks. There are three core components of therapeutic alliance: bond (mutual liking, trust and attachment in the therapeutic relationship), tasks (agreement on joint tasks and strategies used in therapy), and goals (agreement on the targeted areas for change).

LIST OF ABBREVIATIONS

CBT Cognitive behavior therapy
F2F Face-to-face
MET Motivation enhancement training

INTRODUCTION

Over the past decade, as the need for specific cannabis interventions has increased, research has focused on both developing and maximizing the impact of available treatments for cannabis use disorder (Copeland, 2004; Gibbons et al., 2010). Available evidence suggests the benefits of psychological and behavioral therapies in reducing cannabis consumption (Pacek & Vandrey, 2014). Current guidelines for clinicians specifically working with cannabis users (Copeland et al., 2009), emphasize engagement and building a strong therapeutic alliance as critical when working with cannabis clients.

WHY IS ENGAGEMENT PARTICULARLY IMPORTANT FOR CANNABIS USERS?

Early treatment studies have indicated that fewer than 10% of cannabis users access treatment for their cannabis use; a lower proportion than for any other drug type (Stephens et al., 2007; Copeland & Allsop, 2014). In 2008, only 2% of past-year cannabis users in New Zealand received help for cannabis use, yet 13% reported daily consumption (MoH, 2010). Typically, cannabis users do not approach traditional services for fear of being stigmatized (Copeland & Swift, 2009; Gates et al., 2012), or because of a view that they can successfully navigate a quit attempt, and maintain abstinence on their own (Pacek & Vandrey, 2014).

Most cannabis users do not access treatment until their mid-30s, often over concerns about related mental health or lifestyle problems (Teesson et al., 2012), or earlier via coercion from family members or the legal system (Carroll et al., 2006; van der Pol et al., 2013). They have typically used cannabis near-daily for over 10 years and have made more than six serious but unsuccessful attempts to reduce or stop (Swift & Copeland, 2012). When they do seek help, few express a desire to stop using cannabis completely, and opt for reduction goals (Carroll et al., 2006). Many clinicians report reluctance to address cannabis use, and others may not have the expertise required to deliver integrated treatment packages that address the issues most pertinent to cannabis users (Kay-Lambkin et al., 2004). Once they enter treatment, cannabis users have also been shown to have the highest rates of noncompletions (67%) when compared to alcohol (32%) and other illicit substances (ranging from 13 to 34%, Myers et al., 2010).

Importantly; however, the existing literature highlights the benefits of engaging cannabis users in treatment, with unsuccessful cannabis quit attempts being associated with lower exposure to formal treatment programs (Rooke et al., 2011). In addition, a comparison of 87 successful and 78 unsuccessful cannabis quitters attributed the lack of success to a strong desire to quit, but failure to adequately plan strategies for coping with a quit attempt (Rooke et al., 2011).

Brief (one-two) session interventions have demonstrated effectiveness in reducing the quantity and frequency of cannabis use, and associated harms (eg, Swift & Copeland, 2012), especially over the shorter term (eg, 3 months). However, recent research indicates the potential of extended (multisession) cognitive behavioral treatments over and above that achieved by a brief intervention for cannabis use problems. This is somewhat different from people with alcohol use disorders, for whom brief interventions can be highly effective (Heather, 2002) and as effective as longer psychological treatments (Baker et al., 2014). For example, Baker et al. (2009) compared outcomes for 248 participants with alcohol or cannabis use disorders across three clinical trials of psychological treatments consisting of brief advice, one session of motivation enhancement training (MET), or ten sessions of integrated MET and cognitive behavior therapy (CBT). The results indicated that brief interventions (advice or one session) were significantly more effective for alcohol misuse, while more intensive MET/CBT was more effective in reducing cannabis use, even with the inclusion of participants with comorbid mental health diagnoses, such as, major depression and psychosis (Baker et al., 2009). For cannabis users across these studies, effect size changes between baseline and 6-month follow-up were 0.56 (medium) for those in the 10-session MET/CBT intervention, compared to 0.16 (small) for cannabis users who received the brief interventions ($p = 0.03$). Although the longer interventions

were recognized as optimal for cannabis users, the study does not suggest that brief interventions are ineffective. In fact, 50% of cannabis users reduced their use somewhat, and maintained the reduction at 6-month follow-up after receiving a brief intervention, suggesting that it may be a helpful first step into treatment (Baker et al., 2009). However, following brief intervention, cannabis use remained high, with 71% remaining above a cannabis use threshold requiring treatment (Baker et al., 2009). This indicates the potential value of working to optimize engagement and retention of cannabis users in longer-term treatment programs.

WHAT IS UNIQUE ABOUT CANNABIS USERS THAT MAY INFLUENCE TREATMENT ENGAGEMENT?

Physiological Effects of Cannabis

The intoxication and long-term physiological effects of cannabis may in part explain why this group may be unique and challenging to engage and retain in traditional substance use counseling treatments. Euphoria, relaxation, time distortion, perceptual alterations, intensified sensory experience, difficulties with the formation and retrieval of memories, and attention difficulties are all associated with the short-term effects of cannabis use (Hall et al., 1994; NIDA, 2010). These physiological effects may make it difficult for people to remember and regularly attend appointments, and to take on board psychological treatment strategies.

Influence of Cannabis Use on People Presenting for Treatment

Cannabis use also has psychological impacts on anxiety, panic, depressive symptoms, and psychosis, usually associated with chronic consumption (Johns, 2001), and which may affect the ways in which they engage with and respond to treatment. Integrated data across three large longitudinal studies in Australia and New Zealand revealed consistent and clear associations between daily cannabis use before the age of 17 and significantly reduced odds of high school completion, 17–18 times the odds of later cannabis dependence, 7–8 times the risk of other illicit drug use, and 6–7 times the risk of a suicide attempt (Silins et al., 2014). In a 15-year longitudinal cohort study from adolescence to age 29 years, Degenhardt et al. (2013) revealed a strong association between daily cannabis use in adolescence and 2–3 times the odds of developing an anxiety disorder at 29 years.

Cannabis use may also be associated with subthreshold problems that may impact on the therapeutic relationship. In a recent study of 274 people with depression and comorbid alcohol, cannabis, or stimulant use problems (Kay-Lambkin et al., 2011), those who were above threshold for cannabis use (at least once weekly use for the month prior to baseline assessment) were compared to those below threshold on a range of presenting characteristics. People were recruited from the general community in response to media campaigns advertising a treatment trial for people with comorbidity. People who were above threshold for cannabis use in this study reported significantly higher psychoticism (*"the idea that something is wrong with your mind,"* $F(1220) = 6.056$, $p = .015$), interpersonal sensitivity (*"feeling inferior to others,"* $F(1218) = 5.313$, $p = .022$), somatization (*"faintness or dizziness,"* $F(1220) = 8.091$, $p = .005$), paranoid ideation (*"not getting proper credit for your achievements,"* $F(1220) = 11.144$, $p = .001$), obsessive-compulsiveness (*"having to check and double-check what you do,"* $F(1220) = 6.125$, $p = .014$), and phobic anxiety (*"feeling uneasy in crowds,"* $F(1220) = 6.125$, $p = .014$) on the brief symptom inventory (Derogatis & Melisaratos, 1983) than did people who were not above the treatment threshold for cannabis use. No significant differences were found between above and below threshold cannabis users on the subscales of depression (*"feeling no interest in things"*), hostility (*"urges to break or smash things"*), or anxiety (*"feeling tense or keyed up"*). Together, these differences in people using cannabis at thresholds requiring treatment suggest a unique set of interpersonal challenges that may affect their ability to engage with and follow through on a program of psychological treatment with a therapist relative to users of alcohol or other drugs and may result in them feeling disconnected from the treatment process. As further evidence of this, people who were above threshold for cannabis use attended significantly fewer treatment sessions than did those who were below threshold (4.95 sessions vs 6.10 sessions, $F(1,206) = 4.361$, $p = .038$). This emphasizes the particular need to focus on better engaging cannabis users in psychological treatments.

Motivation

Controversially, *"amotivational syndrome"* has also been suggested to be associated with chronic cannabis use, generally defined *"as the diminished or absent drive to engage in typically rewarding activitie"* (NIDA, 2010, p. 7). The syndrome is typically described as an inability to concentrate and carry out long-term plans, and a loss of desire to work or be productive (Cherek et al., 2002). Although there is limited research to support *"amotivational syndrome"* in cannabis use, the impact of cannabis use on decreased motivation is documented throughout the available literature (eg, Hall et al., 1994; NIDA, 2010). By way of example, in a sample of 274 people with depression and alcohol or cannabis use problems

(Kay-Lambkin et al., 2011), baseline data on readiness to change revealed differences between people using alcohol at above threshold levels, and those using cannabis at above threshold levels. Average readiness and motivation to change alcohol use was significantly higher (and closer toward the action stage of change) than it was for cannabis use [67.91 vs 40.95, t(117) = –23.31, p = 0.000]. This is despite people presenting for the research program for help with alcohol or cannabis use and depression. These results suggest that ambivalence to change cannabis use most likely needs to be considered in the early stages of engaging and managing cannabis use.

Risk Perceptions

Risk perceptions play a strong role in decisions to stop using substances (Romer & Jamieson, 2001). Behavioral theories highlight the role of perceived harms in predicting initiation and continuation of substance use. For example, in a 30-year longitudinal study of US adolescents, higher levels of substance use were observed in participants with lower perceptions of risks for that substance (Johnston, O'Malley, Backman, & Schulenberg, 2005). This has also been demonstrated in Australia, where higher levels of cannabis use in particular, was associated with lower perceived risk of harms related to the drug, and continued use (Thornton et al., 2013).

Cannabis has often been viewed as a relatively benign illicit drug, with the majority considering their use sporadic, of limited duration and without major consequences (Thornton et al., 2012a). A key barrier to treatment engagement is the widespread perception that cannabis is a *"safe, harmless, natural substance"* and few cannabis users want to quit or cut down (Akre et al., 2009) (Box e20.1).

A large scale comparison of cannabis users in the United States explored a range of presenting characteristics among treatment seeking versus nontreatment-seeking cannabis users (Pacek & Vandrey, 2014). Across both samples, cannabis users did not endorse reasons for initiating a quit attempt that involved concerns around the deleterious health effects of cannabis use (eg, knew others who had suffered with health problems due to cannabis, worried about reduced life expectancies; Pacek & Vandrey, 2014). Hence, treatment seeking in cannabis users may be more related, for example, to the impact of use on a person's ability to perform their desired roles, rather than to concerns about the health impacts of the drug.

In a qualitative study involving cannabis, tobacco and alcohol users, participants reported that cannabis was a drug to be used and enjoyed without an impact on functioning (Thornton et al., 2012b). On the other hand, tobacco use was considered to be a drug associated with a range of significant negative physical consequences, and was unanimously nominated by participants as the most harmful substance of cannabis, alcohol, and tobacco (Thornton et al., 2012b).

Despite their attitudes, cannabis users frequently report use patterns that increase the likelihood of long-term use, polydrug use, and exposure to cannabis-related harms (Swift & Copeland, 2012). Ambivalence about the negative effects of cannabis use, the multiple perceived positive effects, and relatively low cost, all contribute to the documented difficulties in encouraging cannabis users to modify their consumption of the drug (Budney & Stanger, 2012).

BOX e20.1

RISK PERCEPTIONS ASSOCIATED WITH CANNABIS USE ARE LOW

"Most blokes who go home from work and have a couple of cones or a joint, they tinker in their shed, or play the guitar, or whatever and next they get up and go to work and perform as necessary with absolutely no problems."

(Thornton et al., under review)

Therapeutic Alliance

The importance of the therapeutic alliance as a component of engagement in substance use treatment is well established. Therapeutic alliance refers to the collaborative relationship between a healthcare professional and a client or patient (Bordin, 1979). Alliance is the most studied process factor in psychotherapy research, because of its positive influence on treatment outcomes (Shelef & Diamond, 2008). The importance of engaging clients in treatment and establishing a strong therapeutic alliance is highlighted throughout the substance abuse treatment literature, and is found to positively influence treatment outcomes (Miller et al., 2005; Tetzlaff et al., 2005). Miller and Wilbourne (2002) suggest that 50–66% of outcome in alcohol treatment is attributable to the therapeutic alliance. In particular, the client's experience of the quality of alliance early in treatment is thought to impact on substance use outcomes (Diamond et al., 2006).

Therapeutic alliance consists of three core components: bond, tasks, and goals (Bordin, 1979, see Table e20.1). It is argued that all three components need to be present, and in agreement between client and therapist in order to maximize engagement and treatment outcomes.

One core dimension of therapeutic alliance, *bond*, describes the personal feelings a client has toward a therapist, characterized by acceptance, support, and

TABLE e20.1 The Three Core Components of the Therapeutic Alliance; the Collaborative Relationship Between a Client and Therapist (Bordin, 1979)

Therapeutic alliance domain	Interpretation
Bond	Mutual liking, trust, and attachment between client and therapist
Tasks	Agreement between client and therapist about the strategies and activities of therapy
Goals	Agreement between client and therapist about the targeted areas of change for therapy (including the order in which targets should be addressed)

understanding in the relationship. In a study of 77 clients presenting to a public drug and alcohol service in Australia, Healey et al. (2013) reported that cannabis users reported significantly poorer perceptions of bond, relative to users of other drugs. This was supported by their therapists, who also rated the bond with their cannabis-using clients significantly lower than they did clients using other drugs. There was evidence for the client's perception of the bond to worsen as the severity of cannabis use increased, and cannabis use remained a significant independent predictor of client-related bond, when age and gender were taken into account (Healey et al., 2013). Given that the bond dimension incorporates the emotional attachment between the client and therapist, perhaps a combination of lower interpersonal sensitivity, heightened paranoia, phobic anxiety, and psychoticism that have been associated with cannabis use (NIDA, 2010; Healey et al., 2013) led to clients feeling emotionally numbed or disconnected from their therapist during treatment. These factors are suggestive of the potential difficulties cannabis users face in engaging in substance use treatment—leading to poor treatment seeking and premature treatment dropout (Fig. e20.1).

WHAT CAN BE DONE TO MINIMIZE THE IMPACT OF THESE CHALLENGES ASSOCIATED WITH CANNABIS USE?

Internet/Computerized Treatments—the "Tasks" of Therapy

One of the most promising new areas of intervention research is the development of innovative models of intervention delivery to reduce the barriers to treatment provision. One way of improving engagement and the therapeutic alliance in cannabis users, given their heightened paranoia and interpersonal sensitivity relative to users of other drugs, may be the use of non-face-to-face (non-F2F) treatments. In addition, a desire for self-reliance is the most important treatment barrier reported by the majority of cannabis users, followed by a preference for informal help seeking (van der Pol et al., 2013). Thus, non-F2F approaches may be an ideal treatment-client match for this group of illicit drug users. Such treatments include bibliotherapy, computer-delivered, Internet-delivered, and phone-based modalities.

In recent years, as the demand for drug and alcohol services has remained strong, leading to the integration of non-F2F treatments into the substance abuse literature and practice as a potentially cost-efficient strategy that can be available to a large number of people (Copeland, 2011). In a comparison of computer-based interventions with traditional psychotherapy, similar

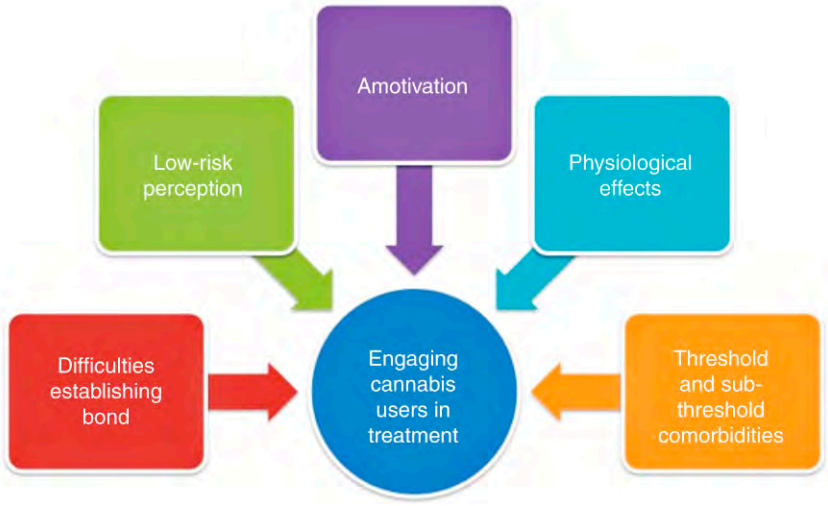


FIGURE e20.1 **Potential threats to the engagement of cannabis users in treatment.**

improvements in substance use outcomes were reported, lending weight to the idea that a computer-assisted treatment model could augment the treatment provided by clinicians working in substance use services (Carroll et al., 2008).

Research is emerging regarding the efficacy of non-F2F interventions in cannabis use disorders. For example, in a synthesis of data across two randomized trials of online self-guided versus telephone delivered interventions for cannabis use, Rooke et al. (2014) reported stronger treatment effects in telephone-delivered treatment than for web-based treatment, along with higher rates of treatment engagement.

With the widespread availability of the Internet, there are many benefits to Internet-delivered treatments for substance use, such as the absence of waiting lists and appointment times, and the freedom of the location and timing of treatment. Moore et al. (2011) conducted a systematic review into computer-based interventions for drug use disorders. Results across five randomized controlled trials revealed that computer-based treatments are associated with high client satisfaction, and were no different in terms of client retention and engagement when compared to therapist-delivered interventions (Moore et al., 2011).

Previous research has indicated that cannabis users are less anxious than people using other drugs about the idea of integrating computers or the Internet into their treatment (Healey et al., 2013). In addition, the same study reported that a higher proportion of cannabis users had previously used the Internet to access treatment or information about cannabis use; a level higher than users of any other drug (Healey et al., 2013). This perhaps points to a potential advantage of this treatment modality for cannabis users in that they may be willing to at least try alternative modes of delivery of cannabis use treatment.

A recent metaanalysis conducted across 10 randomized trials of Internet or computer-based interventions for cannabis use indicated the efficacy of these treatment modalities, at least over the short term, in terms of reduced cannabis use (Tait et al., 2013). For example, in a trial of a 6-session web-based treatment program for cannabis use disorder, 225 treatment seeking cannabis users were randomized to receive web-based treatment versus an attentional control (web-based; Rooke et al., 2013). Results at 3-month follow-up indicated those in the web-based cannabis intervention reported significantly fewer days of cannabis use in the past month, lower quantity of cannabis use and significantly fewer symptoms of cannabis abuse relative to controls (Rooke et al., 2013).

There is also emerging evidence that computerized treatments for cannabis use are at least as effective, if not more so, than are F2F treatments. For example, the clinical efficacy of the SHADE (self-help for alcohol and other drug use and depression) computer treatment program has been examined in two trials among people with depression and comorbid alcohol or cannabis use, relative to therapist-delivered treatment (Kay-Lambkin et al., 2009, 2011). Both SHADE computerized and therapist-delivered treatments yielded similar improvements in substance use and depression, at posttreatment and at 12-month follow-up. SHADE was most efficacious for people with cannabis use, with cannabis users exposed to SHADE computerized treatment reporting large effect size changes from baseline to 12-month follow-up assessment (Kay-Lambkin et al., 2009). An examination of therapeutic alliance for participants in the SHADE trial revealed no significant differences for those above and below threshold for cannabis use on the therapeutic alliance dimension of bond overall [$F(1,178) = 0.700$, $p = .404$], and for participants in the SHADE computerized treatment condition [$F(1,63) = 0.009$, $p = .925$].

These findings generally highlight the potential of non-F2F treatments, particularly those involving computer/the Internet in supporting treatments for cannabis users. These modalities can potentially increase the reach of therapy outside an individual treatment session, and can overcome logistical and emotional barriers to attending regular treatment appointments with a therapist that have been previously reported by cannabis users. Given cannabis users might additionally find it difficult building an emotional bond with their therapist, report paranoid ideation and interpersonal sensitivity, Internet/computer based treatments may be one potential way to engage, transition, or retain this growing population in treatment.

Target Issues of Concern/Risk—the "Goals" of Therapy

Pacek and Vandrey (2014) have previously reported that the biggest disparity between treatment seekers and nontreatment seekers for cannabis use were related to self-image and positive life change (like myself better, control of life, increase energy). Cannabis users in their sample who sought treatment endorsed reasons for treatment seeking that favored improved self-image. Other research has indicated that the majority of cannabis users (up to 91%) also smoke tobacco (Norberg et al., 2012), which they universally perceive as a toxic and addictive substance (Akre et al., 2009). Importantly, the majority of cannabis users report a clear intent to stop tobacco use (Akre et al., 2009), providing an opportunity for engaging them in broad discussions about their current lifestyle.

Preliminary evidence suggests the potential benefit of a lifestyle-focused intervention in cannabis users. For example, a recent "Healthy Lifestyle" trial among 252 tobacco smokers with psychosis (Baker et al., 2011; Baker et al., 2015), engaged a sample who were, on average,

daily cannabis users at baseline. Cannabis users in this study who received a telephone-delivered tobacco intervention reported an average reduction in cannabis use of daily to weekly use from baseline to follow-up assessment ($p = 0.033$). This is despite cannabis not being specifically targeted in the treatment.

A second study is underway using an online lifestyle intervention in the general community. Of the 92 participants engaged to date, 14 (15%) are daily cannabis users. Initial data analyses indicate the potential benefit of the online intervention in reducing tobacco use, cannabis use, depressive symptoms, and improving quality of life in both users and nonusers of cannabis. In particular, at baseline, 6 (43%) of the cannabis users reported mixing tobacco with cannabis, and all of these participants had stopped this practice at 6-month follow-up. The reduction in cannabis by the small sample of users might be indicative of the benefits of capitalizing on the high motivation to quit tobacco in cannabis users, and previous work suggesting the serendipitous benefits of multicomponent Internet-delivered treatments (Kay-Lambkin et al., 2013).

By focusing cannabis treatment in the early stages on issues of perceived harm and risk, there is a real potential to encourage cannabis users to consider other aspects of their lifestyle that might impact on these risky behaviors (eg, cannabis use). This approach is consistent with a nonconfrontational, motivational approach, which may also assist with higher engagement and retention in treatment, and a "foot-in-the-door" to cannabis users addressing their cannabis use down the track. For example, when addressed first, tobacco cessation treatment enhances engagement in substance use treatment. The important secondary prevention opportunity to minimize tobacco- and other lifestyle-related harms in this treatment strategy cannot be overstated.

Specialized Cannabis Clinics—the "Bond" in Therapy

The success of cannabis interventions is still largely dependent on client engagement, retention and adherence to treatment long enough to produce positive change (Baker et al., 2009; Gibbons et al., 2010). Evidence suggests that cannabis users do not identify with users of other substances, and accordingly are reluctant to engage with traditional substance use treatment services for fear of stigmatization. In response to this, in Australia, some publicly funded substance use agencies have established specialized cannabis clinics, for exclusive engagement of cannabis users, and located separately from the mainstream drug services.

Initial research conducted with the population seeking treatment for cannabis use via specialized cannabis clinics indicates the benefits of this approach, with these clinics attracting and engaging a greater proportion of treatment naïve cannabis users than nonspecialized drug and alcohol clinics (Copeland & Allsop, 2014). Importantly, clients who engaged with the specialized cannabis clinic attended an average of 10 weeks of treatment (10.95 weeks).

Research conducted among a drug and alcohol service incorporating a specialized cannabis clinic revealed that the differential perceptions of bond by cannabis users versus users of other drugs is not evident in clients attending the specialized clinic (Healey et al., 2013). Perhaps this provides initial support for the idea of specialized cannabis clinics, engaging clients specifically interested in managing their cannabis use, which allows the therapist to focus more on the establishment of a collaborative partnership with the client in therapy as opposed to a focusing on working toward agreeing on a primary focus of treatment. Staff in these specialized clinics receive additional training and supervision around engaging and managing people with current cannabis use problems; attesting to the importance of these support packages for therapists working exclusively with cannabis using clients. This is particularly true, given reports by cannabis clinic therapists in the study by Healey et al. (2013), who reported significantly lower confidence and optimism in therapy than did therapists in the traditional drug and alcohol services.

MINI-DICTIONARY

Cannabis users In this chapter, the term "cannabis users" refers to people who use cannabis, and is not meant to convey prejudice or offense toward people engaging in this form of drug use.
Comorbid/comorbidity Refers to the concurrent occurrence of more than one disorder within the one person, for example, a substance use problem and a mental health problem. Includes subthreshold presence of symptoms related to multiple disorders.
Engagement Refers to the factors associated with increased attendance and participation in treatment, including therapeutic alliance, individual client factors, and individual therapist factors.
Psychological and behavioral treatment Refers to treatment that is nonpharmacological in nature, and includes a focus on changing behaviors, psychological responses to key events, thoughts, feelings, and social factors. Types of treatment include, but are not limited to, motivation enhancement training, and cognitive behavior therapy.
Therapeutic alliance Refers to the relationship between a client and a therapist who are engaged in a program of treatment. The core components of therapeutic alliance are bond, tasks and goals.

References

Akre, C., et al. (2009). Cannabis and tobacco use: where are the boundaries? *Health Education Research and Clinical Studies in Headache, 25*(1), 74–82.

Baker, A., et al. (2009). The long and the short of treatments for alcohol or cannabis misuse among people with severe mental disorders. *Addictive Behaviors, 34*(10), 852–858.

Baker, A., et al. (2011). Study Protocol: a randomized controlled trial investigating the effect of a healthy lifestyle intervention for people with severe mental disorders. *BMC Public Health*, *11*, 10.

Baker, A. L., et al. (2014). Randomized controlled trial of MICBT for co-existing alcohol misuse and depression: outcomes to 36-months. *Journal of Substance Abuse Treatment*, *46*(3), 281–290.

Baker, A. L., et al. (2015). Randomised controlled trial of a healthy lifestyle intervention among smokers with psychotic disorders. *Nicotine and Tobacco Research*, *17*(8), 846–854.

Bordin, E. S. (1979). The generalizability of the psychoanalytic concept of the working alliance. *Psychotherapy*, *16*, 252–260.

Budney, A. J., & Stanger, C. (2012). Cannabis use and misuse. *IACAPAP e-textbook of child and adolescent mental health substance use disorders#*. Geneva, Switzerland: J. M. Rey.

Carroll, K. M., et al. (2006). The use of contingency management and motivational/skills building therapy to treat young adults with marijuana dependence. *Journal of Consulting and Clinical Psychology*, *74*(5), 955–966.

Carroll, K. M., et al. (2008). Computer-assisted delivery of cognitive behavioral therapy for addiction: a randomized trial of CBT4CBT. *American Journal of Psychiatry*, *165*(7), 881–889.

Cherek, D. R., et al. (2002). Possible amotivatioal effects following marijuana smoking under laboratory conditions. *Experimental and Clinical Psychopharmacology*, *10*, 26–38.

Copeland, J. (2004). Developments in the treatment of cannabis use disorder. *Current Opinion in Psychiatry*, *17*, 161–167.

Copeland, J. (2011). Application of technology in the prevention and treatment of substance use disorders and related problems: opportunities and challenges. *Substance Use and Misuse*, *46*, 112–113.

Copeland, J., & Allsop, D. (2014). Dedicated 'cannabis only' treatment clinics in New South Wales, Australia: client and treatment characteristics and associations with first-time treatment seeking. *Drug and Alcohol Review*, *33*, 565–571.

Copeland, J., & Swift, W. (2009). Cannabis use disorder: epidemiology and management. *International Review of Psychiatry*, *21*(2), 96–103.

Copeland, J., et al. (2009). *Management of cannabis use disorder and related issuses: a clinician's guide*. University of NSW, Australia: National Cannabis Prevention and Information Centre, ISBN: 9780733428074.

Degenhardt, L., et al. (2013). The persistence of the association between adolescent cannabis use and common mental health disorders into young adulthood. *Addiction*, *108*, 124–133.

Derogatis, L. R., & Melisaratos, N. (1983). The brief symptom inventory: an introductory report. *Psychological Medicine*, *13*, 595–605.

Diamond, G. S., et al. (2006). Early therapeutic alliance as a predictor of treatment outcome for adolescent cannabis users in outpatient treatment. *American Journal on Addictions*, *15*(Suppl. 1), 26–33.

Gates, P., et al. (2012). Barriers and facilitators to cannabis treatment. *Drug and Alcohol Review*, *31*, 311–319.

Gibbons, C. J., et al. (2010). Treatment processes, alliance and outcome in brief versus extended treatments for marijuana dependence. *Addiction*, *105*, 1799–1808.

Hall, W., et al. (1994). *The health and psychological consequences of cannabis use*. Canberra: Australian Government Publishing Service, National Drug Strategy Monograph Series 25.

Healey, A., et al. (2013). Avoiding emotional bonds: an examination of the dimensions of therapeutic alliance among cannabis users. *Frontiers in Psychiatry*, *4*.

Heather, N. (2002). Effectiveness of brief interventions provide beyond reasonable doubt. *Addiction*, *97*, 293–299.

Johns, A. (2001). Psychiatric effects of cannabis. *British Journal of Psychiatry*, *178*, 116–122.

Johnston, L.D., O'Malley, P.M., Backman, J.G., & Schulenberg, J.E. (2005). Monitoring the future national survey results on drug use, 1975–2004: Volume I, Secondary school students (NIH Publication No. 05-5727). Bethesda, MD: National Institute on Drug Abuse.

Kay-Lambkin, F., et al. (2004). The 'co-morbidity roundabout': a framework to guide assessment and intervention strategies and engineer change among people with co-morbid problems. *Drug and Alcohol Review*, *23*(4), 407–424.

Kay-Lambkin, F., et al. (2009). Computer-based psychological treatment for comorbid depression and problematic alcohol and/or cannabis use: a randomized controlled trial of clinical efficacy. *Addiction*, *104*, 378–388.

Kay-Lambkin, F., et al. (2011). Clinician-assisted computerised versus therapist-delivered treatment for depressive and addictive disorders: a randomised controlled trial. *Medical Journal of Australia*, *195*, S44–S50.

Kay-Lambkin, F., et al. (2013). The impact of tobacco smoking on treatment for comorbid depression and alcohol misuse. *International Journal of Mental Health and Addiction*, *11*(6), 619–633.

Miller, W. R., & Wilbourne, P. L. (2002). Mesa Grande: a methodological analysis of clinical trials of treatment for alcohol use disorders. *Addiction*, *97*.

Miller, W. R., et al. (2005). Evidence-based treatment: why, what, where, when and how? *Journal of Substance Abuse Treatment*, *29*(4), 267–276.

Ministry of Health. (2010). *Drug use in New Zealand: key results of 2007/08 New Zealand Alcohol and Drug Use Survey*. Wellington, New Zealand: Ministry of Health.

Moore, B. A., et al. (2011). Computer-based interventions for drug use disorders: a systematic review. *Journal of Substance Abuse Treatment*, *40*(3), 215–233.

Myers, B. J., et al. (2010). Correlates of substance abuse treatment completion among disadvantaged communities in Cape Town, South Africa. *Substance Abuse Treatment Prevention and Policy*, *5*(3), 5–64.

NIDA. (2010). *Research report series: "Marijuana Abuse"*. Bethesda, MD: National Institute on Drug Abuse, US Department of Health and Human Services, NIH Pub Number: 10-3859. Available from http://www.drugabuse.gov/publications/research-reports/marijuana-abuse

Norberg, M. M., et al. (2012). Quantifying cannabis use with the Timeline Followback approach: a psychometric evaluation. *Drug and Alcohol Dependence*, *121*, 247–252.

Pacek, L. R., & Vandrey, R. G. (2014). Cannabis use history and characteristics of quit attempts: a comparison study of treatment-seeking and non-treatment-seeking cannabis users. *Experimental and Clinical Psychopharmacology*, *22*(6), 517–523.

Romer, D., & Jamieson, P. (2001). The role of perceived risk in starting and stopping smoking. In P. Slovic (Ed.), *Smoking, risk, perception and policy* (pp. 64–80). Thousand Oaks, CA: Sage.

Rooke, S. E., et al. (2011). Successful and unsuccessful cannabis quitters: comparing group characteristics and quitting strategies. *Substance Abuse Treatment Prevention and Policy*, *6*, 30.

Rooke, S. E., et al. (2013). Effectiveness of a self-guided web-based cannabis treatment program: randomized controlled trial. *Journal of Medical Internet Research*, *15*(2), e26.

Rooke, S. E., et al. (2014). Applying technology to the treatment of cannabis use disorder: comparing telephone versus internet delivery using data from two completed trials. *Journal of Substance Abuse Treatment*, *46*, 78–84.

Shelef, K., & Diamond, G. M. (2008). Short form of the revised Vanderbilt therapeutic alliance scale: development, reliability, and validity. *Psychotherapy Research*, *18*(4), 433–443.

Silins, E., et al. (2014). Young adult sequelae of adolescent cannabis use: an integrative analysis. *Lancet Psychiatry*, *1*(4), 286–293.

Stephens, R. S., et al. (2007). The marijuana check-up: promoting change in ambivalent marijuana users. *Addiction*, *102*(6), 947–957.

Swift, W., & Copeland, J. (2012). Cannabis use disorder: epidemiology, management and future directions. In J. Canales (Ed.), *Emerging targets for drug addiction treatment*. New York, NY: Nova Publishing.

Tait, R. J., et al. (2013). Internet and computer based interventions for cannabis use: a meta-analysis. *Drug and Alcohol Dependence*, *133*, 296–304.

Teesson, M., et al. (2012). Prevalence, correlates and comorbidity of DSM-IV cannabis use and cannabis use disorders in Australia. *Australian and New Zealand Journal of Psychiatry*, *46*(12), 1182–1192.

Tetzlaff, B. T., et al. (2005). Working alliance, treatment satisfaction, and patterns of posttreatment use among adolescent substance users. *Psychology of Addictive Behaviors*, *19*(2), 199–207.

Thornton, L., et al. (2013). Perceived risk associated with tobacco, alcohol, and cannabis use among people with and without psychotic disorders. *Addictive Behaviors*, *38*, 2246–2251.

Thornton, L., Baker, A., Johnson, M., & Kay-Lambkin, F. (2012a). "sometimes cigarettes have been my only friend": a qualitative study of substance use among people with mental health problems. *Drug and Alcohol Review*, *31*, 28.

Thornton, L. K., Baker, A. L., Johnson, M., & Lewin, T. J. (2012b). Attitudes and perceptions towards substances among people with mental disorders: a systematic review. *Acta Psychiatrica Scandinavica*, *128*(2), 87–105.

van der Pol, P., et al. (2013). Facilitators and barriers in treatment seeking for cannabis dependence. *Drug and Alcohol Dependence*, *133*(2), 776–780.

Index

A
AA. *See* Arachidonic acid (AA)
α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), 803
  receptor, 330
Abdominal viscera, sensory innervation, 440
Ab initio protein folding, 684
Aboriginal and Torres Strait Islanders (ATSI), 105
Abstinence, 489, 1097
Abstinence rates, e199
Abstinence violence effects (AVE), 1062
Abuse screening
  urine sample, use of, 988
    chromatographic screening, 988
    immunoassay, 988
AC. *See* Amygdaloid complex (AC)
Academic performance
  chronic marijuana use, impact of, 290
ACC. *See* Anterior cingulate cortex (ACC)
Acetaminophen, 453
Acetylcholine (ACh), 830
Acetyl-CoA, 15
  carboxylase, 15
Acetylhydrolase (AChE), 830
ACh. *See* Acetylcholine (ACh)
AChE. *See* Acetylhydrolase (AChE)
Achievement goal
  cannabis use, relation with, 289
Acidic precursor, 708
ACP-CoA transacylase, 15
Acquired immunodeficiency syndrome (AIDS), 106, 199
ACR. *See* American College of Rheumatology (ACR)
Action outcome associations (A-O associations), 268
Acute cannabis exposure, 380
  driving ability, 380
  laboratory studies, 381
    attention, 381
    brain imaging, 386
    decision making, 381
    motor inhibition and impulsivity, 381
    time perception, 381
    working memory, 381
  on-road studies, 387
  simulator studies, 386
Acute postoperative pain, 451
  mechanism, 451
Acyl-activating enzyme (AAE), 15
Acyl-CoA synthetase, 15
AD. *See* Alzheimer disease (AD); *See also* Alzheimer's dementia (AD)
Adaptive Poisson–Boltzmann Solver (APBS), 686
ADB. *See* Aggressive, disruptive behavior (ADB)
ADCA. *See* Alcohol and other Drugs Council of Australia (ADCA)
Addiction, 715
  molecular mechanisms, 715
Additive effects, 211, 212
Adenylyl cyclase, 560
ADHD. *See* Attention-deficit/hyperactivity disorder (ADHD)
Adjunct therapy, 824
Adolescent
  antidrug media campaign targeting, 216
  cannabis use, 311
  levels of endocannabinoids and cannabinoid receptors, 30
  as a phase of vulnerability for addiction, 143
  prevention and intervention program, 1037
  substance abuse, 1049
  vulnerability for THC effects in, 144
Adult Memory and Information Processing Battery Form A and B, 71
Advanced cancer
  symptoms
    anorexia/early satiety, 860
    constipation, 860
    dry mouth, 860
    dyspnea, 860
    fatigue/lack of energy, 860
    nausea/vomiting, 860
    pain, 860
Adverse drug reaction (ADRs)
  associated with cannabinoids, 203
Adverse health effects, increased risk, 30
AEA. *See* Anandamide (AEA); *See also* *N*-Arachidonoylethanol-amine (AEA)
AER. *See* Ascending enteric reflex (AER)
2-AG. *See* 2-Arachidonoylglycerol (2-AG)
Age
  at first exposure to cannabis, 30
  of first use is related to cannabis use disorder risk, 35
Aggressive, disruptive behavior (ADB), e19
  in elementary school leading to later cannabis use, e21
    analyzed data collected from Dutch adolescents as part of TRAILS, e23
    empirical support, e21, e22
    theory, e21
    using data from the Raising Health Children (RHC) study, e23
  leading to ADB in middle and high school, e24
    empirical support, e25, e26
    theory, e24
  leading to cannabis use in middle and high school, e23
    empirical studies supporting, e24
    theory, e23
Agoraphobia, 65, 68
Agouti-related protein (AgRP), 640
Agricultural status, 8
AgRP. *See* Agouti-related protein (AgRP)
Airway dynamics, 495
AjA. *See* Ajulemic acid (AjA)
Ajulemic acid (AjA), 676
AKT1 gene, 64, 82
Alcohol, 80, 321
Alcohol and other Drugs Council of Australia (ADCA), 1078
Alcohol dependence, 1026
Alcohol-IAT, 270
Alcohol-induced steatosis, e123
Alcohol, smoking and substance involvement screening test (ASSIST), e171–e178
  psychometric performance, e178
Alcohol use disorders identification test (AUDIT), e169
Alcohol withdrawal, 873
Alkylindole compound, 984
Alkylresorcinol moiety, 15
Allergic contact dermatitis, 545
Allergic reaction, 518
Allodynia, 906
Allosteric agonism, 577
Alpha-7 nicotinic receptors, 745
ALS pharmacology, e103
  binge pattern, e105
  and cannabis, e105
    animal studies, e105–e107
    human studies, e107–e108
  dopaminergic deficits, e105
  exchange-diffusion model, e104
  hyperthermia, e105
  mephedrone, e105
  METH-binging activate, e105
  methylone, e105
  neurotoxicity, e105
  serotoninergic deficits, e105
Alveolar macrophage number, 498
Alzheimer disease (AD), 405, 734, 803, 805, 963
Alzheimer's dementia (AD), 829
American Academy of Neurology, 910
American College of Rheumatology (ACR), e159
American court responses to shared use, 194
American Criminal Justice System, 189, 195

American drug court model, 194
American soldier, drug abuse, e151
Amidosulfone, 600
γ-Aminobutyric acid (GABA), 559, 806
4-Aminopyridine (4-AP), 797
5-Aminosalicylic acid (5- ASA), 932
Amisulpride, 873, 887
Amitriptyline, e161
Amotivational syndrome, 66, e204, 289
AMP. *See* Amphetamine
AMPA. *See* α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)
AMPA glutamate receptor, 734
AMPH. *See* Amphetamine
Amphetamine (AMPH), e102, e151, 181, 321, 1008
  as Benzedrine, e103
  biological properties, e103
  chemical structures, e103
  dopaminergic neuronal activity, e104
  physiological concentrations, e104
  treatment for, attention-deficit/hyperactivity disorder (ADHD), e103
Amplified fragment length polymorphisms (AFLP), e7
AMPs. *See* Antimicrobial peptides (AMPs)
Amygdala, e54
Amygdala reactivity, 360
Amygdaloid complex (AC), e141
Amyloid precursor protein (APP), 829
β-Amyloid protein, e137
AN. *See* Anticipatory nausea (AN)
Analgesia, 456, 823
  cannabinoid, effect of, 961
Analysis of biological samples, 986–995
  blood, 986–987
  other matrixes, 991–995
  urine, 988–991
Analysis of plant material, 985
Analysis of pure substance, 985
Analytical challenge, 984–985
Analytical issue, 1010
  immunoassay, related to, 1010
  mass spectrometry (MS), related to, 1010
  other cannabinoid, related to, 1010
  THC, related to, 1010
Analytical technique, 982–983
Anandamide (AEA), 452, 512, 593, 617, 672, 789, 872, 878, 943
  biosynthesis, 618
  effects, 617
  levels, 415
  metabolism, 617
  of network lipids, 620
  receptors, 621
  REM, effect on, 878
  sleep induction
    adenosine, molecule, activation of, 879
    role in, 879
  sleep modulation, effect on, 878
  SWS, effect on, 878
  targets, 621
Ancient Egypt
  historical perspectives, for cannabis usage, 111
Androgen receptor activity
  regulation
    insulin-like growth factor-I, 866
    keratinocyte growth factor, 866
Anger, 182
Angiogenesis, e118
Animal models, e132, 538, 705, 709
  of multiple sclerosis (MS)
    CBD, effect of, 899–900
    experimental autoimmune encephalomyelitis (EAE) model, 899
    Theiler's murine encephalomyelitis virus (TMEV) induced model, 899
  of Parkinson's and Alzheimer's disease, 49
Animal studies, 507
ANS. *See* Autonomic nervous system (ANS)
Antagonists, 535
  HcrtR1, 535
  HcrtR2, 535
Anterior cingulate cortex (ACC), e54
Anticholinergics, 47
Anticipatory nausea (AN), 704
Anticonvulsant effects, 434
Anticonvulsant properties, 432
Antidepressants, 47
Antidrug campaigns, e156
  Not in My Corps, e156
Antidrug task forces, 124
Antiepileptic drug (AED), 433, 435
  pharmacoresistant epilepsy, 433
Antiexcitotoxic and antiinflammatory properties, 49
Antimicrobial peptides (AMPs), 544
Antinociception, 741
Antiobesity agents, 654
Antioxidant, 49
Antioxidant capacity, e125
  by electron transfer, e125
  by hydrogen transfer, e127
Antiproliferative activity of cannabinoids
  intracellular mechanisms underlying, e114
    actions beyond CB1/CB2 receptors, e116
    antitumoral action of cannabinoids in vitro, e114
    mechanism of cannabinoids proapoptotic action–inhibition of prosurvival pathways, e114–e115
      ceramide in cannabinoid signaling, e115
      contribution of ER-stress and autophagy to cannabinoids-induced cell death, e117
      inhibition of prosurvival pathways in antitumoral action of cannabinoids, e116
    role of ER stress and autophagy in cannabinoid-induced cell death, e115–e116
Antipsychotic doses, 47
Antiretroviral therapy, 911
Anti-TNFα. *See* Antitumor necrosis factor alpha (anti-TNFα)
Antitumor necrosis factor alpha (anti-TNFα), 932
Antiviral immunity, 509
Anxiety, e132, 182, 824
  cannabis, 311
  disorders, 299, 872
    cannabis, 304–305
    course of cannabis use, effects of, 305
    dependence, 301
    incidence of CUD, risk of, 305
    lifetime abuse, 301
    marijuana, effect of, 872
    prevalence of past-year cannabis use, 301
    three-year incidence lifetime cannabis user *vs.* nonuser, 302
  like behaviors, e137, 651
Anxiolytic effects, e134
4-AP. *See* 4-Aminopyridine (4-AP)
Apathy evaluation scale, 289
  motivation score, 290
APBS. *See* Adaptive Poisson–Boltzmann Solver (APBS)
Aphrodisiac, 181
APINACA *N*-(4-hydroxypentyl), 985
Apoptosis, e114, e116, e119
  cannabinoid, effect of MAPKs stimulation, 865
APP. *See* Amyloid precursor protein (APP)
Appetite, 643, 655
Appetitive behavior, 269
Apprehended driver
  finding, 851
  synthetic cannabinoid variants, detection of, 852
Arachidonate-based lipids anandamide (AEA), 283
Arachidonic acid (AA), 731
Arachidonoyl ethanolamine, 878
N-Arachidonoylethanol-amine (AEA), 631
2-Arachidonoylglycerol (2-AG), 81, 407, 452, 556, 607, 617, 641, 732, 878
  biosynthesis, 618
  effects, 617
  metabolism, 617
  receptors, 621
  targets, 621
Arterial hypotension, 512
Arvanil, 924
5- ASA. *See* 5-Aminosalicylic acid (5- ASA)
ASC. *See* Ascorbic acid (ASC)
Ascending enteric reflex (AER), 932
Ascorbic acid (ASC), e127
ASSIST. *See* Alcohol, smoking and substance involvement screening test (ASSIST)
Associative memory, 268
AT. *See* Attention
Atherosclerosis, 490
Atomoxetine, 1033, 1034
Atopic dermatitis, 546
Atrial fibrillation, 489
Attention, 71, 155, 157, 262
Attention-deficit/hyperactivity disorder (ADHD), e65, e103
  associations between ADHD, ODD/CD, and cannabis use, e66
  and cannabis use comorbidity, e65
  causality, e66
  childhood ADHD, e66
  cross-sectional research, e65
  externalizing disorders, e66

impact of THC AND CBD content, e67–e69
mediating factors, e67
retrospective studies, evidence for, e66
Attention deficits, cannabis users, e65
Attitude, 217, 218
AUDIT. *See* Alcohol use disorders identification test (AUDIT)
Australia
alcohol use increases during cannabis abstinence, 103
among young adults, cannabis use, 102
attitudes toward cannabis in 1994, 102
cannabis cultivation, 102
cannabis law in, brief history of, 105
cannabis law reform in, 105
cannabis use prevalence in states and territories, 103
cigarette use increases during cannabis abstinence, 103
effects of cannabis use on Australian communities, 107
indigenous cannabis use, 105
epidemiology, 105
medicinal cannabis in, 106
cannabinoid replacement therapy (CRT), 106
cannabis withdrawal symptoms, 107
mid-1990s, cannabis, illicit drug in, 102
National Cannabis Policy (NCP), 106
National Task Force on cannabis, 105
patterns of cannabis usage in relation to other substance misuse, 102
prevalence of CUD, 132
strategies for reducing misuse, 107
cannabis public information initiatives, 107
types of cannabis products: availability, price, and purity, 104
Australian Capital Territory (ACT), 105
Australian Institute of Criminology (AIC), 107
Australian Institute of Health and Welfare (AIHW), 102, 105
Authoritative style, 219
characterized by, 219
Autonomic nervous system (ANS), 320
Auto-oxidation, 15
Autophagy, e115, e117
Autosome, e4
Auxins, 10
Axial diffusivity (AD), 393
Axon
fiber connectivity, investigation of, 393
axonal integrity, measures of, 396
DW-MRI data, 393, 394
novel network-based statistic, 394
right fimbria of hippocampus and, 395
streamlines interconnecting right precuneus with, 396
white matter integrity perturbations, 394
white matter, whole-brain investigation of, 396
whole-brain tractography, 395
hippocampus, morphology in, 397
mechanisms of disturbance, 397
Azathioprine, 932

B

Baclofen, 773, 943, 1032, 1071
BACS symbol coding. *See* Brief assessment of cognition in schizophrenia (BACS) symbol coding
BAD. *See* Bipolar affective disorder (BAD)
Barbiturates, 456
Basal ganglia disorder
cannabis, use of, 917
Huntington disease, 917
Parkinson disease, 917
Basal ganglia–thalamocortical circuit, 919
Basophil activation test (BAT), 524
principle, 524
BAT. *See* Basophil activation test (BAT)
Bath salts, e153
Bayley Scales of Infant Development, 165–167
BBB. *See* Blood brain barrier (BBB)
B-cells, 518, 932
epitopes, 518
BD. *See* Bipolar disorder (BD)
BDE_{OH}. *See* Bond dissociation energy (BDE_{OH})
BDL rats. *See* Bile duct-ligated (BDL) rats
BDNF. *See* Brain-derived neurotrophic factor (BDNF)
BDS. *See* Botanical drug substance (BDS)
Bed nucleus stria terminalis (BNST), e135
Behavioral learning, 318
Behavioral methods, 249
cue-induced craving, 250
reaction times, 249
Behavioral tests, 742
Behavior-specific responses, 456
Benton-visual-retention-test (BVRT), 888
Benzimidazoles, 600
Benzodiazepines, 47, 456
antagonist flumazenil, 775
chlordiazepoxide, 775
Benzoylindoles, 721
Betula verrucosa, 519
Bhageerath algorithm, 685
Bicyclic cores, 600
benzimidazoles, 600
bicyclic pyrazoles, 602
imidazopyridines, 602
naphthyridines, 602
pyrazolopyridines, 602
quinolones, 602
Bicyclic pyrazoles, 602
Bile duct-ligated (BDL) rats, 512
Bioactive lipids, 617
Biological matrices, 238
Biological receptors, 672
Biological stressor, 282
Biological vulnerability factors, 62
Bipolar affective disorder (BAD), 788
Bipolar disorder (BD), 209, 300
age at onset, medication use, and suicide attempts in, 425
anecdotal reports, 424
cannabis use disorders, 423
and symptoms, 427
brain structure and functioning, 427
cognition, 427
childhood trauma, 427
clinical and epidemiological studies, 424
consequences of cannabis use, 427
dose–response relationship between cannabis use and age at onset, 425, 426
genetic vulnerability models, 426
environmental mediation of genetic influences, 426
gene-environment interaction, 426
genetic confounding, 426
hypomanic/manic episode, 424
neurobiology of cannabis in, 425
cortical glutamate levels, role in, 425
psychoactive compounds, 425
pathophysiology and genetic risk, 424
prevalence of cannabis use and use disorders, in United States, 423
psychotic disorders, and cannabis use, 423
psychotic symptoms, 427
relationship between cannabis use disorders and, 424
self-medication hypothesis, 424, 427
sociodemographic factors, 427
symptoms and course of, 427
types of, 422
Blood alcohol concentration (BAC), 239
Blood brain barrier (BBB), 432, 643
Blood-retinal barrier (BRB), 763
Blood screening method
reversed phase LC coupled to mass spectrometry, use of, 986
sample preparation
liquid–liquid extraction, use of, 986
BMI. *See* Body mass index (BMI)
BNST. *See* Bed nucleus stria terminalis (BNST)
Body mass index (BMI), 589, 933
after 3 months of cannabis treatment, 934
at study inception, 934
Body packers, 123
demographics of, 124
Body packing, 123
clinical perspective, 124
acute toxicity, 125
bowel obstruction, 125–126
intestinal perforation, 126
ethical considerations, 126–127
legal considerations, 124
as means of smuggling, 123
security considerations, 127
Body stuffing, 123
Boidae snake, e143
Bond dissociation energy (BDE_{OH}), e124
Botanical drug substance (BDS), 960
Botany of *Cannabis*, 4
macroscopical features, 4
microscopical features, 5
BPD. *See* Bipolar disorder (BPD)
Brain
development, 152
dopaminergic pathway, 280
Brain changes and cognitive functioning, in psychotic cannabis users over time, e60
Brain-derived neurotrophic factor (BDNF), 717
Brazilian research Protocol for Illegal Drugs, e73

BRB. *See* Blood-retinal barrier (BRB)
Breast cancer cells
  prolactin downregulation, 866
Breast carcinoma, 865
Breastfeeding, 528
  initiation and continuation, factors influencing, 528
  levels of neonatal exposure, 529
Brief assessment of cognition in schizophrenia (BACS) symbol coding, 1088
Brief strategic family therapy (BSFT), 1049
Broaden and Build theory of positive emotion, 313
Bronchoalveolar lavage, 498
BSFT. *See* Brief strategic family therapy (BSFT)
Bullous lung disease, 501
Buproprion, 941, 943, 1031
Burden of disease study methods, details of, 90
  case definition, 90
  DisMod-MR modeling, 91
  systematic reviews, 90
Bush cannabis, 104
Buspirone, 943, 1032, 1071

C
CAGE-AID. *See* Cut down-annoyed-guilty-eye opener-adapted to include drug (CAGE-AID)
Calcitonin gene-related peptide (CGRP), 780
Calcium channels, 567
California verbal learning test-II, 295
  learning and memory performance, 295
Callus culture, 10
cAMP. *See* Cyclic adenosine monophosphate (cAMP)
Canada, past year cannabis use by age group, 39
Cancer
  cannabinoid, treatment with, 963
Cancer anorexia–cachexia syndrome, 863–864
  anabolic factor, effect of, 863
    growth hormone, 863
    insulin growth factor, 863
    thyroid hormone, 863
  leptin regulation system, role of, 863
  proinflammatory cytokines, role of, 863
  skeletal muscle breakdown, 863
Candida albicans, e73
CANDIS treatment program. *See* Cannabis disorder (CANDIS) treatment program
Cannabaceae, e2, 3
Cannabichromene (CBC), 104, 959
Cannabichromenic acid synthase (CBCAS), 15, 19
Cannabidiol (CBD), 25, 63, 65, 80, 104, e123, e132, 418, 432, 607, 672, 706, 751, 788, 796, 814, 829, 895, 940, 959, 1010
  activity of fatty acid amide hydrolase (FAAH), inhibition of, 940
  adenosine signaling, enhancement, 898
  animal model, effect on, 880
  antianxiety properties, e132
  antiepileptic effects, in animal models, 798
  antioxidant property, 895
  as antipsychotic drug, 789
  antipsychotic effects, 790
  anxiolytic effects, e136
  arousal, effect on, 879
  biotransformation, 899
  cannabinoid receptors, affinity with, 940
    CB1, 895, 940
    CB2, 895, 940
  cellular targets, 901
  derivatives, e124
  δ opioid receptors, effect on allosteric modulation, 899
  dream retrieval, effect on, 879
  electronic properties, e126
  endocannabinoid signaling system, effect on, 895
  excretion, 899
  functional activity modulation of amygdala-hippocampus complex, 940
    cingulate cortex, 940
    parahippocampal gyrus, 940
    paralimbic regions, 940
    temporo-limbic structure, 940
  glial modulating effects, 920
  glutamate-mediated neurotoxicity, effect on, 895
  hangover effect, 879
  5-$HT1_A$ serotonin receptor, interaction with, 898
  intraventricular administration, 880
  inverse agonist of CB2 receptor, 898
  lateral hypothalamus infusion, 880
  mechanisms of action, 789, 796, 895–899, 940
  neuroprotective effect, 898
  nucleoside-transporter-1, binding with, 898
  μ opioid receptors, effect on allosteric modulation, 899
  orphan receptor GPR55, antagonism of, 899
  peroxisome proliferator-activated receptors (PPARs), effect on, 899
  pharmacokinetics, 899
  pharmacology, 895–899
  potential beneficial effects in human, 901
  property
    anticraving, 940
    antidepressant, 940
    anxiolytic, 940
    mood stabilizer, 940
    sedative, 940
  psychoactivity, lack of, 940
  regional cerebral blood flow (rCBF), effect on, 940
  reuptake of endocannabinoid anandamide, inhibition of, 940
  schizophrenia, use in, 959
  side effects, 792
  sleep, effect on, 879–880
  sleep-wake cycle, effect on, 879
  social anxiety disorder (SAD), effect on, 940
  structure, e124
  total sleep time, effect on, 879
  toxicity, 899
  transient potential vanilloid receptor type-1 (TPVR-1), interaction with, 898
  uptake of dopamine, inhibition of, 941
  vs. cannabis, 943–944
  vs. nicotine, 942–943
  vs. opiates/opioids, 941
  vs. stimulants, 941–942
  wakefulness, effect on, 879
Cannabidiolic acid (CBDA), e2, 7, 708
Cannabidiolic acid synthase (CBDAS), e4, 15, 19
Cannabidivarin (CBDV), 21, 607
Cannabidivarinic acid (CBDVA), 21
Cannabigero, glaucoma treatment, use in, 880
Cannabigerol (CBG), 104, 672, 709, 751, 959
  α2-adrenoceptor, effect on, 960
  anorexia-cachexia syndrome induced weight loss, treatment of, 964
  anxiolytic effect, 964
  cancer cell line
    cell proliferation inhibition, 964
  cannabinoid type-1 (CB1R), effect on, 960
  cannabinoid type-2 (CB1R), effect on, 960
  λ-carrageenan-evoked hypersensitivity, effect on, 961
  COX inhibition, role in, 961
  G-protein-coupled-receptor, effect on, 960
  human oral epitheloid carcinoma, inhibition of, 964
  intra ocular pressure reduction, 965
  keratinocyte proliferation, inhibition of, 965
  medical use, 961–965
    analgesia, 961–962
    cancer, 963–964
    inflammation, 961–962
    inflammatory bowel disease (IBD), 962–963
    mood disorders, 964
    neuroinflammation in multiple sclerosis, 963
    other, 964–965
  mesenchymal stem cell stimulation, 965
  metabolism, 959
  pharmacokinetic, 959–961
  pharmacology, 959–961
    actions, 961
    targets, 961
  phospholipase-A2 (PLA2), effect on, 960
  plasma and brain pharmacokinetic, 959
  prostate cancer cell, effect on, 964
  rat feeding behavior, effect on, 964
  Staphylococcus aureus, activity against, 965
  testosterone synthesis in rat, inhibition of, 965
  therapeutic application, 962
  transient receptor potential (TRP) channels, effect on, 960
  uptake of dopamine, inhibition of, 960
Cannabigerolic acid biosynthesis, 17
Cannabigerolic acid synthase (CBGAS), 15
Cannabigerovarinic acid (CBGVA), 21
Cannabimimetic compound, 982
  detection time, 982
  psychoactive effect, 982
Cannabimimetics, 554
Cannabinoid, 473, 536, 547, 554, 593, 718, 723, 749, 761, 771, 940
  allergic skin diseases treatment, therapeutic potential, 547

analgesics, as, 933
antitumor effect, 865–866
biological activity, 718
biosynthesis, e2
bone-forming cells, e78
bone physiology, impact on, e73
bone-resorbing cells, e78
cannabis smoke-generating apparatus, smoke exposure, e74
Cannabis smoke, impact of, e77
cell mediated immunity, effect on, 933
chronic neuropathic pain, effect on, 933
effect on bone metabolism, e78
endocannabinoids, e72
endogenous, 593
host response evaluation, in cell cultures, e73
impact on immune cell activity, e78
inflammatory pain, effect on, 933
intraocular pressure, 749
lipopolysaccharide (LPS), e72
LPS-evoked macrophage activation, inhibition, 933
main component
cannabidiol (CBD), 878
tetrahydrocannabinol (THC), 878
mast cell, inhibition, 933
molecular targets for, 753
CB1, 753
CB2, 754
neurobiological mechanisms, 536
in pathophysiological states, 474
colorectal cancer, 476
functional disorders of lower GI tract, 474
inflammatory bowel diseases, 475
pharmacology, 595
in physiological states, 473
receptors, e73, 556
cell physiology, 559
discovery of, 556
endogenous ligands, 556
function, 559
location, 559
studies. *See* Histomorphometric study, in rats
synthetic, 721
THC, 940
Th1 cytokine production, effect on, 933
Th2 cytokine production, effect on, 933
therapeutic potential in ocular disorders, 761
use in cancer, 859
vascular inflammatory diseases, influence on, e73
visceral sensory nerve, effect on, 933
Cannabinoid based medicines (CBMs)
adverse effect, 890
neuropsychological performance, effect on, 890
Cannabinoid hyperemesis syndrome (CHS), 952
Cannabinoid receptor 1 (CB1), 90, 269, 327, 392, 415, 442, 462, 495, 554, 565, 575, 609, 651, 667, 840, 848, 871, 932, 940
agonists, 565
allosteric ligands, 575
allosteric modulation
dimerization, 579
regulation, 577
signaling, 577
allosteric modulators, 575
advantages, 575
binding of orthosteric ligands, 576
proposed, 579
antagonism, 512
antagonists, 646, 651, 667
associated ion channel modulation, 567
calcium channels, 567
potassium channels, 567
in axonal growth, 397
brain dispersion, 861
canonical signaling, 566
CB1-D2 heterodimer, 579
constitutive signaling, 568
function, 861
$G\alpha_i$ protein coupling, 566
G protein coupling promiscuity, 568
internalization, 853
inverse agonists, 653
knockout mice, 920, 932
location, 918
mediated anandamide signaling, 872
mood regulation, role in, 871
neutral antagonist, 651
orexin 1 receptor heterodimer, 580
receptors polymorphism, 283
regulation, 569
arrestin recruitment and signaling, 570
desensitization, 569
internalization and degradation, 570
reward and habit development, role in, 269
signaling, 682
transductions, 560
stimulation of, 393
structural characteristics, 556
Cannabinoid receptor 2 (CB2), 415, 442, 498, 554, 565, 584, 840, 848, 932, 940
polymorphisms. *See* CB2 polymorphisms
signal transductions, 560
structural characteristics, 556
Cannabinoid receptors, 632, 723, 740
cannabinoid CB-1 receptor. *See* Cannabinoid receptor 1 (CB1)
cannabinoid CB-2 receptor. *See* Cannabinoid receptor 2 (CB2)
transmembrane receptor
G protein-coupled receptor, 848
Cannabinoid replacement therapy (CRT), 106
Cannabinoids, 3, 14, 182, 185, 672, 932
analgesic effect, 864
anandamide, 697
antiemetic effect, 862
antitumor mechanisms, 865, 866
biosynthesis of, 18
cannabichromen, 948
cannabidiol, 948
cannabidiolic acid, 948
cannabidivarin, 948
cannabigerol, 948
clinical symptom moderation
biochemical pathway, 860, 861
dopamine receptors, effect on, 862
effects on anxiety, 865
emesis inhibition, 953
enterochromaffin cells, effect on, 862
for epilepsy, 436
intoxication, 317
neuroprotectant effect, 873
pathway, 18
pharmacodynamic, 860
pharmacokinetic, 860
potential therapeutic application, 953–955
precursor biosynthesis, 15
with propyl side chains, 21
psychological effects, 865
serotonin receptors, effect on, 862
side effect, 913
dizziness, 913
dry mouth, 913
feelings of intoxication, 913
gastrointestinal effects, 913
hunger, 913
loss of balance, 913
sedation, 913
specific receptor, 878
substance
extracted from cannabis, 880
synthetic compound, 880
system, in psychiatric disorders, 62
tetrad, 693
Δ^9-tetrahydocannabivarin, 948
and their precursors, 14
therapeutic use
CINV, 862
vagal stimulation, effect on, 862
and visceral pain, 441
gastrointestinal tract, 442
small/large intestine, 444
stomach, 444
genitourinary tract, 445
endometrium, 446
kidney and ureter, 445
ovary, 445
prostate, 446
testes and scrotum, 446
urinary bladder and urethra, 445
heart, 441
liver, 444
pancreas, 444
Cannabinoids on brain function, acute effects of, e44
during cognitive tasks, e44
acute administration of delta-9-THC/CBD/placebo condition, e44
attentional salience processing, e47
emotional faces processing task, e46
neuroimaging studies of acute administration of THC and CBDin naïve cannabis users, e45
response inhibition task, e46
sensory processing task, e46
genetic vulnerability to the acute effects of cannabis, e47
acute effects on dopamine release, e48
response inhibition task (Go, No-Go Task) and AKT1 polymorphism, e47
verbal learning task, e47
during resting state, e44
after acute administration of delta-9-THC or CBD, e44

Cannabinol (CBN), 104, 719, 751, 814, 880
molecular composition, 719
optical activity, 719
psychoactive, 719
Cannabis, 48, e102, 358, 373, 478, 488, 495, 660, 740, 771
abstinence, 1030
abuse, 943
dependence, 41
acute effects on movement, 373
addiction
development and maintenance, 1058–1059
compulsive use, 1059
high risk situations (HRS), 1058, 1059
predisposing factor, 1058
addicts, 67
airway dynamics, acute effects, 495
allergens, 520
allergy. *See* Cannabis allergy
antineoplastic mechanism, 860
in Arabic medicine, 111
arteritis, 483
associated stroke, 490
association
with lower respiratory infection, 500
with pneumothorax, pneumomediastinum, and bullous lung disease, 501
based medicinal extract (CBME), 910
based medicine administration
sleep in human protocols, effect on, 881
cannabinoid receptor, effect on, 269
cannabis abuse screening test. *See* Cannabis abuse screening test (CAST)
cessation, 1037
triadic theory of influence, 1038
cessation, preparation for, 1061
chemical compound
tetrahydrocannabinol (THC), 840
chronic pain management, 840
cigarette, 373
classification of, 5
in clubbing drug users
rates of using, 174
on cognition, effects of, 71
and cognition in psychosis
methodological issues to take into account, 46
and cognitive deterioration, 65–66
cognitive effects, in nonpsychosis individuals, 360
and cognitive functions, comprehensive reviews for, 73
on cognitive functions, tolerance to effects of, 72
consumption, 122
effect of intervention, 1088
lifetime progression, 1075
progression
comparative data of main prevalence, 1075
side effect
dependence, 940
euphoria, 940
mental health side effect, 940
consumption duration, 47
craving, 1031, 1058, 1059
diary example, 1063
scale, 1064
daily-use
family socioeconomic status, effect of, 972
dependence, 943, 1038, 1048, 1097
face to face treatment
cognitive behavioral therapy (CBT), 1049
contingency management, 1049
motivational interviewing, 1049
family therapy, 1048
brief strategic family therapy (BSFT), 1049
functional family therapy (FFT), 1049
MDFT, 1049
multisystemic therapy (MST), 1049
problem behavior, associated, 1048
treatment, 1030
evaluated medications, 1032
dependent youth
multidimensional family therapy, 1049
treatment need, 1048
detection and traffic, 238–239
dopamine system, effect on, 269
effects of higher exposure of, 29
endocannabinoid signaling, 269
and executive functioning. *See* Cannabis on executive functioning, effects of
extract, mental disorder, treatment, 878
functional imaging studies, 361
herbal leaf, 478
higher exposure, effects of, 29
hybrid cultivars, 7
hyperemesis syndrome. *See* Cannabis hyperemesis syndrome (CHS)
induced psychosis, 48, 337
genetic aspects, studies examining, 337
ischemic stroke, 487
learning function, effect on, 1057
long-term use, changes in movement, 374
and lung cancer, 498
lung, chronic effects on, 495
alveolar macrophage number, 498
chronic respiratory symptoms, 495
human bronchial pathology, 498
lung function, 496
lung pathology, 498
thoracic high-resolution computed tomography, 497
in Medieval Arab World, 111
memory, effect on, 1057
and mental disorders, 210
negative reinforcing effect, 1038
patient reason, for not using, 1063
patients with schizophrenia, 361
cognitive performance, 361
neurofunctional alterations, 367
positive affect, 1038
potency, 25, 26, 28
and exposure to THC, 26
preparation
hashish, 840
marijuana, 840
prevention. *See* Cannabis prevention
prolonged use
memory loss, 860
psychomimetic effects, 358
and psychosis, 66, 82. *See also* Cannabis, and psychosis correlation
outcomes, 358
recreational use, 281, 840, 849
related disorders (CRD), 730
psychosocial treatment, CBT, use of, 1057
related neural substrate
linking associative effect, 269
related problems, 25, 30
resin usage, 478
screening, 388
biological matrices for, 388
self-initiated cessation
adolescent, 1037
cultural values, role of, 1043
decision-making, role of, 1039
emerging adult, 1037
ethnic culture, role of, 1043
family cohesion, role of, 1040
future intervention
cultural/attitudinal stream-derived intervention, 1043
intrapersonal stream-derived intervention, 1043, 1044
goal orientation, role of, 1040
parental bonding, role of, 1042
sibling influences, role of, 1042
social support system, role of, 1041
spousal interaction, role of, 1042
strategies, 1042, 1043
distraction, 1042
stimulus removal, 1042
victimization, role of, 1040
self-medicating benefit, 1039
and sexual behavior. *See* Cannabis and sexual behavior
smoking, 823
lung contamination with, 1018
synaptic effects of endogenous cannabinoid, effect on, 1057
for therapeutic purposes. *See* Cannabis for therapeutic purposes (CTP)
and tobacco (CTS), 495
treatment. *See* Cannabis treatment
trichomes classification, 6
triggers psychosis, 49
use. *See also* Cannabis use (CU)
accidental problem, 1057
addiction, development of, 1057
in adolescents, 74–75. *See also* Adolescent
appetite stimulation, 933
behavioral consequence, 1037
codependence to illicit substances, 1057
cognitive distortion, 1058
energy metabolism, effect on, 933
impulse control problem, 1057
initiation of, 34
as a learned behavior, 1058–1059
learning model, 1058
mental disorder, development of, 1057
motivating factor
conformity, 1057

emotional regulation, 1057
enjoyment, 1057
experimentation, 1057
feeling high, 1057
getting rid of boredom, 1057
relaxation, 1057
social enhancement, 1057
psychological consequence, 1037
regulation of appetite, 933
sexually risky behavior, 1057
weight gain stimulation, 933
use disorder. *See* Cannabis use disorder (CUD)
use in adolescents, 74–75
use on cognitive functions
gender differences for effect of, 76
use, social norms, and legal status, 38
withdrawal symptom, 133, 1038, 1067–1068, 1070
N-acetylcysteine, effect of, 943
agonist replacement therapy, 943
buccal spray, use of, 943
anxiety, 944
clinical implication, 1070–1071
pharmacological treatment, 1071
psychosocial treatment, 1070
DSM-5, according to, 1067–1068
anger, 1067
decreased appetite or weight loss, 1068
depressed mood, 1068
insomnia, 1067
irritability, 1067
nervousness, 1067
other symptoms, 1068
physical symptom, 1068
restlessness, 1068
sleep difficulty, 1067
vivid unpleasant dream, 1068
effects of CBD, 944
gabapentin, effect of, 943
insomnia, 944
lithium, effect of, 943
lofexidine, effect of, 943
mirtazapine, effect of, 943
nefazodone, effect of, 943
physical symptom
fever, 1068
stomach pain, 1068
sweating, 1068
tremor, 1068
variations in time course, 1068–1070
demand characteristic, 1069–1070
zolpidem, effect of, 943
withdrawal symptoms, 1030
Cannabis, e132
effects on brain, and impact on cognition, e54
genome, e4
heterozygous state (B_T/B_D), e5
homozygous B_T/B_T genotype, e5
problematic use, e169, e170, e178–e180
prevalence estimation, general population, e169–e170
problems, e169
psychosocial hazards, e169
related problems
screening instrument, e169, e170, e178, e181
screening instruments, e170
self-administrable, e172
self-report
prevalence data, e188
Australian general population, e188
European general population, e188
global drug survey, e188
United States general population, e188
youth, e188
validity, e188–e190
affecting points, e189
concordance with biological measurements, e189–e190
smoking, e169
Cannabis abuse screening test (CAST), e178–e179, 972–973
application, 972–973
Italian experience, 974–975
computing a score
Italian validated version, use of, 973
independent variable, 974
questionnaire, 973
statistical analyses
main evidence, 975–977
Cannabis allergy, 520
clinical manifestations, 521
diagnosis, 522
natural history, 525
prevalence, 520
routes of exposure, 520
sensitization, 520
treatment, 525
Cannabis, and psychosis correlation, 414
causality, 416–418
causes of, 417
CBD/THC ratio and the risk of, 418
chronic disorders, 419
component cause of, 416, 417
dopaminergic transmission, 416
endocannabinoid signaling, 416
environmental factors, 418
metaanalysis estimated in young adults, United Kingdom, 419
exposure predicted severity of, 415
general population studies, effect of, 414
genes and environment interact, 418
genetic vulnerability, 417
psychotogenic effects of, 418
schizophrenia, risk of, 417
Cannabis and sexual behavior, 182–185
main studies addressing, 184
positive and negative effects, 184
proposed explanations, for positive effects of cannabis at low doses, 185
Cannabis compound, other
β-caryophyllin, 880
cannabigerol, 880
cannabinol, 880
dronabinol, 880
nabilone, 880
sleep, effect on, 880
Cannabis consumers, e102
estimated number of people, e102
gateway drug, e103
genetic factors, e103
Cannabis dependence and mental disorders, 64
anxiety disorders, 65
panic, 65
posttraumatic stress disorder, 65
social phobia, 65
bipolar disorder, 65
depression, 64–65
Cannabis disorder (CANDIS) treatment program, e194–e196
aims, e195
case report, e197
delivery by
type of clinician, e195
exclusion criteria, e195
inclusion criteria, e195
study phase
I, e198
II, e199
therapy sessions, e196, e197
treatment modules, e195–e196
cognitive-behavioral strategies (CBTs), e195–e196
motivational enhancement therapy (MET), e195
problem solving training, e196
Cannabis expiation notice (CEN), 105
Cannabis for therapeutic purposes (CTP), 312–313
analgesic effect, 312
antiemetic property, 312
appetite-stimulating, 312
cancer-related chemotherapy, 312
future research direction, 314
multiple sclerosis, 312
pain-related anxiety, 313
Parkinson's diseases, 312
Cannabis hyperemesis syndrome (CHS), 466
complications, 468
diagnosis, 468
features, 467
pathogenesis, 467
treatment, 468
Cannabis indica, e2, 5, 873
Cannabis intoxication acute, physical effects of, 81
Cannabis on executive functioning, effects of, 73
attention, 74
planning and decision-making, 74
verbal fluency, 74
verbal learning and memory, 74
working memory, 74
Cannabis picture presentations
behavioral studies, 251
classical conditioning, 251
event-related potential research, 253
fMRI research, 253
orbitofrontal cortex (OFC), 254
orbitofrontal cortex in BESA brain space, 254
spatial resolution, 251
temporal resolution, 250, 251

Cannabis prevention
natural history of cannabis consumption, effect of, 1074–1076
school-based program
affective-focused program, 1076
best practice, 1078–1083
Australian program, 1078
characteristic, 1081–1082
European program, 1078
identification process, 1081
North American program, 1078
other intervention and evaluation detail, 1082–1083
characteristic, 1076–1077
individual component, 1077
booster session, 1077
program facilitator, 1077
program intensity, 1077
timing of delivery, 1077
interactive program, 1076
intervention and evaluation detail, 1080
knowledge-focused, 1076
main characteristic, 1077
noninteractive program, 1076
resistance skills training, 1076
skills-focused program, 1076
type, 1076
selective or targeted, 1076
universal, 1076
Cannabis Problems Questionnaire for adolescents (CPQ-A-S), e179
Cannabis-related knowledges
and attitudes in context of medical use of, 203–204
cannabis use and views, 200
drug prevention program at school, 203
gender differences, and risk perception among Polish students, 205
relationship between self-reported cannabis use prevalence and risk perception
among European students, 200
risk and protection factors influencing, 203
sources of information on illicit drugs
reported by European young people, 202
substance use prevention programs, 201
Cannabis ruderalis, e2, 5
Cannabis sativa, 3, e72, 432, 441, 452, 554, 593, 607, 849, 860, 873, 877, 932
publication distribution, 878
sleep, effect on, 879
sleep latency, decrease in, 879
sleep-wake cycle, effect on, 879
Cannabis sativa hexanoyl-CoA synthetase 1 (CsHCS1 or CsAAE1), 15
Cannabis sativa hexanoyl-CoA synthetase 2 (CsHCS2 or CsAAE3), 15
Cannabis sativa subsp. *indica* var. *indica* (domesticated), 5
Cannabis sativa subsp. *indica* var. *kafiristanica* (wild), 5
Cannabis treatment, 1026, 1094
barrier, 1026–1028
low perceived need, 1026
negative views from community, 1026
treatment availability, 1026
treatment cost, 1026
barrier, implications of, 1028
computer-based intervention, 1028
self-guided intervention, 1028
smartphone app, 1028
cognitive behavioral therapy, 1028
family systems approach, 1028
motivational enhancement therapy, 1028
Cannabis use (CU), 138
above threshold
interpersonal sensitivity, e204
obsessive-compulsiveness, e204
paranoid ideation, e204
phobic anxiety, e204
psychoticism, e204
somatization, e204
absenteeism, 290
across age groups, 36
across the lifespan, trends in, 35
age at first CU and CUD onset and the course of CUD, 148
by age group in US (percentage), 37
among older adults, 39
anxiogenic effect, 311
associative memory assessment, 269–270
on brain structure and function, chronic effects of, e48
functional neuroimaging studies, e48
under cognitive tasks, e49
resting state, e48
genetic vulnerability to chronic cannabis use
and its effect on brain structure, e49
structural effects, e48
challenges associated, impact minimization, e206–e208
bond in therapy, e208
concern/risk
target issues, e207–e208
goals of therapy, e207–e208
internet/computerized treatment, e206–e207
specialized cannabis clinics, e208
depressive outcome
adjusted odds ratios (AORs), 303
confidence intervals (CIs), 303
early
in CUD risk in the context of existing models of, 145
as a risk factor for CUD, 143
escalation of, 143
euphoric effect, 309
higher rates of suspension, 290
high-risk age periods for, 139
implicit association test, 269–270
later
childhood adversity, association with, 292
novelty-seeking behavior, association with, 292
parents with prior criminality, association with, 292
socially disadvantaged background, association with, 292
lower school satisfaction, 290
important features of earlier CU as a risk factor for CUD, 144
negative association, 311
poor school performance, 290
primary motive
CTP, 310
recreational, 310
problematic use motivation in early onset CU, 146
as a risk factor for psychosis, e30
risk of DSM-IV cannabis dependence by age at first CU, 140
salutary effect, 309
self-report, e185
methods, e186–e187
global drug survey (GDS), e187
guidelines in Europe, e186–e187
surveys in Australia, e187
surveys in United States, e187
youth surveys, e187
young age at CU onset and its association with CUD risk, 139
Cannabis use disorder (CUD), 34, 131, e194, e195, e198, e199, 299, 823, 940, 1026, 1030, 1057, 1087
adolescent-community reinforcement approach (A-CRA), 133
age at onset, medication use, and suicide attempts in BD patients with and without, 425
anecdotal reports, 424
approach, 1059
brain structure and functioning, 427
clinical features, 132–134, 1060
clinical studies, 425
and cognition, 427
cognitive behavioral therapy, use of
case example, 1062–1064
successful outcome, 1063–1064
therapeutic alliance, 1062
unsuccessful outcome, 1062–1063
comorbid disorder
pharmacotherapy, 1033–1034
comorbidities, challenge of, 428, 1059
diagnosis, 1059
disorder, treatment of, 76
dopamine and glutamate, 425
dose–response relationship, age at onset in bipolar disorder, 426
dysphoria and negative psychological experiences, link between, 424
environmental mediation, genetic influences, 426
evaluation, 1059
factor models, 427
gender differences in severity markers, 133
genetic vulnerability models, 426
high-risk age periods for incidence, 139
integrative model of role of younger age at CU onset in CUD risk, 146–148
male to female ratio of lifetime cannabis use, 132
mesolimbic dopaminergic pathways, 425
modular treatment
CANDIS program, e193
scientific evaluation, e198–e199

neurobiological mechanisms, 425
pharmacotherapy, 1032–1033
antidepressants and anxiolytic, use of, 1032
cannabinoid agonist, use of, 1032
glutamatergic agents, use of, 1033
other medication, use of, 1033
prevalence of cannabis use, 131
prodromal phase, 424
psychiatric disorder
anxiety disorder, 1033
bipolar disorder, 1033
depression, 1033
quality of life, daily functioning, and, 428
relationship between BD and, 423, 424
schizophrenia, 424
self-medication hypothesis, 424
stage
cognitive behavioral approach, 1061–1062
sobriety, maintainance, 1061
symptoms and course of BD, 427
technology-assisted intervention, 1094
computer-based intervention, 1094
high technology based, 1094
interactive voice response intervention, 1094
low technology based, 1094
mobile-based interventions, 1094
telemedicine, 1094
telephone counseling, 1094
transition from cannabis first use to, 132
treatment
CBT method, 1060–1061
cognitive restructuring, 1060–1061
cognitive-behavioral approach, 1059
effectiveness, 135
services, 134–136
in youth, effective treatment, 1048–1049
Cannabis use disorders identification test (CUDIT), e170, e179–e180
Cannabis use intervention
computer-based, 1096
computer-based counselor-guided, 1095
high-tech delivery, 1095
limitation, 1095
lost to follow-up, 1095
participant anonymity breach, risk of, 1095
vs. low-tech delivery, 1098
low-tech delivery, 1097–1098
limitation, 1098
participant dropout, 1098
participant self-report, risk of bias in, 1098
short follow-up assessment period, 1098
telecounseling intervention, 1097
technology-assisted
age, effect of, 1098
comparison of, 1098
gender, effect of, 1098
Cannabis use problems identification test (CUPIT), e180–e181
Cannabis users, 1026
axonal fiber connectivity, investigation of, 393
DW-MRI data, 393, 394
novel network-based statistic, 394
right fimbria of hippocampus and, 395
streamlines interconnecting right precuneus with, 396
white matter integrity perturbations, 394
white matter, whole-brain investigation of, 396
whole-brain tractography, 395
chronic
psychosocial stress
dopamine response, to, 281
evidence for dysconnectivity, 393
adult and adolescent, white matter microstructural alterations in, 393
corpus callosum, 393
DW-MRI studies, 393
white matter abnormalities, 393
evidence, 397
foot-in-the-door, e208
future directions, 397
non-face-to-face (non-F2F) treatment, e206
schizophrenia, development of, 392
treatment engagement, e202
importance, e203
influence by, e204–e205
cannabis use, e204
motivation, e204
physiological effects of cannabis, e204
risk perceptions, e205
therapeutic alliance, e205
potential threats, e206
premature treatment dropout, e205
Cannabis withdrawal syndrome
abstinence-induced sleep disruption, 1032
pharmacotherapy, 1031–1032
antidepressant, use of, 1031
cannabinoid agonist, use of, 1031
evaluated medication, 1031
mood stabilizer, use of, 1031
other medication, use of, 1032
sedative hypnotic, use of, 1032
Canonical signaling, 566
CapOpus trial, 1087
result, 1089
Capsaicin, 924
Capsazepine, 924
Carbon tetrachloride-induced hepatic damage, 509
autoimmune hepatitis, 509
liver fibrosis, effect of cannabinoids, 510
Carboxyhemoglobin, 490
Carboxylation, 15
9-Carboxy-THC, 529
Cardiac embolism, 489
Cardiomyopathy, 482
CART. *See* Cocaine, and amphetamine-regulated transcript (CART)
β-Caryophylline, 878
antiinflammatory effect, 880
Case-control studies, 585
Caspases, e114
CAST. *See* Cannabis abuse screening test (CAST)
CAT. *See* Catalase (CAT)
Catalase (CAT), 782
Catalepsy, 330, 651, 730
Catalepsy-like immobilization, 327
THC-induced, 327
involvement of
CB1 receptor, 327
glutamatergic neuronal system, 330
nucleus accumbens, 328
serotonergic neuronal system, 329
Catechol-*O*-methyltransferase (COMT), 336
inhibitor, 943
Cathinone, e103
chemical structures of, e104
CB1. *See* Cannabinoid receptor 1 (CB1)
CB2. *See* Cannabinoid receptor 2 (CB2)
CBC. *See* Cannabichromene (CBC)
CB2 cannabinoid receptor gene *(CNR2)*, 586
CBD. *See* Cannabidiol (CBD)
CBDA. *See* Cannabidiolic acid (CBDA)
CBDA synthase, e3
CBDA-synthase gene, e6
DNA sequencing electropherogram of a portion, e6
CBD/THC ratio, e4, e5
CBDV. *See* Cannabidivarin (CBDV)
CBG. *See* Cannabigerol (CBG)
CB-GABA interactions, 773
CBG-free-CBG-BDS, 960
CB_1-knockout mice, e137
CBM. *See* Cognitive behavioral model (CBM)
CBME. *See* Cannabis, based medicinal extract (CBME)
CBMs. *See* Cannabinoid based medicines (CBMs)
CBN. *See* Cannabinol (CBN)
CB2 polymorphisms, 586
and bipolar disorder, 589
and bone disorders, 588
and cardiovascular disorders, 587
and CNS disorders, 588
and eating disorders, 589
and immune system disorders, 586
and liver disorders, 587
and schizophrenia, 589
CB2 Q63 polymorphism, 588
and alcoholism, 588
and depression, 589
CBT. *See* Cognitive behavioral therapy (CBT)
CCD. *See* Cross-reactive carbohydrate determinants (CCD)
CCl_4-induced cirrhosis, 512
CD. *See* Celiac disease (CD); *See also* Crohn's disease (CD)
CD activity index (CDAI), 933
CDAI. *See* CD activity index (CDAI)
cDNA. *See* Complementary DNA (cDNA)
cDNA/EST library, 15
CEDS. *See* Clinical endocannabinoid deficiency syndromes (CEDS)
Celiac disease (CD), 587
Cellular pathways
cannabinoid, effect of, 865
neurotransmitters release inhibition, 865
Central nervous system (CNS), e105, e159, 534, 584, 593, 714, 796
Central neuropathic pain, by multiple sclerosis, 909–911

Central sensitization, 906
Ceramide, e114, e115, e119
  in cannabinoid signaling, e115
Cerebellar motor impairment, 408
Cerebellum, 71
Cerebral vasculitis, 490
Cerebral vasoconstriction, 490
Cerebrospinal fluid (CSF), 64, 283, 789
Cerebrovascular effects, 483
CF. *See* Cognitive functions
CFC models. *See* Contextual fear conditioning (CFC) models
CGRP. *See* Calcitonin gene-related peptide (CGRP)
Chalcone synthase (CHS), 15
CHC. *See* Chronic hepatitis C (CHC)
Chemical phenotype, e3
Chemokines, 544
Chemopreventive agents, 818
Chemoreceptor trigger zone (CTZ), 862
Chemotaxonomic classification, 7
Chemotherapy-induced nausea and vomiting (CINV), 860, 862–863, 953
  neurotransmitters, role of, 862
    dopamine, 862
    histamine, 862
    muscarine, 862
    serotonin, 862
  pathogenesis, 862
Chibouque, 111, 119
Childhood trauma
  age at onset, substance abuse and childhood trauma abuse dose effect, 212
  history link between cannabis use and severe mental disorders, 211
  interaction between childhood sexual abuse, cannabis abuse, and, 212
  and mental disorders, 209
  prone to illicit drug use, 210
  questionnaire (CTQ), 211
  substance abuse and childhood trauma abuse dose effect, 211
Chinese hamster ovary K1 (CHO K1) cell line, 566
CHIP assay. *See* Chromatin immunoprecipitation (CHIP) assay
Chloroplast DNA (cpDNA), e8
Chlorpromazine (CPZ), 730, 862
CHO K1 cell line. *See* Chinese hamster ovary K1 (CHO K1) cell line
Cholinergic systems, 741
Chromatin immunoprecipitation (CHIP) assay, 724
Chronic cannabis, 490
  abuse, 463
    effects, 81
Chronic hepatitis C (CHC), 587
Chronic obstructive pulmonary disease (COPD), 496
Chronic pain model, 451
  mechanism, 451
Chronic pelvic pain syndrome, 446
Chronic prostatitis, 446
CHS. *See* Cannabinoid hyperemesis syndrome (CHS)
Ciliary muscle, 756
CINV. *See* Chemotherapy-induced nausea and vomiting (CINV)
Cirrhotic mesenteric arteries, 512
Cirrhotic rats, 512
Cisplatin-induced delayed emesis, 953
Citalopram, 329
Client satisfaction questionnaire (CSQ), 1088
Climate schools model (CSM) program, 1078, 1082
Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) project, 48
Clinical endocannabinoid deficiency syndromes (CEDS), e162
Clinical test of impairment (CTI), 851
Clinical trials, 453
  acute postoperative pain treatment with cannabinoids, 453
Clubbing community, reasons for cannabis use in, 176
  gateway theory, 176–177
  short-term effects, 176
Clubbing culture, 171
Clubbing drugs, 172, 176
Club drugs, e12, e15
CM. *See* Contingency management (CM)
Cannabinoids, 585
CNR2. *See* CB2 cannabinoid receptor gene *(CNR2)*
CNR1, CNR2, and FAAH genes, 64
CNR1 polymorphisms, 62
CNS. *See* Central nervous system (CNS)
Cocaine, 48, 162, 172, 181, 182, 321, 373, 1008
  and amphetamine-regulated transcript (CART), 780
  dependence, 281
    marijuana, effect of, 874
  smuggling, 123
Cognition, 48, 122, 361
  in psychosis, 46
Cognitive areas, studied in psychosis related to cannabis use, 46
Cognitive assessment, 47
Cognitive behavioral model (CBM), 1058
Cognitive behavioral therapy (CBT), 135, e194–e196, e203, 1057, 1087, 1094, 1103
  coping with cravings and urges, e196
  marijuana use treatment, 1103
  principle, 1060
  psychoeducation, e195
  refusal/social competence training, e196
  relapse prevention (RP), e196, 1057
  sobriety state, effect on, 1057
  target day preparation, e196
  understanding consumption patterns, e196
Cognitive deficits, 44
Cognitive disorder, 874
  marijuana, effect of, 874
Cognitive domains, 225
Cognitive effects, 823
  cannabis use in healthy subjects, 45
  long-term use of cannabis in psychotic patients, 45
  nicotine, 47
Cognitive functions, 47, 48, 50, e54, e55, e59, e60, 71, 262
  in cannabis users, 48
  in current IQ, verbal memory, working memory span, and planning ability, 49
  key facts of, 70
  psychotic patients, using cannabis, 48. *See also* Explicative hypothesis
  related with cannabis use
    risk factors and treatment of impairments in, 76
  in schizophrenia, 49
Cognitive impairments, 45
Cognitive independence model, 1060
Cognitive inhibitory control, 293
Cognitive performance, 45, 47
  THC, effect of, 874
Cognitive restructuring method
  behavioral, 1061
  cognitive, 1060–1061
  emotional, 1061
Colonic transit
  Δ^9-tetrahydrocannabinol, effect of, 950
Colorectal cancer (CRC), 476
Communication, 219
Comorbid, 47, 48, 75
  attention deficit hyperactivity disorder marijuana, effect of, 874
  drug usage, 47
  major depression, 1026
Companionship, 310
Comparative risk assessment (CRA), 91
Compatible trial, regions of activity within group, 274
Complementary DNA (cDNA), 556
Component resolved diagnosis (CRD), 524
Composite International Diagnostic Interview (CIDI), 47
COMT. *See* Catechol-O-methyltransferase (COMT)
COMT gene, 64, 66, 337, 418
  and cannabis use interactions, and the effect on risk for psychosis, e32
    evidences from case-only and case-control studies, e33
    evidences from experimental and experience sampling method based studies, e37
    evidences from longitudinal studies, e32
    evidences from neuroimaging studies, e37
  cannabis use interaction, studies on, e37
    environmental factors interaction, e38
    genetic interactions and other biological mechanisms, e38
    methodological concerns and challenges, e38–e39
    pleiotropic biological and behavioral effects of *COMT* gene, e37
  and effect of Val158Met polymorphism on COMT activity, e32
  molecular variability and the risk for psychosis, e30
COMT inhibitor. *See* Catechol-O-methyl transferase (COMT) inhibitor

COMT Val158Met polymorphism, 337
Conceptual model of achievement
  effects on substance use during adolescence, 291
Conditioned gaping, 705
  acute nausea, 705
  anticipatory nausea, 705
Conditioned place preference (CPP), 666
Condition interaction
  significant regions of activity, 275
Conduct disorder (CD), e23, e66
Confounding effect, 224
Confounding factor, 311
  cannabis use assessment, 311
  polydrug use, 311
  sample selection, 311
Confounding variables, 48
Conservation initiatives, 8
Constitutive signaling, 568
Consultant-participant alliance, 1087
Contact allergic inflammation, 545
  proinflammatory chemokines, secretion of, 545
Content of successful drug prevention program at school, 203
Contextual fear conditioning (CFC) models, e135
Contingency management (CM), e194
Continuous performance test (CPT), e55, 1088
Contrave, clinical trial, 650
Controlled drug
  identity determination
    chemical structure
      infrared spectroscopy (IR), 982
      mass spectrometry (MS), 982
      nuclear magnetic resonance spectroscopy (NMR), 982
Controlled Substances Act (CSA), e155
COPD. *See* Chronic obstructive pulmonary disease (COPD)
Cortisol-awakening response, 280
COX. *See* Cyclooxygenase (COX)
COX-2. *See* Cyclooxygenase-2 (COX-2)
Cox regression, 499
C3-phytocannabinoids, 21
C5-phytocannabinoids, 21
CPP. *See* Conditioned place preference (CPP)
C-prenylation of divarinic acid (DA), 21
CPZ. *See* Chlorpromazine (CPZ)
CRAFFT, e179
Cranial mesencephalon, e145
Craving
  cannabis, 317
  cue-reactivity, 320–321
  environment stimuli, 319
  memory encoding disruption, 322
  reason for relapse, 317
  theoretical etiology, 318–319
  theoretical issue, 321–322
CRC. *See* Colorectal cancer (CRC)
CRD. *See* Cannabis, related disorders (CRD)
Criminal justice system, 216
CRIP1a, 683
  3D structure prediction, 683
CRIP1a-CB_1R model, 685
Critical tracking task (CTT), 380
Crohn's disease (CD), 475, 932
  enhanced endocannabinoid, 954
  gastrointestinal (GI) tract, effect on, 932
  marijuana smoking, effect of, 954
Cross-reactivity, 518
  carbohydrate determinants (CCD), 520
  syndrome, 521
Cross-sectional data
  limitation, 302
  significance, 302
Cross-sectional studies, 46
CRT. *See* Cannabinoid replacement therapy (CRT)
Cryptotis parva, 953
Crystallization, 687
CSA. *See* Controlled Substances Act (CSA)
CSF. *See* Cerebrospinal fluid (CSF)
CSM program. *See* Climate schools model (CSM) program
CSQ. *See* Client satisfaction questionnaire (CSQ)
CTP. *See* Cannabis for therapeutic purposes (CTP)
CTQ. *See* Childhood trauma, questionnaire (CTQ)
CTS. *See* Cannabis, and tobacco (CTS)
CTT. *See* Critical tracking task (CTT)
CTZ. *See* Chemoreceptor trigger zone (CTZ)
CU. *See* Cannabis use (CU)
CUD. *See* Cannabis use disorder (CUD)
CUDIT. *See* Cannabis use disorders identification test (CUDIT)
Cue-elicited craving, 320
Cue-reactivity
  cannabis study, 322–323
  paradigm, 321
  virtual reality experiment, 323
Cultivation techniques, of *Cannabis*, 9
  indoor cultivation, 9
  outdoor cultivation, 9
  in vitro micropropagation, 9
Cultural/attitudinal predictor
  attitudes, 1041
  cognitive processing, 1040–1041
  emerging adulthood (EA), 1040–1041
  larger social environment, 1040
  pro-drug use myth, 1041
CUPIT. *See* Cannabis use problems identification test (CUPIT)
Current *Cannabis* varieties, 7
Current drug dependence diagnosis, 47
Current scientific classification, of *Cannabis*, 7
Cut down-annoyed-guilty-eye opener-adapted to include drug (CAGE-AID), e169
CVS. *See* Cyclic vomiting syndrome (CVS)
Cyclic adenosine monophosphate (cAMP), 593
  accumulation, 579
    assays, 586
  dependent protein kinase A, 566
  inhibition, 822
Cyclic vomiting syndrome (CVS), 467
Cycloalkylcarboxamide group, 600
Cyclobenzaprine, e161
Cyclohexylphenol (CP) synthetic cannabinoid, 841
Cyclooxygenase (COX), 796
  inhibition assay, 960
  inhibitor indomethacin, 512
Cyclooxygenase-2 (COX-2), 724, 803
  pathway, 731
Cyclopropanoylindoles, 721
CYP. *See* Cytochromes P450 (CYP450)
CYP1A1 inhibitors, 814
CYP1A2 inhibitors, 814
CYP1 enzymes
  active-site cavity, 817
  inhibition, 816
    significance, 818
Cysteinyl leukotrienes, 512
Cytochromes P450 (CYP450), 621, 814
  enzymes, e123
Cytokinins, 10

D

DAGL. *See* Diacylglycerol lipase (DAGL)
d-amphetamine, 941
D1 and D2 receptors, 64
DAST-10. *See* Drug abuse screening test-10 (DAST-10)
DAST-28. *See* Drug abuse, screening test (DAST-28)
DAT. *See* Divided attention task (DAT)
DBSNP. *See* Single nucleotide polymorphism database (DBSNP)
DC. *See* Dendritic cell (DC)
Decision-making (DM), 71
Deep layers of the superior colliculus (dlSC), e141
Degree of tolerance, 456
ΔEiso. *See* Stabilization energy (ΔEiso)
Delinquency, 216, 219
Delta-9-Tetrahydrocannabinol, effect of *vs.* cannabidiol, 875
Delusions, 44
Dementia, 829, 874
  marijuana, effect of, 874
Demographics
  characteristics, 54
  of drug trade, 123
Dendritic cell (DC), 518
Dendritic spines, 734
Density Functional Theory (DFT) methodology, e124
Department of Health and Human Services (DHHS), e187
Depolarization-induced suppression of inhibition (DSI), 632
Depression, 182
  cannabis, 311
  cannabis dependence, 311
Depressive symptoms, 415
Descriptive epidemiology of aggressive/disruptive behavior, e20
Descriptive epidemiology of cannabis use, e20
Designer drugs, e151, 841
Detection of drivers under the influence of cannabis (DUIC), 387
Developmental periods, risk factors for cannabis use during, e21
Deviant social milestones, 58

DFT methodology. *See* Density Functional Theory (DFT) methodology
DHHS. *See* Department of Health and Human Services (DHHS)
Diabetes mellitus (DM), 780
Diabetic dyslipidemia, 780
Diabetic neuropathy, 912
Diacylglycerol lipase (DAGL), 283, 618
  inhibition, 624
Diagnostic and Statistical Manual of Mental Disorders (DSM), e170, 299, 1067
Diaphoresis, 467
Diazepam, 775
Dibenzopyran-numbering system, 14
Diffeomorphic registration algorithm (DARTEL), e55
Differential association theory, 189, 190
Diffusion tensor imaging (DTI), 347, 393
  in adolescent cannabis use, 348
  in early phase psychosis, 347
Diffusion-weighted imaging (DWI), 491
Digital necrosis, 483
Digit-symbol-substitution task, 773
Dihydrocodeine, 912
2, 5-Dimethoxy-4-iodoamphetamine (DOI), 329
2, 4-Dinitro-1-fluorobenzene (DNFB), 545
Diphenhydramine, 912
1, 1-Diphenyl-2-picryhydrazyl (DPPH), e127
Disability-adjusted life years (DALYs), 95–98
Disability assessment schedule, 1088
Disability weights, 91
  calculation of YLDs, YLLs and DALYs, 91
  comorbidity adjustments, 91
  comparative risk assessment (CRA), 91
  data on regular (weekly or more frequent) cannabis use in the past year, 92
DISC1 gene. *See* Disrupted-in-schizophrenia-1 (DISC1) gene
Disrupted-in-schizophrenia-1 (DISC1) gene, 346
Divalproex, 943, 1031
Divided attention task (DAT), 380
dlSC. *See* Deep layers of the superior colliculus (dlSC)
DM. *See* Decision-making (DM)
DMV. *See* Dorsal motor nucleus of the vagus (DMV)
DNA banding, e3
DNA methylation, 726
DNA microarray, 726
DNFB. *See* 2, 4-Dinitro-1-fluorobenzene (DNFB)
Doctor–patient relationship, 531
DOI. *See* 2, 5-Dimethoxy-4-iodoamphetamine (DOI)
Domeperidone, 862
Doose syndrome, 799
Dopamine (DA), 181, 254, 279, 425, 535, 714
  craving development, 322
  D2/D3 receptor, 321
  examination, 290
  mesolimbic release, 319
  mesolimbic reward system, 293
  mesolimbic system, 289, 321
  neuronal firing, 416
  neurons, e30
  pathway, 319
    ventral tegmental area (VTA), 319
  producing neurons, 918
  release amphetamine-induced, 281
  reward-sensitive stimuli, 320
  synthesis capacity, 290
  uptake blocker and releaser, 941
  ventral striatum level, 321
Dopamine cells, 741
Dopaminergic agonists, 788
Dopaminergic hyperactivity, 824
Dopaminergic mesocorticolimbic system, 534
Dopaminergic system, 714
DOPE scoring, 685
Dorsal motor nucleus of the vagus (DMV), 948
Dorsal portions of periaqueductal gray (DPAG), e135
Dorsal raphe nucleus (DRN), 705
Dorsolateral striatum (putamen), 269
Dorsomedial striatum (caudate), 269
Dose–response cannabis effects, 30, 49, 415
Dose–response relationships, 47
Dose–response theory, 312
DPAG. *See* Dorsal portions of periaqueductal gray (DPAG)
DPPH. *See* 1, 1-diphenyl-2-picryhydrazyl (DPPH)
Dravet syndrome, 434–436
  clinical trial on cannabidiol, 436
Driving ability, 380
  attention, 380
  critical tracking, 380
  decision-making, 380
  reaction time, 380
Driving under influence of cannabis
  blood/serum concentrations of synthetic cannabinoids, 850
  epidemiology of, 235
  fatal crash studies, 237–238
  hospital studies, 237
  roadside surveys, 235–237
Driving-under-the-influence-of-drugs (DRUID), 849, 1003
DRN. *See* Dorsal raphe nucleus (DRN)
Dronabinol, 528, 912, 943, 953, 1071
  awoke less often, effect on, 880
  sleep-wake cycle, effect on, 880
  synthetic CB1 receptor agonist, 880
Drug abuse, e151
  craving, function of, 318
  dependence development, 281
  in females, 182
  screening test (DAST-28), 973
  side effects of, 181
Drug abuse screening test-10 (DAST-10), e181
Drug addicts, 182
  THC analysis, chromatogram, 1023
Drug control and treatment of addiction fund (DCTAF), 119
Drug courts, 195
Drug dependence, 873
Drug detection kits, 999
Drug discrimination, 771
  interoceptive effects, 772
  neuropharmacological interactions, 772
Drug-free children, best practices for raising, 220
Drug interactions, 47, 437
Drug readministration, 667
Drug recognition expert (DRE) protocol, 849
Drug-related activities, 124
Drug-relevant stimuli, 320
Drug replacement, 57
Drugs and sexual behavior, 181
  type of, 182
Drug-seeking behavior, 318
  effect of CBD, 941
Drugs, for pregnant and nonpregnant women, 168
Drug substitution, 54, 58, 59
Drug testing, e152
Drug use disorders, cannabidiol, treatment with, 940
Drug use disorders identification test (DUDIT), e170
Drug use in general population, 172–174
DRUID. *See* Driving-under-the-influence-of-drugs (DRUID)
DSI. *See* Depolarization-induced suppression of inhibition (DSI)
DSM-5. *See* Diagnostic and Statistical Manual of Mental Disorders (DSM)-5
Δ^9-Tetrahydrocannabinol (Δ^9-THC), 3, 62, 71, 80, 373, 433, 440, 462, 467, 472, 660, 704, 714, 723, 740, 782, 789, 796, 803, 814, 829, 940
  cannabidiol effects on brain stimulation reward, 660
  chemical structure of, 720
  in dyslipidemia, 782
  effect on CPP, 666
  in food intake and energy homeostasis, 783
  functional analogs, 719
  immunosuppressive effects, 783
  metabolic route, 463
  metabolites excretion in urine, 784
  narcotic properties, 720
  in oxidative stress, 782
  physical dependence, 667
  role in reward and dependence, 661
  in secretion of insulin, 782
  self-administration, 665
  self-administration in experimental animals, 665
  three-dimensional shape, 719
Δ^9-THC. *See* Δ^9-Tetrahydrocannabinol (Δ^9-THC)
DTI. *See* Diffusion tensor imaging (DTI)
Dual pathology, 67
DUDIT. *See* Drug use disorders identification test (DUDIT)
DUIC. *See* Detection of drivers under the influence of cannabis (DUIC)
Duloxetine, e161
Duration, frequency, and amount of cannabis usage, 73
DWI. *See* Diffusion-weighted imaging (DWI)

Dyskinesia, 824, 833
cannabinoid induced, 922
L-dopa-induced, 922
effect of cannabinoids, 923
human study, 922
nonhuman primates study, 922
Dysthymia, 1033

E
EA. *See* Emerging adulthood (EA)
EAE. *See* Experimental autoimmune encephalopathy (EAE)
Early onset cannabis use, 73
EAS. *See* Encephalic aversion system (EAS)
ECA. *See* Epidemiological catchment area (ECA)
eCBs. *See* Endocannabinoids (eCBs)
Ecological momentary assessment (EMA), 1103
Ecological momentary intervention (EMI), 1103
mobile technology, 1108
usage
addictive behavior change, 1103
alcohol dependence, 1103
mental health disorders, 1103
self-monitoring, 1103
smoking cessation, 1103
ECS. *See* Endogenous cannabinoid system (ECS)
Ecstasy, e102, 162, 172, 373
EDDRA. *See* Exchange on drug demand reduction action (EDDRA)
EDM. *See* Electronic dance music (EDM)
EDTA. *See* Ethylenediaminetetraacetic acid (EDTA)
Education, 48
EEG activity. *See* Electroencephalographic (EEG) activity
EETs. *See* Epoxyeicosatrienoic acid (EETs)
Effect sizes, 47
Egypt
cannabis abuse in selected population groups, 115
cannabis use in
patients with drug overdose, 115
patients with other medical conditions, 116
psychiatric patients, 116
school and university students, 115
cannabis, source of, 113
cigarette smoking and cannabis, 118
current status of cannabis use in, 113
gender and demographic differences in cannabis abuse, 116
hashish during 18th–20th Centuries, 111
initiation of drug/cannabis abuse, 117
drug abuse pattern among different categories, 119
Upper Egypt, 117
prevalence of factors associated with, and affecting, cannabis abuse, 114
relevance of cannabis use to educational level and marital status in Lower Egypt, 118
social and economic factors, 118
Eicosanoids, 617
Electroencephalographic (EEG) activity, 433, 611
Electronic dance music (EDM), e15
Elevated T-maze (ETM), e132
Eligible Studies
cases-control, key characteristics, e84
Cohort, key characteristics of, e83
pooled approach/re-analysis of secondary data, e81
ELISA. *See* Enzyme-linked immunosorbent assays (ELISA)
EMA. *See* Ecological momentary assessment (EMA); *See also* European Medicines Agency (EMA)
EMCDDA. *See* European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)
Emerging adulthood (EA), 1040
characteristic
endless possibilities, belief on, 1040
feeling in-between, 1040
lifestyle, 1040
own identity establishment, 1040
self-focused, 1040
EMI. *See* Ecological momentary intervention (EMI)
Emotional regulation, 62
Emperical article
summary of finding, 1042
EMT. *See* Endocannabinoid membrane transporters (EMT)
Encephalic aversion system (EAS), e141
Endocannabinoid, e72, e136, e141, e162, 753
2-arachidonoylglycerol (2-AG), e141
arachidonylethanolamide, 753
2-arachidonylglycerol, 753
N-arachidonoyl ethanolamide, e141
O-arachidonoylethanolamine, e141
other receptor, binding with vanilloid-1 receptor (TRPV1), 878
palmitoylethanolamide, 753
in pre- and postsynaptic neurons, e31
receptor
CB1, 878
CB2, 878
Endocannabinoid-hydrolysis inhibitors, 607
Endocannabinoid membrane transporters (EMT), 473
Endocannabinoid metabolism, 621
Endocannabinoids anandamide, 327
Endocannabinoid signaling system (ECS), 282–283, 416
component, 284
HPA function, mediation of, 283
psychosis risk, 283–284
stress regulation, role in, 283–284
stress-responsive neural circuit, 282
Endocannabinoids
N-arachidonoylethanolamine, 617
Endocannabinoids (eCBs) system, 63, 64, 81, 392, 405, 416, 452, 512, 609, 631, 651, 672, 741, 789, 860, 982
anandamide, 840
in anxiety, 63
2-arachidonoylglycerol, 840
in bipolar disorder, 63
composition
biochemical apparatus, 878
endogenous agonist, 878
receptor, 878
in depression, 62
implicated in the pathogenesis of depression, 63
link between stress, continuous cannabis use, and psychosis, 282–283
in psychosis, 64
schematic representation, 610
sensory innervation, 440
sleep modulation, role in, 878
sleep, relation with, 878–879
Endocannabinoid system (ECS), 641, 651
Endogenous acetylcholine, 746
Endogenous cannabinoid system (ECS), 71, e162, 472, 544, 948. *See also* Microglia
2-arachidonoylglycerol, e162
cannabinoid agonists, direct effect on receptors, 405–407
abnormal-cannabidiol receptors (abn-CBD), 406
neurodegenerative diseases, treatment in, 405
peroxisome proliferator-activated receptor (PPAR), 406
potential sites, sensitive to cannabinoid compounds, 406
synthetic cannabinoid analogs, 406
transient receptor potential cation channel (TRP), 406
cannabinoid receptors, e162
CB1 receptors, 948
CB2 receptors, 948
downstream effector of ECS deregulation, 408–410
downregulation and desensitization of, 408
effect of THC exposure in the neuronal circuit, 408
experimental data on impact of subchronic exposure to THC and withdrawal, 410
experimental data supporting, 409
microglial reactivity in cerebellum, 408
gut innervation controlling motility, role in, 948
ligands anandamide, e162
localization of components in gut, 472
in neurodegenerative diseases, 405
nonclassical receptor, 948
peroxisome proliferator-activated receptor, 948
transient receptor potential vanilloid-1 (TRPV1) channel, 948
Endometriosis, 446
eNOS protein expressions, 513
ENS. *See* Enteric nervous system (ENS)
Entacapone, 943
Enteric nervous system (ENS), 472
Enzyme inhibitors, 754
Enzyme-linked immunosorbent assays (ELISA), 988

Enzymes involved in cannabinoid biosynthesis, 16
Epidemiological catchment area (ECA), 299
Epidemiological studies, 415
  cannabis and psychosis, 82
  migration and psychosis, 83
Epigenetics, 718
Epilepsy, 437, 607, 796. *See also* Epileptic seizures
  risks/benefits, 432
    cannabis safe and well tolerated in patients, 437
    pro- and anticonvulsant effects of, 434
      compounds in established epilepsy, 434–436
      people without epilepsy, 434
    seizure, pathophysiological basis of, 432–434
  syndromes, 436
  treatment, 608
Epileptic seizures, 432, 433, 436
  cannabis use and effects, 435
  clinical signs and symptoms, 432
  first seizures, stratification of, 433
  treatment algorithm, 434
Episodic memory, 71
Epoxyeicosatrienoic acid (EETs), 621
EPS. *See* Extrapyramidal side effects (EPS)
EPSPs. *See* Excitatory postsynaptic potentials (EPSPs)
EQ-5D health survey, 934
ERα. *See* Estrogen receptor α (ERα)
ERK. *See* Extracellular-signal-regulated kinases (ERK)
EROD assay. *See* 7-Ethoxyresorfin *O*-deethylase (EROD) assay
Escitalopram, 943, 1032, 1071
ESPAD. *See* European School Survey Project on Alcohol and Other Drugs (ESPAD)
Estrogen, 672
Estrogen receptor α (ERα), 724
Estrogen signaling pathways, 726
Ethanol, 665
Ethnicity, 83, 84
Ethological models, e144
7-Ethoxyresorfin *O*-deethylase (EROD) assay, 814
  comparison of K_i values for inhibition, 815
  inhibition, kinetic parameters for, 815
Ethylenediaminetetraacetic acid (EDTA), 766
ETM. *See* Elevated T-maze (ETM)
Euphoria, 122
Eurobarometer telephone-based survey, 199
Europe
  age as a predictor of cannabis use, 34
  emphasis on cannabis control, 38
  past month cannabis use by age group and country, 37, 40
  past year cannabis use by age group, 39
  percentage of past year cannabis use by age group, 36
European Medicines Agency (EMA), e160, 651
European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 25, 33, e170, 841, 972, 984, 1078
European School Survey Project on Alcohol and Other Drugs (ESPAD), e178, e187, 199
Exchange-diffusion model, e104
Exchange on drug demand reduction action (EDDRA), 1078
Excitatory postsynaptic potentials (EPSPs), 632
Excitotoxicity, 803, 804, 829
Executive functions (EF), 47, e55, e59, e65–e67, 71, 152, 153, 262
  impairment of, 144
Exhaled breath
  after smoking of cannabis
    mean concentration of THC, 1022
  after smoking of THC
    THC, individual concentration, 1021
  alcohol testing, use for, 1019
  amphetamine presence, 1018
  analytical aspect, 1022
  cannabinoid presence, 1018
  cannabinoid, studies on, 1021–1022
  collection
    electrostatic filter technology, 1019
  component collection
    tenax tube, use of, 1019
  drug testing
    liquid chromatography-mass spectrometry (LC-MS), use of, 1019
    sampling device, 1020
    workflow, 1023
  drug testing, use for, 1019
  11-hydroxy-THC presence, 1019
  lipid, presence of, 1019
  nonvolatile component, presence of, 1019
  particle, presence of, 1019
  protein, presence of, 1019
  sampling, 1022
    collection time, 1022
    exhaled breath condensate, 1022
    impactor technology, 1022
  specimen for
    drugs of abuse testing, 1019
    toxicology investigation, 1019
  THC presence, 1018
    carboxylic acid, 1019
Exogenous cannabinoid
  CB1 receptor stimulation, 284
Experimental autoimmune encephalopathy (EAE), 673
Explanatory models, 366
  lower vulnerability hypothesis, 368
  neuro-protection hypothesis, 366
  social hypothesis, 368
Explicative hypothesis
  of cognitive functioning related to cannabis use in psychotic disorders, 48
Ex situ conservation in gene banks, 8
Externalizing disorders, 133
Extracellular domain, 558
Extracellular-signal-regulated kinases (ERK)
  cell differentiation, role in, 865
  phosphorylation, 566
Extrapyramidal side effects (EPS), 327
Extrinsic sensory neurons, 473

F

FA. *See* Fractional anisotropy (FA)
FAAH. *See* Fatty acid amide hydrolase (FAAH)
FAAH-related activities, 445
Face-to-face (F2F) treatment, e207
5F-APINACA *N*-(4-hydroxypentyl), 985
Fatty acid amide hydrolase (FAAH), 283, 510, 556, 594, 609, 619, 675, 724, 940, 948
  enzyme inhibitors, 63
  FAAHinhibitor URB597, 445
  pharmacological blockade
    colonic propulsion, delay in, 950
    GI transit, delay in, 950
Fatty acid derivatives, 655
FDA. *See* Food and Drug Administration (FDA)
Feddan, 113
Federal Analogue Act, e155
Feeding behavior, 643
Feeding inhibitors, 646
FEP. *See* First-episode psychosis (FEP)
FES. *See* First-episode schizophrenia (FES)
FEV1. *See* Forced expired volume in 1s (FEV1)
FFT. *See* Functional family therapy (FFT)
F2F treatment. *See* Face-to-face (F2F) treatment
Fibromyalgia, e159
  pathogenesis, e159
  symptoms, e159
  treatment, e160
    nonpharmacological therapies, e160
    pharmacological therapies, e160
Fibromyalgia (FM), 824, 872
Fibromyalgia Impact Questionnaire (FIQ), e163
FIQ. *See* Fibromyalgia Impact Questionnaire (FIQ)
First cannabis use, univariate logistic regression, 975
First-episode psychosis (FEP), e55, 257
  adherence and functionality, 260
  cannabis use and adherence, 260
  cannabis use and functionality, 261
  cannabis use as a risk factor for psychosis, 258
  high rate of cannabis use among, 258
  impact of cannabis use on cognitive functioning in, 262
  influence of cannabis use on clinical and functional outcomes, 259
  role of depressive symptoms in, 259
  structural magnetic resonance studies in patients and cannabis use, e56
  treatment for cannabis users, 263
  ventricular alterations in cannabis users with, e59
First-episode schizophrenia (FES), e55
First synthetic cannabinoids, chemical structures, 841
FLAIR imaging, 491

Fluorescence resonance energy, 579
Fluoxetine, 943, 1033, 1071
Fluvoxamine, 814
fMRI. *See* Functional magnetic resonance imaging (fMRI)
Focal cerebral ischemia (FCI), 407
Food and Drug Administration (FDA), e103, 941
Food intake, 473
Forced expired volume in 1 s (FEV1), 496
Forced swimming test, 964
Forced vital capacity (FVC), 496
Fourier transform infrared spectroscopy (FT-IR)
  functional group determination, 985
Fractional anisotropy (FA), 347, 393
Friends, influencing marijuana use, 190
  early adolescence, 190
  early adulthood, middle adulthood, and beyond, 193–194
  emerging adulthood, 192–193
  late adolescence, 191
  late childhood, 190
  middle adolescence, 191
Frontotemporal dementia (FTD), 829
FTD. *See* Frontotemporal dementia (FTD)
Functional family therapy (FFT), 1049
Functional implications, of cannabis use, 155–156
Functional magnetic resonance imaging (fMRI), e135, 270, 361, 940
  cognitive process, neural activation, 270
FVC. *See* Forced vital capacity (FVC)

G

GABA. *See* γ-Aminobutyric acid (GABA)
GABAergic inhibition, 635
GABAergic interneurons, 536
GABAergic neurons, 824
GABAergic pathways, e144, 721
GABAergic transmission, 374, 833
Gabapentin, e161, 943, 1033, 1071
Gabapentinoids, e161
GABA receptors, 771
  allosteric modulation, 774
  global CNS elevation, 772
  tiagabine-induced, 774
GAD. *See* Generalized anxiety disorder (GAD)
$G\alpha_i$ protein coupling, 566
Galvanic skin-conductance response, 320, 321
Gamma-aminobutyric acid (GABA), 62–64, e161, 1033
Ganja goo ball, e15
Gas chromatography (GC), 982
  drugs of abuse analysis, 982
Gas chromatography mass spectrometry (GC-MS), 239, 982, 999
  golden standard for qualitative analysis, 985
GASP1. *See* GPCR-associated sorting protein 1 (GASP1)
Gastric emptying, Δ^9-tetrahydrocannabinol, effect of, 950
Gastroesophageal reflux disease (GERD)
  cannabinoid, effect of, 953
  mechanism
    transient lower esophageal sphincter relaxations (TLESRs), 953
  symptom
    heartburn, 953
    regurgitation, 953
    retrosternal pain, 953
Gastrointestinal (GI), 472
  inflammation
    endogenous cannabinoids, role of, 951
    exogenous cannabinoids, role of, 951
    TRP channel, role of, 962
  motility, 474
    cannabinoid, effect of, 949–952
  motor function
    cannabinoid, effect of, 948
    cisplatin-treated rat
      WIN55, 212-2, effect of, 954
    endocannabinoid system
      role of, 951
      targetting drug, effect of, 952
    extrinsic neural control, 951
  muscle contraction
    CB1receptor agonists, effect of, 949
  origin, cannabinoid involvement in visceral pain, 443
Gastrointestinal (GI) tract, 442, 948
  small/large intestine, 444
  stomach, 444
Gatehouse project (GP), 1081
GC. *See* Gas chromatography (GC)
GC-MS. *See* Gas chromatography mass spectrometry (GC-MS)
GDS. *See* Global Drug Survey (GDS)
Gene–environment interaction (GxE), e31, 426
Gene expression, 714
Generalized anxiety disorder (GAD), e161, 304
General substance use, common liability to, 177
Genetic and environmental risk factors (GxE), 336
Genetic confounding, 426
Genetic markers, for forensic identification, e7
Genetic mouse models, 338
Genetic polymorphisms, 585, 714
Genetics, of *Cannabis*, 7
"Gene x environment" (GxE) interaction model, 82
Genitourinary tract, 445
  endometrium, 446
  kidney and ureter, 445
  ovary, 445
  prostate, 446
  testes and scrotum, 446
  urinary bladder and urethra, 445
Genome, of *Cannabis sativa*, 7
Genome-wide association studies (GWAS), 585
Genotypes and chemical phenotypes of cannabis, e7
Genotyping, 585
Genuine cannabis allergy, 521
Geographical distribution, 3, 8
Geranyl diphosphate, 14
  biosynthesis of, 16
GERD. *See* Gastroesophageal reflux disease (GERD)
German version of the auditory verbal learning test (VLMT), 888
Germplasm Resources Information Network (GRIN) database, 8
GH. *See* Growth hormone (GH)
GHB (gamma-hydroxybutyrate), 172
Ghrelin (GHRL), 643, 780
  basal levels, 643
  central administration, 644
  interaction with peripheral CB1 receptors, 644
  receptors, 644
GHRH. *See* Growth hormone releasing hormone (GHRH)
GHRL. *See* Ghrelin (GHRL)
GI. *See* Gastrointestinal (GI)
Gilles de la Tourette syndrome (GTS), 884
  alternative forms of treatment, 885
  cannabinoid based medicines (CBMs), treatment with, 885, 886
    adverse effect, 890
  cannabinoids, treatment with, 885, 886
    performance-enhancing effect, 887
  Cannabis sativa, treatment with, 886–887
    case report, 886
    controlled trials, 887
    prospective survey, 887
  causes
    environmental factor, 884
    multiple genetic susceptibility factor, quantitative effect of, 884
  comorbid condition
    attention deficit hyperactivity disorder (ADHD), 884
    obsessive compulsive behavior (OCB), 884
  diagnosis, 884
  etiology, 884
  fatty acid amide hydrolase (FAAH) inhibitor, effect of, 887
  features
    motor tics, 884
    vocal tics, 884
  pathophysiology, 884
    endocannabinoid system and neurotransmitter pathways, role of, 885
    imbalances in the dopaminergic (DA) system, 884
      cortico-striato-thalamo-cortical (CSTC) circuitry, 884
  THC, treatment with, 887–888
    adverse effects, 888
    randomized controlled trials, 888
    uncontrolled single case study, 887
  treatment, practical aspect, 890–891
  treatment strategies, 885
    alternative medicine, 885
    behavioral therapy, 885
    deep brain stimulation, 885
    pharmacological therapy, 885

GIRK. *See* G-protein-coupled inwardly-rectifying K^+ channels (GIRK)
GI tract. *See* Gastrointestinal (GI) tract
Glandular trichome, e3
Glaucoma, 749, 761
  intraocular pressure, 749
  intraocular pressure lowering, 761
  retinal neuroprotection, 762
  treatment, 749
Glial fibrillary acidic protein (GFAP), e105
Glial tumors, e112
  multimodal action of cannabinoids against, e117, e118
    antitumoral action of cannabinoids in vivo, e117
    effects of cannabinoids on angiogenesis, cell migration, and invasion, e118–e119
Glioblastoma multiforme (GBM), e112, 866
  cannabinoid, effect of, 866
  expression of CB2 receptor in human glioblastoma, e113
Gliomas
  alterations of major components of cannabinoid system in, e113
  cell culture
    cannabinoid, effect of, 866
  therapeutic potential of cannabinoids against human gliomas, e119
    results from a pilot clinical study, e119
    risks and constrains, e119
    safety profile of therapeutic cannabinoids, e119
Global burden of disease (GBD), 90
Global drug survey (GDS), e187
Globus pallidus, 918
Glucocorticoid, 672
Glucose homeostasis, 782
Glutamate, 321, 425
Glutamate receptors, 631
Glutamatergic neuronal system, 330
Glutamatergic neurons, 330
Glutamatergic synapses, 330
Glutathione peroxidase (GPx) enzymes, 782
Glycogen synthase kinase 3β(GSK-3β), 805
Goza, 113
GPCR. *See* G protein-coupled receptor (GPCR)
GPCR-associated sorting protein 1 (GASP1), 560
GPCR kinase (GRK), 566
GPR55, orphan G-protein linked receptor, e114
G-protein-coupled inwardly-rectifying K^+ channels (GIRK), 560
G-protein coupled receptor (GPCR), 558, 565, 574, 584
  allosteric modulation, 574
  signaling, 683
G protein coupling promiscuity, 568
G-proteins. *See* Guanine-nucleotide-binding proteins (G-proteins)
GPx enzymes. *See* Glutathione peroxidase (GPx) enzymes
Gray matter (GM), e54, e55, 153
  brain structural analysis displaying clusters of significant decreased gray matter, e58
GRK. *See* GPCR kinase (GRK)
Grooved pegboard test, 376
Growth hormone (GH), 643
Growth hormone releasing hormone (GHRH), 645
GSK-3β. *See* Glycogen synthase kinase 3β(GSK-3β)
GTS. *See* Gilles de la Tourette syndrome (GTS)
GTS-CGI. *See* Tourette's syndrome clinical global impression scale (GTS-CGI)
Guanine-nucleotide-binding proteins (G-proteins), 558
Gut disorder, cannabis, use of, 948
GWAS. *See* Genome-wide association studies (GWAS)
GxE. *See* Genetic and environmental risk factors (GxE)

H

Habit formation
  associative memory processes, role of, 269
  neuroscience of dual systems, role of, 269
Habitual cannabis, 66
HADDOCK. *See* High Ambiguity Driven DOCKing (HADDOCK)
HADS. *See* Hospital Anxiety Depression Scale (HADS)
Hallucinations, 44, 80
Haloperidol, 330, 862
Harvey–Bradshaw index, 933
  result, 935
Hashish during 18th–20th Centuries, 111
HBCC. *See* Human breast cancer cell (HBCC)
HBSC. *See* Health behavior in school-aged children (HBSC)
HD. *See* Huntington's disease (HD)
Head and neck cancers
  alcohol and tobacco use, e96
    mutagenic effects of, e96
    risk factors, e97
  case-control study in, Boston, e96
  INHANCE Consortium, e97
  marijuana use and, e96
    epidemiological studies, e98
    potential relation, e96
  sexual behavior, confounding by, e96
Health behavior in school-aged children (HBSC), e187
Health risk, 30
Healthy life style trial
  tobacco smokers with psychosis, e207
Heart, 441
  disease, 489
HEIA. *See* Homogeneous enzyme immunoassay (HEIA)
Heme oxygenase-1 enhanced portal hypertension, 513
Hemp, e2, e4, e5
  varieties, 8
Hepatic CYP isozymes, 437
Hepatic stellate cells (HSCs), 506
Herbal cannabis, THC and CBD levels, 25
Herbal smoking blend. *See also* Smoking
  synthetic cannabinoid, use of
    chemical structures, 844
Herbal smoking mixture, 840
hERG potassium channel assay, 653
Heroin self-administration
  effect of CBD, 941
Heterocycles, 655
Heterodimerization, 574, 579, 725
Heteromers, 579
Heterosynaptic LTD, 633
HETEs. *See* Hydroxyeicosatetraenoic acid (HETEs)
Hexanoyl-CoA synthetase, 15
HFS. *See* High frequency stimulation (HFS)
5-HIAA. *See* 5-Hydroxyindoleacetic acid (5-HIAA)
High Ambiguity Driven DOCKing (HADDOCK), 685
Higher exposure, to THC and risk of adverse health effects, 28
Higher potency cannabis, and plasma THC levels, 26
Highest occupied molecular orbital (HOMO), e124
High frequency stimulation (HFS), 633
High-resolution computed tomography (HRCT), 497
High resolution mass spectrometry (HR-MS), 983
  advantage, 983
    nontargeted compound, data-dependent acquisition of, 983
  metabolic patterns of synthetic cannabinoid, use in, 983
  urine biomarker identification, use in, 983
Hippocampus, e54, 71
Histomorphometric study, in rats
  activity of osteoblasts, e75
  bone formation, e75
  bone inflammation around teeth, e76
  bone loss, during ligature-induced periodontitis, e76
  bone resorption, e75
  bone-to-implant contact, reduction in, e76
  cannabinoid receptors (CB1 and CB2), activation, e75
  cannabis smoke inhalation, e76
  cannabis smoking, cancellous bone healing impact of, e73
  degree of bone-to-implant contact, e73
  effect of marijuana inhalation, e76
  endocortical osteoblast number, enhancement, e76
  furcation region of rat molar, e77
  immune targets, cannabinoid-based drugs, e75
  periodontal inflammation, regulation of, e76
  periodontitis, effects of cigarette smoking, e73
  photomicrographic illustration, bone healing in implant surface, e74, e75
  retention device for, oral microorganisms, e76

risk factor for, e76
tetrahydrocannabinol (THC) detection in, urine samples, e75
THC effect in, inhibiting CB2 expression, e76
thirty Wistar rats, e73
titanium implants, decrease bone mass around, e73, e75
HIV painful neuropathy (HIV-PN), 911
HIV-PN. *See* HIV painful neuropathy (HIV-PN)
^{1}H-MRS. *See* Proton magnetic resonance spectroscopy (^{1}H-MRS)
Homicide rates, 127
HOMO. *See* Highest occupied molecular orbital (HOMO)
Homodimeric enzyme, 15
Homogeneous enzyme immunoassay (HEIA), 988
Homosynaptic LTD, 634
Homovanillic acid elevation, 282
Homozygous THC producing *BtBt* genotypes, 8
Hopkins verbal learning test, 1088
Hospital Anxiety Depression Scale (HADS), e162
HPA. *See* Hypothalamic-pituitary-adrenal axis (HPA)
HPMC. *See* Hydroxypropyl methyl cellulose (HPMC)
HPT-axis. *See* Hypothalamic-pituitary-thyroid axis (HPT-axis)
HRCT. *See* High-resolution computed tomography (HRCT)
HR-MS. *See* High resolution mass spectrometry (HR-MS)
HSCs. *See* Hepatic stellate cells (HSCs)
5-HT. *See* 5-Hydroxytryptamine (5-HT)
5-HT$_{1A}$ receptors. *See* 5-Hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptors
5-HTP. *See* 5-Hydroxy-l-tryptophan (5-HTP)
HU-210, 920
HU-308, 920
Human breast cancer cell (HBCC), 723
Human bronchial pathology, 498
Human colon adenocarcinoma cell line HT29 CBG, 960
Human functional imaging technique, 270
Human ghrelin, chemical structure, 643
Human in vivo neuroimaging techniques, 346
Human motor cortex, 374
Human studies, 506
Human trials, 749
Humulus lupulus (hop), 14
Huntington's disease (HD), 405, 434, 806, 829
cannabinoid signaling, 924
characteristic
behavioral deficits, 918
cognitive, 918
motor dysfunction, 918
effect of cannabinoids, 924
clinical data, 924
genetic model, 926–927
effect of cannabinoids, 927
Hydromorphone, 771
Hydrophilic prodrug derivatization, 766
Hydrophobic interactions, 685
11-Hydroxy-delta 9-tetrahydrocannabinol (11-OH-THC), 1010, 1011, 1013
6-Hydroxydopamine-induced apoptosis, 920
6-Hydroxydopamine lesioned rat, 920, 922
Hydroxyeicosatetraenoic acid (HETEs), 621
5-Hydroxyindoleacetic acid (5-HIAA), 706
Hydroxylation, 238
5-Hydroxy-l-tryptophan (5-HTP), 329
N-(4-Hydroxyphenyl)-arachidonamide, 920
Hydroxypropyl methyl cellulose (HPMC), 766
11-Hydroxy-THC (11-OH-THC), 238
5-Hydroxytryptamine (5-HT), 329
5-Hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptors, 704
agonists, 706
antagonists, 706
Hyperalgesia, 906
Hyperemesis, 467
Hyperglycemia, 780
Hyperpolarization, 577
Hypertelorism, 163
Hyperthermia, e105
Hypocretinergic system, 538
Hypocretin system, 534
addictive properties of drugs abuse, role in, 535
cannabinoid dependence, role in, 538
mRNA levels, 538
potential therapeutic utility, 536
Hypokinesia
haloperidol-induced, 887
Hypolocomotion, 741
Hypomania, 422
Hypomotility, 651
Hypothalamic-pituitary-adrenal axis (HPA), 279
long-term cannabis use, effect of, 952
Hypothalamic-pituitary-thyroid axis (HPT-axis), 462
Hypothermia, 651, 741
Hypoxic-ischemic brain injury, 803

I

IBD. *See* Inflammatory bowel disease (IBD)
IBS. *See* Irritable bowel syndrome (IBS)
Ibuprofen, 824
IC. *See* Inferior colliculus (IC)
ICD. *See* International classification of diseases (ICD)
ICD-10 criteria, 1087
ICH. *See* Intracerebral hemorrhage (ICH)
ICSS paradigm. *See* Intracranial self-stimulation (ICSS) paradigm
IgE-mediated reaction, 518
primary, 518
principles, 518
secondary, 518
ILAE. *See* International League Against Epilepsy (ILAE)
Ill-being, cannabis, 311
Illegal drug usage, e155
Illegal recreational drug
cannabis, 940
stimulant, 940
Illicit Drug Reporting System (IDRS), 104
Illicit drugs, e12, 131, 161, 171, 201
Illness-inducing agent, 705
Imidazopyridines, 602
Immune cells, 559
Immune thrombocytopenic purpura (ITP), 587
Immunoallergic vasculitis, 490
Immunoassay screening
urine screening, 982
Immunocytochemical analyses, 559
Immunohistochemical analyses, 444
Immunohistochemistry, e113
Immunological methods, 239
Immunosuppression, 437
Implicit association test (IAT), 269
attitude examination, 269
behavior-related associative memory structure, examination of, 269
imaging, 270
INCANT. *See* International cannabis need of treatment trial (INCANT)
Incarceration, 124
Incidence rate ratio
CapOpus group
vs. TAU, 1089
Incompatible trial
regions of activity within group, 274
Indole, 593
biological data, 595
chemical structures, 596
Indoor cultivation, of *C. sativa*, 9
Inducible nitric oxide synthase (iNOS), 963
Inferior colliculus (IC), e141
Inflammatory bowel disease (IBD), 475, 932, 953, 962
cannabis, treatment with, 918
beneficial effects, 921
hospitalization risk, 922
side effect, 922
surgery risk, 922
cannabis user *vs.* nonusers of cannabis, 922, 927
Crohn disease (CD), 954
patient, beneficial effects of cannabis, 925
treatment
inhaled cannabis, 921
option, 932
self-administration of cannabis, 921
ulcerative colitis (UC), 954
Inflammatory steatohepatitis, 587
Inheritance of chemical phenotype, in *C. sativa* "codominant monogenic control, ", 8
Inhibition, 152, 153, 156
iNOS. *See* Inducible nitric oxide synthase (iNOS)
In situ conservation as in vitro gene banks, 9
Insomnia, 824
marijuana, use of, 872

Instrumental conditioning, 318
Instruments, for assessing premorbid IQ, 226
Instruments selection
  from the literature, e170–e171
Intellectual quotient (IQ), 224
  Flynn–Dickens model, 227
  longitudinal studies on premorbid IQ, 227–229
  premorbid IQ and cannabis
    findings from longitudinal studies, 229
    risk, 229–231
Intensive forms, of drug use, 973
Internalizing disorders, 133
International cannabis need of treatment trial (INCANT), 1048, 1050, 1051
  flow diagram, 1051
  outcomes, 1051
  treatment site characteristic, 1050
International classification of diseases (ICD), 90, e170, 299
International League Against Epilepsy (ILAE), 607
International Lung Cancer Consortium, e95
Interpersonal predictor, 1041–1042
  family influence, 1042
  peer influence, 1041
Interpretation of result, 1014–1015
  historical use, 1015
  medicinal use, 1015
  proof of use, 1015
  recent use, 1014
Inter simple sequence repeat amplification (ISSR), e7
Intervention, 1087–1088
  CapOpus trial, 1087–1088
  treatment as usual (TAU), 1088
Intervention design
  marijuana use reduction, 1104
Intervention group
  time to first psychiatric emergency room contact, 1090
  time to first psychiatric hospital contact, 1091
Intestinal inflammatory condition
  cannabinoid, effect of, 953–955
Intoxications, 224, 433
Intracerebral hemorrhage (ICH), 488
Intracranial self-stimulation (ICSS) paradigm, 660, 941
Intraocular pressure, 749
  cannabinoid-induced reduction, cellular mechanisms, 755
    ciliary epithelium, 755
    trabecular meshwork, 755
Intrapersonal predictor, 1038–1039
  cognitive processing, 1039
  neurobiological, 1038–1039
  self-image and deviant behavior, 1039
In vitro micropropagation of leaf-derived calli from *C. sativa* L., 10
Ion channel transient receptor potential A1 (TRPA1), 445
Ionization potential (IP), e124
Ionomycin, 962
Iowa gambling task (IGT), 293
  performance, differences in
    between chronic marijuana users and control, 294
  PET activation, differences in
    between chronic marijuana users and control, 294
IP. *See* Ionization potential (IP)
IQ scores, e59
Irritable bowel syndrome (IBS), 953
  characteristic
    abdominal discomfort, 954
    altered bowel habits, 954
    bloating, 954
IRT analysis. *See* Item response theory (IRT) analysis
Ischemia, 407
  reperfusion injury, 507
Ischemic ulcers, 483
Islets of Langerhans, 780
Item response theory (IRT) analysis, 318
ITP. *See* Immune thrombocytopenic purpura (ITP)

J
Jam band performance, e14
Joints, e169
JWH-018, 841
JWH-081, 982
JWH-122, 982
JWH-210, 982
JWH chemical compounds, e153

K
Kaempferol, 814
Kainic acid model, 611
Karyotype, e4
Keratinocytes, 453, 544
  atopic-like inflammation, regulation of, 547
  endogenous cannabinoid system, 544
  proinflammatory chemokines, secretion of, 546
Ketamine, 172, 173, 175, 636, 790
Ketoprofen, 453
Khat plant, e103
K2/Spice, synthetic cannabinoids, 441, 849
Kupffer cells, 513

L
l-α-Lysophosphatidylinositol (LPI), 407
Lapse situation
  intervention strategy, 1061–1062
Lateral septum (LSV), 339
Laxatives, 123
LC. *See* Liquid chromatography (LC)
Learning theory, 318
Leeds Sleep Evaluation Questionnaire (LSEQ), e165
Left inferior frontal gyrus, 270
Legal considerations, 124
Legal drugs, e153
Legal highs, e151, 841, 854
Legislation, 239
  approaches to, 239
  Australia, 240
  Canada, 240
  European Union, 240
  impairment legislation, 239
  United States, 240
  zero-tolerance laws, 239
Lennox–Gastaut syndrome, 436, 799
Leonotis leonurus, 840
Leonurus sibiricus, 840
Levonantradol, 862
Leydig cell, 965
LFP. *See* Local field potential (LFP)
Libido, 181
Life satisfaction, 289
Life skills training (LST), 1078, 1082
Likert-type scale, 1028
Limbic-motor interface regulating stress response, 872
Limits of detection (LOD), 854
Lipopolysaccharide (LPS), 405
Lipoxin A4, 579
Lipoxygenases (LOX), 621
  pathway, 15
Liquid chromatography (LC), 982, 986, 995
  aqueous biological sample analysis, 982
  bioanalysis, 982
Liquid chromatography coupled to mass detector (LC-MS or LC-MS/MS), 239, 999, 1019, 1022
Literatures
  regarding impairment after long-term use of cannabis, 29
  regarding impairment of acute cannabis effects, 28
Lithium, 1071
Lithium carbonate, 1031
Liver, 444
  disorders, 587
Liver cirrhosis
  effect of cannabinoids, 510
  hemodynamic changes, 512
Local field potential (LFP), 797
LOD. *See* Limits of detection (LOD)
Lofexidine, 1031, 1032, 1071
Longitudinal studies, 35
  on premorbid IQ, 227–229
Long-term depression (LTD), 714, 734
Long-term marijuana use. *See also* Marijuana
  brain, effect on, 874
Long-term potentiation (LTP), 714, 734
Long-term video-EEG, 436
Lorcaserin, 650
Lower GI tract, functional disorders, 474
Lower urinary tract symptoms (LUTS), 441
Lower vulnerability hypothesis, 368
Lowest unoccupied molecular orbital (LUMO), e124
LSD (lysergic acid diethylamide), 172
LSEQ. *See* Leeds Sleep Evaluation Questionnaire (LSEQ)
LST. *See* Life skills training (LST)
LSV. *See* Lateral septum (LSV)
LTD. *See* Long-term depression (LTD)
LTP. *See* Long-term potentiation (LTP)

LUMO. *See* Lowest unoccupied molecular orbital (LUMO)
Lung adenocarcinoma
  mice animal model, 865
Lung cancer, 498
  and alcohol use, e81
  case control study in New Zealand, e82
  data from, Maghrebian region, e95
  family history, e82
  histological changes in, tracheobronchial epithelium, e82
  logistic-regression models, e95
  marijuana use in late adolescence/early adulthood, e81, e95
    epidemiological studies, e98
  risk of, e95
  and tobacco use, e81

M

Mad hatter, 988
MAGL. *See* Monoacylglycerol lipase (MAGL)
Magnetic resonance imaging (MRI), e59, 433
Magnetic resonance spectroscopy (MRS), 350, 375
  in cannabis use, 351
  in early psychosis, 351
Main myenteric neuronal population
  endocannabinoid system, components of, 950
Major depressive disorder (MDD), 300, 310
Major histocompatibility complex (MHC), 894
Malignant cardiac arrhythmia, 482
Malondialdehyde (MDA), 782
Malonyl-CoA, 15
Manchester short assessment of quality of life (MANSA), 1088
Mania, 422
Manolis study from 1983, 1019
MANSA. *See* Manchester short assessment of quality of life (MANSA)
MAPKs. *See* Mitogen-activated protein kinases (MAPKs)
Marijuana, e2, 3, e12, 25, 39, e80, 162, 181, 189, 194, 481, 527, 730
  abuse, 1070
  and alcohol use, e81
  Cannabis sativa, 440
  cardiovascular consequences, 482
    cardiomyopathy, 482
    cerebrovascular effects, 483
    myocardial infarction, 482
    myocardial ischemia, 482
    peripheral vascular disease, 483
  cigarette, 818
  consumption, 181
  desire, 1102
  detection in biological specimen, 1008
  development of cancer, e81
  electronic dance music (EDM), e15–e16
  eligible articles, e81
  friends impact use, 189
  immunoassay test, 982
  inhalation route, 527
  jam band subculture, e14–e15
  legalization, 1102
    in the United States, 195
  motherhood, legal concerns, 531
  physiological effects, 482
  picture, 271
  polydrug use, 1102
  primary care, brief treatment, 1102
  recreational use, 1102
  smoker, 1019
  smoking, in form of blunts within hip-hop subculture, e12, e13
  structural brain change, 1102
  substance use disorder, 1102
  and tobacco use, e81
  trajectories, use for those in early adulthood, 193
  treatment project, 1030
  usage, e81
    adolescent, 1102
    among jazzmen, e12
    behavioral study, 269, 1102
    contextual factor, 1102–1103
    disorder, 871
    effect
      apathy, 289
      lack of motivation, 289
      lethargy, 289
    habit-related change, 274
    implicit association toward, 271
    intervention
      counseling plus mobile technology, use of, 1104
    mean momentary desire, 1107
    momentary factor, 1102–1103
    performance on a motivational task, 294
    positive subjective-effect rating, increase in, 1102
    sense of euphoria production, 1102
    social anxiety, effect of, 1103
    social environment, effect of, 1103
    young adult, 1102
  user *vs.* nonuser, 269
  withdrawal symptoms, 871
Marijuana-IAT imaging, 270–271
  incompatible trial
    temporal layout of, 272
  inferior frontal gyrus, 272
  interaction effect, 273
  orbitofrontal cortex, 272
  paradigm, 271–274
Marijuana plant
  cannabidiol (CBD), 872
  delta-9-tetrahydrocannabinol (THC), 872
Marijuana substitute, 840
  bay bean, 840
  blue lotus, 840
  honeyweed, 840
  Indian warrior, 840
  lion's tail, 840
  maconha brava, 840
Marinol, 862, 1010
Mass spectrometry (MS)
  clinical application, 982
  forensic analysis, 982
Mast cell, 932
Master's-level university librarian (TF), e81
Maternal breast milk, 529
Matrix metalloproteinase 2 (MMP-2) activity, 509
Maximal finger tapping, 375
MCF-7 cells, 724
MD. *See* Molecular dynamics (MD)
MDA. *See* Malondialdehyde (MDA)
MDFT. *See* Multidimensional family therapy (MDFT)
MDMA. *See* 3, 4-Methylenedioxymethamphetamine (MDMA)
MDMA (3, 4-methylenedioxy-*N*-methylamphetamine), 172
  reinforcing effects, e105
Mean diffusivity (MD), 393
Meconium, 162
Median raphe nucleus (MRN), 705
Medicago sativa, 15
Medical cannabis, 34
  well being, direct effect on, 310
  well being, indirect effect on, 310
Medical marijuana, 869–871. *See also* Marijuana
  acute immune deficiency syndrome (AIDS), effect on, 870
  antiemetic properties, 870
  central nervous system effect, 870
  epilepsy, effect on, 870
  insomnia medicine, 870
  mental disorder, effect on, 871
  multiple sclerosis (MS), effect on, 870
  short-term physical effect, 870
Medication, 823
  compliance, 203
Medicinal properties, of cannabis plant, 451
Medicines and Healthcare Products Regulatory Agency (MHRA), 822
Mental health
  human endocannabinoid system, effect of, 301
Mental illness
  cannabis use, cooccurrence with, 299–302
    cross-sectional data, 302
  cannabis user, 301
  concurrent cannabis use mechanism, 301
  prevalence of cannabis use, 300
5-MeODMT. *See* 5-Methoxy-*N, N*-dimethyltryptamine (5-MeODMT)
6-Mercaptopurine, 932
Meristemoid formation, 10
Mesolimbic dopamine system, 289
Mesolimbic/mesocortical DA system, 282
MET. *See* Motivational enhancement therapy (MET)
Metabolic disturbances, 433
Metabotropic glutamate receptors (mGluRs), 631
METH. *See* Methamphetamine
Methadone, 940
  improved substance abuse outcome, 940
Methadone induction, 874
Methamphetamine (MA), 48, 172, 181, 373, 941

Methamphetamine (METH), e102
annual prevalence of, in United States, e103
as crystal/chalk/ice, e103
dopaminergic neuronal activity, e104
Methionine (Met) allele, 418
Methodological limitations, 50
Methotrexate, 932
5-Methoxy-*N*, *N*-dimethyltryptamine (5-MeODMT), 329
Methylenedioxymethamphetamine (MDMA), e12, e15
3, 4-Methylenedioxymethamphetamine (MDMA), e15, 655
cathinone, e103
consumption, e104
dopaminergic neuronal activity, e104
dopaminergic stimulation, e103
dysphoric symptoms of, ecstasy, e103
psychostimulant drugs, e103
in synthesis of styptic drugs, e104
Methylphenidate, 771, 885, 941
1-Methyl-4-phenyl pyridinium [MPP(+)]
induced cell death, 920
1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)
neurotoxic effect, 920
Metochlopramide, 862
MGL. *See* Monoacylglycerol lipase (MGL)
mGluRs. *See* Metabotropic glutamate receptors (mGluRs)
MHC. *See* Major histocompatibility complex (MHC)
MHRA. *See* Medicines and Healthcare Products Regulatory Agency (MHRA)
MI. *See* Motivational interviewing (MI)
Mice xenograft model, 865
Michigan alcohol screening test, e181
Microglia, 674
activation process
activated state, 402
as active sensors, 402
antiinflammatory effects, 404
cannabinoid receptors, 404
chemokine-receptor, 404
effector cells, 403
expression of "Off" signals, 403, 404
motility control depends on ATP, 402
"On" signals, triggering process, 403, 404
proinflammatory genes activation, 403
purinergic 2X ionotropic receptors, 403
P2Y metabotropic receptors, 403
resting state, 402
signal regulatory protein α (SIRPα) receptor, 404
toll-like receptors (TLR), 403
endogenous cannabinoid system
cannabinoid agonists, direct effect of, 405
cannabinoid agonists, direct effect on receptors, 405–407
downstream effector of ECS deregulation, 408–410
endocannabinoids (eCBs), 405
in neurodegenerative diseases, 405
hematopoietic origin, 402
im central nervous system (CNS), 402
Microscopic photographs of *C. sativa* trichomes, 5
Midazolam, 775
Midbrain tectum (MT), e143
Migration phenomena, 83
cannabis use, and psychosis onset, 85
causing psychotic disorders, 84
risk factors for first episode psychosis in migrants, 85
risk of first episode psychosis (FEP) in populations, 84
types of, 83, 84
Military substance use, e154
media coverage, e154
Milnacipran, e161
Mineralocorticoid, 672
Miniature inhibitory postsynaptic currents (mIPSCs), 578
mIPSCs. *See* Miniature inhibitory postsynaptic currents (mIPSCs)
MIS. *See* Multifocal arterial stenosis (MIS)
MIST. *See* Montreal imaging stress task (MIST)
Mitochondria dysfunction, 831
Mitogen-activated protein kinases (MAPKs), 452, 560, 632, 682
MMP-2 activity. *See* Matrix metalloproteinase 2 (MMP-2) activity
Mobile technology
ecological momentary intervention (EMI), delivery of, 1108
marijuana use reduction, 1104
Modafinil, 941
Modeling, earlier age of onset of schizophrenia, 92
additional burden attributable to cannabis use as a risk factor for schizophrenia, 93
burden of cannabis dependence, 93
country-level DALYs, 97
estimated DALYs attributable to regular cannabis use as a risk factor for schizophrenia, 2010, 98
estimated DALYs for cannabis, by sex and region, 2010, 96
estimated prevalence and number of cases of cannabis dependence in 2010, by sex and region, 95
global cannabis dependence DALYs (all YLDs) by age and sex, 95
implications of findings, 96
limitations, 99
modelling increased severity of Schizophrenia, 92
pooled regional prevalence of cannabis dependence, 2010, 94
prevalence of cannabis dependence, 92
proportion of DALYs due to cannabis dependence relative to, 98
Modulatory sites, 574
Molecular classification, 7
Molecular dynamics (MD), 686
MOLPROBITY, 684
Momentary self-monitoring and feedback + motivational *en*hancement therapy (MOMENT) intervention, 1103–1108
component, 1103, 1104
design, 1104
formative research, 1104
MET sessions
field note, 1107
mobile technology, use of, 1104
momentary reporting, 1105
pilot study, 1104
data collection, 1105
pilot study phase
momentary, daily, and individual outcome
differences in, 1106
individual-level mean, 1106
standard deviation, 1106
potential challenges to implement, 1108
randomized controlled trial, 1105
responsive messaging, 1105
Monoacylglycerol lipase (MAGL), 283, 340, 556, 609, 618, 631, 948, 960
Monoamines dopamine (DA), e103
Monocyclic cores, 597
biological data, 596
chemical structures, 597
pyridines, 598
pyrimidines, 598
sulfamoyl benzamides, 598
Monte Carlo simulation, 684
Montreal imaging stress task (MIST), 280–281
Mood disorder, 871
cannabinoids, effect of, 864
cannabis, 302–303
course of
cannabis use, effects of, 303
and insomnia, 864–865
marijuana, effect of, 871
serotonergic system, effect of, 864
three-year incidence
lifetime cannabis user *vs.* nonuser, 302
tryptophan, effect of, 864
Morphine, 455
brain reward function
effect of CBD, 941
reward-facilitating effect
effect of CBD, 941
Morphine-dependent rodent
abstinence scores
effects of CBD, 941
naloxone-precipitated withdrawal, 941
withdrawal
effects of CBD, 941
Morphine withdrawal symptoms
naloxone-precipitated, 874
Motherhood, 531
Motivation, 319
for achievement
during high school, 290
theory, 291–292
cannabis use, educational under-achievement lead, 292

educational under-achievement, cannabis use lead, 291
problem behavior theory, 292
during university, 291
cannabis intoxication, effect of, 293
cannabis use, 310
chronic cannabis user, 289
laboratory measure, 289, 293–295
acute effect, 293
effects of chronic use, 293–295
marijuana use, effect of, 289
research
limitation, 292
cultural difference, 292
multiple forms of substance use, 292
self-report, 289–290
dopamine examination, 290
Motivational enhancement therapy (MET), 135, e195, 1032, 1094, 1103, 1104, 1107, 1108
Motivational index score
chronic alcohol user
vs. healthy volunteer, 295
Motivational inhibitory control, 293
Motivational interviewing (MI), e194, 1104
Motivation enhancement training (MET), e203
Motor disorders, 824
Motor expressions, 607
clonic, 608
myoclonic, 608
tonic, 608
tonic-clonic, 608
Motor global scale, 888
Mouse cerebellar granule cell, 920
Mouse model of ileus
cannabidiol, effect of, 955
MPP(+). *See* 1-Methyl-4-phenyl pyridinium [MPP(+)]
MPTP. *See* 1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)
MPTP-induced parkinsonism, 921
MPTP-marmoset model, 921
MPTP-treated rhesus monkey, 921
MRN. *See* Median raphe nucleus (MRN)
mRNA second messenger, 456
MRS. *See* Magnetic resonance spectroscopy (MRS)
MS. *See* Multiple sclerosis (MS)
MST. *See* Multisystemic therapy (MST)
MS therapy pipeline, 901
MT. *See* Midbrain tectum (MT)
Multidimensional family therapy (MDFT), 1049–1050
certified therapist
requiremen, for, 1053
effects of, 1050–1051
implementation in Europe, 1051–1054
accreditation, 1051–1052
innovation, 1053–1054
licensing, 1053
number of team, 1053
team requirement, 1053
training, 1053
treatment center manager, 1052
intervention, 1050
outpatient treatment program, 1053
vs. comparison treatment, 1052
Multifocal arterial stenosis (MIS), 487
Multimodal therapy, 453
Multiple choice vocabulary test (Mehrfachwahl-Wortschatztest, MWT-B), 888
Multiple sclerosis (MS), 106, 405, 806, 894, 963
antiinflammatory treatment, 895
characteristic
autoimmune disease, 894
demyelination, 894
multifocal inflammation, 894
neuronal injury
brain, 894
optic nerve, 894
spinal cord, 894
chronic inflammatory demyelinating disease, 963
first-line medication
glatiramer acetate, 895
immunomodulatory medication, 895
immunosuppressive medication, 895
interferon-β, 895
microglial activation, 963
neuropathic pain
CBD/THC combination, effect of, 899
patient
expanded disability status scale (EDSS), 894
risk factor
environmental, 894
Epstein–Barr virus infection, 894
Herpesvirus 6 infection, 894
multiple genetic, 894
Theiler's murine encephalomyelitis virus (TMEV) model, 963
treatment, 896
cannabidiol (CBD), use of, 895
sativex compounds, use of, 895
Multiple substance use, rates of, including cannabis, 174
Multisystemic therapy (MST), 1049
Multivariate multinomial analysis, 977–978
Murine colitis
dinitrobenzene sulfonic acid (DNBS) model, 963
Muscimol, 775
Mutagen-activated protein kinases (MAPKs)
apoptosis, role in, 865
Mutation, e3
Myeloperoxidase, 804
Myenteric neuron
endocannabinoid system, relation with, 949
Myocardial infarction (MI), 482, 587
Myocardial ischemia, 482

N

NAA. *See N*-Acetyl-aspartate (NAA)
NAAA. *See N*-Acylethanolamine-hydrolyzing acid amidase (NAAA)
Nabilone, 822, 862, 872, 912, 943, 953, 1071
chemical structures, 822
chronic insomnia, effect on, 880
comorbid posttraumatic stress disorder, effect on, 880
fibromyalgia, effect on, 880
synthetic CB1 receptor agonist, 880
Nabiximols, 910, 1071
NAc. *See* Nucleus accumbens (NAc)
N-Acetyl-aspartate (NAA), 350
N-Acetylcysteine, 1033, 1071
N-Acylethanolamine acid amidase (NAAA), 960
N-Acylethanolamine-hydrolyzing acid amidase (NAAA), 619
N-Acylethanolamines (NAEs), 619
N-Acyl phosphatidylethanolamine phospholipase (NAPE), 283
NADA. *See N*-Arachidonoyl dopamine (NADA)
NAEs. *See N*-Acylethanolamines (NAEs)
NAFLD. *See* Nonalcoholic fatty liver disease (NAFLD)
NAPE. *See N*-Acyl phosphatidylethanolamine phospholipase (NAPE)
Naphthoylindoles, 692, 721, 982
Naphthoylpyrroles, 721
Naphthylmethylindenes, 692
Naphthylmethylindoles, 692
Naphthylpyrroles, 692
Naphthyridines, 602
N-Arachidonoyl dopamine (NADA), 510, 632, 878
N-Arylamide oxadiazoles, 600
NASH. *See* Nonalcoholic steatohepatitis (NASH)
Nasopharyngeal cancers, e96
National adult reading test, 1088
National Campaign against Drug Abuse (NCADA), 105
National Cannabis Policy (NCP), 106
National Cannabis Prevention and Information Centre (NCPIC), 107
National Center for Biotechnology Information (NCBI), 586
National comorbidity survey (NCS), 299
National Council for Addiction Treatment and Prevention (NCATP), 119
National Drugs Campaign (NDC), 108
National Drug Strategy Household Survey (NDSH), 102, e187
National Epidemiologic Survey on Alcohol and Related Conditions (NESARC), 299
National Fibromyalgia and Chronic Pain Association (NFCPSA), e163
National Institute on Drug Abuse (NIDA), 199, 463, 1000
National Registry of Evidence-based Programs and Policies (NREPP), 1078
National Survey on Drug Use and Health (NSDUH), e95, e187, 300
National Survey on Mental Health and Wellbeing (NSMHWB), 301
Nausea, 705
Nausea and emesis
cannabinoid, effect of, 953
Nausea and vomiting
pathogenesis, 862

Navy Drug Screen, e154
NCBI. *See* National Center for Biotechnology Information (NCBI)
NDD. *See* Neurodegenerative disorders (NDD)
NDSHS. *See* National drug strategy household survey (NDSHS)
Nefazodone, 1031, 1032
Neonatal fecal levels, 529
Neonate, 530
  marijuana exposure, long-term effects, 530
  marijuana exposure, short-term effects, 530
NESARC. *See* National Epidemiologic Survey on Alcohol and Related Conditions (NESARC)
Neural network model, 269
Neuregulin 1 (NRG1), 336
Neuroadaptation, 321, 456
Neuroanatomical analysis, 348
Neurobiological stimulation, 318
Neuroblastoma cells, 566, 920
Neurocognitive abnormalities, 157
Neurocognitive deficits, in emerging adult cannabis, 153
Neurocognitive effects, 152, 157, 224
Neurocognitive implications, of cannabis use, 152
Neurodegenerative disorders (NDD), 804
Neurodevelopmental impairment, 231
Neuroimaging studies, methodological limitations of, e50
Neuroimaging technique, 250, 320, 375
  event-related potentials, 250–251
  functional magnetic resonance imaging, 251
  functional magnetic resonance imaging (fMRI), 281
  positron emission tomography (PET), 281
Neuroinflammation, 673, 831
Neuroinflammatory disorders, 673
Neuromaturational changes, 157
Neuromodulation, 714
Neuronal maturation, 346
Neuropathic pain, 823
  approach to the management, 910
  cannabinoids, role of
    future clinical study, 913
  cannabinoids, use of, 864, 909
  common causes, 906
  mechanism, 906
  pathogenesis
    L-glutamate, role of, 864
  pathological process, 906, 907
    diabetic polyneuropathies, 906
    postherpetic neuralgia, 906
    postsurgical/traumatic neuropathies, 906
    spinal cord injury, 906
    trigeminal neuralgia, 906
  pharmacological treatment, 908, 909
  symptoms and sign, 906
Neuropathy
  diabetic distal symmetrical sensorimotor polyneuropathy, 912
  HIV infection, due to, 911
  spinal cord injury, 912
Neuropeptide (NPY), 640
Neurophysiological tests, 742
Neuro-protection hypothesis, 366
Neuroprotective effect, 49
Neuropsychological assessment battery maze, 1088
Neuropsychological deficits, 225
Neuroticism, 310
Neurotoxicity, 225
Neurotransmission, 445, 830
  modulation, 830
  systems, 66
Neurotransmitter
  pleasurable experience, 319
New psychoactive substances (NPS), e103, 172, 173, 841
  bath salts, e104
  discovery of, e103
  exchange-diffusion model, e104
  indirect monoaminergic agonists, e104
  neurobiological interactions, e103
  plant fertilizers, e104
  rates of cannabis use in users of, 175
  reported to the EMCDDA, 842
  representative packaging of, e104
New synthetic cannabinoids, 843
New Zealand Cancer Registry, e96
NFCPSA. *See* National Fibromyalgia and Chronic Pain Association (NFCPSA)
NF-κB. *See* Nuclear factor kappa B (NF-κB)
Nicotine, 48, 321, 665
  consumption, 162
  dependence, Fagerström test, e169
NIDA. *See* National Institute on Drug Abuse (NIDA)
Nigrostriatal damage
  6-hydroxydopamine induced
    effect of cannabinoids, 921
  1-methyl-4-phenyl pyridinium [MPP(+)] induced
    effect of cannabinoids, 922
  1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) induced
    effect of cannabinoids, 922
Nigro-tectal pathway activity, e145
Nitric oxide (NO), 796
  synthase, 472, 498
3-Nitropropionic acid (3NP), 806
NK cell, 932
NMDA. *See N*-Methyl-D-aspartate (NMDA)
NMDA receptor
N-Methyl-D-aspartate (NMDA)
  induced neuronal death, 873
  receptor, 330, 762, 803
    antagonists, 330
      amantadine, 330
      MK-801, 330
NO. *See* Nitric oxide (NO)
Nocturnal motor activity
  THC, effect on, 874
Nonaffective psychosis, 46
Nonalcoholic fatty liver disease (NAFLD), 587
Nonalcoholic steatohepatitis (NASH), 587
Noncontrolled clinical trials, e163
Non-F2F treatment. *See* Non-face-to-face (non-F2F) treatment
Nongastrointestinal origin
  cannabinoid involvement in visceral pain, 443
Nonmilitary drug use, e155
Nonrandomized clinical trials, e163
Nonspecific lipid transfer protein (ns-LTP), 519
Nonsteroidal antiinflammatory drugs (NSAIDs), e161, 441, 624, 730
11-Nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH), e189, 238, 1010, 1011, 1013
Not for human consumption, e155
3NP. *See* 3-Nitropropionic acid (3NP)
NPS. *See* New psychoactive substances (NPS)
NPY. *See* Neuropeptide (NPY)
NREPP. *See* National Registry of Evidence-Based Programs and Policies (NREPP)
NRG1. *See* Neuregulin 1 (NRG1)
NRG1-cannabis interactions, 336, 339
NRG1 signaling pathway, 346
NRS. *See* Numeric Rating Scale (NRS)
NRs. *See* Nuclear receptors (NRs)
NSAID-induced gastric hemorrhages, 444
NSAIDs. *See* Nonsteroidal antiinflammatory drugs (NSAIDs)
NSDUH. *See* National survey on drug use and health (NSDUH)
ns-LTP. *See* Nonspecific lipid transfer protein (ns-LTP)
NSMHWB. *See* National Survey on Mental Health and Wellbeing (NSMHWB)
NTS. *See* Nucleus of the solitary tract (NTS); *See also* Nucleus of tractus solitarius (NTS)
Nuclear factor kappa B (NF-κB), 733
Nuclear magnetic resonance spectroscopy (NMR), 982, 985
Nuclear receptors (NRs), 672
Nucleus accumbens (NAC), 328, 534, 743
Nucleus of the solitary tract (NTS), 862
Nucleus of tractus solitarius (NTS), 642
Numeric Rating Scale (NRS), e162

O

OAS. *See* Oral allergy syndrome (OAS)
Obesity, 473, 650
Obsessive compulsive disorder (OCD), e132
OCD. *See* Obsessive compulsive disorder (OCD)
Ocular pain and inflammation, 762
Ocular tumors, 762
Odds ratio (OR), 92, 211, 212
OEA. *See* Oleoylethanolamide (OEA)
OF. *See* Oral fluid (OF)
11-OH-THC. *See* 11-Hydroxy-delta 9-tetrahydrocannabinol (11-OH-THC)
Oleoylethanolamide (OEA), 646
Olivetolic acid, 14, 15
Olivetolic acid cyclase (OAC), 16

Olivetol synthase (OLS), 15, 16
OLS protein, 15
Ondasetron, 863
One-leg stand (OLS) test, 851
On-site device
  cutoffs for cannabis, 1000
  sensitivity for, cannabis in oral fluid, 1002
  specificity for, cannabis in oral fluid, 1002
  three matrices, comparison of results, 1003
  two matrices, comparison of results, 1003
On-site drug testing, 999
  alere DDS V Test, 1001
  detection limit, 1000
  matrix of choice, 999–1000
  on-site oral fluid testing, 1002
  on-site urine testing, 1001
  use of
    emergency department, 1003
    forensic autopsies, 1002–1004
    primary health care, 1004
    schools, 1003
Opiates/opioids, 182, 665
  animal exposure
    effects of CBD, 942
  drug-related death, 941
Opiates use disorder
  treatment strategy
    methadone use, 940
μ−Opioid receptors, 941
Opioids, 452
Opioid withdrawal symptoms
  THC, effect of, 874
Oppositional defiant disorder, e66
Oral allergy syndrome (OAS), 519
Oral fluid (OF), 1008
  analysis
    gas chromatography–mass spectrometry (GC/MS), 1010
    immunoassay screening, 1010
    liquid chromatography with tandem mass spectrometry (LC-MS/MS), 1010
    time of flight (TOF) detection, 1010
  collection device, 1008–1009
    cannabinoid recovery, 1011
    desirable characteristic, 1008
      collection pad, 1009
      transportation buffer, 1009
    THC recovery, 1009
  composition, 1008
    cholesterol, 1008
    electrolyte, 1008
    enzyme, 1008
    epithelial cell, 1008
    mucin, 1008
    protein, 1008
    vitamin, 1008
    water, 1008
  disposition of cannabinoid, 1010–1013
    marijuana smoking, 1011
    oral administration, 1013
    smoking and oral administration, 1013
  drug stability, 1008–1009
  marijuana recovery, 1008–1009
  sample
    from frequent marijuana smoker
      concentration of cannabinoid, 1012
      median concentration of cannabinoids, 1013
    marijuana positive
      cannabinoid composition, 1014
    sativex user
      median cannabinoid ratios, 1015
  THC recovery, 1009
  THC stability, 1009
Oral mucosal disease, e73
Oral tongue cancers, e97
  marijuana use and, e97
Orchialgia, chronic, 446
Orexigenic effect, 644
Orexin system, 534
  addictive properties of drugs abuse, role in, 535
  cannabinoid dependence, role in, 538
  potential therapeutic utility, 536
ORG27569 allosteric modulators, 575
  binding site, elucidation of, 578
  in vivo studies, 578
ORG27759 allosteric modulators, 575
ORG29647 allosteric modulators, 575
Organon compounds, 575
  ORG27569, 575
  ORG27759, 575
  ORG29647, 575
Oromucosal spray administration, 895
Oropharyngeal cancers, e97
Orthosteric ligands, 574
Otenabant, 653
  development program, 653
Overweight, 650
Oxidative stress, 780, 831
  beta cells dysfunction, 781
Oxytocin, 1071

P

PAG. *See* Periaqueductal gray matter (PAG)
PAHs. *See* Polycyclic aromatic hydrocarbons (PAHs)
Pain control
  endogenous cannabinoids, role of, 909
  opioids, role of, 909
Pain Disability Index (PDI), e162
Painful neuropathy, 912
Pain management, 824, 864
  cannabinoids, use of, 864
  pharmacotherapy class
    dorsal horn inhibitory mechanism, 908
    membrane stabilizing agents, 908
Palliative cancer, symptoms relief
  approved cannabinoid, 862
Palmitoylethanolamide (PEA), 675
N-Palmitoylethanolamine, 626, 951
Pancreas, 444, 780
Pancreatic polypeptide (PP), 780
Panic attack-like behaviors, e142
PANSS. *See* Positive and negative syndrome scale (PANSS)
Papyrus, 112
Paralytic ileus
  cannabinoid, effect of, 955
  symptom
    bloating, 955
    distension, 955
    emesis, 955
    gas and secretion accumulation, 955
    visceral pain, 955
Parental communication and children's substance use, 219
Parental influence, on adolescent cannabis use, 216–217
  future directions in, 220
Parental monitoring, 972
Parental warmth
  and monitoring, 218
  and parenting style, 219
Parenting style, 219
Parkinson's disease (PD), 792, 805, 918, 963
  cannabinoids, effect of, 919
  characteristic
    bradykinesia, 918
    postural instability, 918
    rigidity, 918
    tremor, 918
  endocannabinoid system, 918
  6-hydroxydopamine model, 920
  L-dopa treatment, 918
  1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine model, 920
  primate models, 921
  rotenone model, 920
Past month cannabis use
  by age group and country, 37
  by age group, Europe and the United States, 40
Past year cannabis use
  by age group, Europe, Canada, and the United States, 39
Pathogenesis, 467
Patient flow
  through the study, e198
Pavlovian conditioning, 318
PBC. *See* Primary biliary cirrhosis (PBC)
PBE. *See* Poisson–Boltzmann equation (PBE)
PCE. *See* Prenatal cannabis exposure (PCE)
PCU. *See* Problematic cannabis user (PCU)
PD. *See* Parkinson's disease (PD)
PDI. *See* Pain Disability Index (PDI)
pDomTHREADER, 683
PEA. *See* Palmitoylethanolamide (PEA)
Pedicularis densiflora, 840
Peer influence, nature of, 189–190
Peers *vs.* friends, 189
Pelvic viscera, sensory innervation, 440
Pentylenetetrazole (PTZ) model, 797
Pepcan-12, 579
Pep pills, e152
Peptide YY(PYY), 780
Perceived behavioral control, 218–220
Percentage
  of current age cohorts and corresponding age of first use for cannabis, 37
  of past year cannabis use by age group and country, 36
Perception of drugs, 201

Periaqueductal gray matter (PAG), e141
Perinatal exposure, 741
Periodontal disease, e77
Peripheral signals, 640
Peripheral vascular disease, 483
Peroxisome proliferator-activated receptor (PPAR)
  alpha agonist, 744
Peroxisome proliferator-activated receptor-γ (PPARγ), 406, 805, 960
Personality disorders, 66
Personalized medicine, 590
Pertussis toxin (PTX), 566, 732
PET. *See* Positron emission tomography (PET)
PGE2. *See* Prostaglandin E2 (PGE2)
Pharmacokinetic activity, 162
Pharmacokinetic (PK) models, 387
Pharmacological studies
  in rats, e105
Pharmacology, 822
Pharmacotherapy trial, 1032
Phencyclidine, 665
Phenobarbital, 665
Phenothiazines, 456
Phentermine, 650
Phenylacetylindoles, 692, 721
Phosphatidylinositide-3-kinases (PI3K), 560, 566
Phospholipase C, 631
Phosphorylation, 566
  of ERK by CB_1, 566
Phytocannabinoids, 14, 555, 607, 609, 654, 706, 749
  chemical structures, 555
  effects, 609
  intraocular pressure, effect on, 750
    cat, 750
    dog, 751
    human, 749
    monkey, 751
    rabbit, 750
    rat, 751
PI3K. *See* Phosphatidylinositol-3-kinase (PI3K)
Pilocarpine and pentylenetetrazol (PTZ), 608
Pittsburgh Sleep Quality Index (PSQI), e163
PKC. *See* Protein kinase C (PKC)
PK models. *See* Pharmacokinetic (PK) models
PL. *See* Prelimbic frontal cortex (PL)
Placebo, 824
Plasma cells, 518
Pleasure pathway, 319
P42/44 MAP kinase, 756
PND. *See* Postnatal day (PND)
Pneumomediastinum, 501
Pneumothorax, 501
POC. *See* Point-of-collection (POC)
Point-of-collection (POC), 999
Poisson–Boltzmann equation (PBE), 686
Poisson regression analysis, e3
Polycyclic aromatic hydrocarbons (PAHs), 498
Polydipsia, 467
Polydrug, 162, 203
  problems associated with, 175–176
Polyketide pathway, 15
Polyketide synthases (PKSs), 15
Polysubstance dependence, 1058
Polysubstance use, 75
POMC. *See* Proopiomelanocortin (POMC)
Popular media, e154
POSIT. *See* Problem-oriented screening instrument for teenagers (POSIT)
Positive and negative syndrome scale (PANSS), 29, 792, 1088
Positive well-being
  cannabis, 309–310
    future directions, 313
    limitations, 313
  component
    happiness, 309, 310
    life satisfaction, 309, 310
Positron emission tomography (PET), 293, 375, 789, 918
Posterior cingulate cortex (PCC), e55
Postnatal day (PND), 634
Postoperative pain, 453
  multidimensional model for pathophysiology, 452
Postsynaptic density protein 95 (PSD-95), 734
Posttraumatic stress disorder (PTSD), e132, 872
  marijuana, effect of, 872
  nightmares, 825
Potassium channels, 567
Potency, 25, 26
PP. *See* Pancreatic polypeptide (PP)
PPAR. *See* Peroxisome proliferator-activated receptor (PPAR)
PPARα receptors, 473
PPARγ. *See* Peroxisome proliferator-activated receptor-γ (PPARγ)
Preclinical studies, 773
Preclinical work, 775
Prefrontal cortex (PFC), e54, 71, 152
Pregabalin, e161
Pregnancy, 162, 531
  characteristics of women, using cannabis during, 162
Prelimbic frontal cortex (PL), e135
Premammillary hypothalamic nucleus, e143
Premorbid IQ, and cannabis
  findings from longitudinal studies, 229
  possible explanation of link to cannabis use, and psychosis, 230
Premorbid neuropsychological abilities, 224
Prenatal cannabis exposure (PCE), 161
  detection methods and accuracy of prevalence estimates, 162
  effects on offspring, 163–166
    Bayley scales of infant development, 165
    behavioral alterations in children prenatally exposed to cannabis, 167
    cardiovascular changes associated with, 163
    gestation and infant mortality, 165
    neurocognitive development, 166
    size effects associated with, 164
  neuroimaging studies, 165
  prevalence and behavioral patterns across pregnancy, 161
    SAMHSA report, 161
  Stanford-Binet Intelligence Scale, 165
Pretreatment dropout, 1026
Prevalence of dual pathology, in cannabis addicts in treatment, 67
  age for cannabis use *vs.* mental disorders, 68
  current diagnoses of axis I disorders assessed by the MINI, 67
  distribution of lifetime comorbid mental disorders, 67
  distribution of personality disorders in patients with lifetime cannabis misuse, 67
  Madrid study on, 67
Primary biliary cirrhosis (PBC), 587
Primary dystonias, 833
Problematic cannabis user (PCU), 974
  negative variables, with
    multivariate multinomial logistic regression, 977
    variables positive, with
      multivariate multinomial logistic regression, 978
Problematic use of cannabis (PUC), e181
Problematic use of marijuana (PUM), e181
Problem behavior theory (PBT), e19
Problem-oriented screening instrument for teenagers (POSIT), 973
Processing speed, e67, 74, 262
Proconvulsant effects, 434
  of cannabis, 434
  medically administered CBD, 434
Prodromal nausea, 467
Progesterone, 672
Program agenda for the drug court model, 194
Proinflammatory cytokines, 559
Proliferator-activated receptors (PPARs), 744
Proopiomelanocortin (POMC), 640
Propyl cannabinoids, 21
Prostacyclin, 731
Prostaglandin biosynthesis
  cannabigerol, role of, 963
Prostaglandin E2 (PGE2), 724, 730, 796
Prostaglandin production pathway
  CBG, effect of, 960
Prostaglandins, 731
Prostate carcinoma, 866
  pathogenesis, 866
Prostatectomy, 455
Prostatic serum antigen (PSA), 866
Protein folding, 829
Protein kinase A (PKA), e104
Protein kinase C (PKC), e104, 534
Protein–protein docking, 685
Proteome, e3
Proton magnetic resonance spectroscopy (^{1}H-MRS), 350
Prunus persica, 519
PSD-95. *See* Postsynaptic density protein 95 (PSD-95)
PSQI. *See* Pittsburgh Sleep Quality Index (PSQI)
Psychiatric comorbidity, 75, 133
Psychiatric treatment
  level, CapOpus, effects of, 1090
Psychoactive drugs, e151

Psychoactive substances, 41
Psychological and relational problems, experienced by substance users, 183
Psychological effects
of acute cannabis intoxication, 81
present in drug addicts, 183
Psychopathological symptoms, 48
Psychopharmacological effects, 54
Psychosis, 25, 29, 80, 414, 873
cannabis use, effect of, 283
dopamine release
stress-induced, 281–282
ECS, effect on, 283
gene–environment interaction, 282
inducing effects of cannabis, 30
self-medication by marijuana, 872
Psychosocial derailment, and problem accumulation, 146
Psychosocial stress, 281. *See also* Psychosis
Psychostimulants, 535, 665
Psychotic disorder, e30
marijuana, effect of, 873
Psychotic illness, 47, 48
Psychotic patients, 49
Psychotic spectrum disorder, 281
Psychotic symptoms, 66, 415
Psychotomimetic effects, 49
Psychotropic effects, 414
PTSD. *See* Posttraumatic stress disorder (PTSD)
PTX. *See* Pertussis toxin (PTX)
PTZ. *See* Pilocarpine and pentylenetetrazol (PTZ)
PUC. *See* Problematic use of cannabis (PUC)
Pulsatility indices, 163
PUM. *See* Problematic use of marijuana (PUM)
Purkinje cell excitability, 408
PyMOL molecular graphics system, 686
Pyrazolopyridines, 602
Pyrazolylidene, 600
Pyridine, 598
Pyrimidine, 598
PYY. *See* Peptide YY(PYY)

Q

QLIP. *See* Quality of Lfe Impairment by Pain (QLIP)
Qsymia, 650
Quality of Lfe Impairment by Pain (QLIP), e162
QUARK algorithm, 684
Quetiapine, 943, 1032
Quinolones, 602

R

RADAR. *See* Register of Australian Drug and Alcohol Research (RADAR)
Radial diffusivity (RD), 348, 393
Radioimmunoassay, 48
Random amplified polymorphic DNA (RAPD), 7
Randomized clinical trials (RCT), e161
Randomized controlled clinical trials, e164
Randomized controlled trials, 434
Rapid eye movement (REM), 878
Rave culture, e15
ecstasy, e15–e16
ideology of PLUR, e15
RCT. *See* Randomized clinical trials (RCT)
RCVS. *See* Reversible vasoconstriction syndrome (RCVS)
Reaction time (RT), 381
Reactive oxygen species (ROS), 677, 803
Recency of parents' cannabis use and children's initiation, 217
Recent-onset schizophrenia (ROS), e55
Receptor–agonist interactions, 718
Receptor trafficking, 734
Recreational drug
online available, 844
Recreational drugs, 162
Recreational use, cannabis, future research directions, 314
Reference spectrum, 985
Register of Australian Drug and Alcohol Research (RADAR), 1078
Relapse prevention (RP), e194, 1057, 1061
strategies, 1062
cannabis craving, understanding of, 1062
cognitive distortion, 1062
cognitive skill acquiring, 1062
emotion, 1062
psychoeducation, 1062
role play, 1062
REM. *See* Rapid eye movement (REM)
Resin (hash), 25
Resin-type cannabis, e3
Resistance indices, 163
Response inhibition, e67
Restriction fragment polymorphisms (RFLP), 7
Retinal ganglion cell (RGC), 761
Retinal pigmented epithelium (RPE), 763
Retinoid X receptor (RXR), 672
Reversible cerebral cannabis arteriopathy, 490
Reversible vasoconstriction syndrome (RCVS), 487
Reviews and metaanalyses on cannabis and cognition, 225
Reynaud phenomena, 483
RGC. *See* Retinal ganglion cell (RGC)
Rhesus monkeys, 697
Rho-GTPase signaling pathways, 565
Right censoring, 139
Right inferior frontal gyrus (RIFG), 274
Rimonabant, 473, 651, 697, 920, 964
antihypokinetic activity, 920
glutamate release, effect on, 920
Risk and protection factors influencing substance use, 203
Risperidone, 887
RMSD. *See* Root mean square deviation (RMSD)
RMSF. *See* Root mean square fluctuation (RMSF)
Roadside Testing Assessment project, 1000
Road traffic accident
cause, driving under the influence (DUI) of drugs, 849
Robetta's algorithm, 684
Rodents, e142, 741
behavior, e142
Romberg's test, 851
Root mean square deviation (RMSD), 687
Root mean square fluctuation (RMSF), 687
ROS. *See* Reactive oxygen species (ROS)
RP. *See* Relapse prevention (RP)
RPE. *See* Retinal pigmented epithelium (RPE)
RT. *See* Reaction time (RT)
RXR. *See* Retinoid X receptor (RXR)

S

SAD. *See* Social anxiety disorder (SAD)
Saliva, biochemical composition, 1008
Salting out technique (SALLE), 991
SAMHSA. *See* Substance Abuse and Mental Health Services Administration (SAMHSA)
Sativex, 943, 1010, 1071
oromucosal spray
spasticity, treatment, 959
Sativex compound, 895
SBIRT. *See* Screening, brief intervention, and referral to treatment (SBIRT)
SC. *See* Synthetic cannabinoids (SC)
SCBs. *See* Synthetic cannabinoids (SCBs)
Schedules for, clinical assessment in neuropsychiatry (SCAN) interview, 1087
Schizophrenia, 47, 66, 80, 224, 336, 346, 358, 414, 788, 1087
attribution style, 358
candidate genes, 336
and cannabis, 337
cognition, 358
deficits in social knowledge, 358
diagnoses, 336
disorders, 361
emotion recognition, 358
functional imaging studies, 361
gene–cannabis interactions, 337
catechol-*O*-methyltransferase, 337
neuregulin 1, 338
gene–environment interactions, 336
heritability for, 80
marijuana, effect of, 873
negative symptoms, 788
anhedonia, 788
apathy, 788
social withdrawal, 788
neurofunctional Alterations, 360
and nonaffective psychosis, 46
patients, 46
prevalence, 336
rates of, 80
related disorder
amphetamine-induced DA release, 282
spectrum psychosis, 1087
symptoms, 336
anhedonia, 336
cognitive, 336
impairment of sensorimotor gating, 336
social withdrawal, 336
theory of mind, 358
Schizophreniform psychosis, 414
School-based drug prevention program, 201

School-oriented programs, 202
SCN1A gene mutation, 434
Screening, brief intervention, and referral to treatment (SBIRT), 1102
Screening instruments
  characteristics, e171–e181
  process of selection and analysis, e171
Screening tests, by immunoassay, 239
SDS-PAGE analysis, 15
Seized material analysis, GC-MS, use of, 985
Selective serotonin and norepinephrine reuptake inhibitor (SSNRI), e161
Selective serotonin reuptake inhibitors (SSRIs), e161, 490, 885
Self-help for alcohol and other drug use and depression (SHADE), e207
Self-medication, 303
  hypothesis, 415, 424
  theory, 312
Self-report
  of adult population
    cannabis use prevalence, e187
  and biological measurements
    concordance between, e186
    concordance with
      armed forces, e190
      driving population, e189
      drug users in treatment, e190
      emergency department patients, e190
      HIV-infected youth, e190
      pregnant women, e190
      university students, e189
Self-validation, 310
Sensation seeker, 56
Sensorimotor gating, 337
  deficits, 338
Serotonergic neuronal system, 329
Serotonergic systems, 329
Serotonin$_{1A}$, 705
Serotoninergic agents, 721
SERS. *See* Surface enhanced Raman spectroscopy (SERS)
Set-shifting, 46
Sexual
  attitudes, 182
  behavior, 181, 182
    effect of drugs on, 183
  desire, 182
  difficulties, 184
  disorders, 181
  excitement, 182
  expectations, 185
  function, 182
  performance, 182, 183
  pleasure, 183
  satisfaction, 181
  violence, 182
Sexually transmitted diseases, 183
SF-36. *See* Short Form 36 Health Survey (SF-36)
SF-36 health survey
  results, 934
SHADE. *See* Self-help for alcohol and other drug use and depression (SHADE)
Shapiro Tourette syndrome severity scale (STSSS), 888
Shisha, 115
Short Form 36 Health Survey (SF-36), e163
Short inflammatory bowel disease questionnaire (SIBDQ) score, 918
Short inventory of problems-alcohol and drugs (SIP-AD), e181
SIBDQ score. *See* Short inflammatory bowel disease questionnaire (SIBDQ) score
Siblings, 46, 49
SIDS. *See* Sudden infant death syndrome (SIDS)
Signaling pathways, 577
Simulation of the public speaking test (SPST), e134
Single nucleotide polymorphism (SNP) assay, e4
Single nucleotide polymorphism database (DBSNP), 586
Single nucleotide polymorphisms (SNPs), 336, 340, 585
  nonsynonymous, 586
Single photon emission computed tomography (SPECT), e134, 940
Sinsemilla (skunk), 25, 29, 418
SIP-AD. *See* Short inventory of problems—alcohol and drugs (SIP-AD)
Skills, 49
  based programs, 201, 202
Skin, 544
Skin lesions, 546
Skin prick tests (SPT), 522
Sleep disorders, 872–873
  marijuana, use of, 872
  THC, effect of, 865
Sleep latency, acute administration of marijuana, effect of, 872
Sleep, physiological role of endocannabinoid, 878
Sleep-wake cycle, 535
  cannabis, effect of, 878
SLF. *See* Superior longitudinal fasciculus (SLF)
Slow transit constipation
  cannabinoid, effect of, 955
Slow wave sleep (SWS), 878
Smokers, effects of CBD, 943
Smoking inhibition
  CBD inhaler, use of, 942
  CBD, use of
    mechanism of action
      CB1 receptor antagonist, 942
      FAAH inhibition, 942
  use of low dose CBD, 942
Smoking, lead to increased total exposure of THC, 30
Smoking, synthetic cannabinoids, 445
Smuggling, 122
SNc. *See* Substantia nigra pars compacta (SNc)
SNpr. *See* Substantia nigra pars reticulata (SNpr)
SNPs. *See* Single nucleotide polymorphisms (SNPs)
SNPSTR database, e7–e8
SNr. *See* Substantia nigra pars reticulata (SNr)
Social anxiety disorder (SAD), e134, 872
Social cognition, 46
Social development model, 292
Social drug, 189, 194
Social hypothesis, 368
Social network during adolescence, 192
Social phobia, 434
SOD. *See* Superoxide dismutase (SOD)
Sources of information on illicit drugs, reported by European young people, 202
Specialized addiction treatment, 1027
SPECT. *See* Single photon emission computed tomography (SPECT)
Spice, 840, 849, 986
  diamond, 986
  neurologic effects, 844
  psychoactive component identification, 841
  sympathomimetic effects, 844
  variant
    spice diamond, 840, 841
    spice gold, 841
Sprague Dawley rats, 653, 660
SPST. *See* Simulation of the public speaking test (SPST)
SPT. *See* Skin prick tests (SPT)
SR141716A
  rapid eye movement (REM), effect on, 878
  slow wave sleep (SWS), effect on, 878
  wakefulness induction, role in, 878
Src activation pathways, 580
SSNRI. *See* Selective serotonin and norepinephrine reuptake inhibitor (SSNRI)
SSRIs. *See* Selective serotonin reuptake inhibitors (SSRIs)
Stabilization energy (ΔEiso), e124
Staphylococcus aureus, 965
Steatosis, 587
Stilbene synthase (STS), 15
Stimulants, e152
  abuse and dependence treatment
    dopamine uptake blockers and releasers, use of, 941
  abuse potential, 941
  amphetamine, 940
  animal exposure
    effects of CBD, 942
  cocaine, 940
  dependence
    pharmacotherapies, development of, 941
  dopamine level, increase in, 941
  euphoria, 941
  methamphetamine, 940
Stimulus–response (S-R) association, 268
STN. *See* Subthalamic nucleus (STN)
Street drug, 869
Streptozotocin (STZ), 780

Stress, 62
brain response, 280
DA-related mental illnesses, 279
drugs use disorders, 279
evaluation, 280
induction, 280
and mental disorder, 279–280
neuropsychiatric disorder, 279
schizophrenia, 279
psychosis illnesses, 280
related HPA activity, 279
study, 280
animal models, 280
Stressful interviewing protocol, 280
Striatal damage
3-nitropropionic acid-induced, 924–926
quinolinic acid-induced, 926
in vitro study, 926
STR locus, e7
Stroke, 489
onset, 489
Structural implications, cannabis use of, 153–155
gray matter, 153
moderating factors, 155
potential confounding, 155
white matter, 154
STSSS. *See* Shapiro Tourette syndrome severity scale (STSSS)
STZ. *See* Streptozotocin (STZ)
Subcortical dementias, 833
Subjective effects, 823
Subjective norms, 218
Substance Abuse and Mental Health Services Administration (SAMHSA), 1051, 1078
guideline, 985
Substance abuse and mental health services administration (SAMHSA), e187
Substance abuse prevention, 1078
Substance-related problem, 317
Substance use
controlled, e169
harmful, e169
nonproblematic, e169
problematic, e169
risky, e169
Substance use disorder (SUD), 62, 873–874, e169, e170, 972, 1057, 1059, 1087
cognitive behavioral therapy, use of, 1058
marijuana, role of, 873
role of early CU in CUD risk in context of, 143
self-monitoring, 1103
Substance use intervention, 1102
Substance use problem, 1026
Substance use treatment, 1028
Substantial problem, e154
Substantia nigra pars compacta (SNc), 918
Substantia nigra pars reticulata (SNpr), e144, 918
Subthalamic nucleus (STN), 918
Sudden infant death syndrome (SIDS), 530
Sulfamoyl benzamides, 598
Suncus murinus, 953
Superior longitudinal fasciculus (SLF), 347
Superoxide dismutase (SOD), 782
Surface enhanced Raman spectroscopy (SERS), 1022
Survey of young people in Egypt (SYPE), 114
Swallowers, 123
SWS. *See* Slow wave sleep (SWS)
Sympathetic nervous system (SNS), 279
Synaptic plasticity, 633
Synaptic potentiation, 635
Synaptic stripping, 402
Synopsis of *C. sativa* sectional species, subspecies, and varieties, 6
Synthetic analogs, 593
Synthetic cannabinoids (SCBs), 54, e151, 173, 607, 632, 692, 751, 982
aminoalkylindoles, 752, 982
WIN55212-2, 752
behavioral effects, 693
anxiety, 692
confusion, 692
psychosis, 692
speech, 692
blood concentration in suspected driver, 851–852
brand names, 55
cannabis-like effects, 849
classical, 751
BW146Y, 751
HU-211, 751
nabilone, 751
clinical consequence, 842–844
clinical effects, 692
hypertension, 692
hyperventilation, 692
mydriasis, 692
nausea, 692
reddened conjunctivae, 692
vomiting, 692
clinical presentation, of intoxication, 850
concentration in biological media, 984
consumption effect, typical performance deficit, 849
demographics associated with, 57
detection, analytical challenge, 853
discriminative stimulus effects, 696
cross-tolerance, 697
tolerance, 697
driving skills, effect on, 853
drug aficionado, 59
drug testing, 845
immunoassays, 845
liquid chromatography-mass spectrometry, 845
standard urine drug testing procedures, 845
effects, 611
of legislation, 841–842
safe driving, interfere with, 851
experimental user, 58
frequently detected
AM-2201, 850
JWH-018, 850
habitual user, 59
herbal smoking blend, use as, 985
isobaric compound
JWH-015, 984
JWH-019, 984
JWH-073, 984
JWH-122, 984
separation of, 984
K2, 849
legal responses to, 56
legislation, 854
metabolite, 985
nonclassical, 752
abnormal cannabidiol, 752
profile of users, 57
psychoactive effect
calmness, 842
euphoria, 842
relaxation, 842
reasons for usage, 54–56
receptor agonist, 982
recreational use, 986
replacement user, 58
research chemicals, 985, 988
seized product, 988
serum or blood analysis quantitative method, 989
spice, 849
structure, 983
summary of the four profiles, 56
5th generation
APINACA, 982
5F-PB-22, 982
UR-144, 982
timeline of major legal and media events in the United States, 56
tolerance, 853
urine analysis
quantitative method, 992
users, types, 54
Synthetic cathinones, e105, e151
Synthetic Drug Abuse Prevention Act (2012), e153

T

Tail-flick assay, 445
TAs. *See* Traffic accidents (TAs)
TAU. *See* Treatment as usual (TAU)
Tau hyperphosphorylation, 830
Tauopathy, 831
Tautomerization, 676
TCAs. *See* Tricyclic antidepressants (TCAs)
T2D. *See* Type 2 diabetes (T2D)
TEAC. *See* Trolox equivalent antioxidant capacity (TEAC)
Telescoping effect, 132
Temporal perception, 185
Tenax collection method, 1019
Terpenophenolic, 3
constituents, 14
Tesofensine, 650
Testicular germ cell tumors
logistic regression model, e97
marijuana use and, e97
multivariate adjustment, e97
nonseminoma, e97
pure seminoma, e97
Test of memory malingering, 295
Testosterone, 182
lowering medicaments (TLM), 965

Tetrahydrocannabinol (THC), e189, 299, 321, 415, 593, 763, 959, 1008, 1018, 1019, 1021, 1031, 1068, 1087. *See also* Δ^9-Tetrahydrocannabinol (THC)
anxiety-like symptom, 304
awakenings, effect on, 879
bicyclic analogs, 593
changes in concentrations in blood samples, 27
chemical structures, 841
concentrations in biological samples, 27
content in different plants varies according to a number of factors, 25
detection, gas chromatographic-mass spectrometric method, use of, 1019
drowsiness, effect on, 879
ocular delivery, 763
challenges, 763
positive driver, 850
psychomimetic effect, 281
sleep efficiency, effect on, 879
sleep latency, effect on, 879
sleep-wake cycle, effect on, 879
standard substance, 1019
treatment
vs. placebo
STSSS score, changes in, 889
TS-CGI score, changes in, 890
YGTSS score, changes in, 889
tricyclic analogs, 593
Δ^9-Tetrahydrocannabinol (Δ^9-THC), e127, e132, e141
Tetrahydrocannabinolic acid (THCA), e2, 7
Tetrahydrocannabinolic acid synthase (THCAS), 15, 19
Tetrahydrocannabivarian (THC-V), 21, 104
Tetrahydrocannabivarinic acid (THCVA), 21
Tetramethylcyclopropy, 598
TGB. *See* Thyroxin-binding-globulin (TGB)
Thaumatin-like proteins (TLP), 521
THC. *See* Δ^9-tetrahydrocannabinol (THC); Δ^9-tetrahydro-cannabinol (THC)
THCA synthase (THCAS), e2, e4, e5
activity, e6
THC carboxylic acid (THCCOOH), 1019, 1021, 1022
THC/CBD ratio, 25, 26
THC/CBD ratios, e58
THCCOOH. *See* THC carboxylic acid (TH-COOH)
THC-COOH. *See* 11-nor-9-carboxy-delta 9-tetrahydrocannabinol (THC-COOH)
Theories and empirical support, e20
Theories of synergism, 455
Theory of planned behavior, 218
Theory of triadic influence (TTI), 1037
Therapeutic alliance domain, e206
Therapeutic Goods Administration (TGA), 106
Thiazolidinediones (TZDs), 673
Thiazolylidene, 600
Thromboxane, 512, 731
Thymic stromal lymphopoietin (TSLP), 546
Thyroid function tests, 463
Thyroid-stimulating hormone (TSH), 462
Thyroxin-binding-globulin (TGB), 463
TIA. *See* Transient ischemic attack (TIA)
Tiagabine, 775
Timeline follow-back (TLFB), 1088
Titration of THC intake during smoking, 26
TLESRs. *See* Transient lower esophageal sphincter relaxations (TLESRs)
TLFB. *See* Timeline follow-back (TLFB)
TLP. *See* Thaumatin-like proteins (TLP)
TLR4-dependent pathway, 473
TM. *See* Trabecular meshwork (TM)
TNF. *See* Tumor necrosis factor (TNF)
Tobacco, 80
cigarette smoking, 495
TOC. *See* α-Tocopherol (TOC)
α-Tocopherol (TOC), e127
Toll-like receptors (TLR), 403
Tourette's syndrome clinical global impression scale (GTS-CGI), 888
Tourette syndrome, 833
Tourette syndrome global scale (TSGS), 887
Tourette syndrome symptom list (TSSL), 888
Towards No Drug Abuse (TND), 1081, 1082
Trabecular meshwork (TM), 749
Traffic accidents (TAs), 235
Global Status Report on Road Safety, report, 235
risk factors, 235
and synthetic cannabinoid, 852–853
vehicular risk factors, 235
Trail making test, 1088
Transcranial magnetic brain stimulation, 374
Transcription factor, 538
Transcriptomes, e5
Transient ischemic attack (TIA), 486, 487
Transient lower esophageal sphincter relaxations (TLESRs), 953
Transient receptor potential cation channel (TRP), 406
Transient receptor potential cation channel ubfamily V member 1 (TRPV1), e141
Transient receptor potential (TRP) channels, e137
Transient receptor potential vanilloid type-1 (TRPV1) channels, 607, 612, 632
Transmembrane domains (TMs), 558
Transporter inhibitors, 755
Treatment as usual (TAU), 1087, 1088
Treatment attrition, 1095
Treatment facility, contact with, 1089
T regulatory cells, 509
Triaryl derivatives, 600
Triazolam, 771
Trichomes, 3
Tricyclic antidepressants (TCAs), e161
Tricyclic cores, 602
Trolox equivalent antioxidant capacity (TEAC), e127
TRP. *See* Transient receptor potential cation channel (TRP)
TRPV1
TRPV1 channels. *See* Transient receptor potential vanilloid type-1 (TRPV1) channels
TSGS. *See* Tourette syndrome global scale (TSGS
TSH. *See* Thyroid-stimulating hormone (TSH)
TSLP. *See* Thymic stromal lymphopoietin (TSLP)
TTI. *See* Theory of triadic influence (TTI)
Tumor necrosis factor (TNF), 796
Tumor-promoting effects, 500
Two-option experimental task, 293
nonwork option, 293
work option, 293
Type 2 diabetes (T2D), 673
TZDs. *See* Thiazolidinediones (TZDs)

U

UC. *See* Ulcerative colitis (UC)
Ulcerative colitis (UC), 475, 932, 954
Unambiguous structure elucidation
NMR, use of, 985
United Nations Office on Drugs and Crime (UNODC), 90, 107, 870, 982
United States
past month cannabis use by age group, 40
past year cannabis use by age group, 39
percentage of older adults using cannabis in past year, 41
United States Drug Enforcement Agency, 123
United States Food and Drug Administration (FDA), e160
Univariate logistic regression model, 975
covariate
family/friendly/scholastic environments, 977
lifestyle and use of leisure time, 977
self-opinion, 977
UNODC. *See* United Nations Office on Drug and Crime (UNODC)
Unprovoked seizure, 433
Urinalysis assessments, e154
Urinalysis testing, e151
Urine drug screening (UDS), 162
Urine sample analysis
enzyme-linked immunosorbent assays (ELISA), use of, 988
homogeneous enzyme immunoassay (HEIA), use of, 988
Users of new psychoactive substances
rates of cannabis use in, 175
US National Survey on Drug Use and Health (NSDUH) data, 39

V

Vagal nodose ganglia, 644
Vagotomy, 643
Vagus nerve, 644
role in ghrelin actions, 644
Valine (Val) allele, 418
Val66Met BDNF gene polymorphism, 717
Vanilloid-1 receptor (TRPV1), 878
VAS. *See* Visual Analog Scale (VAS)
Vascular imaging, 490
Vasoconstriction effect, 483
Venlafaxine, 943, 1033, 1071
Ventral tegmental area (VTA), 534
Ventricular alterations, e54

Verbal learning, 74
VGCCs. *See* Voltage-gated calcium channels (VGCCs)
Videobronchoscopy, 498
Visceral cancer pain, 441
Visceral hypersensitivity, 441
Visceral pain, 441
  anatomic and functional features of, 442
  cannabinoids, 441
Visual acuity, 122
Visual analog scale (VAS), 773
Visual reaction time, 71
Visuomotor tracking, 373
Visuospatial memory, 152
Voltage-gated calcium channels (VGCCs), 1033
Voxel-based morphometry (VBM), e55
VTA. *See* Ventral tegmental area (VTA)
Vulnerability
  general heritable, for disinhibitory behavior and psychopathology, 146
  as a phase of vulnerability for addiction, in adolescence, 143
  for THC effects in adolescence, 144

W

Walk-and-turn (WAT) test, 851
WAR. *See* Wistar audiogenic rats (WAR)
Warrant treatment, 1027
WAT. *See* White adipose tissue (WAT)
Weight-loss drugs, 650
White adipose tissue (WAT), 651
White House Office of National Drug Control Policy, 869
White matter, 154
WHO. *See* World Health Organization (WHO)
WIN55, 212-2
  induced gastric emptying delay, 951
  neuroprotective effect, 920
  repeated administration in rat, effect of, 951
  in vivo effect in rat, 950
Wisconsin Card Sorting Test (WCST), e54
Wistar audiogenic rats (WAR), 608
Wistar rats, e145, 666
Withdrawal symptom
  newly abstinent drug user, 321
WM. *See* Working memory
Working memory, 46, 47, 49, e67, 71, 155–157
World Health Organization (WHO), 92, 113, e171, 860, 1008
  traffic accidents estimate, 235

X

X chromosome, e4
Xerostomia, e73
X-ray structure, of active center THCAS, 21

Y

Yale global tic severity scale (YGTSS), 888
Y chromosome, e4
Years lived with disability (YLDs), 90
Years lost due to premature mortality (YLLs), 90
YGTSS. *See* Yale global tic severity scale (YGTSS)
YMRS. *See* Young mania rating scale (YMRS)
Young age at CU onset and its association with CUD risk, 139
  empirical evidence, 139
  epidemiological studies on earlier onset of CU in CUD risk, 141
  important methodological aspects of studies on early onset of substance use and substance use disorder risk, 143
  methodological considerations, 139
  possible explanations for association between a younger age at first CU and CUD risk, 140
Young mania rating scale (YMRS), 792
Youth cultures, e12
Youth interventions foundation, 1051

Z

ZNF804A gene, 347
Zolpidem, 1032
Zornia latifolia, 840

Page left intentionally blank

Handbook of
Cannabis and Related Pathologies

EDITED BY
VICTOR R. PREEDY

Handbook of Cannabis and Related Pathologies: Biology, Pharmacology, Diagnosis, and Treatment is the first book to take an interdisciplinary approach to the understanding of cannabis use and misuse. Recent worldwide trends toward decriminalizing marijuana for medical use have increased legal use of the drug and recreational use remains high, making cannabis one of the most commonly used drugs. Cannabis has a wide range of adverse neurological effects, and use and abuse can lead to physical, social, and psychopathological issues that are multifarious and complex. Effective understanding and treatment requires knowledge of the drug's effects from across scientific disciplines.

This book provides an overview of the biological and pharmacological components of the cannabis plant; outlines its neurological, social, and psychopathological effects; assists in diagnosis and screening for use and dependency; and aids researchers in developing effective treatments for cannabis-related issues and disorders. Fully illustrated, with contributions from internationally recognized experts, *Handbook of Cannabis and Related Pathologies* is the go-to resource for neuroscientists, pharmacologists, pathologists, public-health workers, and any other researcher who needs an in-depth and cross-disciplinary understanding of cannabis and its effects.

Key Features
- Each chapter includes an abstract, key facts, mini-dictionary of terms, and summary points
- Comprehensively illustrated with numerous figures, tables, and diagrams per chapter
- The only publication to provide a "one-stop-shopping" synopsis of everything to do with cannabis and its related pathology, from chemicals and cells, to individuals and communities, to diagnosis and treatment
- The interdisciplinary approach offers an integrated and informed synopsis of the complex issues surrounding cannabis as a substance, its use, and its misuse

About the Editor
Victor R. Preedy, BSc, PhD, DSc, FRSB, FRSH, FRIPHH, FRSPH, FRCPath, FRSC is a senior member of King's College London. He is also director of the Genomics Centre and a member of the Faculty of Life Sciences and Medicine.

Professor Preedy has longstanding academic interests in substance misuse, especially in relation to health and well being. He was a member of the editorial board of *Drug and Alcohol Dependence* and was a founding member of the editorial board of *Addiction Biology*. In his career, Professor Preedy was Reader at the Addictive Behaviour Centre at the University of Roehampton, and also Reader at the School of Pharmacy (now part of University College London). Professor Preedy is editor of the multivolume seminal works *Comprehensive Handbook of Alcohol Related Pathology* and *The Neuropathology of Drug Addictions and Substance Misuse* (both published by Academic Press-Elsevier).

To his credit, Professor Preedy has published over 600 articles, which include peer-reviewed manuscripts based on original research, abstracts and symposium presentations, reviews, and numerous books and volumes.

ELSEVIER

ACADEMIC PRESS
An imprint of Elsevier
elsevier.com

ISBN 978-0-12-800756-3
9 780128 007563