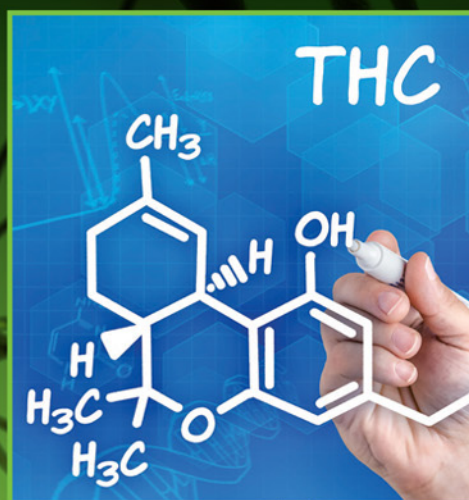
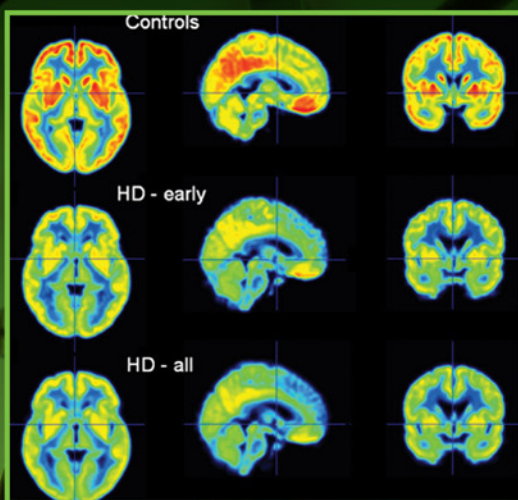


Cannabinoids in Neurologic and Mental Disease

Edited by
Liana Fattore



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Cannabinoids in Neurologic and Mental Disease

Edited by

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Preface

Cannabis sativa has a very long history of use for medical purposes, however this use declined early in the twentieth century due to the growing evidence of marijuana's addictive potential. Recent progress in cannabinoid pharmacology, together with the discovery of the body's endogenous cannabinoid system as a neuromodulatory system comprised of receptors, endogenous ligands (endocannabinoids), and enzymes responsible for the synthesis and degradation of endocannabinoids, has renewed interest in cannabis-based medicines. This allowed novel cannabinoid-based medicines development for treatment of different human pathologies, like Cesamet[®] (nabilone), Marinol[®] (synthetic THC), and Sativex[®] (an equimolecular combination of THC and cannabidiol enriched botanical extracts).

This book is intended to provide a fascinating exploration into the latest evidence of therapeutic potentials of Cannabis derivatives, and to analyze the pros and cons for cannabis-based medications in mental diseases in a systematic and balanced manner. Leading academics and clinicians in the fields of medicine, neuroscience, pharmacology, and psychology illustrate and discuss the most recent findings on involvement of endocannabinoid system in 16 different brain diseases, ranging from rodent studies to clinical trials, from behavioral pharmacology to brain neurotransmission.

By illustrating opinions of prominent experts on current advances in each mental disorder in a clear and readable form, this book provides a balanced synopsis of the role of the endogenous cannabinoid system in: 1) neuroinflammatory and neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, epilepsy, migraine); 2) neurological and psychiatric disorders (schizophrenia, bipolar disorder, Tourette syndrome, post-traumatic stress disorder, drug addiction, appetite dysregulation); and 3) impulsive and compulsive disorders (impulsive behavior, obsessive-compulsive disorder, binge eating disorder, trichotillomania). An introductory chapter on Endocannabinoids in the nervous system health and disease paves the way for the following dedicated chapters, while a comprehensive overview on cannabinoid CB₂ ligands and their therapeutic potential in mental diseases concludes this volume.

This book will likely help unravel why some patients voluntarily consume cannabinoids to relieve symptoms (e.g. multiple sclerosis) while others (e.g. schizophrenic patients) are particularly vulnerable to cannabis exposure, and will shed (scientific) light on common myths concerning the medicinal uses of cannabis.

Liana Fattore

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The ever-expanding world of the endocannabinoids: A concise introduction

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THE ENDOCANNABINOID SYSTEM: DISCOVERY AND EARLY DEFINITIONS

The use of various strains of cannabis as a source of medicinal preparations, as well as of “inspiration” in religious rites and “recreational” psychotropic effects, seems to have originated in ancient China, more than 4000 years ago. Although the earliest written reference to the use of hemp against pain and inflammation is the Chinese *Rh-Ya* (1500 BC), this plant had been mentioned much earlier in the book *The Herbal*, attributed to the “red emperor,” Shen Nung (2838–2698 BC), who is considered the precursor of all herbalists (Wood, 1997). More recent evidence for the use of cannabis against various ailments can be found in the ancient Egyptian, Indian, Greek, and Roman pharmacopeias and in medieval Islamic medicine. The Irish physician William O’Shaughnessy is credited with the introduction of the therapeutic use of cannabis into Western medicine in the 1830s (O’Shaughnessy, 1839). Despite the millennial, albeit mostly anecdotal, history of medicinal use of cannabis, it was only with the explosion, in the 1960s, of the abuse of marijuana, one of its most popular preparations in Western countries, that major efforts were started with the aim of understanding the molecular mechanisms of the psychotropic activity of cannabis strains used for such a preparation. Thus, of the three most important uses of cannabis by mankind (one should not forget that hemp has provided strong fiber for ropes and fabric much earlier than cotton), it was the one that had become the most abused, for recreational use, that led first to the discovery of the psychotropic component of cannabis, the “cannabinoid” (–)- Δ^9 -tetrahydrocannabinol (THC) (Gaoni and Mechoulam, 1964a), and later to the identification of specific plasma membrane, G-protein coupled receptors for this compound, named “cannabinoid receptors” (Matsuda et al., 1990; Munro et al., 1993). Subsequently, endogenous ligands for cannabinoid receptors, the “endocannabinoids,” and metabolic enzymes for such ligands, were also identified (Devane et al., 1992; Di Marzo et al., 1994; Mechoulam et al., 1995), thus revealing a complete signaling system, the “endocannabinoid system.” This is currently regarded

as a fundamental regulatory apparatus involved in nearly all physiological and pathological aspects of mammalian biology (Di Marzo and Petrosino, 2007).

CANNABINOIDS

One year before the molecular characterization of THC, another abundant cannabis component, cannabidiol (CBD), had already been identified (Michoulam and Shvo, 1963), whereas other chemically related terpenophenolic compounds, including cannabichromene (CBC) and cannabigerol (CBG), were isolated from *Cannabis sativa* soon thereafter (Gaoni and Mechoulam, 1964b; Gaoni and Mechoulam, 1966). When it became clear that these natural products are nearly unique to the cannabis plant, or maybe simply because they were first identified from this source, these compounds were collectively named “cannabinoids.” Thus, the name “cannabinoid” indicates any secondary metabolite from various strains of cannabis with biogenetic origin from a terpene, normally geranyl pyrophosphate, and a phenol, i.e., olivetol or olivetolic acid. It is now established that cannabinoids are produced by the plant flowers as their corresponding carboxylic acids, which are then decarboxylated following heating or desiccation. Interestingly, a compound similar to CBG acid, but clearly not directly derived from olivetol, was recently isolated from South African plants of the *Helichrysum* genus (Lourens et al., 2008). Thus, cannabinoids might not be unique to the cannabis plant, although their biosynthesis in other plants might follow different routes. Furthermore, of all the natural cannabinoids that were initially tested, THC was shown to be the only one responsible for the “recreational” properties induced by the smoking of marijuana in humans. These properties can be described as generally mood and sensory altering effects leading to, among others, euphoria and sedation (Panagis et al., 2008). Later, a definition of THC-like activity was given as the ensemble of “central” pharmacological effects that THC induces in non-human primates, dogs (ataxia), and rodents. In mice, it was proposed that the concomitant induction of: (1) immobility in a square box, (2) catalepsy on a ring, (3) analgesia in the hot plate or tail flick tests, and (4) hypothermia, known as the “tetrad” of tests for “cannabimimetic” activity, would be a good predictor of THC-like pharmacological activity (Martin et al., 1991).

CANNABINOID RECEPTORS

It was through the screening of a series of “orphan” (i.e., without a known ligand) G protein-coupled receptors (GPCRs) that a specific receptor for THC, or “cannabinoid receptor,” was molecularly characterized in 1990, and shown to be most abundant in the brain (Matsuda et al., 1990). Previously, enantioselective binding sites for THC analogues with stronger psychotropic activity had been identified in the rodent brain and shown to be coupled to inhibition of adenylate cyclase (Devane et al., 1988; Bidaut-Russell et al., 1990). Thus, the name “cannabinoid receptor” was used for this GPCR to underline the fact that it was selective for

the “most famous” cannabinoid, i.e., THC, versus many other tested exogenous or endogenous molecules with different chemical structures. This receptor was most likely responsible for the “central” pharmacological activity of THC and cannabis and was then renamed “cannabinoid receptor type-1” (CB₁) in 1993, after the discovery, by homology cloning, of a second GPCR for THC, known thereafter as “cannabinoid receptor type-2” (CB₂) (Munro et al., 1993). However, it soon became clear that: (1) CB₂ receptors, which are present in very low amounts in the brain, are not responsible for the psychotropic effects of THC, whereas CB₁ receptors are also present in peripheral tissues and non-neuronal cells, and coupled to non-psychotropic effects (Mackie, 2008); (2) THC is not the only pharmacologically active cannabinoid component of cannabis, and compounds such as CBC, CBG, and, particularly, CBD are endowed with interesting and potentially therapeutically useful pharmacological actions (De Petrocellis et al., 2011); and (3) neither CB₁ nor CB₂ is efficaciously activated by any of these “non-psychotropic” cannabinoids—on the other hand, both natural and synthetic compounds with non-cannabinoid chemical structures (last but not least the endocannabinoids) have been shown to activate these two GPCRs (Gertsch et al., 2010), without necessarily disdaining to interact with other targets. Therefore, the definition of “cannabinoid receptors” no longer denotes the capability of being specifically activated by any cannabinoid, or associated with THC-like central, or “cannabimimetic,” activity, and CB₁ and CB₂ should, instead, be defined as “THC receptors.” Yet, the definition of “cannabinoid receptor” has persisted to the present date. Conversely, if a new definition of “cannabinoid receptors” as targets specific and exclusive also for other non-THC cannabinoids is to be used, this will have to wait until such proteins are discovered.

Indeed, since the cloning of CB₂ there have been several reports suggesting, based almost uniquely on pharmacological and biochemical approaches, the existence of other cannabinoid receptors. Other “orphan” GPCRs, first of all GPR55, first appeared and then disappeared from the list of putative targets for THC action (Pertwee, 2010). Interestingly, some of these GPCRs are currently suggested to mediate some of the effects of non-THC natural or synthetic cannabinoids, and hence be included in a wider definition of “cannabinoid receptors.” At any rate, no homologue of CB₁ and CB₂, nor any other specific THC receptor, has been identified to date. Accordingly, the effects of THC in the “tetrad” of tests for cannabimimetic activity is abolished in CB₁, but not in CB₂, null mice (Zimmer et al., 1999), whereas some of the immunomodulatory effects of this cannabinoid are instead absent in CB₂, but not in CB₁, null mice (Buckley et al., 2000).

The activation of CB₁ and CB₂ receptors, usually via stimulation of G proteins of the G_{i/o} type, results in numerous intracellular responses that are often cell type and agonist specific and include the inhibition of various voltage-gated Ca²⁺ channels, the activation of K⁺ channels (in the case of CB₁ only), the inhibition of adenylate cyclase activity resulting in lower cAMP levels, and the activation of MAPK pathways (Howlett, 2002). Other second messenger pathways, in some cases occurring also through the activation of G proteins of the G_{q/11} type, have

been revealed for the two receptors, including the phospholipase C/inositol-trisphosphate/intracellular Ca^{2+} mobilization, the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (PKB), the Jun N-terminal kinases 1 and 2 (JNK1 and JNK2), and the ceramide cascades (Howlett, 2002). Finally, apart from the several regulatory proteins that interact with cannabinoid receptors as well as other GPCRs, it is worthwhile mentioning the two specific cannabinoid receptor-interacting proteins 1a and 1b (CRI1a and 1b), which are thought to play important roles in the regulation of intracellular trafficking, desensitization, down-regulation, signal transduction, and constitutive activity of CB_1 receptors (Howlett et al., 2010).

ENDOCANNABINOIDS

By definition, “endocannabinoids” are agonists of cannabinoid receptors present in the same organisms that express such receptors, that is, they are “endogenous.” As suggested by other examples of endogenous mediators discovered from studies on the mechanism of action of xenobiotic molecules, first of all the endorphins, endocannabinoids are unlikely to have a cannabinoid-like chemical structure, although their 3D structure would be expected to share some of the features of THC and of the cannabinoid receptor pharmacophore. The first endocannabinoid was identified in 1990 as *N*-arachidonoyl-ethanolamine, and this compound was baptized “anandamide” from the Sanskrit word *ananda* for “internal bliss” to emphasize its activity as a THC mimic and CB_1 agonist (Devane et al., 1992). Indeed, we now know that anandamide, in agreement with its name, is a much less efficacious agonist at CB_2 than it is at CB_1 receptors, but also interacts with other molecular targets that have little to do with the psychotropic and mood altering actions of THC and marijuana, thus in contradiction with the etymology of this name. The second endocannabinoid to be discovered, and perhaps the most selective for, and efficacious at, both CB_1 and CB_2 receptors, is 2-arachidonoyl-glycerol (2-AG) (Mechoulam et al., 1995; Sugiura et al., 1995). Both anandamide and 2-AG contain an arachidonate acyl chain and a polar alcohol function in their chemical structure and as such have physicochemical properties in common with THC such as their lipophilicity, and the presence of an *n*-pentyl chain and of a hydroxyl group, which are sterically organized as in the cannabinoid. Unlike THC, however, anandamide and 2-AG also contain an amide and an ester bond, respectively, which are prone to enzymatic hydrolysis, thus possibly explaining why both compounds exert effects in the “tetrad” of tests only at doses much higher than those needed for THC to exhibit the same actions. This possibly explains why the effects of anandamide in the “tetrad” of tests for cannabimimetic activity are not abolished in CB_1 null mice (Di Marzo et al., 2000). However, this finding can also be explained by the fact that anandamide also interacts with other receptors and ion channels (see below).

Other endogenous, lipophilic, fatty acid derivatives have been suggested to activate CB_1 and CB_2 receptors at low/submicromolar concentrations.

Among these, three more arachidonic acid derivatives, 2-arachidonyl glyceryl ether (noladin ether) (Hanus et al., 2001), *N*-arachidonoyl-dopamine (NADA) (Huang et al., 2002), and *O*-arachidonoyl-ethanolamine (virodhamine) (Porter et al., 2002), have been studied from the pharmacological standpoint. However, evidence suggesting that these compounds act as endocannabinoids under physiological or pathological conditions is still scant and controversial. In particular: (1) no biosynthetic mechanism has been identified for noladin ether, (2) NADA is present in tissue at concentrations usually very near to the detection limit and possibly insufficient to activate cannabinoid receptors, and (3) virodhamine was shown to be formed in a non-enzymatic manner from anandamide. Other fatty acid derivatives that have been occasionally proposed to act as endocannabinoids include the primary amide of oleic acid, oleamide (Cravatt et al., 1995) and other long chain polyunsaturated *N*-acylethanolamines (Fontana et al., 1995), which, however, have much lower affinity for both CB₁ and CB₂ receptors than does anandamide (see below). More recently, some hemoglobin-derived peptides have been shown to act as orthosteric CB₁ ligands, in most cases as inverse agonists (Heimann et al., 2007; Gomes et al., 2009; Bauer et al., 2012), whereas other lipophilic compounds have been shown to behave instead as allosteric ligands at the CB₁ receptor, by either enhancing, as in the case of lipoxin-A₄ (Pamplona et al., 2012), or antagonizing, as in the case of pregnanolone (Vallee et al., 2014), its effects. Future studies are now needed to understand the physiopathological role of these compounds as endogenous cannabinoid receptor modulators, in particular by investigating the mechanisms and conditions through and under which their tissue levels change concomitantly with those of anandamide and 2-AG. In fact, in order to exert their actions, endogenous blockers and allosteric modulators at a given receptor require the co-presence of the endogenous agonists at that receptor.

SPECIFIC AND LESS SPECIFIC METABOLIC ROUTES AND TRAFFICKING MECHANISMS FOR ENDOCANNABINOIDS

Like all chemical signals, endocannabinoids also require specific mechanisms for the regulation of their tissue levels, that is, anabolic and catabolic reactions for their biosynthesis and inactivation, respectively, as well as enzymes catalyzing such reactions. Anandamide belongs to the family of long chain *N*-acylethanolamines (NAEs). The enzymatic formation and degradation of the saturated and monounsaturated members of this family had already been elucidated in the 1970s (Schmid et al., 1996). Therefore, it was straightforward to demonstrate that also anandamide, similar to, for example, *N*-palmitoylethanolamine, is produced from the hydrolysis of the phosphoester bond of the corresponding *N*-acylphosphatidylethanolamine (NAPE), i.e., *N*-arachidonoyl-phosphatidylethanolamine (NArPE), a minor membrane phospholipid belonging to this family of phospholipids that had escaped detection in previous studies (Schmid et al., 1996). It also appeared likely that anandamide would be inactivated through the hydrolysis of

its amide bond by an amidase, which had been identified in the 1970s, but never molecularly characterized. The products of this reaction, arachidonic acid and ethanolamine, are not active at cannabinoid receptors, and, therefore, the enzymatic hydrolysis by this amidase, later cloned and named fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996), represents a true inactivation step for anandamide. Likewise, it was not farfetched to predict that 2-AG, a common intermediate in (phospho)glyceride metabolism, could be produced from the hydrolysis first of phosphoglycerides, such as phosphatidylinositols, to *sn*-2-arachidonoyl-containing diacylglycerols (DAGs) by phospholipase C, followed by the hydrolysis of DAGs by a DAG lipase (Bisogno et al., 1997; Stella et al., 1997). It was also shown that, like other monoacylglycerols (MAGs), 2-AG is inactivated by enzymatic hydrolysis to arachidonic acid and glycerol (Stella et al., 1997; Di Marzo et al., 1999a; Goparaju et al., 1999).

Despite the fact that the identification of endocannabinoid metabolic pathways took relatively little time from the discovery of anandamide and 2-AG, the enzymes catalyzing the corresponding reactions, with the only exception of FAAH, were only characterized in the 2000s [in fact, the MAG lipase later shown to be responsible for 2-AG inactivation (MAGL) (Dinh et al., 2002) had been cloned as early as 1997, but not yet associated with endocannabinoid signaling (Karlsson et al., 1997)]. In 2003, two diacylglycerol lipases selective for the hydrolysis of the ester bonds in the *sn*-1 position of DAGs, and named DAGL α and DAGL β , were cloned and shown to be responsible for the biosynthesis of 2-AG in both the perinatal and adult brain (Bisogno et al., 2003). Finally, the hydrolase responsible for the biosynthesis of anandamide and other NAEs from NArPE and other NAPEs, previously denoted as NAPE-selective phospholipase D (NAPE-PLD), was identified and cloned in 2004, and shown to be a protein with no homology with other phospholipases D, and belonging instead to the cholesteryl-glycine hydrolase family, with structural and functional similarity to acid ceramidase (Okamoto et al., 2004). The DAGLs and NAPE-PLD, although Ca²⁺ sensitive, are not Ca²⁺ dependent, and this led to the suggestion that the reactions that they catalyze do not represent the rate-limiting steps in 2-AG and anandamide biosynthesis, respectively, which occurs “on demand” usually after elevation of the intracellular concentration of Ca²⁺. Instead, phospholipases C (in particular phospholipase C β), which form DAGs from various phospholipids, and the *N*-acyltransferase responsible for NArPE biosynthesis from phosphatidylethanolamine, which has not yet been cloned, are Ca²⁺ dependent. This suggests that it is the biosynthesis of the direct precursors of the endocannabinoids that dictates the rate of their formation. However, as discussed in the next section, at least for anandamide, the scenario is complicated by the existence of several NArPE-processing enzymes.

All the enzymes that were initially assigned to endocannabinoid biosynthesis (i.e., DAGL α , DAGL β , and NAPE-PLD) and inactivation (i.e., FAAH and MAGL) are hydrolytic enzymes belonging to the superfamily of the serine hydrolases, although within different subfamilies (FAAH is an amidase, DAGL and

MAGL are esterases, and NAPE-PLD, as mentioned above, is a cholesterylglycerol hydrolase). This made it, at the same time, easy to find inhibitors for these enzymes, but difficult to render such inhibitors selective for each of these enzymes versus the others or other serine hydrolases. Thus, some of the inhibitors originally developed for FAAH were found to inhibit also phospholipase A₂ enzymes and, later, MAGL and DAGL (Deutsch et al., 1997). It was only at the turn of the century that truly selective inhibitors for FAAH or MAGL (reviewed in Feledziak et al., 2012) and DAGLs (Bisogno et al., 2006) could be designed, and only very recently inhibitors selective for DAGL α vs. DAGL β and vice versa were identified (Hsu et al., 2010; Baggelaar et al., 2013), whereas no such inhibitor exists to date for NAPE-PLD. These compounds, with the limitations due to the redundancy of endocannabinoid metabolic pathways and molecular targets (see below), and together with genetically modified mice lacking one enzyme or the other (Cravatt et al., 2001; Gao et al., 2010; Zhong et al., 2011; Aaltonen et al., 2014), have proven very valuable tools to interrogate the role of anandamide and 2-AG in many physiological and pathological aspects of rodent biology.

Enzymatic hydrolysis is certainly the most important type of endocannabinoid catabolic reaction, but other transformations were also shown to occur, at least in experiments *in vitro*. In particular: (1) oxidation by enzymes of the arachidonate cascade, including cyclooxygenase-2 (COX-2) (Kozak et al., 2002), 12-lipoxygenase (12-LOX) (Ueda et al., 1995), and cytochrome p450-containing oxygenases (cp450) (Bornheim et al., 1995); and (2) esterification into di- and tri-acylglycerols and phospholipids (Di Marzo et al., 1999b) have all been demonstrated, to a varying extent, not only for anandamide and/or 2-AG but also for noladin ether (Fezza et al., 2002) and NADA (Prusakiewicz et al., 2007). In general, while the former type of reactions occur, with different efficacy, for both ester/ether-type (2-AG, noladin, virodhamine) and amide-type (anandamide/NADA) endocannabinoids, and leads to products that are either inactive (when due to COX-2) or still active (when due to 12-LOX and cp450) as CB₁ and CB₂ ligands (Edgemond et al., 1998; Snider et al., 2010), esterification has so far been demonstrated only for 2-AG and noladin ether, and only in intact cells, and occurs via as yet unidentified enzymes (Di Marzo et al., 1999b; Fezza et al., 2002). The biological relevance of some of these reactions under physiological or pathological conditions *in vivo* is only now emerging (see below).

In view of: (1) the extracellular (although embedded in the membrane bilayer) location of both CB₁ and CB₂ binding sites; (2) the intracellular location of the endocannabinoid anabolic and catabolic enzymes mentioned above; (3) the high lipophilicity of anandamide and 2-AG; and (4) anandamide and 2-AG being presumably released “on demand” only after *de novo* biosynthesis, and not in a vesicle-mediated manner (Di Marzo et al., 1994), it has long been debated whether or not these two mediators require a passive transporter for their passage through the plasma membrane according to the gradient of their concentrations, both after their biosynthesis and before their enzymatic transformation (Fowler, 2013). Although strong indirect evidence has been gained in favor of the

existence of such a transporter even very recently (Ligresti et al., 2010; Chicca et al., 2012), this controversy will not be resolved until such a protein or mechanism is molecularly characterized. Several proteins involved, more or less selectively, in the intracellular trafficking of anandamide have been identified (Kaczocha et al., 2009; Oddi et al., 2009; Fu et al., 2012) and shown to participate in anandamide cellular uptake. However, these proteins, as well as FAAH, only account for part of anandamide transport across the membrane (Leung et al., 2013), and only in one direction (i.e., that of reuptake and not of release), thus not ruling out the presence of a membrane carrier.

REDUNDANCY AND COMPLEXITY IN ENDOCANNABINOID TARGETS AND METABOLIC ENZYMES

As already mentioned in the previous section, studies carried out during the last 10 years have revealed the potential existence of a high degree of redundancy for both the molecular targets and metabolic routes, and corresponding enzymes, of the endocannabinoids. The role of CB₁ and CB₂ receptors and enzymes such as FAAH, MAGL, and DAGL α in various aspects of endocannabinoid signaling have been supported by many studies using selective pharmacological tools and genetically modified mice made null for these proteins. On the other hand, similar studies have not always managed to confirm a role for NAPE-PLD (Leung et al., 2006) and DAGL β (Gao et al., 2010) in anandamide and 2-AG biosynthesis, respectively. Indeed, apart from the direct hydrolysis of NArPE by NAPE-PLD, at least three more routes have been suggested to mediate the multi-step conversion of this phospholipid into anandamide (Okamoto et al., 2007): (1) the sequential action of α,β -hydrolase-4 (ABHD4) and glycerophosphodiesterase-1 (GDE1), catalyzes the conversion of NArPE first into lyso-NArPE, then glycerophosphoanandamide and, finally, anandamide (Simon and Cravatt, 2008); (2) formation of lyso-NArPE can occur also through the action of a soluble phospholipase A₂, followed by direct conversion into anandamide by a lyso-PLD (Sun et al., 2004); and (3) an as-yet-unidentified phospholipase C, followed by the action of various phosphatases (such as protein tyrosine phosphatase N22 or SH2 domain-containing inositol phosphatase), can convert NArPE first into phosphoanandamide and then anandamide (Liu et al., 2008). Of these three additional biosynthetic routes, the former might apply to anandamide biosynthesis *in vivo* together with the NAPE-PLD-catalyzed pathway (as well as other routes), as suggested by studies carried out with double NAPE-PLD/GDE1 null mice (Simon and Cravatt, 2010). The other two pathways have not been investigated further to date.

A certain degree of redundancy has also been described for 2-AG enzymatic hydrolysis. Although studies carried out in mouse brain homogenate have demonstrated that more than 80% of 2-AG enzymatic hydrolysis is accounted for by

MAGL (Blankman et al., 2007), these also evidenced for the first time the role of two other hydrolases, α , β -hydrolase-6 and -12 (ABHD6 and 12), and confirmed that FAAH can also hydrolyze this endocannabinoid (Di Marzo et al., 1999b; Goparaju et al., 1999). Studies focusing on specific cell types and tissues or brain areas, and using selective inhibitors, have highlighted that ABHD6 (Marrs et al., 2011; Navia-Paldanius et al., 2012) and FAAH (Jhaveri et al., 2006; Maione et al., 2006) can indeed contribute to 2-AG inactivation under physiological or pathological conditions. Furthermore, alternative, and PLC β -independent, biosynthetic pathways have been proposed also for 2-AG (Bisogno et al., 1999; Carrier et al., 2004), but their physiopathological relevance *in vivo* is not known.

Although initially only shown to occur in cell-free homogenates or intact cells *in vitro*, recent studies have shown how the COX-2-mediated transformation (in conjunction with the subsequent action of specific prostaglandin synthases) of either anandamide (Gatta et al., 2012; Silvestri et al., 2013) or 2-AG (Alhouayek et al., 2013; Valdeolivas et al., 2013) into the corresponding prostaglandin-ethanolamides (prostamides) and prostaglandin-glycerol-esters (PGEs) can occur also *in vivo*. These metabolites act as lipid mediators in several physiological or pathological conditions via specific, non-cannabinoid and non-prostanoid, GPCRs (Nirodi et al., 2004; Liang et al., 2008). Indeed, cascades of chemical signals with different molecular targets, often producing effects that are in opposition to each other, and being produced by sequential chemical reactions, are typical of lipid mediators. Another recent example of this is lysophosphatidylinositol, which acts at GPR55, and is hydrolyzed to lysophosphatidic acid, which acts at several GPCRs showing some homology to CB₁ receptors.

Redundancy is a hallmark not only of endocannabinoid metabolic routes but also of endocannabinoid targets. In particular, anandamide has been shown to interact with several other GPCRs and ion channels at low/submicromolar concentrations (reviewed in Piscitelli and Di Marzo, 2012). Among these channels, the most studied one is the transient receptor potential vanilloid type-1 (TRPV1) cation channel, which anandamide activates (Zygmunt et al., 1999) at concentrations ranging from 0.2 to 20 μ M depending on the experimental conditions, and it has been shown to participate in several biological effects of endogenous anandamide *in vivo*, especially when the levels of this endocannabinoid are increased above physiological ones following inhibition of FAAH (Maione et al., 2006). Anandamide-induced activation of cannabinoid receptors and TRPV1 channels can produce effects that are in the same or opposite direction, depending on the cell or tissue type and the physiopathological context (Di Marzo and De Petrocellis, 2012). Other direct molecular targets for relatively low (≤ 10 μ M) concentrations of anandamide, which have been reported, although so far only *in vitro*, by at least two research groups, are TASK-1 K⁺ and T-type Ca²⁺ channels (both of which inhibited) and peroxisome proliferator-activated receptor- γ (PPAR- γ) (activated) (Di Marzo and De Petrocellis, 2012; Piscitelli and Di Marzo, 2012). Evidence has emerged recently to suggest that also 2-AG, which was believed to be selective for cannabinoid receptors until only a few years ago,

activates other targets, and, in particular, the $\beta(2)$ subunit of the GABA_A receptor (Baur et al., 2013) and TRPV1 channels (Zygmunt et al., 2013). However, such evidence awaits confirmation by groups other than those that originally reported it. Clearly, even without considering the redundancy of endocannabinoid catabolic pathways, the “promiscuity” of endocannabinoid molecular targets complicates the use of inhibitors of their inactivation as a selective strategy for the indirect activation of CB₁ and CB₂ receptors.

ENDOCANNABINOID-RELATED MEDIATORS AND THEIR TARGETS

Another potential complication arising from the pharmacological inhibition of endocannabinoid biosynthesis and inactivation arises from the fact that no metabolic pathway has been elucidated so far that is specific for anandamide or 2-AG versus their respective congeners, the NAEs and the MAGs. This fact did not appear as a problem until it was clear that non-endocannabinoid NAEs and MAGs are bioactive molecules *per se*, acting in most cases at non-cannabinoid receptors. Thus, inhibition of the biosynthesis or degradation of anandamide and 2-AG through enzymes such as the NAPE-PLD and DAGLs or FAAH and MAGL, respectively, is very likely to affect also the tissue levels of these “endocannabinoid-related mediators” (ERMs), and the activity of their targets. Among NAEs, *N*-palmitoylethanolamine (PEA) and *N*-oleoylethanolamine (OEA) are the most studied ones and many of their pharmacological actions *in vitro* and *in vivo* have been demonstrated to occur via activation of TRPV1 and, particularly, peroxisome proliferator-activated receptor- α (PPAR- α) (Ahern, 2003; Lo Verme et al., 2005). Furthermore, evidence exists suggesting that PEA and OEA also activate GPR55 and GPR119 (Overton et al., 2008; Godlewski et al., 2009; McKillop et al., 2013). Unsaturated 2-acylglycerols, namely 2-oleoyl- and 2-linoleoylglycerol, also activate GPR119 (Hansen et al., 2011). Regarding the bioactive NAEs, a certain degree of selective pharmacological manipulation of their levels can be obtained thanks to the existence of amidases different from FAAH that are more specific for PEA, as in the case of the *N*-acylethanolamine acid amidase (NAAA) (Ueda et al., 2001), or OEA, as in the case of the FAAH homologue, FAAH-2 [which, however, is not expressed in rodents (Wei et al., 2006)]. Things are even more complicated with the observation that FAAH can also catalyze the hydrolysis of other types of fatty acid amides (hence its name), including: (1) the fatty acid primary amides, such as the sleep-inducing factor oleamide (Cravatt et al., 1996), (2) the *N*-acyltaurines, which exert actions via TRPV1 and its homologue, TRPV4 (Saghatelian et al., 2006), and (3) the *N*-acylglycines (Huang et al., 2001), which, like anandamide, may act as inhibitors of T-type Ca²⁺ channels (Cazade et al., 2014), but, unlike anandamide, are also agonists at GPR18 (Kohno et al., 2006). Thus, FAAH inhibition can elevate the tissue levels

also of non-NAE bioactive metabolites. Additionally, it can result in alternative metabolic pathways for anandamide, such as those leading to the aforementioned prostamides or to TRPV1-activating 15-LOX derivatives (Starowicz et al., 2013), thus potentially leading to the modulation of the activity of several types of molecular targets.

Other ERMs that are less, if at all, prone to FAAH-mediated hydrolysis have also been identified in tissues. They include the *N*-acyl-dopamines (Bisogno et al., 2000; Huang et al., 2002), which again activate, either directly or indirectly, TRPV1 channels and inhibit T-type Ca^{2+} channels (Ross et al., 2009), and the *N*-acyl-serotonins (Verhoeckx et al., 2011), which, when polyunsaturated, inhibit FAAH and antagonize TRPV1 channels (Bisogno et al., 1998; Maione et al., 2007). Finally, amides between fatty acids and other amino acids, for example serine (Milman et al., 2006) and GABA (Huang et al., 2001), have also been identified and collectively named “lipoamino acids,” and their varied mechanisms of action are currently under investigation. These compounds should, in principle, pose fewer problems when it comes to predict the consequences of inhibiting FAAH, although at least in one case, i.e., *N*-arachidonoyl-dopamine, evidence exists suggesting that this amidase may instead catalyze the biosynthesis of this compound (Hu et al., 2009) via a direct condensation reaction.

CONCLUSIONS: THE “ENDOCANNABINOIDOME” AND HOW TO MANAGE IT

The ensemble of the several possible ERMs (only the lipoamino acids might be more than 80 different chemical entities, if one thinks of all the possible amides between amino acids and fatty acids), their metabolic enzymes (which they share with the two endocannabinoids only in some cases), and their previously known or novel and yet to be fully characterized molecular targets, constitutes, altogether, a quite high number of metabolites (>100) and proteins with encoding genes (>25), and might be viewed as an “ome” in its own right. Therefore, in the future, this large set of small and large molecules could be referred to as the “endocannabinoidome.” Given the redundancy of enzymes and promiscuity at targets mentioned above, managing this complex signaling system using pharmacological or genetic tools targeting these proteins might not be sufficient to fully distinguish the function of endocannabinoids from those of ERMs, or to develop new therapeutic drugs to treat disorders in which these mediators play an important protective or counterprotective role. The introduction of further sophistication in these tools can help in both these tasks, but is not always possible. For example, the development of inhibitors selective for the COX-2-catalyzed oxygenation of endocannabinoids versus arachidonic acid has revealed the role of this enzyme in the inactivation of that portion of brain anandamide that plays a role in toning down anxiogenic responses (Hermanson et al., 2013). This strategy, however, can eventually only be applied for those enzymes that have a

binding mechanism that clearly distinguishes among different substrates. FAAH might be one such example since it has already been modified, through site-directed mutagenesis, in a way to distinguish between NAEs and *N*-acyl-taurines (Mckinney and Cravatt, 2006). Alternatively, brain-impermeant pharmacological tools, i.e., CB₁ inverse agonists and FAAH inhibitors (Kunos and Tam, 2011; Moreno-Sanz et al., 2013), have been used to dissect the role played by enzymes and receptors in the central nervous system from that in peripheral organs, and in some cases are being proposed also as more selective, and hence safer, therapeutic tools because they are devoid of “central” unwanted effects. More selectively, the use of “conditional knockout” mice, in which genes encoding for receptors and enzymes are inactivated only in a limited number of cells and at a given time—a strategy so far applied only to CB₁ receptors (Marsicano et al., 2003; Corbille et al., 2007; Metna-Laurent et al., 2012)—might help understanding whether a given enzyme/receptor prefers a certain substrate/ligand in a time- and cell-dependent manner because of the potential abundance of that over other congeners. Indeed, all metabolites of the “endocannabinoidome” are derived from fatty acids, and their relative concentrations are likely to reflect in some way the relative abundance of the corresponding fatty acids in membrane phospholipids. Thus, another way that could be used in the future to manipulate the tissue levels of certain endocannabinoids and ERMIs instead of others might be through changes in the fatty acid composition of the diet. For example, prolonged feeding with dietary n-3 polyunsaturated fatty acids has already been reported to selectively manipulate the levels of anandamide and 2-AG over other NAEs and MAGs, and preferentially in peripheral organs versus the brain (Artmann et al., 2008; Banni and Di Marzo, 2010; Piscitelli et al., 2011).

It is also possible that, rather than developing ultra-selective tools, new medicines will be obtained in the future by deliberately designing “multi-target” compounds, or exploiting those obtained from natural sources. It has already been shown in animal models that dual “FAAH-TRPV1” blockers can produce potentially more efficacious and safer analgesic and anxiolytic drugs (Micale et al., 2009; Costa et al., 2010; Maione et al., 2013), and efforts are being made to develop also dual “FAAH-COX” inhibitors (Favia et al., 2012; Cipriano et al., 2013). On the other hand, PEA (in Normast[®] and, in combination with polydatin, Pelvilen[®]) and CBD (in combination with THC in Sativex[®]) offer two examples of how compounds with multiple molecular mechanisms of action can produce very efficacious treatment and/or enlarge the therapeutic window of co-administered drugs, and at the same time reach the pharmaceutical market.

In conclusion, despite it being undoubtedly made of light and shadow, the endocannabinoid system (and now the “endocannabinoidome”), with its many proven and potential roles in animal physiology and pathology, has not discouraged all those who have been fascinated and captured by the basic and applied aspects of its study. The following chapters in this book provide evidence of how investigations of this system are, in fact, still alive and flourishing.

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PART

Role of the
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Cannabinoids for the treatment of neuroinflammation

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NEUROINFLAMMATION

Neuroinflammation has emerged as a relatively new concept and relates to the inflammatory response occurring within the brain, which has distinct features in comparison with peripheral inflammation. In the periphery, the majority of cells engaged in inflammation are cells of hematopoietic origin, macrophages and lymphocytes, that extravasate from the vascular beds invading the harmed tissue. The complexity of the inflammatory response is still being unraveled. It aims to combat pathogens, which are sensed as intruders, eliminating damaged cells and all sorts of irritants or damaging molecules. In the case of neuroinflammation, the major players are glial cells, including astrocytes and microglial cells, along with blood-derived lymphocytes, monocytes, and macrophages. One of the more evident consequences of glial activation is the synthesis and release of a great number of cytotoxic molecules (such as the complements, cytokines, chemokines, glutamate, interleukins, nitric oxide, and reactive oxygen species), which are detrimental for neurons, although sometimes the beneficial consequences of neuroinflammation have been disregarded. The final objective is to eliminate the possible pathogens or cell debris from dead neurons, and culminate in brain tissue repair. Indeed, this is the case, as several anti-inflammatory cytokines are released, while growth factors and tissue repairing molecules also restore brain homeostasis. The complex inflammatory response is context dependent, e.g., it depends on the actual trigger of the reaction, the brain region where it occurs, and the age of the subject. This process is very dynamic since it evolves by changing over time, but should be limited both in space and in time. This is the case, for example, following an infection or after an acute injury. However, in neurodegenerative diseases, inflammation is sustained, albeit of lower intensity, thus contributing to neurodegeneration. This has led to the proposal that chronic inflammation is a causative factor to the pathogenesis of neurological diseases and disorders (Minghetti, 2005).

Nowadays, it is considered that many neurological and mental diseases involve some kind of neuroinflammation, whether primary in the disease, or secondary to an overt neurodegeneration. Furthermore, even during normal aging there is glial activation and in age-associated degenerative conditions this can be exacerbated. Therefore, the use of molecules with anti-inflammatory properties in their treatment is warranted. In that respect, cannabinoids are agents with a very interesting pharmacological profile, since they have shown in a wide spectrum of paradigms that they are neuroprotective (Grundy et al., 2001; van der Stelt and Di Marzo, 2005; Gowran et al., 2011) and have demonstrated anti-inflammatory activity as well (Correa et al., 2005; Croxford and Yamamura, 2005; Klein, 2005).

As already mentioned, for years neuroinflammation has been considered detrimental to the brain. Indeed, it has been modeled by the administration of a lipopolysaccharide (endotoxin, LPS), a bacterial membrane component, or a β -amyloid peptide ($A\beta$), a major constituent of senile plaques in Alzheimer's disease (AD), which increases the release of cytotoxic molecules including nitric oxide (NO), superoxide anion, arachidonic acid, and proinflammatory cytokines, such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), or IL-6. Microglia, the resident macrophage of the brain, mediate multiple facets of neuroinflammation, including cytotoxicity, repair, regeneration, and immunosuppression due to their ability to acquire diverse activation states, or phenotypes. Indeed, microglial cells when activated by these former agents release such molecules, showing what we now call the M1 activation state, which has a predominantly cytotoxic profile. However, there are activated microglia that release anti-inflammatory cytokines, such as IL-4 or IL-10, expressing the enzyme arginase-1, which is involved in arginine degradation like nitric oxide synthase (NOS), and other repairing molecules, which are considered to be alternatively activated or in the M2 state. Some authors (see Chhor et al., 2013) even describe intermediate phenotypes: M2a with an alternate activation and involved in repair and regeneration, M2b with an immunoregulatory phenotype, or M2c with an acquired-deactivating phenotype.

ENDOCANNABINOID SYSTEM ELEMENTS IN GLIAL CELLS

The endocannabinoid system consists of two established (CB₁ and CB₂) and some candidate metabotropic receptors including the GPR55, and endocannabinoid signal molecules including arachidonylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), along with their synthesizing and degrading enzymes (Piomelli, 2005; Di Marzo and De Petrocellis, 2012; Pertwee, 2012). However, different cannabinoids can also interact with other receptors, for instance transient receptor potential vanilloid 1 (TRPV1). All types of cells present in the brain bear cannabinoid CB₁ receptors (CB1Rs). Indeed, CB1Rs are

very well represented in the brain (very high density and widespread distribution), and thus initially were named as the *central* cannabinoid receptor, while the CB2Rs were considered the *peripheral* receptor, due to their predominant presence in immune cells and organs. Neurons express CB1Rs, as is very well known, but there is increasing evidence that some neurons can express also CB2Rs, as shown by *in situ* hybridization, immunohistochemistry, and different functional responses with selective CB2R agonists (van Sickle et al., 2005; Atwood and Mackie, 2010; Lanciego et al., 2011; Andó et al., 2012; den Boon et al., 2012).

As mentioned above, glial cells also bear CB1Rs and CB2Rs as well. First, the existence of CB1Rs in cultured astrocytes was reported (for reviews see Stella, 2004, 2010), and was followed by immunocytochemical evidence in brain sections (Rodriguez et al., 2001; Navarrete and Araque, 2008; Duarte et al., 2012). In regard to CB2Rs, the first report of their astrocytic existence was pharmacologic, as a selective CB2R antagonist blocked AEA effect (Molina-Holgado et al., 2002), whose expression was confirmed later in human astrocytes (Sheng et al., 2005) and in gliosome preparations from adult rats (Bari et al., 2011). Importantly, astrocytes in culture also show endocannabinoid synthesizing and degrading enzymes, and may release endocannabinoids upon stimulation of different neurotransmitter receptors (Walter et al., 2002; Stella 2004, 2010). In fact, astrocytes in culture from different species (mouse, rat, and human) synthesize AEA and other related acylethanolamides, as measured by chemical ionization gas chromatography/mass spectrometry, which are released by a calcium-dependent process (Walter et al., 2002). If results obtained in neonatal astrocytes in culture cast some doubts, they have been confirmed in gliosomes from adult animals (Bari et al., 2011), which bear all the endocannabinoid system armamentarium. Taking into account all these findings, one may conclude that astrocytes are both sources and targets of cannabinoids, which may alter different astrocytic functions. Moreover, endocannabinoids released from astrocytes may modulate other cell types (see below).

Similarly, microglial cells also have all of these endocannabinoid system elements (Stella, 2009). Indeed, the presence of both cannabinoid receptors has been shown in primary microglial cells or different cell lines in culture (Cabral and Marciano-Cabral, 2005; Cabral et al., 2008; Stella, 2010). Microglial cells in culture produce AEA and 2-AG, with ionomycin and millimolar concentrations of adenosine triphosphate (ATP) selectively increasing 2-AG production (Walter et al., 2003; Carrier et al., 2004). They have efficient ways to metabolize endocannabinoids either by fatty amide hydrolase (FAAH) or by monoacylglycerol lipase (MGL). In addition, Stella's group also identified another enzyme responsible for the degradation of 2-AG by microglia, the serine hydrolase α/b -hydrolase domain-containing 6 (ABHD6; Marrs et al., 2010).

In different works, the modulation of these elements, in particular inflammatory conditions *in vitro* or in pathological conditions *in vivo*, has been observed but their description goes beyond the scope of this chapter.

GLIAL FUNCTIONS AND THEIR MODULATION BY CANNABINOIDS

ASTROCYTE FUNCTIONS

Astrocytes are involved in a vast array of very important functions in the maintenance of brain homeostasis (Verkhatsky and Butt, 2007). They are in charge of the removal of ions and neurotransmitters such as glutamate (Glu), regulation of synaptic transmission, and impediment of rising excitotoxic concentrations. They have a major metabolic role in energy supply to neurons and in metabolism. Their protective role is exerted through the synthesis and release of several trophic factors and the expression of antioxidant enzymes which detoxify oxidant molecules. However, when activated following either an injury or an insult, they can release proinflammatory cytokines and therefore are involved in neuroinflammation.

Astrocytes in culture respond to different inflammatory challenges by releasing proinflammatory cytokines and NO (nitric oxide). Cannabinoids are able to reduce the release of these kinds of molecules by a CB1R- and/or CB2R-mediated effect. One of the earliest reports was that AEA decreased either endotoxin or Theiler's murine encephalomyelitis virus (TMEV) infection induced release of NO or TNF- α in the culture medium (Molina-Holgado et al., 1997). Both CB1Rs and CB2Rs were involved in the inhibitory effects of cannabinoids on LPS-induced NO release in astrocyte cultures (Molina-Holgado et al., 2002). Moreover, AEA potentiated the release of IL-6, which may show anti-inflammatory properties as well, in TMEV-infected astrocytes in a CB1R-dependent manner (Molina-Holgado et al., 1998). In human astrocytes activated by IL-1 β , addition of cannabinoids inhibited the expression of NOS and NO release, along with several chemokines (CXCL10, CCL2, and CCL5) and TNF- α , which were sensitive to CB1R or CB2R antagonists (Sheng et al., 2005).

Activation of CB1Rs on rat astrocytes in culture increases the rate of glucose oxidation and ketogenesis (Sánchez et al., 1998), two mechanisms involved in the energy supply of the brain. Recently, glucose metabolism was shown to be regulated by cannabinoids both in cultured neurons and in cultured astrocytes (Duarte et al., 2012). These authors reported that CB1Rs are able to control hippocampal intermediary metabolism in both neuronal and glial compartments, which suggests new alternative mechanisms by which CB1Rs may control cell physiology and afford neuroprotection. Because perivascular astrocytes are pivotally located and involved in supplying energy from blood to neurons in a stimulus-dependent manner, and as inflammatory conditions may alter this arrangement and the blood–brain barrier integrity, intermediary metabolism could be altered and cannabinoids may offer some kind of regulation. However, the effects of cannabinoids under inflammatory conditions have so far not been studied.

As already mentioned, astrocytic modulation of Glu uptake is central to Glu transmission. Cannabinoids have been shown to regulate Glu release from nerve terminals. Brown and colleagues studied Glu release and uptake from striatal

sections and found that both processes were inhibited by THC, resulting in a reduction of synaptic transmission. Since astrocytes express several Glu transporters responsible for its uptake, the effect of cannabinoids was directly assessed in cultures (Brown et al., 2003). Several cannabinoids (AEA, CP55,940, and WIN 55,212-2) inhibited transport of ^3H -D-Asp, a non-metabolizable Glu analogue, in a concentration- and stereoselective-dependent manner, which was partially inhibited by CB₁-selective blockade (Shivachar, 2007). The mechanism proposed was inhibition of uptake by cannabinoids resulting in increased extracellular Glu, which in turn would interact with presynaptic Glu receptors that would decrease its own release thus inhibiting its synaptic activity.

It should be noted that Glu could be released from astrocytes by reverse function of Glu transporters or by depolarization-evoked exocytotic Glu release, which is driven by reversal of the membrane $\text{Na}^+/\text{Ca}^{2+}$ exchanger and Ca^{2+} entry. This astrocyte exocytotic release was modulated by the stable AEA analogue methanandamide, as shown in an elegant recent work (Bari et al., 2011). Interestingly, by using selective cannabinoid agonists at CB₁R, CB₂R, and TRPV1, respectively, it was demonstrated that potentiation of [^3H]D-aspartate release was due to CB₁R, while its inhibition was due to CB₂R and TRPV1 activation. Given that inflammatory signals, such as prostaglandins, chemokines and ATP, all involved in neuroinflammation, can influence this kind of release, it would be worth assessing the possible effects of cannabinoids.

Cannabinoids have been reported to regulate the responses of astrocytes *in vitro* and *in vivo* to A β , which model AD astrogliosis. Interestingly, immunohistochemical and biochemical findings revealed that selective agonism at CB₁Rs and antagonism at CB₂Rs were able to blunt A β -induced reactive astrogliosis with subsequent overexpression of glial fibrillary acidic (GFAP) protein and S100B protein. Moreover, A β induced down-regulation of CB₁Rs together with a reduction of AEA concentration, whereas CB₂ receptors were up-regulated and 2-AG concentration was increased (Esposito et al., 2007).

In summary, although astrocytes in the brain are involved in numerous functions and represent important elements in neuroinflammation, the effects of cannabinoids reported so far are limited, and mostly focused on the responses to different inflammatory challenges such as LPS or TMEV.

MICROGLIAL FUNCTIONS

Production and release of cytotoxic molecules

Since the pioneering work of the group of Cabral, many authors have confirmed and extended the effect of cannabinoids, which is in general inhibitory, on the production and release of cytokines. In fact, cannabinoids prevent microglial activation and decrease NO production (Waksman et al., 1999) and TNF- α expression and release (Puffenberger et al., 2000; Facchinetti et al., 2003), evoked by different agents. In our own work, we have shown that cannabinoids (HU210,

WIN 55,212-2, and JWH133) block A β -induced activation of cultured microglial cells, as judged by mitochondrial activity, cell morphology, and TNF- α release; these effects were independent from the antioxidant action of cannabinoid compounds and exerted by a CB2R-selective agonist (Ramírez et al., 2005). In addition, we have shown that cannabinoids including the phytocannabinoid cannabidiol reduced NO generation, as judged by nitrites in the culture medium, in N13 microglial cells or primary microglia challenged with LPS (Martín-Moreno et al., 2011). Several excellent reviews have already addressed these cannabinoid effects (Cabral et al., 2005, 2008; Benito et al., 2008; Stella, 2010).

Migration

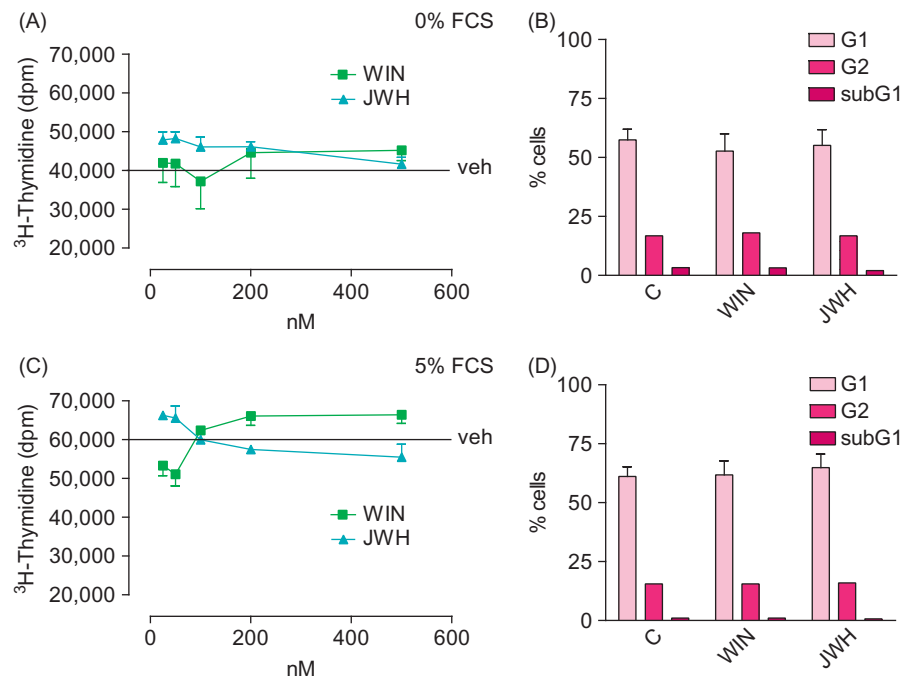
In the adult healthy brain, microglia displays a baseline motility characterized by the continuous extension and retraction of dynamic processes without movements of the cell bodies (Nimmerjahn et al., 2005). This baseline motility allows microglial cells to “sense” microenvironment alterations. Brain damage triggers a rapid microglial response, including an increased motility and chemotaxis to the site of the injured cells (Petersen and Dailey, 2004; Davalos et al., 2005; Hanisch and Kettenmann, 2007). Cannabinoids, whether plant derived, like THC and cannabidiol (CBD), or endocannabinoids (AEA, 2-AG, and other endocannabinoids), have been shown to induce migration of BV-2 microglial cells (Walter et al., 2003) in a concentration-dependent manner. The cannabinoid that promoted migration of microglial cells with the highest potency was 2-AG, and its effect was counteracted by a CB2R-selective antagonist and an abnormal CBD (Abn-CBD) antagonist (Walter et al., 2003). In another study it was shown that palmitoylethanolamide (PEA), which increased following focal cerebral ischemia, potentiated the promigratory effect of AEA in cultured BV-2 microglial cells, although the receptors engaged in PEA effect were not CB1Rs, CB2Rs, or Abn-CBD receptors (Franklin et al., 2003). In our hands, WIN 55,212-2, a mixed CB1R/CB2R agonist, two CB2R-selective agonists, JWH133 and HU308, and the phytocannabinoid CBD, all were effective at promoting migration of N13 cells and rat primary microglia (Martín-Moreno et al., 2011). Interestingly, besides a CB2R component, we also implicated the CB1R in cannabinoid-induced migration based on the effects of selective antagonists. Although several inflammatory challenges promote migration (e.g., A β peptide), and migratory microglia are considered activated, this function is considered positive for the resolution of the inflammation, since microglia travel towards the affected brain area, and it is a prerequisite for phagocytosis. Therefore, the promigratory effect promoted by cannabinoids should be considered beneficial. However, in other contexts cannabinoids have shown the opposite effect. For instance, in a model of prion disease, CBD decreased the migration of microglia towards cultured neurons previously exposed to prion protein and its neurotoxicity as well (Dirikoc et al., 2007). Another example is the decreased migration induced by several cannabinoids (THC, CP55,940, or CBD) when stimulated by Tat, a human immunodeficiency

virus-derived protein (Fraga et al., 2011). This effect was blocked by a CB2R-selective antagonist and knockdown with small interfering RNA. Therefore, it appears that cannabinoids can modify microglial migration, in general resulting in favorable effects for the neuroinflammation, but the actual direction of the effect can go either way (Miller and Stella, 2008).

Proliferation and phagocytosis

Upon neuroinflammation, microglia proliferate and this has been observed in *in vivo* situations where the density of microglial cells is enhanced. The group of Stella also assessed whether cannabinoids (synthetic and endocannabinoids) may induce proliferation of BV-2 microglia, and they found that even at 1 μ M concentration they were unable to induce proliferation, although the combination of LPS with interferon γ (IFN- γ) did so (Franklin et al., 2003). Interestingly, these authors did not observe any effect of the cannabinoids under study on phagocytosis of opsonized beads, even though LPS/IFN- γ addition was effective in that respect (Franklin et al., 2003). These results contrast with the 2-AG proliferation of a rat microglial cell line via a CB2R-dependent mechanism described by Carrier and coworkers (Carrier et al., 2004). It should be taken into account that the cell line was obtained from primary rat microglia grown in the continuous presence of macrophage colony stimulation factor (MCSF) until they began to proliferate and form foci, from which individual colonies were obtained by limited dilution. Therefore, 2-AG further enhanced proliferation of already proliferating cells. Interestingly, the proliferative effect of 2-AG was dependent upon the presence of MCSF (Carrier et al., 2004). We revisited this issue using the microglial cell line N13, in the absence and the presence of fetal calf serum (5% FCS), assessing mitochondrial respiration (MTT assay), actual cell number counts (after trypsinization), and ^3H -thymidine incorporation following treatment with WIN 55,212-2, a mixed CB1R/CB2R agonist, and JWH133, a CB2R-selective agonist, at 100 nM concentration. Cannabinoids modified neither mitochondrial respiration nor actual cell counts (data not published), although the presence of serum did increase the total cell number. As shown in Figure 1.1, incorporation of ^3H -thymidine was similar after cannabinoid treatment in comparison to vehicle in the absence of serum (Figure 1.1A) over a wide range of concentrations. Although the incorporation was higher in the presence of 5% serum (Figure 1.1C), the cannabinoids again failed to modify proliferation. In flow cytometry experiments assessing the proportion of cells in the different phases of cell cycle, it was found that (i) the majority of the cells were in G1 (growth 1 phase of the cell cycle) in N13 cultures treated with vehicle, (ii) 18–20% were proliferating (G2 phase, pre-mitotic phase after DNA replication), and (iii) cannabinoids did not modify this scenario.

In summary, cannabinoids as expected did not induce proliferation of microglial cells, indicating microglial activation, and only proliferation was observed in MCSF-induced proliferating cells.

**FIGURE 1.1**

Cannabinoids do not induce N13 microglial proliferation. For ^3H -thymidine incorporation assays 10^4 N13 cells were seeded in P96 plates, and incubated for 24 h in RPMI 1640 in the absence (A) or presence of 5% FCS (C), along with cannabinoid treatment (10–1000 nM). Then, $0.25 \mu\text{Ci}$ of ^3H -thymidine (20 Ci/mmol specific activity) was added and the cells were further incubated for 5 h. Cells were solubilized in 0.05% SDS, liquid scintillator was added to the wells, and plates were counted (Wallac 1450 Microbeta Trilux). Results are mean $\pm$ SEM ($n = 3$ independent experiments) and are expressed in dpm (A and C). In flow cytometry analysis (B and D), 250,000 cells were seeded in P12 plates, after 24 h in culture they were treated with the cannabinoids (100 nM), and after a further 24 h of incubation the cells were trypsinized, fixed, and labeled with propidium iodide for 15 min, and finally submitted to flow cytometry analysis. Cannabinoids WIN 55,212-2 (WIN) and JWH133 (JWH) showed similar ^3H -thymidine incorporation to vehicle (veh), and proportion of cells in G1, G2, or sub-G1 phases of the cell cycle, in the absence (B) or presence of serum (D).

CONCLUDING REMARKS

Neuroinflammation induces several changes in the functions of glial cells, whether astrocytes or microglia, involved in the inflammatory response along blood-derived cells. So far a great deal of information has been gathered on the reduction of proinflammatory cytokines and NO by cannabinoids both in

astrocytes and in microglial cells in culture. The inhibitory effects have been shown to be CBR dependent, by CB1R and/or CB2R, although some of them appear to be CBR-independent effects. It is quite surprising the scarce information gathered in cultured astrocytes, and the important astrocyte functions that may be altered by inflammatory conditions (e.g., metabolism or Glu uptake), that have not been addressed so far. In regard to microglial cells, there is far more information, with a particular bias towards the effects of cannabinoids on the expression and release of cytotoxic molecules. However, in the case of microglial cells, the effects of cannabinoids on important functions, such as migration, have been reported. On the other hand, given the relevance of the alternative activation state of microglia, it would be very interesting to investigate whether cannabinoids, besides reducing the M1 phenotype (cytotoxic profile), are able to enhance the M2-activated phenotype (anti-inflammatory/reparative profile) boosting anti-inflammatory cytokine production, reparative, and protective effects. Furthermore, cannabinoids may influence the well-known dialogue between brain cells, in particular between astrocytes and microglia.

This chapter has focused on the effects of cannabinoids, of different origins, on glial functions under inflammatory conditions relevant to neuroinflammation. Of course, pathological conditions, including neurodegenerative or mental diseases, in which neuroinflammation has been recognized for years or, on the contrary, just recently, are the subject of different chapters of this book. Synthetic or phytocannabinoids, such as CBD, have demonstrated their efficacy in promoting significant beneficial effects in several diseases. In particular, CB2R-selective agonists have interesting pharmacological features that make them attractive as therapeutic agents. First, they are devoid of psychoactivity, one of the drawbacks of CB1R or mixed agonists, and second, CB2R in glial cells are up-regulated in many of those pathological conditions. Another interesting therapeutic avenue would be that of selective enzyme inhibitors, which would enhance the effects of endocannabinoids and reduce neuroinflammation.

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Endocannabinoids and Alzheimer's disease

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INTRODUCTION

Alzheimer's disease (AD) is a complex age-related neurodegenerative disease characterized by the progressive loss of memory and cognitive function. Approximately 36 million people worldwide suffer from AD and, with an increasingly aged global population, that number is estimated to triple by 2050 (Wilmo and Prince, 2010). Symptoms of the disease include memory loss, difficulty with abstract thinking and completing familiar tasks, confusion, spatial and temporal disorientation, problems with speech, and altered mood (Thies and Bleiler, 2013). Deterioration in the health of patients with AD leads to death within 3 to 9 years and severe dementia can result in medical complications (Querfurth and LaFerla, 2010). Causes of death that increase in those suffering from AD include dementia, dehydration, pneumonia, and decubitus ulcers, which arise from the debilitating nature of the disease (Ganguli et al., 2005). The level of informal, social, and medical care required for AD patients is enormous and in 2010 alone the cost of this disease to the global economy was estimated at \$604 billion (Wilmo and Prince, 2010). The most striking observation in the AD-affected brain is the severe atrophy caused by neuronal loss, most notably in the CA1 region of the hippocampus, entorhinal cortex, and pyramidal cells in lamina II, as well as the shrinkage and loss of neuronal processes (Huang and Mucke, 2012). With no effective treatments yet available this is a serious concern for many healthcare systems. A number of degenerative insults have been identified in the pathogenesis of AD and the picture emerging indicates that the accumulation of misfolded proteins results in chronic activation of the immune response, oxidative damage, and excitotoxicity leading to synaptic dysfunction and neurodegeneration. These triggers worsen the progression of symptoms, functional alterations, and micro-anatomical deficits found in AD. Modulation of the endocannabinoid (eCB) system is emerging as a viable therapeutic target for neurodegenerative diseases such

Table 2.1 The Neuroprotective Effects of eCB Modulation on Neurodegeneration in AD

Alzheimer's Disease	eCB Modulation
Inflammation	↓ microglial activation by WIN 55,212-2 (Marchalant et al., 2009), THC, JWH015 (Ehrhart et al., 2005), CBD (Martin-Moreno et al., 2011), and JWH133 (Aso et al., 2013) ↓ IL-6 by WIN 55,212-2 (Marchalant et al., 2009), CBD (Martin-Moreno et al., 2012), and JWH133 (Aso et al., 2012) ↓ IL-1 β by WIN 55,212-2 (Marchalant et al., 2009) and JWH133 (Aso et al., 2013) ↓ TNF- α by WIN 55,212-2 (Marchalant et al., 2009) and JWH133 (Aso et al., 2013)
Oxidative stress	↑ oxidative damage in CB ₁ knockout mice (Kim et al., 2005) ↓ mitochondrial depolarization by <i>trans</i> -caryophyllene (Choi et al., 2013)
Excitotoxicity	↓ lipid peroxidation by ACEA and JWH133 (Aso et al., 2013) ↓ glutamate release by WIN 55,212-2 (Wang, 2003) ↓ NMDA toxicity by WIN 55,121-22 (Kim et al., 2006a). ↓ intracellular Ca ²⁺ release under high excitability conditions by CBD (Ryan et al., 2009)
Reduced neurogenesis	↓ neurogenesis by ↓ 2-AG (Gao et al., 2010) or CB ₁ knockout (Kim et al., 2006b) ↑ proliferation by HU210 (Jiang et al., 2005) ↑ neurogenesis by WIN 55,212-2 (Marchalant et al., 2009) ↑ bFGF and BDNF in CB ₁ -dependent manner (De March et al., 2008)

as AD (Table 2.1). The ability of this system to regulate many neuronal functions, through cannabinoid 1 (CB₁) and cannabinoid 2 (CB₂) receptors, such as Ca²⁺ buffering, metabolic activity, neurotransmission, and the inflammatory response, is proving very beneficial against the pathogenic characteristics of AD.

ALZHEIMER'S DISEASE

In 1907, when Alois Alzheimer first described AD as a special or unusual illness he noted what are now known as neuritic plaques and neurofibrillary tangles in the postmortem brain of his patient (Alzheimer et al., 1995). Today these lesions are still considered the characteristic hallmarks of AD. Neuritic plaques are formed by the extracellular accumulation of amyloid- β (A β) fibrils entangled with activated microglia, reactive astrocytes, and dystrophic neurites. Monomers of the A β peptide, ranging from 36 to 43 amino acids in length, are normal products of metabolism but longer forms of the peptide are quite prone to aggregation, forming dimers, trimers, oligomers, and insoluble fibrils of β -pleated sheets. The amyloid precursor protein (APP) is a single-pass transmembrane protein with a large extracellular domain whose exact physiological function is still unknown.

Some *in vitro* work has indicated that APP may play a role in cellular adhesion (Soba et al., 2006), neurite outgrowth (Qiu et al., 1995), and neuronal survival (Perez et al., 1997), while overexpression of APP increased cortical neuronal size in transgenic mice (Oh et al., 2009). Cleavage of APP by the enzymatic action of beta-site amyloid precursor protein enzyme 1 (BACE-1), a β -secretase, releases its large ectodomain, and further proteolysis by γ -secretase produces A β monomers. Accumulation of A β results from an imbalance between the production and clearance of the peptide. Early-onset (<60 years) familial AD has been attributed to mutations in three genes—APP, *presenilin-1* (PS1), and *presenilin-2* (PS2)—that affect the A β burden. APP has a direct link to A β production and, interestingly, so do PS1 and PS2 proteins. The catalytic subunit of the APP-cleaving γ -secretase protein contains either PS1 or PS2 and mutations in these corresponding genes have been shown to increase A β production and cause AD pathology (Bertram et al., 2010). While familial AD accounts for less than 1% of cases, the discovery of these mutations has led to the development of transgenic animals vital to AD research, which have further elucidated the pathogenic mechanisms of the disease. The more common sporadic form of AD has a multifactorial etiology with a number of genetic and environmental risk factors.

Apolipoprotein E (ApoE) is a polymorphic protein with three common isoforms, apoE2, apoE3, and apoE4, which are involved in neurite outgrowth as well as cholesterol and A β transport (Huang and Mucke, 2012; Rodrigue et al., 2013). A person with two copies of the ϵ 4 allele has a 10- to 12-fold risk of developing AD compared with ϵ 3 homozygotes (Corder et al., 1993). Age is the most prevalent risk factor for AD: it is estimated that 10% of people over 65 and 25% of people over 80 years of age are afflicted by this debilitating disease, and that number is set to rise to 1 in every 85 people by 2050 (Hebert et al., 2003; Brookmeyer et al., 2007). Next to age, one of the primary environmental risk factors for AD is vascular health. Hypertensive patients in particular have shown that increased levels of A β deposition and elevated blood pressure during midlife are indicators for the development of dementia (Rodrigue et al., 2013). The convergence between the genetic involvement of A β -regulating proteins in familial AD and the molecular evidence regarding A β toxicity has led many to believe that the accumulation and aggregation of A β is the initiating factor in AD.

Another characteristic hallmark of the disease is the formation of intracellular neurofibrillary tangles composed of hyperphosphorylated forms of the microtubule-associated protein, tau. In fact, the number and location of neurofibrillary tangles is one of the most accurate pathogenic markers for the severity of AD (Arriagada et al., 1992; Dickson et al., 1995). The primary function of tau is the stabilization of microtubules, allowing for functional axonal transport of signaling molecules, some cellular organelles, and trophic factors. Hyperphosphorylation of tau by a number of kinases such as glycogen synthase kinase-3 β and cyclin-dependent kinase 5 causes the protein to detach from microtubules and aggregate within the cytosol (Wagner et al., 1996; Plattner et al., 2006; Rankin et al., 2007). It has also been suggested that this may occur as a result of decreased dephosphorylation by the phosphatases

PP1, PP2A, PP2B, and PP2C or because of A β -induced oxidative stress (Schweers et al., 1995; Mailliot et al., 1998; Lee et al., 2001; King et al., 2006). It is important to note, however, that the spatial and temporal occurrence of neurofibrillary tangles does not always coincide with neuritic plaque formation. The pathogenic nature of hyperphosphorylated tau arises from both the loss of normal structural and regulatory microtubule function as well as from the toxic nature of the neurofibrillary tangles, which may sequester functioning cytosolic protein thereby disrupting further normal cellular function (Ballatore et al., 2007).

THE ENDOCANNABINOID SYSTEM AS A THERAPEUTIC TARGET

Targeting the eCB system through the use of the commercially available oromucosal spray Sativex, a combination of the phytocannabinoids cannabidiol (CBD) and Δ^9 -tetrahydrocannabinol (THC), has already proved beneficial for the treatment of neuropathic pain and spasticity in multiple sclerosis (Nurmikko et al., 2007; Notcutt et al., 2012). Furthermore, in a number of clinical trials modulation of the eCB system has improved behavioral symptoms in AD patients. In patients diagnosed with probable AD, a twice daily dose of 2.5 mg dronabinol, a phytocannabinoid derived from THC, was shown to reduce weight loss and improve disturbed behavior with minimal side effects of euphoria, drowsiness, and tiredness (Volicer et al., 1997). A more recent study has shown that in patients with late-stage AD, a single daily 2.5 mg dose of dronabinol improved nocturnal aggression and agitation with no adverse side effects (Walther et al., 2006). A single case study has also reported a reduction in the severity of agitation and resistiveness in a patient with mild AD through the use of nabilone, a CB₁ receptor agonist (Passmore, 2008). Furthermore, ongoing placebo-controlled double-blind phase II clinical trials are being carried out on the safety and efficacy of Namisol, an oral tablet containing THC, in patients suffering from AD and vascular dementia. Measurable outcomes from these two studies include any alteration in neuropsychiatric symptoms, agitation, balance and mobility, pain, quality of life, and episodic memory (Rikkert, 2014a,b). To date, no clinical studies have been carried out on the effectiveness of these drugs on abrogating neurodegenerative processes in AD. There is, however, a wealth of preclinical data outlining the beneficial effects of cannabinoid treatment on neuroinflammation, excitotoxicity, oxidative stress, and neurodegeneration that may be of relevance to AD.

THE ENDOCANNABINOID SYSTEM IN ALZHEIMER'S DISEASE

Over the past two decades a number of studies have characterized the status of the endocannabinoid system in AD, and considering its role in inflammation,

Ca^{2+} buffering, and neurotransmission it is unsurprising that this system is greatly affected. In AD patients, the CB_2 receptor was found to be selectively overexpressed in microglial cells in the entorhinal cortex and parahippocampus (Benito et al., 2003), and increased by as much as 40% around neuritic plaques in cortical brain tissues (Brodman area 10) from AD patients (Benito et al., 2003; Solas et al., 2013). This increase in receptor expression positively correlates with an increase in glial cells and has been proposed to be a response to the highly immunogenic environment as an attempt to down-regulate immune-mediated neurotoxicity (Koppel and Davies, 2010; Solas et al., 2013). Conflicting reports have emerged on the condition of CB_1 receptor density in AD. A variety of methods have been used to quantify CB_1 levels in a number of different brain regions and a consensus is yet to be reached. No change was found in CB_1 receptor expression in the hippocampus, entorhinal cortex, frontal cortex, anterior cingulate gyrus, or caudate nucleus of AD patients when compared to aged-matched controls (Benito et al., 2003; Lee et al., 2010; Mulder et al., 2011). However, in other studies, decreased CB_1 receptor expression was found in the parahippocampus and cortex of AD patients when compared to aged-matched controls (Ramirez et al., 2005; Solas et al., 2013). Recently, Farkas et al. have reported an initial rise, followed by a continuous decline, in CB_1 receptor density in the frontal cortex of AD patients (Farkas et al., 2012). By comparing each of the six stages of AD (Braak stages I–VI) to those in an age-matched control group, they have revealed a possible compensatory mechanism of vulnerable neurons whereby CB_1 receptor expression is increased in neurons, which are then lost due to progressive neuroinflammation and degeneration. Furthermore, CB_1 receptor activity becomes functionally impaired in AD, possibly through nitrosylation, which would affect its G protein coupling and downstream signaling (Ramirez et al., 2005). The eCB signaling molecules anandamide (AEA) and 2-arachidonylglycerol (2-AG) are also greatly affected. Analysis of postmortem tissue from the midfrontal and temporal cortex of AD patients has revealed decreased levels of AEA and its precursor N-arachidonoyl phosphatidylethanolamine, and importantly this inversely correlated with the levels of $\text{A}\beta_{42}$, the primary $\text{A}\beta$ isoform found in neuritic plaques that plays a vital role in plaque formation (Iwatsubo et al., 1994; Jung et al., 2012). Furthermore, an increase in the levels of the AEA-metabolizing enzyme fatty acid amide hydrolase (FAAH) has been reported around neuritic plaques, which may further reduce AEA tone (Benito et al., 2003). Interestingly, this reduction in AEA levels positively correlated with a decline in cognitive function, specifically the speed of information processing and language facility (Jung et al., 2012). While no global changes in 2-AG expression have been found in the brains of AD patients, studies on animal models of the disease have reported increased hippocampal expression (van der Stelt et al., 2006). Indeed, hippocampal samples from AD patients have shown significant alterations in the 2-AG signaling network. The protein expression of both isoforms of the 2-AG synthesizing enzyme diacylglycerol lipase (DAGL), $\text{DAGL}\alpha$ and $\text{DAGL}\beta$, is increased in neurons and microglia surrounding neuritic plaques. Furthermore, the primary 2-AG hydrolyzing

enzyme monoacylglycerol lipase (MAGL) undergoes cellular redistribution from the membrane towards the cytosol with some possible functional ramifications such as reduced 2-AG degradation at the cell membrane resulting in heightened eCB signaling (Mulder et al., 2011). Dissociation of MAGL from the membrane has been proposed to increase depolarization-induced suppression of inhibition thereby prolonging the excitatory neurons' firing periods leading to neuronal hyperactivity. The concomitant increase in cytosolic degradation of 2-AG may also affect its receptor-independent antioxidant capabilities, leaving neurons vulnerable to A β -induced oxidative stress (Mulder et al., 2011).

CANNABINOID AND NEUROINFLAMMATION

Inflammation in the central nervous system (CNS) is a fundamental process in the development of AD. Studies using various *in vitro* and *in vivo* models of inflammation have reported beneficial effects of cannabinoid treatment on reducing the inflammatory burden. Aging is the most prominent risk factor for the development of AD and is associated with increased microglial activation and exaggerated immune response (Nakanishi and Wu, 2009). Postmortem analyses on the brains of AD patients have shown increased numbers of activated microglia and astrocytes surrounding neuritic plaques as well as significantly higher levels of the proinflammatory cytokines IL-1, IL-6, and tumor necrosis factor alpha (TNF- α) (Akiyama et al., 2000; Arroyo et al., 2011). The activation of microglia occurs through the stimulation of a number of pattern recognition receptors such as Toll-like receptors (TLRs), NOD-like receptors (NLRs), and receptors for advanced glycation end products (RAGE) (Akiyama et al., 2000; Salminen et al., 2009). The binding of A β to these pattern recognition receptors results in the induction of microglial phagocytosis and the synthesis and release of proinflammatory cytokines and neurotoxic factors such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Heneka and O'Banion, 2007; Halle et al., 2008; Salminen et al., 2009).

In rats aged 23 months, treatment with the synthetic mixed CB₁/CB₂ receptor agonist WIN 55,212-2 for 21 days significantly reduced the levels of activated microglia in the hippocampus and dentate gyrus and this was shown to be both CB₁ and CB₂ receptor dependent. Furthermore, the same treatment reduced the mRNA levels of the proinflammatory cytokine interleukin 6 (IL-6) while increasing levels of the anti-inflammatory cytokine interleukin-1 receptor antagonist (IL-1ra). Protein levels of TNF- α and interleukin-1 beta (IL-1 β) were also decreased (Marchalant et al., 2009). In primary microglial cultures, treatment with interferon gamma (IFN- γ) increased the expression of CD40, a marker for microglial activation and inducer of TNF- α release. THC and JWH015, a selective CB₂ receptor agonist, suppressed this up-regulation by inhibiting the phosphorylation of signal transducer and activator of transcription 1 (STAT1) thereby impeding the janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway (Ehrhart et al., 2005).

In models of AD, cannabinoid treatment has been shown to effect microglial activation and migration, proinflammatory cytokine release, cognitive function, and neurotoxicity. [Ramirez et al. \(2005\)](#) have reported increased microglial activation in rats after intracerebroventricular (i.c.v.) injection of A β along with reduced spatial learning and neuronal markers indicating neuronal cell loss. Co-administration with WIN 55,212-2 ameliorated these effects and further *in vitro* study revealed the neuroprotective capabilities of WIN 55,212-2 were both CB₁ and CB₂ receptor dependent ([Ramirez et al., 2005](#)). The mobilization of intracellular Ca²⁺ in response to extracellular ATP signaling from dying cells is a seminal event in the activation of microglia and the induction of the inflammatory response. The cannabinoids CBD, WIN 55,212-2, and the CB₂ receptor selective agonist JWH133 were all shown to decrease the ATP-induced rise in intracellular Ca²⁺ concentration in the N13 microglial cell line and in primary microglial culture ([Martin-Moreno et al., 2011](#)). The effects of WIN 55,212-2 and JWH133 were fully reversed by the selective CB₂ antagonist SR 144528, indicating a CB₂ receptor-mediated effect; however, this reversal was not seen in CBD-treated cells suggesting that CB₂-independent mechanisms may also be at work. Treatments with CBD and WIN 55,212-2 also resulted in a significant reduction in the six-fold A β -induced rise in IL-6 *in vivo* ([Martin-Moreno et al., 2011](#)). Interestingly, CBD, WIN 55,212-2, JWH133, and HU308 were all shown to promote migration of N13 microglial cells and primary microglia.

The use of transgenic animals has allowed researchers to closely mimic the amyloidosis and neuroinflammation found in AD. In Tg APP2576 mice carrying the Swedish mutation, a missense mutation before the A β region of APP causes increased production of A β resulting in cognitive impairments, microglial activation, and the release of proinflammatory cytokines ([Haass et al., 1995](#); [Martin-Moreno et al., 2012](#)). In an attempt to mimic clinical circumstances, WIN 55,212-2 and JWH133 were administered orally for 4 months from when the mice were presymptomatic at 7 months of age ([Martin-Moreno et al., 2012](#)). Neither treatment affected cognitive function in wild-type mice but object recognition was improved in Tg APP2576 mice receiving either WIN 55,212-2 or JWH133. Microglial activation was increased by 53% in the transgenic mice, which was fully reversed by treatment with JWH133 but not WIN 55,212-2. Other inflammatory parameters found to be increased in Tg APP2576 mice were the expression of COX-2 protein, TNF- α mRNA, and soluble A β levels, all of which were significantly reduced by both cannabinoid treatments indicating a role for both the CB₁ and CB₂ receptors.

In a series of experiments using double transgenic APP/PS1 mice, Aso et al. further elucidated the specific role of these two receptors in neuroinflammation and their neuroprotective capabilities ([Aso et al., 2012, 2013](#)). The Tg APP/PS1 mice were treated with either arachidonyl-2-chloroethylamide (ACEA), a synthetic specific CB₁ receptor agonist, or JWH133 for 5 weeks at presymptomatic or early symptomatic stages. The preventive treatment with both cannabinoids restored cognitive function in all tests performed. However, while the cannabinoid

action of both agonists at the early stages of disease progression was successful in improving object recognition, no significant difference was found against vehicle control in the active avoidance test. Behavioral improvements cannot be attributed to a reduced A β burden as no difference in A β was measured in the neocortex and hippocampus. Stimulation of the CB₁ receptor at both the presymptomatic and early symptomatic stages was shown to have no effect on microglial activation but the number of plaque-associated astrocytes and IFN- γ was significantly reduced. Interestingly, no CB₁ receptors were localized on the astrocytes and the CB₁ receptor-mediated effects were thought to be as a result of neuronal–astroglial communication. Stimulation of the CB₂ receptor was successful in reducing microglial activation and the release of the proinflammatory cytokines IL-1 β , IL-6, and TNF- α as well as the anti-inflammatory cytokine IL-10.

A number of studies have identified the peroxisome proliferator-activated receptor γ (PPAR γ) as a key mediator of the cannabinoid anti-inflammatory effect. The PPAR family is a group of nuclear hormone receptors known to be involved in gene expression, lipid and glucose metabolism, and the inflammatory response. In cultured rat astrocytes A β induces the release of nitric oxide (NO), IL-1 β , TNF- α , and S100B, an effect that is reversed with co-treatment with CBD. Addition of the PPAR γ antagonist GW9662 inhibits the anti-inflammatory effect of CBD, suggesting a role of PPAR γ in cannabinoid signaling. Furthermore, hippocampal fractions from rats have revealed that CBD reduces the A β -induced rise in inducible nitric oxide synthase (NOS), glial fibrillary acidic protein, and S100 calcium-binding protein B (S100B) mediated by the inhibition of the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cell (NF- κ B). Antagonism of PPAR γ stops this inhibition and thereby affects CBD's anti-inflammatory capabilities (Esposito et al., 2011). Fakhfour et al. (2012) have further elucidated the relationship between cannabinoids and PPAR γ *in vivo* and have identified that A β , when administered intrahippocampally to adult rats, increased PPAR γ transcriptional activity and protein expression is observed, which is further increased as a result of i.c.v. administration of WIN 55,212-2 treatment. Furthermore, an A β -induced increase in NF- κ B nuclear translocation, caspase-3, and TNF- α protein expression was significantly reduced by WIN 55,212-2 treatment. Interestingly, the beneficial effects caused by WIN 55,212-2 were halted by the antagonism of PPAR γ by GW9662 (Fakhfour et al., 2012).

The role of immune response is to remove the A β burden as well as necrotic cells, which may cause further damage. While microglia can recognize and engulf A β fibrils, they are unable to degrade the peptide and phagocytosis is ineffective against neuritic plaques that have been formed. It is this inability to remove the A β burden and the steady release of proinflammatory cytokines that results in the chronic and self-propagating activation of the immune response and the accumulation of ROS and RNS to toxic levels. However, the immune response in the CNS is a dynamic process offering both anti-inflammatory and trophic support, as well as proinflammatory and apoptotic support, depending on the molecular triggers received from neighboring cells. The accumulating evidence indicates that activation

of the eCB system drives an anti-inflammatory and pro-survival environment whether it is through direct inhibition of microglial activation and proinflammatory cytokine release or through the indirect modulation of neuronal–astroglial signaling.

CANNABINOIDS AND OXIDATIVE STRESS

Oxidative stress is one of the earliest and most prominent features of AD, which can be caused by activation of the immune response, excitotoxicity, and A β . Indeed, the accumulation of A β is an upstream event to oxidative stress in the pathogenesis of AD followed by Ca²⁺ dysregulation and mitochondrial dysfunction (Texel and Mattson, 2011). The resulting mitochondrial dysfunction and the accumulation of ROS and RNS can initiate a number of intracellular apoptotic cascades. ROS are normal by-products of the mitochondrial respiratory chain and play a crucial role as signaling molecules, but markers for toxic increases in ROS levels such as lipid peroxidation, protein oxidation, and DNA/RNA oxidation are found even in the earliest stages of AD (Pratico and Sung, 2004; Wang et al., 2013). Activated microglia are a major source of ROS and RNS in the brain, primarily through NADPH oxidase, which is highly active in the AD brain (Shimohama et al., 2000), but also through the action of intracellular peroxidases and oxidative processes of the mitochondria (Wilkinson and Landreth, 2006; Block et al., 2007).

Application of FeCl₂ is a common model for oxidative stress, which induces severe cell death in neuronal cultures. The toxic effect is potentiated in neuronal cultures from CB₁ knockout mice and lesion size is increased in these mice when compared to wild-type controls indicating that CB₁ receptor signaling mitigates FeCl₂-induced cell death. Indeed, treatment with WIN 55,212-2 prevents much of this oxidative damage in a CB₁ receptor-mediated fashion. To further elucidate the downstream signaling events that invoke this protective effect, Kim et al. (2005) co-treated FeCl₂ and WIN 55,212-2 with either dibutyl-*cyclic* adenosine monophosphate, which activates protein kinase A (PKA), or wortmannin, an inhibitor of phosphoinositide-3-kinase (PI3K). Activation of PKA but not the inhibition of PI3K prevented the WIN 55,212-2-mediated neuroprotection indicating a CB₁ receptor-PKA-dependent mechanism. The viability of this treatment was further tested against two other inducers of oxidative stress, H₂O₂ and buthionine sulfoximine, with the same result (Kim et al., 2005).

In rat neuronal-glial cultures, the phytocannabinoid *trans*-caryophyllene has been shown to increase neuronal viability through a reduction of oxidative stress, mitochondrial depolarization, and the release of cytochrome c as well as through an increase in the expression of brain-derived neurotrophic factor (BDNF). That study identified CB₂ receptor activation as a mechanism for enhancing the phosphorylation of AMP-activated protein kinase and cAMP responsive element-binding (CREB) protein and increasing expression of the CREB target protein, BDNF (Choi et al., 2013). The phosphorylation of tau has also been attributed to A β -induced oxidative stress (Quintanilla et al., 2005) and small amounts of this characteristic AD pathology are found in transgenic APP and double transgenic

APP/PS1 mice. Treatment with WIN 55,212-2 was successful in normalizing this pathology almost to control levels (Martin-Moreno et al., 2012). Both ACEA and JWH133 treatment of Tg APP/PS1 mice are successful in counteracting the increased tau phosphorylation around neuritic plaques. Furthermore, CB₂ receptor stimulation decreased lipid peroxidation and increased superoxide dismutase-1 and -2 immunoreactivity around neuritic plaques (Aso et al., 2013).

CANNABINOID AND EXCITOTOXICITY

Excitotoxicity is the pathological process of damaging and killing neuronal cells as a result of excessive stimulation of ionotropic receptors, such as N-methyl-D-aspartate (NMDA) receptors, by glutamate and similar substances (Mehta et al., 2013). A β has been shown to directly activate NMDA receptors and reduce the astrocytic uptake of glutamate (Sonkusare et al., 2005; Texido et al., 2011). NMDA receptor activation allows Ca²⁺ influx to the cytosol and, combined with the A β -mediated formation of Ca²⁺ permeable pores on membrane bilayers (Alarcon et al., 2006) and increase in voltage-dependent Ca²⁺ channel activity (MacManus, 2000), excessive amounts of this excitotoxic mediator enter the cell. In an attempt to reduce the cytosolic Ca²⁺ load, neurons expend considerable energy using ion pumps on the endoplasmic reticulum, plasma membrane, and mitochondria, reducing ATP levels and causing excitotoxic lesions (Beal, 2000). Furthermore, excessive mitochondrial uptake of Ca²⁺ results in the generation and release of ROS, which inhibit key mitochondrial enzymes (Lipton, 2006). Increased cytosolic Ca²⁺ also activates a number of Ca²⁺-dependent apoptotic effector enzymes such as calcein and cathepsin (Hajnoczky et al., 2003; Dong et al., 2009).

Endocannabinoids are most commonly synthesized in a Ca²⁺-dependent fashion in response to depolarization and are known to reduce excitotoxic damage by returning Ca²⁺ homeostasis. Indeed, both *in vitro* and *in vivo* studies have reported the synthesis and release of eCBs as a result of Ca²⁺ ionophore application or through kainic acid (KA)-induced membrane depolarization (Cadas et al., 1996; Marsicano et al., 2003). Furthermore, a number of neuroprotective mechanisms against excessive glutamate release, excitotoxicity, and Ca²⁺ dyshomeostasis have been reported as a result of direct CB receptor stimulation. In cultured rat synaptosomes, 4-aminopyridine opens voltage-gated Ca²⁺ and induces the release of glutamate by blocking K⁺ channels. Pre-incubation with WIN 55,212-2 inhibits this glutamate release in a CB₁-dependent manner. This inhibition was shown to act through pertussis toxin-sensitive G_i/G_o proteins and independently of the cAMP–PKA pathway. CB₁ receptor stimulation was shown to act upon N- and P/Q-type voltage-gated Ca²⁺ channels to prevent the influx of Ca²⁺, thereby stopping glutamate release (Wang, 2003). Stimulation of murine cortical cultures with NMDA for 24 hours results in approximately 70% cell death, the majority of which (65%) is rescued through co-incubation with WIN 55,212-2. This neuroprotective action was prevented by CB₁ receptor antagonism in wild-type neuronal cultures and in primary neurons cultured from CB₁ knockout mice.

Interestingly, a 50% increase in NOS was observed in the knockout animals and inhibition of NO synthesis returned toxicity levels to those observed in the wild-type mice. Furthermore, NMDA receptor stimulation was shown to increase NO production ($\approx 160\%$), an effect that was blocked by WIN 55,212-2 and then restored by antagonism of the CB₁ receptor (Kim et al., 2006a).

Another neuroprotective mechanism attributed to eCB signaling is the induction of the immediate early genes *c-fos* and *zif268* as well as increased expression of BDNF. Hippocampi isolated from CB₁ knockout mice after KA-induced excitotoxicity revealed a significant reduction in the phosphorylation of p-42 and p-44 extracellular-regulated kinases (ERK) as well as *c-fos* and *zif268*, two genes known to play a protective role against excitotoxicity and to be at least partially induced through ERK phosphorylation. Expression of BDNF was also found to be reduced in the hippocampus of CB₁ knockout animals (Marsicano et al., 2003). Stimulation of the CB₁ receptor can also affect Ca²⁺ homeostasis by regulating its uptake and release from intracellular stores. In cultured hippocampal neurons, NMDA-induced excitotoxicity was reported to cause a concomitant rise in intracellular Ca²⁺, both of which were reversed upon treatment with WIN 55,212-2. Similarly, ryanodine, an agent known to selectively bind to the ryanodine receptor Ca²⁺ channels that regulate Ca²⁺ release from intracellular Ca²⁺ stores, was successful in reducing the NMDA-induced rise in intracellular Ca²⁺ and was shown to have an additive inhibitory effect when co-administered with WIN 55,212-2 (Zhuang et al., 2005). Pretreatment with the CB₁ receptor antagonist SR141716A had no effect on the ryanodine-mediated inhibition, indicating that both ryanodine and WIN 55,212-2 were affecting the same channels. Inhibition of PKA reversed the effect of WIN 55,212-2 on Ca²⁺ release, suggesting that the CB₁ receptor agonist acts via cAMP-dependent PKA to block the release of Ca²⁺ from ryanodine-sensitive stores upon induction of NMDA-induced excitotoxicity (Zhuang et al., 2005). The bidirectional regulation of mitochondrial stores of Ca²⁺ in both neurons and glia has also been attributed to CB receptor signaling. Indeed, under physiological conditions CBD caused a subtle rise in cytosolic Ca²⁺, while under high excitability conditions CBD reduced cytosolic Ca²⁺ and prevented Ca²⁺ oscillations. Through the use of specific channel blockers targeted at other Ca²⁺ stores such as the endoplasmic reticulum and other mitochondrial channels, Ryan et al. (2009) revealed that the CBD-mediated Ca²⁺ regulation was achieved via the mitochondrial Na⁺/Ca²⁺ exchanger (Ryan et al., 2009). Excessive glutamate stimulation, excitotoxicity, and Ca²⁺ dysregulation have a profound effect on the alteration of behavior and neurodegeneration in the progression of AD, and the eCB system has demonstrable ability to alleviate these neuronal stressors.

CANNABINOIDS AND NEUROGENESIS

Reduced neurotrophic support, which promotes proliferation, differentiation, and survival of neurons and glia, is also an emerging issue in AD research. During disease progression, neurotrophin receptors on cholinergic neurons

(Boissière et al., 1996) and the expression of BDNF (Connor et al., 1997) are markedly reduced. Furthermore, treatments to enhance both BDNF and nerve growth factor in animal models of AD have resulted in improved cognition, neuronal survival, and memory (Cooper et al., 2001; Nagahara et al., 2009). Adult neurogenesis is important for structural plasticity and network maintenance and alterations to this process in AD may aggravate neuronal vulnerability and memory deficits. Conflicting reports have emerged on whether neurogenesis is impaired (Crews et al., 2010) or enhanced (Jin et al., 2004a) in AD with the latter more recently being attributed to glial and vasculature-associated changes (Boekhoorn et al., 2006). Molecular evidence has indicated that a number of molecules associated with AD such as ApoE, PS1, and A β negatively affect proliferation, differentiation, and neurogenesis (Rodríguez et al., 2008; Gadadhar et al., 2011; Yang et al., 2011).

The eCB system has been closely linked to the process of adult neurogenesis. Indeed, both embryonic and adult rat neural stem cells are immunoreactive for CB₁ receptor, and stimulation by HU210 has been shown to increase proliferation, but not differentiation, in the hippocampus of adult rats. Furthermore, mediation of this neurogenic effect was attributed to G proteins and ERK signaling (Jiang et al., 2005). On the other hand, genetic ablation of key eCB signaling proteins has been shown to have a detrimental effect on adult neurogenesis. The eCB 2-AG appears to be a key modulator of neurogenesis. In DGL α and DGL β null mice an 80% and 50% reduction in 2-AG, respectively, can be seen. These transgenic mice were shown to have impaired neurogenesis attributed to the loss of 2-AG-mediated transient suppression of GABAergic transmission at inhibitory synapses (Gao et al., 2010). Furthermore, mice lacking CB₁ receptors displayed an almost 50% reduction in neurogenesis in the dentate gyrus and subventricular zone when compared to wild type (Jin et al., 2004b; Kim et al., 2006b). In murine neuronal cortical culture, WIN 55,212-2 increased incorporation of the thymidine analogue 5-bromo-2-deoxyuridine (BrdU), indicating enhanced neurogenesis. This CB₁ receptor-mediated effect was attributed to the inhibition of NO synthesis (Kim et al., 2006b; Marchalant et al., 2009). Neuronal precursor cell proliferation and the number of migrating neurons in neurogenic regions are increased after ischemia, seizure, and excitotoxic and mechanical lesions suggesting a possible contributing factor in the repair of lesioned circuits (Gould and Tanapat, 1997; Arvidsson et al., 2001; Parent et al., 2002; Lie et al., 2004). Kainic acid (KA)-induced excitotoxicity also provokes the proliferation of neural progenitor cells *in vivo*. CB₁-deficient mice do not retain this neurogenic capability and PCR analysis has revealed a reduction in the trophic factor basic fibroblast growth factor (bFGF) in the brains of these mice. KA was shown to produce bFGF in a CB₁ receptor-dependent manner and this neurotrophic withdrawal is believed to affect the neurogenic capacity of CB₁-deficient mice (Aguado et al., 2007). Two weeks post-excitotoxic lesion in rats, a transient up-regulation in BDNF expression is observed in an attempt to rescue the striatal neuronal population. Interestingly, this neuroprotective event coincides with, and may result from, higher bind

activity and protein expression of CB₁ receptor (De March et al., 2008). Indeed, CB₁ receptor activation has been associated with neural precursor proliferation and neurogenesis, while CB₁ and CB₂ receptor activation is involved in neural progenitor cell proliferation, both of which are vital to the generation and survival of new neurons (Palazuelos et al., 2006; Aguado et al., 2007).

CONCLUSIONS

AD is a debilitating illness that culminates in the progressive loss of cognitive function and death. Severe atrophy of the brain has been attributed to a number of neurodegenerative insults, namely, inflammation, oxidative stress, excitotoxicity and reduced neurotrophic support. The interplay between these processes results in the self-propagating and degenerative characteristics of AD. The eCB system is an evolutionarily conserved neuromodulatory system involved in inflammation, Ca²⁺ buffering, neurogenesis, and neurotransmission and is altered in the pathogenesis of AD. An increasing number of preclinical studies have identified the anti-inflammatory, antioxidant, and neurogenic capabilities of eCB signaling, while clinical studies have already reported positive behavioral effects. Targeting this system had become a viable therapeutic approach for AD but further clinical studies elucidating the efficacy of cannabinoid treatment are required.

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Cannabinoids in Parkinson's disease

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PARKINSON'S DISEASE

Parkinson's disease (PD) is a progressive neurodegenerative disorder that manifests primarily as a motor impairment characterized by slowness of movement, rigidity, and tremor. Neuropathologically, this disease is classically associated with intraneuronal accumulation of α -synuclein-immunopositive inclusions termed Lewy bodies and progressive degeneration of the nigrostriatal dopaminergic pathway. One of the tragedies of this condition is that, by the time a patient presents with initial symptoms, over half of their nigrostriatal neurons have already degenerated, and current symptomatic therapies can do nothing to prevent further cell loss. Indeed, all pharmacotherapies for PD are purely symptomatic and are also limited by the development of severe and disabling side effects. Thus, there are serious unmet clinical needs in the pharmacological treatment of this condition. There is mounting evidence to suggest that the endocannabinoid system may have multiple therapeutic benefits in the treatment of PD. Evidence suggests that cannabinoid drugs have the potential to alleviate the symptoms of the condition as well as to ameliorate the debilitating motor side effects associated with current pharmacotherapies. Moreover, the endocannabinoid system may also have the potential to modify the progression of the disease by affecting nigrostriatal neurodegeneration, Lewy body formation, and neuroinflammation.

This chapter will start with an overview of the endocannabinoid system in the normal basal ganglia and its dysregulation in the Parkinsonian brain, as well as an overview of data that have been generated thus far in clinical trials of cannabinoid drugs in PD patients. The mounting evidence for the symptomatic and disease-modifying potential of this system for PD will then be described. Overall, we aim to demonstrate that the endocannabinoid system is worth pursuing as a potential therapeutic target for the treatment of this debilitating neurodegenerative disorder.

THE ENDOCANNABINOID SYSTEM IN THE NORMAL BASAL GANGLIA

The endocannabinoid system comprises endogenous ligands, including *N*-arachidonoylethanolamide (anandamide) and 2-arachidonoylglycerol (2-AG), enzymes that biosynthesize or degrade the endocannabinoids, and two $G_{i/o}$ protein-coupled receptors, cannabinoid₁ (CB₁) and cannabinoid₂ (CB₂). The endocannabinoid system, in addition to dopaminergic neurotransmission, GABAergic and glutamatergic signaling, is intricately involved in the basal ganglia circuitry, which is the network responsible for motor control. Normal, coordinated movement manifests as a result of a controlled balance of direct striatopallidal and indirect striatopallidal GABAergic signaling. This balance is facilitated by excitatory D₁ receptors in the direct pathway and inhibitory D₂ dopamine receptors in the indirect pathway, in a corticostriatopallidal loop (reviewed in Grillner et al., 2005). An imbalance in this circuitry occurs in PD, due to a loss of striatal dopamine, causing increased inhibition of the motor thalamus, leading to development of typical Parkinsonian symptoms.

CB₂ receptors located on microglia and astrocytes do not appear to play a role in modulating the corticostriatopallidal loop. Rather these are up-regulated primarily in response to neuroinflammatory and cytotoxic injury (Ternianov et al., 2012). The CB₁ receptor, however, is highly expressed in the basal ganglia, and is in fact most dense in the substantia nigra, findings which indicated a likely role in motor control (Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992). Although the nigrostriatal neurons themselves do not express CB₁ receptors, they do express vanilloid TRPV1 receptors, which are activated by the endocannabinoid anandamide (Mezey et al., 2000). The dense localization of CB₁ receptors in the substantia nigra is due to their expression on the presynaptic terminals of medium spiny GABAergic neurons in the striatum that project to the substantia nigra *pars reticulata* and the globus pallidus (Hohmann and Herkenham, 2000). CB₁ receptors are also present at other levels of the “motor loop,” including on corticostriatal glutamatergic terminals in the striatum and on GABAergic projections from the globus pallidus to the subthalamic nucleus (Brotchie, 2003; Benarroch, 2007).

CB₁ receptors have a functional role in the modulation of the basal ganglia motor circuit and locomotor suppression is one of the classic tetrad of behavioral effects of CB₁ receptor agonists in rodents (Smith et al., 1994). In the striatum, CB₁ receptors are co-expressed with D₁ and D₂ receptors on GABAergic medium spiny neurons (Hermann et al., 2002), and CB₁ receptor stimulation inhibits D₁ receptors, whereas D₂ receptor activation stimulates anandamide release (Giuffrida et al., 1999; Hermann et al., 2002). The importance of CB₁ receptor signaling is illustrated by reduced basal motor activity and striatal dopamine content observed in CB₁ knockout mice (Zimmer et al., 1999; Li et al., 2009). The CB₁ receptor is also involved in regulation of glutamatergic input into the striatum. The CB₁ receptors expressed on corticostriatal glutamatergic terminals modulate a form of synaptic plasticity at these synapses (Gerdeman and Lovinger, 2001; Kreitzer and

Malenka, 2007). The mechanism of action remains to be fully elucidated; however, evidence suggests that release of endocannabinoids in the striatum following D₂ receptor stimulation activates CB₁ receptors on the corticostriatal glutamatergic terminals to induce long-term depression of glutamatergic activity in the striatum (Gerdeman and Lovinger, 2001; Kreitzer and Malenka, 2007). Indeed, genetic ablation of the CB₁ and CB₂ receptor has resulted in increased motor deficits in several mouse models of Huntington's disease and Parkinson's disease (Mievis et al., 2011; Pérez-Rial et al., 2011; Bouchard et al., 2012). Thus, there is considerable evidence that the endocannabinoid system plays a crucial role in the normal functioning of the basal ganglia motor circuitry, and that dysregulation of the endocannabinoid system is implicated in the motor dysfunction observed in conditions such as Parkinson's disease.

ENDOCANNABINOID SYSTEM DYSREGULATION IN PD

Several studies have investigated changes in cannabinoid receptors and their endogenous ligands in basal ganglia structures of Parkinson's disease patients (Table 3.1).

The first such study demonstrated an increase in CB₁ receptor binding in the caudate nucleus and putamen of postmortem PD patients (Lastres-Becker et al., 2001). Subsequent postmortem studies were somewhat contradictory to this, however, and revealed either a decrease in CB₁ receptor mRNA levels in the caudate nucleus, anterior dorsal putamen, and external segment of the globus pallidus (Hurley et al., 2003) or no change in CB₁ receptor density in these structures (Farkas et al., 2012). These studies, however, are confounded by variability in the disease course and drug treatment regimes. Only one study thus far has attempted to address these limitations by using MRI and PET imaging to detect CB₁ receptor availability in PD patients at various disease stages undergoing different L-DOPA treatment regimens (Van Laere et al., 2012). This study revealed a reduction in CB₁ receptors in the substantia nigra, which was unaffected by L-DOPA treatment. Although largely unexplored in clinical PD studies, there is an indication that the CB₂ receptor may also be altered in the condition. The only study addressing CB₂ receptor changes in PD identified decreased CB₂ receptor mRNA expression in the cerebellum and hippocampus of both early and late PD patients (Grünblatt et al., 2007). Unfortunately, however, analysis was not performed on basal ganglia structures in this study. Generally, findings from clinical studies indicate that the nigrostriatal neurodegeneration associated with PD is accompanied by an increase in endocannabinoid levels (Pisani et al., 2005, 2010, 2011). A study investigating anandamide levels in newly-diagnosed, non-medicated PD patients, and patients with mild or advanced disease following medication washout, showed that levels of anandamide were increased two-fold in patients regardless of clinical progression (Pisani et al., 2005). A subsequent

Table 3.1 Clinical Reports of Changes in the Endocannabinoid System in Parkinson's Disease Patients

Measure	Patient Groups	Drug Treatment	Findings	Reference
CB ₁ receptor density and activity in the basal ganglia by [3H]CP-55 940 binding assay	Idiopathic PD (<i>n</i> = 7) Age-matched controls (<i>n</i> = 5)	All PD patients were receiving L-DOPA	Increased CB ₁ receptor density and downstream GTP-binding in caudate nucleus and putamen in PD patients. No change in lateral globus pallidus or substantia nigra	Lastres-Becker et al., 2001
CB ₁ mRNA levels postmortem in the basal ganglia by RT-qPCR	Idiopathic PD (<i>n</i> = 10) Age-matched controls (<i>n</i> = 8)	All PD patients were receiving L-DOPA and/or direct dopamine agonists	Decreased CB ₁ receptor expression in the caudate nucleus, anterior dorsal putamen, and external globus pallidus	Hurley et al., 2003
CB ₁ , D ₂ /D ₃ receptor density postmortem in the basal ganglia by autoradiography	Idiopathic PD (<i>n</i> = 8) Age-matched controls (<i>n</i> = 5)	All chronic L-DOPA	CB ₁ receptor density unaltered in PD patients. D ₂ /D ₃ receptor density reduced in the caudate nucleus and putamen	Farkas et al., 2012
CB ₁ receptor availability in the substantia nigra by MRI and PET	Idiopathic PD (<i>n</i> = 29) Age-matched controls (<i>n</i> = 12)	All PD patients were receiving various L-DOPA treatment regimes	Decreased CB ₁ receptor availability in the substantia nigra, which was unaffected by L-DOPA treatment	Van Laere et al., 2012
CB ₂ mRNA levels postmortem in the hippocampus and cerebellum by GeneChip hybridization array and RT-PCR	Idiopathic PD (<i>n</i> = 8–9)	No information provided on patient treatment regimens prior to death	Decreased CB ₂ receptor expression in the cerebellum and hippocampus of PD patients	Grünblatt et al., 2007
Anandamide levels in the CSF by HPLC	Idiopathic PD (<i>n</i> = 16) Age-matched controls (<i>n</i> = 14)	Patients underwent drug washout	Increased anandamide compared to controls. Increase independent of disease stage or UPDRS	Pisani et al., 2005
Anandamide levels in the CSF by HPLC	Idiopathic PD (<i>n</i> = 56) Age-matched controls (<i>n</i> = 37)	Patients underwent drug washout	Increased anandamide in <i>de novo</i> and L-DOPA “washout” patients compared to controls	Pisani et al., 2010

Abbreviations: UPDRS: Unified Parkinson's Disease Rating Scale.

study by the same group found that anandamide levels in untreated patients were more than double that of controls, and chronic dopaminergic replacement therapy was capable of restoring anandamide to control levels (Pisani et al., 2010).

Interestingly, there is also clinical evidence to suggest that the endocannabinoid system may play a role in the non-motor symptoms of PD. For example, the risk of depression (which affects up to 50% of PD patients (Costa et al., 2012)) has been found to be reduced in PD patients with specific genetic polymorphisms in the CB₁ receptor gene (Barrero et al., 2005), while polymorphisms in the fatty acid amide hydrolase (FAAH) gene have been associated with PD-related musculoskeletal pain (Greenbaum et al., 2012). The phytocannabinoid cannabidiol has also demonstrated some efficacy in reducing psychotic symptoms in PD patients (Zuardi et al., 2009).

The studies described above suggest dysregulation of the endocannabinoid system in the brains of patients with PD, which may play a role in both the motor and non-motor symptoms of this condition. Interestingly, these clinical findings were preceded by, or paralleled by, a small number of preliminary clinical studies investigating cannabinoid drug use in PD patients, which will be discussed in the following section.

CLINICAL TRIALS OF CANNABINOID DRUGS IN PD PATIENTS

The few clinical reports of cannabinoid drug use in PD patients have focused on CB₁ receptor agonists as modulators of motor symptoms and/or as adjuncts to L-DOPA therapy to reduce L-DOPA-induced dyskinesia (Table 3.2). L-DOPA-induced dyskinesias are abnormal involuntary movements induced by chronic L-DOPA treatment that affect 90% of patients within 10 years of continued treatment (Ahlskog and Muentner, 2001).

The first study to investigate the effect of cannabinoid drugs on L-DOPA-induced dyskinesias was quite promising, with the CB₁/CB₂ receptor agonist nabilone reducing this debilitating side effect of dopamine replacement therapy in a randomized, double-blind, placebo-controlled crossover trial in a small number of patients (Sieradzan et al., 2001). Subsequent, randomized, controlled trials did not provide encouraging results, with either cannabis (Carroll et al., 2004) or the CB₁ receptor antagonist/inverse agonist SR141716A (Mesnage et al., 2004) providing relief from motor symptoms or L-DOPA-induced dyskinesias. Despite these disappointing results from randomized, controlled trials, other studies have indicated positive anti-Parkinsonian or anti-dyskinetic effects of cannabinoid receptor agonists in patient surveys (Venderová et al., 2004) and open-label trials (Zuardi et al., 2009).

In addition to these clinical studies, numerous preclinical studies have investigated the endocannabinoid system as a potential symptomatic and disease-modifying therapeutic target for PD and these will be reviewed in the next section.

Table 3.2 Clinical Reports of Cannabinoid Drug Use in Parkinson's Disease Patients

Measure and Patients	Trial Design	Drug Treatment	Findings	Reference
L-DOPA-induced dyskinesias in idiopathic PD patients ($n = 7$) all receiving chronic L-DOPA	Randomized, double-blind, placebo-controlled, crossover trial	Nabilone (0.03 mg/kg, split dose at 12 h and 1 h prior to L-DOPA)	The cannabinoid agonist nabilone reduced L-DOPA-induced dyskinesia	Sieradzan et al., 2001
L-DOPA-induced dyskinesias in idiopathic PD patients ($n = 19$) all receiving chronic L-DOPA	Randomized, double-blind, placebo-controlled, crossover trial	Cannabis extracts (<0.25 mg Δ^9 -THC/0.125 mg cannabidiol per day for 4 weeks)	Cannabis extract did not reduce L-DOPA-induced dyskinesia	Carroll et al., 2004
Motor disability and L-DOPA-induced dyskinesias in idiopathic PD patients ($n = 24$) all receiving chronic L-DOPA	Randomized, double-blind, placebo-controlled trial	SR141716A (20 mg/day 1 h prior to L-DOPA for 16 days)	The CB ₁ receptor antagonist SR141716A did not improve motor ability or L-DOPA-induced dyskinesia	Mesnage et al., 2004
Motor disability and L-DOPA-induced dyskinesias in PD patients ($n = 339$)	Self-reported patient survey	Chronic oral cannabis consumption (cannabis users ($n = 85$), non-users ($n = 254$))	Cannabis improved motor symptoms and L-DOPA-induced dyskinesia	Venderová et al., 2004
Motor disability in idiopathic PD patients ($n = 6$)	Open-label pilot study	Cannabidiol (flexible dose (started at 150 mg/day) for 4 weeks)	The phytocannabinoid cannabidiol improved motor ability	Zuardi et al., 2009

POTENTIAL OF THE ENDOCANNABINOID SYSTEM AS A TARGET FOR ALLEVIATION OF MOTOR SYMPTOMS

As indicated above, the progressive loss of nigrostriatal dopaminergic neurons and the consequential loss of striatal dopamine that occurs in PD create an imbalance in the corticostriatopallidal loop, which manifests as the motor symptoms characteristic of the condition (Grillner et al., 2005). Because of the functional role of the endocannabinoid system in the basal ganglia circuitry, this system has been suggested as a potential target for correction of the aberrant signaling that occurs in PD. However, overall, the complexity of endocannabinoid signaling in the basal ganglia as well as the complexity of the endocannabinoid system changes that occur in PD have resulted in conflicting evidence, with drugs that both enhance and reduce endocannabinoid signaling having anti-Parkinsonian motor effects (Table 3.3).

Table 3.3 Preclinical Reports of the Anti-Parkinsonian Motor Effects of Cannabinoid Drugs

Species and Model	Cannabinoid Drug(s)	Cannabinoid Drug(s) Effect on Motor Function	Reference
Rat Reserpine	SR141716A (1 mg/kg)	The CB ₁ receptor antagonist SR141716A potentiated dopamine agonist-induced locomotion	Di Marzo et al., 2000
Cynomolgus monkey MPTP	SR141716A (0.001–10.0 mg/kg) Levonantradol (0.01, 0.1 mg/kg) Δ^9 -THC (0.01, 0.1, 1 mg/kg)	The CB ₁ receptor antagonist SR141716A had no effect on motor deficits The cannabinoid agonists levonantradol and Δ^9 -THC tended to worsen motor function	Meschler et al., 2001
Rat Intra-nigral 6-OHDA	SR141716A (0, 0.5, 1, 1.5 μ g/ μ l)	Direct intra-striatal and intra-pallidal injections of the CB ₁ receptor antagonist SR141716A reduced motor asymmetry	El Banoua et al., 2004
Rat Intra-nigral 6-OHDA	AM404 (1.0–5.0 mg/kg)	The endocannabinoid uptake inhibitor AM404 improved akinesia and sensorimotor orientation These effects were blocked by the CB ₁ receptor antagonist AM251	Fernandez-Espejo et al., 2004

(Continued)

Table 3.3 Preclinical Reports of the Anti-Parkinsonian Motor Effects of Cannabinoid Drugs *Continued*

Species and Model	Cannabinoid Drug(s)	Cannabinoid Drug(s) Effect on Motor Function	Reference
Marmoset MPTP	SR141716A (0.3, 1.0, 3.0 mg/kg)	The CB ₁ receptor antagonist SR141716A increased range of movement at two highest doses	van der Stelt et al., 2005
Rat Intra-nigral 6-OHDA	SR141716A (0.1, 0.5 mg/kg) AM251 (5.0 mg/kg)	The CB ₁ receptor antagonists SR141716A and AM251 improved akinesia, sensorimotor orientation, and forelimb use in severely lesioned rats Direct intra-cerebral injections suggested this was due to effects in the striatum and globus pallidus but not substantia nigra	Fernandez-Espejo et al., 2005
Rat i.c.v. 6-OHDA	SR141716A (0.1, 0.5, 1 mg/kg)	Low doses of the CB ₁ receptor antagonist SR141716A alleviated hypokinesia	González et al., 2006
Rhesus monkey MPTP	CE (0.1, 0.3, 1 mg/kg)	The CB ₁ receptor antagonist CE had no effect on motor deficits	Cao et al., 2007
<i>Drosophila</i> Paraquat	CP55,940 (0.5 mM)	The CB ₁ receptor agonist CP55,940 restored locomotive function	Jimenez-Del-Rio et al., 2008
Rat Intra-MFB 6-OHDA	SR141716A (0.05, 1.0 mg/kg)	The CB ₁ receptor antagonist SR141716A improved contralateral forepaw use	Kelsey et al., 2009
Rat i.c.v. or intra- striatal 6-OHDA	SR141716A (0.1 mg/kg) Δ^9 -THCV (2 mg/kg)	The CB ₁ receptor antagonists SR141716A and THCV enhanced ambulation	Garcia et al., 2011
Mouse MPTP	WIN 55,212-2 (10 μ g/kg) HU210 (10 μ g/kg)	The CB ₁ receptor agonists WIN 55,212-2 and HU210 partially increased the latency to falling in the rotarod test	Chung et al., 2011

Abbreviations: MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MFB: medial forebrain bundle; i.c.v.: intracerebroventricular.

Several studies have demonstrated the efficacy of CB₁ receptor antagonists in alleviating the motor symptoms in PD models. Di Marzo and colleagues were the first to demonstrate that co-administration of the CB₁ receptor antagonist/inverse agonist SR141716A with a dopamine receptor agonist could completely restore

locomotion in dopamine-depleted, reserpinized rats (Di Marzo et al., 2000). Several studies have since supported these findings with evidence that the CB₁ receptor antagonists/inverse agonists SR141716A and AM251 can improve motor performance in 6-OHDA- and reserpine-treated rats (Di Marzo et al., 2000; El Banoua et al., 2004; Fernandez-Espejo et al., 2005; González et al., 2006; Kelsey et al., 2009; García et al., 2011) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated marmosets (van der Stelt et al., 2005). However, there are also conflicting reports in the literature that indicate a lack of efficacy of CB₁ receptor antagonists in alleviating the motor syndrome in MPTP-treated marmosets (Meschler et al., 2001) or rhesus monkeys (Cao et al., 2007). Several studies have also demonstrated the efficacy of CB₁ receptor agonists, or agents that increase synaptic endocannabinoid levels (such as inhibitors of endocannabinoid uptake or catabolism), in alleviating the motor syndrome in PD models. In MPTP-treated mice (Chung et al., 2011) and paraquat-treated fruit flies (Jimenez-Del-Rio et al., 2008) cannabinoid receptor agonists were shown to improve motor function, while in 6-OHDA-lesioned rats, the endocannabinoid uptake inhibitor AM404 led to improvements in functional deficits, which were reversed with a CB₁ receptor antagonist (Fernandez-Espejo et al., 2004).

Because of the complexity of the changes in the basal ganglia corticostriatopallidal loop (including the changes in the endocannabinoid system) that occur in PD, it is difficult to dissect the exact mechanism through which exogenous endocannabinoid drugs might improve motor function in PD. In general, the most consistent finding from the preclinical literature is that CB₁ receptor antagonists can ameliorate motor symptoms (Di Marzo et al., 2000; El Banoua et al., 2004; Fernandez-Espejo et al., 2005; González et al., 2006; Kelsey et al., 2009; García et al., 2011), thus indicating that an overactivity of endocannabinoid signaling at the CB₁ receptor may contribute to the emergence of motor symptoms and restoration of endocannabinoid tone may underlie the functional improvements seen in these studies. Interestingly, this ties in with the clinical finding that levels of anandamide are consistently up-regulated in the cerebral spinal fluid of human Parkinson's disease patients, in addition to an increase in CB₁ receptor density (Hurley et al., 2003; Pisani et al., 2005, 2010, 2011), suggesting that aberrantly elevated endocannabinoid system functioning may contribute to the motor impairments seen. This motor inhibitory effect of anandamide may be due to an inhibition of glutamate release from the corticostriatal glutamatergic terminals that are known to express CB₁ receptors (Gerdeman and Lovinger, 2001; Kreitzer and Malenka, 2007). Other regions in the corticostriatopallidal loop are almost certainly also involved in the anti-Parkinsonian motor efficacy of exogenous cannabinoid agents as a reduction of subthalamic activity has been observed following administration of the cannabinoid receptor agonists Δ^9 -THC, WIN 55,212-2, or anandamide in 6-OHDA-lesioned rats (Morera-Herreras et al., 2011). Moreover, there is also information to suggest that the motor effects of cannabinoid drug administration may involve the serotonergic system with evidence for a role of both 5-HT_{1B} and 5-HT_{1A} receptors in the motor effects elicited by cannabinoid and cannabinoid-like drugs (Fernandez-Espejo et al., 2004; Gomes et al., 2013).

In summary, there is extensive and overwhelming evidence from preclinical studies in PD models that the endocannabinoid system is a valid therapeutic target for the alleviation of the motor dysfunction associated with this condition. That said, the mechanisms underlying these anti-akinetic effects, as well as the site of action in the basal ganglia circuitry, remain to be fully elucidated.

POTENTIAL OF THE ENDOCANNABINOID SYSTEM AS A TARGET FOR ALLEVIATION OF DRUG-INDUCED SIDE EFFECTS

In addition to having therapeutic potential for alleviation of Parkinsonian motor symptoms, cannabinoid drugs have considerable potential as adjuncts to dopamine-replacement therapy to reduce the debilitating side effects of this treatment. Several studies have indicated that the endocannabinoid system may be intrinsically linked to the development of L-DOPA-induced dyskinesias and evidence suggests that cannabinoid drugs may have efficacy in their alleviation. However, as with the evidence from the preclinical anti-akinesia literature, the evidence from the preclinical anti-dyskinetic literature is also conflicting. In one study from our laboratory, dyskinesias were precipitated by the CB₁ receptor antagonist/inverse agonist SR141716A in previously non-dyskinetic, 6-OHDA-lesioned, L-DOPA-treated rats (Walsh et al., 2010). However, the development of L-DOPA-induced dyskinesias was reduced in CB₁ knockout mice (Pérez-Rial et al., 2011). Indeed, the complex role of the endocannabinoid system in L-DOPA-induced dyskinesia is demonstrated by reports that both stimulation and inhibition of CB₁ receptor signaling can reduce the expression of L-DOPA-induced dyskinesias (Table 3.4).

In several studies, cannabinoid receptor agonists have been shown to reduce abnormal involuntary movements (AIMs) in 6-OHDA-lesioned, L-DOPA-treated rats (Ferrer et al., 2003; Morgese et al., 2007, 2009; Walsh et al., 2010; Martinez et al., 2012), reserpine-treated, L-DOPA-treated rats (Segovia et al., 2003), or in MPTP-lesioned, L-DOPA-treated marmosets (Fox et al., 2002). Use of selective CB₁ receptor agonists and antagonists has indicated that this anti-dyskinetic effect is due, at least in part, to CB₁ receptor activation. There is, however, evidence to suggest that CB₁ receptor blockade also has anti-dyskinetic efficacy. The CB₁ receptor antagonist/inverse agonist SR141716A has been shown to reduce L-DOPA-induced AIMs in reserpine-treated rats (Segovia et al., 2003) and MPTP-lesioned marmosets (van der Stelt et al., 2005). The mechanism by which these differential effects manifest may be explained by several points. Experimentally, the choice of species, the neurotoxin used, the extent of the lesion, and so on, are crucial factors that may explain why both CB₁ receptor agonism and antagonism alleviates motor dysfunction. Aside from this, blockade

Table 3.4 Preclinical Reports of the Anti-dyskinetic Effects of Cannabinoid Drugs in PD Models

Species and Model	L-DOPA Treatment	Cannabinoid Drug(s)	Effect of Cannabinoid Drug(s) on L-DOPA-induced Dyskinesia	Reference
Marmoset MPTP	L-DOPA (8 mg/kg)	Nabilone (0.01, 0.03, 0.1 mg/kg)	The cannabinoid agonist nabilone reduced L-DOPA-induced dyskinesias	Fox et al., 2002
Rat Intra-nigral 6-OHDA	L-DOPA (50 mg/kg)	WIN 55,212-2 (1 mg/kg) SR141716A (0.3 mg/kg)	The CB ₁ receptor agonist WIN 55,212-2 reduced L-DOPA-induced oral dyskinesia This anti-dyskinetic effect was reversed by the CB ₁ receptor antagonist SR141716A	Ferrer et al., 2003
Rat Reserpine	L-DOPA (150 mg/kg)	WIN 55,212-2 (0.1–3 mg/kg) SR141617A (0.1–3 mg/kg) AM404 (0.1–1 mg/kg)	Both the CB ₁ receptor antagonist SR141716A and the CB ₁ receptor agonist WIN 55,212-2 decreased L-DOPA-induced abnormal activity The endocannabinoid uptake inhibitor AM404 had no effect on L-DOPA-induced abnormal activity	Segovia et al., 2003
Marmoset MPTP	L-DOPA (8 mg/kg)	SR141716A (0.3, 1.0, 3.0 mg/kg)	The CB ₁ receptor antagonist SR141716A decreased L-DOPA-induced dyskinesia	Van der Stelt et al., 2005
Rat Intra-MFB 6-OHDA	L-DOPA (6 mg/kg)	WIN 55,212-2 (1 mg/kg) AM251 (1 mg/kg)	The CB ₁ receptor agonist WIN 55,212-2 reduced L-DOPA-induced oral dyskinesia This anti-dyskinetic effect was reversed by the CB ₁ receptor antagonist AM251	Morgese et al., 2007

(Continued)

Table 3.4 Preclinical Reports of the Anti-dyskinetic Effects of Cannabinoid Drugs in PD Models *Continued*

Species and Model	L-DOPA Treatment	Cannabinoid Drug(s)	Effect of Cannabinoid Drug(s) on L-DOPA-induced Dyskinesia	Reference
Rhesus monkey MPTP	L-DOPA (50–200 mg)	CE (0.1, 0.3, 1 mg/kg)	The CB ₁ receptor antagonist CE had no effect on L-DOPA-induced dyskinesias	Cao et al., 2007
Rat Intra-MFB 6-OHDA	L-DOPA (6 mg/kg)	WIN 55,212-2 (1 mg/kg)	The CB ₁ receptor agonist WIN 55,212-2 reduced L-DOPA-induced dyskinesias	Morgese et al., 2009
Rat Intra-MFB 6-OHDA	L-DOPA (10 mg/kg)	HU210, (0.5, 5, 50 µg/kg) AM251 (3 mg/kg) SR141716A (3 mg/kg)	The CB ₁ receptor agonist WIN 55,212-2 but not the CB ₁ receptor antagonists AM251 or SR141716A reduced L-DOPA-induced dyskinesia	Walsh et al., 2010
Rat Intra-MFB 6-OHDA	L-DOPA (6 mg/kg)	WIN 55,212-2 (1 mg/kg) AM251 (1 mg/kg)	The CB ₁ receptor agonist WIN 55,212-2 reduced L-DOPA-induced dyskinesia This anti-dyskinetic effect was reversed by the CB ₁ receptor antagonist AM251	Martinez et al., 2012
Mouse Intra-striatal 6-OHDA	L-DOPA (20 mg/kg)	OEA (5 mg/kg) GW6471 (2.5 mg/kg) Capsaicin (1 mg/kg)	OEA attenuated L-DOPA-induced AIMs in lesioned animals This effect was not blocked by GW6471, a PPAR-α receptor antagonist, but was inhibited by capsaicin, a TRPV1 receptor agonist	González-Aparicio and Moratalla, 2014

Abbreviations: OEA: oleoylethanolamide; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MFB: medial forebrain bundle.

of the CB₁ receptor may induce changes in activity downstream of this receptor, potentially altering activity of endocannabinoid degrading enzymes and other cannabinoid and non-cannabinoid receptors.

The TRPV1 receptor, at which anandamide is an endogenous ligand, may also play a role in L-DOPA-induced dyskinesias. In one study, the anti-dyskinetic effects of the endocannabinoid metabolism inhibitor URB597 was only unmasked with co-administration of a TRPV1 receptor antagonist (Morgese et al., 2007). This indicates that anandamide acting at CB₁ and TRPV1 receptors may play opposite roles in L-DOPA-induced dyskinesias with the former having an anti-dyskinetic effect and the latter having a pro-dyskinetic effect. The pro-dyskinetic effect of the TRPV1 receptor was also indicated in a recent study in which the oleoylethanolamine (OEA), a non-cannabinoid drug with structural similarity to anandamide, was shown to have anti-dyskinetic effects in 6-OHDA-lesioned, L-DOPA-treated mice, an effect that was prevented by pretreatment with the TRPV1 receptor agonist capsaicin, indicating antagonistic action of OEA (González-Aparicio and Moratalla, 2014).

Overall, it is likely that CB₁ receptor signaling is a major player in the development of the dyskinetic syndrome after chronic L-DOPA therapy, although the mechanisms involved remain to be identified. Nevertheless, there is substantial, albeit complex and frequently contradictory, evidence to indicate that cannabinoid drugs have considerable potential as adjuncts to L-DOPA therapy in PD.

POTENTIAL OF THE ENDOCANNABINOID SYSTEM AS A DISEASE MODIFYING TARGET

In addition to its potential role in the development of, and therapeutic relief from, Parkinsonian motor symptoms and L-DOPA-induced dyskinesias, the endocannabinoid system has been shown to have considerable potential as a disease-modifying therapeutic target for PD. Classically, this disease is associated with abnormal aggregation of the α -synuclein protein into Lewy bodies, as well as progressive degeneration of the nigrostriatal dopaminergic pathway, and, in more recent years, evidence for chronic neuroinflammation as a pathogenic feature has also emerged. There is evidence that cannabinoid drugs may be capable of reducing Lewy body formation, slowing nigrostriatal neurodegeneration, and suppressing neuroinflammation. The evidence in support of each of these is covered in this section.

THE EFFECT OF CANNABINOID DRUGS ON α -SYNUCLEINOPATHY

The pivotal role of the α -synuclein protein in PD was first identified in two seminal reports in 1997 (Polymeropoulos et al., 1997; Spillantini et al., 1997). The first identified a mutation in the α -synuclein gene that was responsible for a

highly penetrant, autosomal dominant form of PD (Polymeropoulos et al., 1997), while the second identified the protein as a key constituent in Lewy bodies from idiopathic PD patients (Spillantini et al., 1997). Interest in α -synuclein as a driver of PD pathogenesis and as a potential disease-modifying therapeutic target has continued to escalate over the intervening years. The interaction between the endocannabinoid system and the α -synuclein protein, and consequently the potential of the endocannabinoid system as a target for reduction of α -synuclein pathology in PD, has been poorly explored to date. Nevertheless, several studies indicate that this is an area worth exploring in more depth.

The first study to reveal an interaction between the endocannabinoid system and the α -synuclein protein demonstrated widespread CB₁ receptor dysregulation in α -synuclein knockout mice (García-Arencibia et al., 2009). Specifically, CB₁ receptor expression and binding was decreased in the nigrostriatal system of young mice, but increased in the nigrostriatal system of older mice lacking the α -synuclein gene. This finding contrasted with a later study showing that deletion of the α -synuclein gene was associated with overexpression of CB₁ receptors in young mice in other brain regions (hippocampus and amygdala) (Lopez-Jimenez et al., 2013). Overexpression of mutated forms of α -synuclein is also associated with alterations in the endocannabinoid system with overexpression of the A53T mutated form of the protein being associated with a reduction in CB₁ receptor expression and binding in the nigrostriatal system of old mice (Kurz et al., 2010). In addition to these genetic studies, one pharmacological study revealed that α -synuclein accumulation and cell death induced by proteasomal synthase inhibition was inhibited by treatment with the cannabinoid receptor agonist WIN 55,212-2 in an *in vitro* catecholaminergic cell line (PC12 cells) (Jeon et al., 2011). Although more studies are required to fully elucidate the relationship between the endocannabinoid system and the α -synuclein protein, these early studies indicate that there is a functional interaction between them, which may provide a target for reduction of α -synucleinopathy in PD.

THE NEUROPROTECTIVE POTENTIAL OF CANNABINOID DRUGS

As mentioned above, one of the most important unmet clinical needs in PD treatment is the lack of a neuroprotective therapy to slow or halt the neurodegenerative process. In an attempt to address this, the neuroprotective potential of the endocannabinoid system has been the subject of numerous preclinical studies in recent years (Table 3.5).

Initial indications from a study in 2005 by Lastres-Becker and colleagues were that pharmacological targeting of cannabinoid receptors using Δ^9 -THC and cannabidiol resulted in neuroprotection as evidenced by a reduction in 6-OHDA-induced nigrostriatal degeneration (Lastres-Becker et al., 2005). Other cannabinoid receptor agonists including HU210 and WIN 55,212-2 have also been shown to exhibit neuroprotective properties in various PD models, with evidence of direct protection of

Table 3.5 Preclinical Reports of the Neuroprotective Effects of Cannabinoid Drugs in PD Models

Species and Model	Cannabinoid Drug(s)	Neuroprotective Effect of Cannabinoid Drug(s)	Reference
Rat Intra-MFB 6-OHDA	Δ^9 -THC (3 mg/kg) Cannabidiol (3 mg/kg)	Cannabinoid agonist Δ^9 -THC and CB ₁ /CB ₂ receptor inverse agonist cannabidiol protects against neurodegeneration	Lastres-Becker et al., 2005
Rat i.c.v. 6-OHDA	Rimonabant (0.1, 1 mg/kg)	Rimonabant, a CB ₁ antagonist/inverse agonist, enhanced striatal glutamate levels at doses previously shown to alleviate motor symptoms	García-Arencibia et al., 2009
Mouse MPTP CB ₁ KO mice No model	WIN 55,212-2 (4 mg/kg) AM251 (4 mg/kg)	WIN 55,212-2, a non-selective CB ₁ receptor agonist, attenuated nigrostriatal damage and increased dopamine in ventral midbrain WIN 55,212-2 also displayed similar effects in CB ₁ knockout mice AM251 had no effect on WIN 55,212-2-mediated neuroprotection WIN 55,212-2 was neuroprotective to a similar extent in WT and CB ₁ knockout mice	Price et al., 2009
Mouse Intra-striatal LPS Rat Intra-MFB 6-OHDA	Δ^9 -THCV (2 mg/kg) Cannabidiol (3 mg/kg) HU308 (5 mg/kg)	Δ^9 -THCV, a CB ₁ receptor antagonist/CB ₂ agonist, attenuated nigrostriatal dopaminergic loss Cannabidiol attenuated dopaminergic loss also, but to a greater extent than Δ^9 -THCV HU308, a CB ₂ receptor agonist, preserved dopaminergic neuron survival in LPS-lesioned mice	García et al., 2011

(Continued)

Table 3.5 Preclinical Reports of the Neuroprotective Effects of Cannabinoid Drugs in PD Models *Continued*

Species and Model	Cannabinoid Drug(s)	Neuroprotective Effect of Cannabinoid Drug(s)	Reference
Mouse MPTP	WIN 55,212-2 (10 µg/kg) HU210 (10 µg/kg)	Both non-selective CB ₁ receptor agonists WIN 55,212-2 and HU-210 produced a significant increase in nigrostriatal dopamine neuron survival and increased striatal dopamine levels. Both drugs attenuated MPTP-induced oxidative damage and ROS production.	Chung et al., 2011
Rat Intra-nigral LPS	WIN 55,212-2 (5 µg, i.c.v.) HU210 (5 µg, i.c.v.)	Both drugs increased survival of nigral dopamine neurons in addition to inhibiting ROS production.	Chung et al., 2012
Rat Intra-MFB 6-OHDA	WIN 55,212-2 (31.25–250 µg/kg) Δ ⁹ -THC (250–2000 µg/kg)	All agonists inhibited subthalamic hyperactivity associated with the Parkinsonian state.	Morera-Herreras et al., 2011
Mouse MPTP	AEA (50–150 µg, i.c.v.) PEA (10 mg/kg)	AEA exerted a more intense inhibitory effect than other agonists. PEA, a weak cannabinoid agonist capable of enhancing AEA, protected nigrostriatal dopamine neurons from MPTP-induced neurotoxicity.	Esposito et al., 2012
Rat Intra-striatal 6-OHDA	OEA (5 mg/kg)	OEA, a TRPV1 receptor agonist, partially protected against nigral dopaminergic degeneration and reduced oxidative markers.	Gonzalez Aparicio et al., 2013

Abbreviations: LPS: lipopolysaccharide; AEA: anandamide; PEA: palmitoylethanolamide; i.c.v.: intracerebroventricular.

nigrostriatal neurons, restoration of striatal dopamine, and reduction of oxidative stress (Price et al., 2009; Chung et al., 2011, 2012). The mechanism by which cannabinoid drugs, specifically CB₁ receptor agonists, work to exert these neuroprotective effects is most likely not restricted to CB₁ receptor interactions. AM251, a selective CB₁ receptor antagonist, did not alter WIN 55,212-2-mediated neuroprotection in MPTP-treated mice and WIN 55,212-2 conferred neuroprotection in CB₁ null mice (Price et al., 2009). Δ^9 -THCV, a CB₁ receptor inverse agonist/antagonist (which may also act as a partial CB₂ receptor agonist (Pertwee, 2008)), attenuates nigrostriatal damage in lipopolysaccharide (LPS)- and 6-OHDA-treated mice and rats, respectively (García et al., 2011). The phytocannabinoid cannabidiol (a CB₁ indirect agonist with CB₂ inverse agonist properties) acts similarly to Δ^9 -THCV in this model—however, to a much greater extent, indicating non-CB₁ interactions (García et al., 2011). These findings, coupled with the inability of CB₁ receptor antagonists to block neuroprotective effects of non-selective cannabinoid drugs, indicate alternative signaling mechanisms are involved.

In vitro studies corroborate *in vivo* evidence and suggest that the mechanism of cannabinoid-mediated neuroprotection involves anti-oxidative action, as observed in MPP⁺-treated mesencephalic cultures and PC12 cells (Iuvone et al., 2007; Moldzio et al., 2012). Interestingly, one study in 6-OHDA-treated PC12 cells showed that anandamide pretreatment significantly attenuated cell death; however, CB₁, CB₂, and TRPV1 receptor antagonists failed to block these effects, implying non-cannabinoid receptor mediation of the effects of administered anandamide (Mnich et al., 2010).

Further to this, recent evidence shows that systemic administration of OEA, an analogue of anandamide with poor affinity for CB₁ and CB₂ receptors, partially inhibited nigrostriatal degeneration and oxidative stress in the 6-OHDA rat model and attenuated behavioral deficits, effects that were almost entirely reversed by a peroxisome proliferator-activated receptor (PPAR)- α antagonist (Gonzalez-Aparicio et al., 2013). Similarly, palmitoylethanolamide (PEA), another PPAR- α receptor agonist, is neuroprotective following MPTP administration to mice, reducing nigrostriatal damage and reversing MPTP-induced motor deficits (Esposito et al., 2012).

It would appear, given the evidence discussed above, that cannabinoid drugs, specifically CB₁ receptor agonists, exert neuroprotective effects in animal models of PD. The modulation of endogenous cannabinoid signaling and interplay from non-cannabinoid compounds and targets may also represent viable neuroprotective avenues for continued investigation in the search for a disease-modifying, neuroprotective treatment for PD.

THE ANTI-INFLAMMATORY POTENTIAL OF CANNABINOID DRUGS

In addition to the classical neuropathological features of Lewy body formation and nigrostriatal neurodegeneration, PD is associated with chronic neuroinflammation. The first evidence that inflammatory processes are associated with PD came when McGeer et al. (1988) demonstrated the presence of activated

microglia in the substantia nigra of patients at postmortem. Since then, numerous convergent lines of evidence have supported the involvement of neuroinflammation in the disease. Indeed, in recent years, it has become increasingly evident that PD is associated with a self-sustaining cycle of neuroinflammation and neurodegeneration with dying neurons activating microglia, which, once activated, can release several factors that kill further neurons (reviewed in [Glass et al., 2010](#)). Numerous studies have suggested that pharmacological targeting of the endocannabinoid system might be capable of breaking the cycle of neuroinflammation and neurodegeneration associated with PD ([Table 3.6](#)).

The first indication that the endocannabinoid system might have anti-inflammatory potential relevant to PD came when [Lastres-Becker et al. \(2005\)](#) showed that the neuroprotective effect of the cannabinoid receptor agonist HU-210 against the Parkinsonian neurotoxin 6-OHDA was largely dependent on its ability to modify glial cells. More recent studies have further corroborated the anti-inflammatory potential of targeting the CB₁ receptor in animal models of PD. The non-selective CB₁ receptor agonists WIN 55,212-2 and HU 210, respectively, were found to have potent anti-inflammatory effects (reduced microglial activation, reduced expression of proinflammatory cytokines, reduced NADPH oxidase, and reduced reactive oxygen species) in both the MPTP mouse ([Chung et al., 2011](#)) and the LPS-treated rat ([Chung et al., 2012](#)) models of PD. Moreover, these anti-inflammatory effects were associated with protection of nigrostriatal neurons indicating that reducing neuroinflammation can have consequent neuroprotective actions in these models. [Chung et al. \(2011\)](#) identified a specific role for CB₁-mediated effects as selective CB₁ receptor antagonists blocked these effects.

Although several studies exist that support the anti-inflammatory potential of activating the CB₁ receptor, there is a substantial body of evidence to suggest that the CB₂ receptor may be the more promising anti-inflammatory target. The CB₂ receptor is expressed on microglia, the resident immune surveillance cells in the brain, and its expression is strongly up-regulated when these cells are activated (reviewed in [Benito et al., 2008](#)). Moreover, numerous *in vitro* studies have revealed that activation of microglial CB₂ receptors suppresses their release of proinflammatory cytokines, enhances their release of anti-inflammatory cytokines, and reduces their neurotoxicity (reviewed in [Little et al., 2011](#)). These data suggest that pharmacological activation of the CB₂ receptor may be a promising approach for anti-inflammatory intervention in PD.

The first solid evidence for the anti-inflammatory potential of CB₂ receptor agonists in PD models came when [Price et al. \(2009\)](#) demonstrated that pharmacological activation of CB₂ receptors reduced microglial activation in the MPTP mouse model. This anti-inflammatory effect was associated with protection against MPTP-induced nigrostriatal neurodegeneration and motor dysfunction. In the same report, [Price et al. \(2009\)](#) also described an exacerbation of MPTP-induced neurotoxicity in CB₂ receptor knockout mice. Since then, several studies have also reported an anti-inflammatory effect of CB₂ receptors in animal models of PD. Microglial activation and nigrostriatal neurodegeneration were found to be reduced

Table 3.6 Preclinical Reports of the Anti-Inflammatory Effects of Cannabinoid Drugs in PD Models

Species and Model	Cannabinoid Drug(s)	Anti-inflammatory and Neuroprotective Effect of Cannabinoid Drug(s)	Reference
Mouse MPTP	WIN 55,212-2 or HU 210 (0.1–50 µg/kg) AM251 or SR14716A (20 µg/kg)	Both the CB ₁ receptor agonists WIN 55,212-2 and HU 210 reduced inflammatory markers and neurodegeneration. These anti-inflammatory and neuroprotective effects were reversed by the CB ₁ receptor antagonists AM251 and SR14716A.	Chung et al., 2011
Mouse MPTP	WIN 55,212-2 (4 mg/kg) JWH 105 (4 mg/kg)	WIN 55,212-2 and JWH 105, a CB ₂ receptor agonist, reduced MPTP-induced microglial activation.	Price et al., 2009
Mouse LPS	Δ ⁹ -THCV (2 mg/kg) HU 308 (5 mg/kg)	Δ ⁹ -THCV, a CB ₁ receptor antagonist/CB ₂ agonist, and HU 308, a CB ₂ receptor agonist, protected nigrostriatal degeneration induced by the inflammagen, LPS.	García et al., 2011
Rat Intra-MFB 6-OHDA	WIN 55,212-2 (5 µg, i.c.v.) HU-210 (5 µg, i.c.v.)	Both agonists reduced levels of proinflammatory cytokines TNF-α (WIN 55,212-2, HU 210) and IL-1β (WIN 55,212-2 only).	Chung et al., 2012
Mouse MPTP	PEA (10 mg/kg)	PEA reduced alterations indicative of inflammation, including microglial and astrocytic activation.	Esposito et al., 2012

Abbreviations: LPS: lipopolysaccharide; i.c.v.: intracerebroventricular.

in 6-OHDA-lesioned rats after treatment with the CB₁ receptor antagonist/CB₂ receptor agonist Δ⁹-THCV (García et al., 2011), while studies in CB₂ receptor knockout mice have revealed that they are more sensitive to the neurotoxic effects of both LPS (García et al., 2011) and 6-OHDA (Ternianov et al., 2012), and this is associated with a reduction in recruitment of astrocytes and microglia to the lesion site in the 6-OHDA-lesioned mice (Ternianov et al., 2012).

In addition to the CB₁ and CB₂ receptors as potential targets for anti-inflammatory intervention in PD, interest is also mounting in other endocannabinoid receptors (such as TRPV1 and the PPARs as potential anti-inflammatory targets—reviewed in [Alawi and Keeble, 2010](#); [O'Sullivan and Kendall, 2010](#)). Evidence in support of this comes from work demonstrating that PEA had anti-inflammatory and neuroprotective effects in the MPTP mouse model and these effects were reduced in PPAR- α knockout mice ([Esposito et al., 2012](#)).

CONCLUDING REMARKS

Overall, a large number of preclinical studies in various animal models of PD have indicated that targeting the endocannabinoid system may have multiple therapeutic benefits including relief from debilitating motor symptoms and drug-induced motor side effects, as well as disease modification in terms of reduction of α -synucleinopathy, neuroprotection, and dampening of neuroinflammation. These studies are supported by a comparatively smaller number of clinical studies that also indicate a role for the endocannabinoid system in the pathogenesis of PD and suggest that it represents a viable therapeutic target. However, more clinical trials with larger sample sizes and with some of the cannabinoid compounds that have shown promise in preclinical studies are required. This is particularly pertinent given the widely acknowledged limitations of current animal models of PD. Approaches that pursue targets other than the CB₁ receptor also hold considerable promise and are likely to be devoid of the psychoactive side effects associated with CB₁ receptor activation in the brain. In summary, our expanding knowledge of the endocannabinoid system gives cause for optimism for improved understanding and treatment of PD.

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Cannabinoids and Huntington's disease

4

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THE ENDOCANNABINOID SYSTEM

COMPONENTS, WORKING MECHANISM, AND DISTRIBUTION

Marijuana has been used in numerous cultures throughout recorded history. While the folkloric use of marijuana as a medicine has been around since ancient times, it was largely during the 19th century that this substance was assimilated into the armamentarium of medical practice. From about 1850 to 1900, several published testimonials and case histories indicated that marijuana could ameliorate neurological symptoms. In 1964, Gaoni and Mechoulam (Mechoulam and Hanus, 2000) identified delta-9-tetrahydrocannabinol (Δ^9 -THC) as the major psychoactive constituent of marijuana. Since that time, about 60 other cannabinoids and some 260 other non-psychoactive compounds have been identified (Turner et al., 1980), and many hundreds of analogues of Δ^9 -THC have been synthesized in the laboratory (Razdan, 1986). However, it was not until the 1990s that the receptors responsible for many of the actions of Δ^9 -THC were identified (Devane et al., 1988) and cloned (Matsuda et al., 1990). Since then, knowledge on the endogenous cannabinoid system regarding its physiology, pharmacology, and therapeutic potential has expanded enormously.

The endocannabinoid system (ECS) consists of cannabinoid receptors, endogenous ligands, and the proteins for their biosynthesis, degradation, and transport.

To date, two cannabinoid receptors, type 1 (CB₁) and type 2 (CB₂), have been identified by molecular cloning and are unambiguously established as mediators of the biological effects induced by cannabinoids, either synthetically or endogenously produced (Matsuda et al., 1990; Munro et al., 1993). CB₁ and CB₂ receptors are heptahelical transmembraneous G_{i/o}-coupled receptors that share 44% protein identity and display different pharmacological profiles and patterns of expression.

The majority of cannabinoid effects on the central nervous system (CNS) are mediated by the CB₁ receptor. In the brain, the CB₁ receptor is found in areas controlling motor, cognitive, emotional, and sensory functions, i.e., the hippocampus, basal ganglia, cerebellum, cortex, and olfactory bulb (Herkenham et al., 1990; Tsou et al., 1998a). In these regions, the CB₁ receptor is abundantly expressed presynaptically. Evidence of the presence of CB₁ receptors on the dendrites and soma of neurons is not convincing (Freund et al., 2003). CB₁ receptors are additionally found at low levels on various astrocytes, oligodendrocytes, and neural stem cells (Aguado et al., 2005), while in peripheral tissues, CB₁ receptors are expressed in the heart, uterus, testis, liver, and small intestine (Maccarrone et al., 2001; Nong et al., 2001; Klein et al., 2003). The CB₂ receptor seems to be confined to cells of the immune system. High rat spleen binding of [³H]CP55,940, a non-selective CB₁ receptor and CB₂ receptor agonist, was reported in 1994 (Lynn and Herkenham, 1994). This binding is highest in the marginal zone of the spleen, where the CB₂ receptor accounts for ≈ 90% of the cannabinoid binding sites. CB₂ receptor expression has also been described in thymus (Schatz et al., 1997), lymph nodes, Peyer's patches (Lynn and Herkenham, 1994), and immune system-derived cell lines.

In humans, blood cell lines have different degrees of CB₂ receptor expression with the following rank order: B lymphocytes > natural killer cells > monocytes > polymorphonuclear neutrophils > CD8 lymphocytes > CD4 lymphocytes (Galiegue et al., 1995; Fernandez-Ruiz et al., 2007b). The expression level of CB₂ receptors in immune cells can vary in relation to cell differentiation and activation state. CB₂ receptor expression varies depending on the stage of B-cell differentiation with virgin and memory B cells expressing the highest levels of CB₂ receptor mRNA followed by germinal center B cells and centroblasts (Carayon et al., 1998). Carlisle et al. showed that while resident macrophages lack CB₂ receptor expression, thioglycollate-elicited and interferon- γ (IFN- γ)-primed macrophages have high CB₂ receptor levels (Carlisle et al., 2002). CB₂ receptor expression is also observed in osteoblasts, osteocytes, and osteoclasts (Ofek et al., 2006), in preimplantation embryos (Paria et al., 1995), in rat and human skeletal muscle (Cavuoto et al., 2007), in pancreatic islets cells (Cavuoto et al., 2007), in the human ovary (El-Talatini et al., 2009), and in human ventricular myocardium (Weis et al., 2010). In the central nervous system (CNS), CB₂ receptor expression has been described on activated microglia. Where most of the studies describe CB₂ receptor restricted to immune cells in injured brain, other studies also showed evidence of CB₂ receptor in healthy CNS. Van Sickle and colleagues reported the presence of CB₂ receptor mRNA and protein in brainstem neurons (Van Sickle et al., 2005). The group of Onaivi and Gong studied the distribution of CB₂ receptor in healthy adult rat brain and found CB₂ receptor expression in cerebellum, cortical, and subcortical regions (Gong et al., 2006; Onaivi et al., 2006).

By coupling to G_{i/o} proteins, cannabinoid receptors regulate the activity of several membrane proteins and signal transduction pathways. Both CB₁ and CB₂ receptors inhibit cyclic adenosine 5'-monophosphate (cAMP) formation and

activate mitogen-activated protein kinase (MAPK) (Pertwee, 1997). In addition, CB₁ receptors activate ion channels such as A-type and inwardly rectifying potassium channels, and inhibit voltage-sensitive N-type and P/Q-type calcium (Ca²⁺) channels (Deadwyler et al., 1995; Hampson et al., 1995). An important functional consequence of the regulation of ionic currents is the inhibition of neurotransmitter release. Studies have indicated that CB₁ receptor activation decreases the release of glutamate (Grundy et al., 2001), as well as modulates gamma-aminobutyric acid (GABA)ergic transmission in several brain areas by either effects on GABA release or actions on the GABA transporter (Szabo et al., 1998).

The family of endogenous ligands, termed endocannabinoids, is expanding. There are at least five different arachidonoyl derivatives, which can activate the cannabinoid receptors. Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the two best studied and most abundant members. 2-Arachidonoylglycerol ether (Hanus et al., 2001), *O*-arachidonoylethanolamide (Porter et al., 2002), and *N*-arachidonoyldopamine (Huang et al., 2002) have only recently been identified as endogenous ligands and their classification as true endocannabinoids awaits further biochemical and pharmacological characterization. The affinity of AEA and 2-AG for the human CB₁ receptor is between 26 and 209 nM for AEA and even above 10 μM for 2-AG (Steffens et al., 2005). Their affinity for the human CB₂ receptor is within the 0.6–3.5 μM range (Gonsiorek et al., 2000). Besides the activation of these receptors, AEA and 2-AG are also able to activate other molecular targets still under characterization, such as not-well-defined cannabinoid receptors (Breivogel et al., 2001; Begg et al., 2005) and/or a purported cannabinoid type 3 (or GPR55) receptor (Pertwee, 2007). Furthermore AEA, but not 2-AG, behaves as a weak ligand to the transient receptor potential vanilloid 1 (TRPV1) receptor (Jung et al., 1999). TRPV1 is a ligand-gated and non-selective cationic channel, activated by molecules derived from plants, such as the pungent component of “hot” red peppers capsaicin (Jordt and Julius, 2002). Since TRPV1 is expressed in peripheral sensory fibers and also in several nuclei of the CNS (Marinelli et al., 2003), the endovanilloid activity of AEA may also play a role in physiological control of brain function.

Endocannabinoids act as retrograde signals at CNS synapses, as shown in Figure 4.1. In contrast to classical neurotransmitters, endocannabinoids are not stored in vesicles but are produced in dendrites on demand. This is the result of a biosynthetic mechanism relying on the existence of phospholipid precursors of these compounds, and of Ca²⁺-sensitive lipases for the conversion of the precursors in the endocannabinoid products. The biosynthesis of endocannabinoids is immediately followed by their release and the activation of presynaptically located cannabinoid CB₁ receptors. The lifespan of endocannabinoids in the extracellular space is limited by a rapid elimination process consisting of selective uptake into the postsynaptic cell and subsequent degradation. AEA is inactivated by reuptake via the AEA membrane transporter (AMT) and by intracellular enzymatic degradation by fatty acid amide hydrolase (FAAH)-mediated hydrolysis (Giuffrida et al., 2001). 2-AG undergoes similar FAAH-mediated hydrolysis

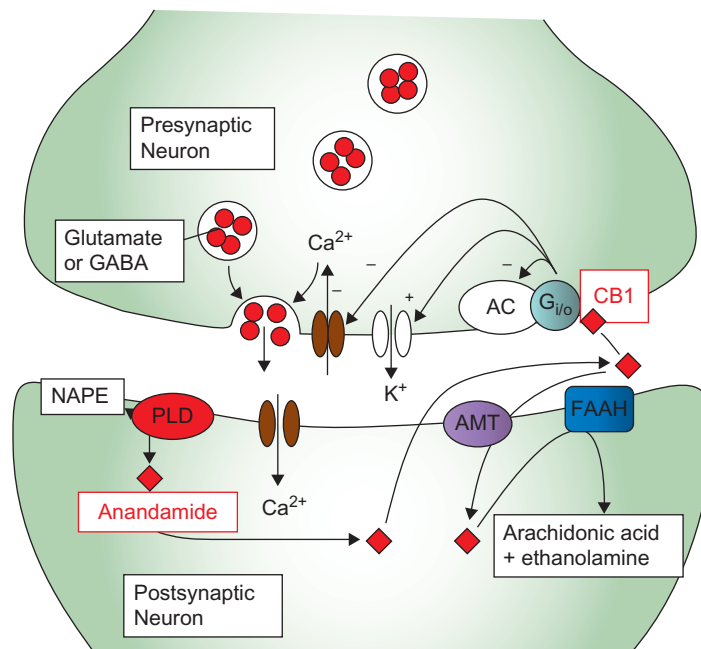


FIGURE 4.1

Schematic representation of endocannabinoid signaling, as illustrated for AEA. Influx of Ca^{2+} in the postsynaptic cells activates phospholipase D (PLD), which acts on *N*-arachidonoyl phosphatidylethanolamine (NAPE) to produce AEA. AEA leaves the postsynaptic cell and activates presynaptic cannabinoid CB₁ receptors. G_{i/o} protein activation leads to inhibition of adenylate cyclase (AC), opening of presynaptic potassium (K⁺) channels, and inhibition of presynaptic Ca^{2+} influx, decreasing the probability of neurotransmitter release. AEA is transported into the cell via a membrane transporter (AMT) and is degraded by FAAH to arachidonic acid and ethanolamine.

Adapted from Benarroch (2007).

(Ueda et al., 2000) and carrier-mediated transmembranal transport (Beltramo and Piomelli, 2000), but recent evidence has demonstrated the existence of another enzyme involved in the degradation of 2-AG, monoacylglycerol lipase (MGL) (Dinh et al., 2002). FAAH is distributed in brain areas in a pattern corresponding that of the CB₁ receptor, i.e., high concentrations in hippocampus, basal ganglia, cerebellum, and cerebral cortex (Tsou et al., 1998b).

Interestingly, COX-2 is also identified in endocannabinoid transmission and forms prostacannabinoids that in turn participate in cell signaling (Kozak et al., 2002). CB₂ receptors have more or less similar pharmacology to CB₁ receptors, which means that most endocannabinoids, synthetic and plant-derived cannabinoids, activate CB₂ receptors, although the affinity can be different to CB₁ receptors (Pertwee, 2005).

PHYSIOLOGICAL ROLE OF THE ECS WITHIN THE CNS

The ECS in the CNS plays an important role in the regulation of brain networks and synaptic transmission, leading to several central actions such as the control of cognition, pain, perception, movement, drug addiction, and memory consolidation (for review see [Di Marzo et al., 1998](#)). Generally, the current view on the ECS is that it acts as a broad-spectrum modulator. This broad-spectrum modulatory effect is largely caused by the interference of endo- and exocannabinoids with classical neurotransmitter signaling via the CB₁ receptor. As mentioned above, the ECS interferes with release or reuptake of neurotransmitters produced by presynaptic terminals, and provides a physiological feedback mechanism able to reduce synaptic inputs onto the stimulated neuron in a highly selective and restricted manner. This “retrograde signaling” of AEA and 2-AG may result in depolarization-induced suppression of inhibition (DSI) at GABAergic synapses, and in depolarization-induced suppression of excitation (DSE) at glutamatergic synapses. The specific role of endocannabinoids in different cell types and brain regions may vary depending upon temporal and spatial patterns of neuronal activity, and kinetics of endocannabinoid biosynthesis, transport, and degradation. In addition to their role in synaptic transmission, CB₁ receptors are endowed with anti-inflammatory and neuroprotective properties ([Drysdale and Platt, 2003](#)).

CB₂ receptors are primarily present in the CNS on activated microglia. Microglia are the only resident hematopoietic cells in the CNS and are seen as resident macrophages of the brain, the main actors of the innate immune response in the CNS. They account for about 5–10% of the entire brain cell population. Microglia have an important protective function in brain injury by removing damaged cells, promoting neurogenesis, inducing the re-establishment of a functional neuronal environment by restoration the myelin sheath, and by releasing neurotrophic factors and anti-inflammatory molecules ([Ziv et al., 2006](#); [Franklin and Ffrench-Constant, 2008](#); [Walter and Neumann, 2009](#)). Although the function of resting microglia in the normal brain has not been fully elucidated yet, upon exposure of the brain to any form of insult, microglia rapidly become activated. They provide the first line of defense in the CNS against infection and injury. Microglia are a very sensitive marker of neuronal insults, since activation often precedes reactions of other cell types in the brain ([Kreutzberg, 1996](#)).

During activation, microglia up-regulate an array of cell surface receptors that may be critical in microglial regeneration and/or degeneration of the CNS. Included among these are immunoglobulin (Ig) superfamily receptors, complement receptors, Toll-like receptors, cytokine/chemokine receptors, opioid receptors, and cannabinoid receptors. The expression of CB₂ receptors is manifest primarily when microglia are in “responsive” and “primed” states of activation, signature activities of which include cell migration and antigen processing ([Figure 4.2](#)). It has been proposed that the role of the CB₂ receptor in immunity in the CNS is primarily anti-inflammatory ([Carrier et al., 2005](#)). In this context, this receptor has the potential to serve as a therapeutic target for appropriately designed CB₂ receptor-specific ligands that could act

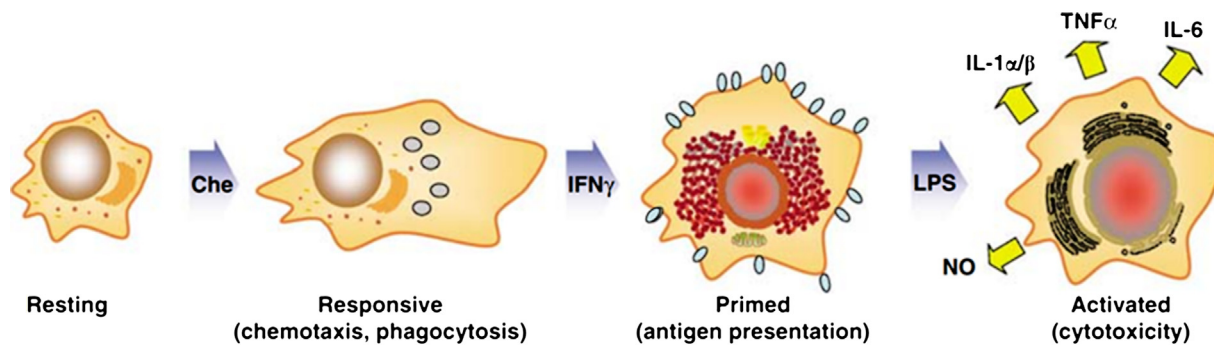


FIGURE 4.2

In vitro model of macrophage/macrogial multi-step activation. Microglia can be activated due to different kinds of signals from a resting state to a responsive, primed, or fully activated state. Each of these states is characterized by differential gene expression and correlative distinctive functional capabilities.

From Cabral et al. (2008).

as anti-inflammatory agents in neuropathological processes. Activation of the CB₂ receptor leads to migration and proliferation of the microglia (Walter and Stella, 2004). It has been demonstrated that microglia express the CB₂ receptor at the leading edge of lamellipodia, consistent with their involvement in cell migration (Walter et al., 2003).

ENDOCANNABINOID SIGNALING AND HUNTINGTON'S DISEASE

As for most neurotransmitter systems, endocannabinoid transmission in the brain is affected by normal senescence (Mailleux and Vanderhaeghen, 1992; Romero et al., 1998). However, as was indicated by *in vitro* and postmortem studies, the degree to which this occurs is supposed to be small compared to the changes observed in the processes of pathological aging. Several *in vitro* and postmortem studies have demonstrated changes in endocannabinoid signaling in the basal ganglia of humans affected by Huntington's disease (Glass et al., 1993, 2000; Richfield and Herkenham, 1994). These changes, with the presence of cannabinoid CB₁ receptors in the basal ganglia and their involvement in movement, have encouraged studying the ECS as a potential therapeutic target for Huntington's disease (HD), especially given the limitations of current therapies. Preclinical data have pointed to a potential role for CB₂ in reducing microglial activation and preventing neurodegeneration (Palazuelos et al., 2009; Sagredo et al., 2009).

HUNTINGTON'S DISEASE

HD is an autosomal dominant neurodegenerative disorder affecting approximately 1 in 10,000 individuals and is characterized by involuntary movements such as chorea, mood and behavioral disturbances, and cognitive impairments. Typically, onset of symptoms is in middle age, but the disorder can manifest at any time between infancy and senescence. HD is caused by a cytosine-adenine-guanine (CAG) repeat expansion within exon 1 of the HD gene (*HTT*) on chromosome 4 (The Huntington's Disease Collaborative Research Group, 1993). Huntingtin (*htt*), the protein encoded by *HTT*, is expressed in all human and mammalian cells, with the highest concentration in the brain (DiFiglia et al., 1995), in particular in the cerebral cortex and cerebellum (Trottier et al., 1995). The physiological role of wild-type *htt* is, as yet, poorly understood. Toxic gain of function of mutant *htt* and loss-of-function of wild-type *htt* have been suggested as possible mechanisms in the pathophysiology of HD (Beal and Ferrante, 2004). Also, the mode of neuronal death in HD continues to be debated, although considerable evidence suggests that apoptosis plays an important role (Hickey and Chesselet, 2003).

HD causes cell loss and atrophy, most prominently in the caudate nucleus and putamen (Vonsattel and DiFiglia, 1998). The striatal medium spiny neurons are the

most vulnerable, while striatal interneurons are generally spared. The atrophy and gliosis of the caudate nucleus and putamen are progressive and marked, but neuropathological changes have also been described in other brain regions, such as the frontal and temporal lobes (Rosas et al., 2003). Nuclear and cytoplasmic inclusions containing mutant htt are apparent in the brain of affected individuals (Davies et al., 1997), even long before symptoms onset (Gomez-Tortosa et al., 2001).

The first clinical symptoms of HD can be psychiatric, motor, or cognitive in nature. Death generally occurs 15–20 years after the first symptoms and usually results from complications of falls, dysphagia, or aspiration. In later stages of the disease, chorea often becomes less prominent, while dystonia and rigidity become more pronounced (Young et al., 1986). Cognitive dysfunction primarily affects executive functions, such as organizing, planning, checking, or adapting alternatives, and delays the acquisition of new motor skills (Walker, 2007).

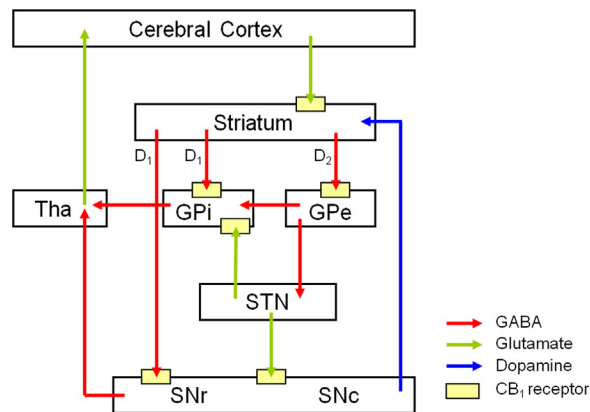
Current therapies are symptomatic, but poorly effective. They include the use of neuroleptics to decrease chorea and the use of psychotropic medications to address depression or behavioral problems (Imarisio et al., 2008).

THE ECS AND THE CONTROL OF MOTOR BEHAVIOR

Marijuana use affects psychomotor activity in humans (Romero et al., 2002). Also in rodents, the administration of plant-derived and synthetic cannabinoids, in particular Δ^9 -THC, affects motor behavior, producing dose-dependent motor impairments in a variety of behavioral tests (for reviews see Sanudo-Pena et al., 1999; Romero et al., 2002). Direct agonists of the CB₁ receptor produce a motor inhibition characterized by decreases in spontaneous locomotor activity and frequency of stereotypes (Navarro et al., 1993; Romero et al., 1995), and development of immobility and catalepsy (Pertwee et al., 1988; Crawley et al., 1993). Direct agonists of the CB₁ receptor also potentiate the action of hypokinetic compounds, such as reserpine or muscimol (Moss et al., 1981; Wickens and Pertwee, 1993), or attenuate the hyperlocomotion caused by amphetamine (Gorriti et al., 1999). In contrast, CB₁ receptor antagonists, such as SR141716 (Rimonabant[®]) are able to reverse the effects of receptor agonists (Souilhac et al., 1995; Di Marzo et al., 2001) and produce hyperactivity (Compton et al., 1996).

Endocannabinoids and CB₁ receptors in basal ganglia structures

In line with the physiological effect of cannabinoids on motor behavior, the basal ganglia exhibit the highest densities of CB₁ receptors in the brain (Herkenham et al., 1990, 1991). The CB₁ receptors are primarily expressed in medium spiny GABA-ergic neurons of the striatum and are concentrated in their presynaptic axon terminals innervating the internal (GPi) and external (GPe) segment of the globus pallidus and the substantia nigra pars reticulata (SNr). There are also presynaptic CB₁ receptors in excitatory glutamatergic cortico-striatal terminals and in excitatory projections from the subthalamic nucleus (STN) to the GPi/SNr (Brotchie, 2003; Fernandez-Ruiz and Gonzales, 2005). CB₁ receptors appear to be

**FIGURE 4.3**

Organization of the basal ganglia circuits and localization of the CB₁ receptors. D₁, dopamine D₁ receptor; D₂, dopamine D₂ receptor; GPi, internal segment globus pallidus; GPe, external segment globus pallidus; SNr, substantia nigra pars reticulata; SNc, substantia nigra pars compacta; STN, subthalamic nucleus; Tha, thalamus.

Adapted from Van der Stelt and Di Marzo (2003).

absent on brain dopaminergic cells (Hermann and Lutz, 2005). The presence of CB₁ receptors on striatal interneurons is currently a matter of debate. Several studies indicated CB₁ receptor protein expression on parvalbumin immunoreactive and cholinergic interneurons (Hohmann and Herkenham, 2000; Fusco et al., 2004). Their endogenous ligands, AEA and 2-AG, are also present in the basal ganglia at higher concentrations than in the rest of the brain (Berrendero et al., 1999; Bisogno et al., 1999; Di Marzo et al., 2000). Endocannabinoids are particularly abundant in the GP and the SN (Di Marzo et al., 2000). These two nuclei also contain a significant amount of FAAH (Desarnaud et al., 1995; Tsou et al., 1998b). The distribution of CB₁ receptors in the different groups of basal ganglia neurons is shown in Figure 4.3.

Functional interaction between ECS and dopamine in basal ganglia circuits

The effects of cannabinoids on motor activity probably originate, in accordance with their distribution, from the ability to interfere with the major neurotransmitters involved in basal ganglia function. Not only release of glutamate and GABA are regulated by the ECS, but there is also substantial evidence supporting a role for the ECS as an indirect modulator of dopaminergic transmission. However, this interaction is remarkably complex.

Administration of exogenous cannabinoids was found to increase dopamine release in the rat nucleus accumbens (Szabo et al., 1999), and to excite dopaminergic neurons in the ventral tegmental area and SN (French et al., 1997).

Chronic treatment with dopamine D₂ receptor antagonists was found to up-regulate CB₁ receptor expression in the rat striatum (Jarrahian et al., 2004). Conversely, microdialysis experiments demonstrated that stimulation of dopamine D₂ receptors *in vivo* increases AEA levels $\approx$ eight-fold over baseline in the striatum (Giuffrida et al., 1999; Beltramo et al., 2000). Additionally, the injection of CB₁ receptor agonists/antagonists into the basal ganglia has been reported to modulate the motor responses of locally administered dopamine D₂ receptor agonists. Moreover, when expressed individually, activation of either the dopamine D₂ or CB₁ receptor inhibits cAMP accumulation, indicating convergence of their signal transduction mechanisms, whereas dopamine D₁ receptor-mediated activation of adenylate cyclase can be completely blocked by CB₁ receptor stimulation (Meschler and Howlett, 2001).

THE ECS AND NEUROINFLAMMATION

Activation of the immune system in the CNS has been described in HD and this amount of activation is correlated to clinical symptoms (Bjorkqvist et al., 2008). Microglial activation is an early process in HD pathogenesis and is present in HD brains before the onset of symptoms (Tai et al., 2007). The number of activated microglia in the striatum and cortex correlates with the extent and progression of neuronal loss (Sapp et al., 2001). Previous studies showed a wide variety of age of onset in HD patients with the same CAG repeat length, suggesting an influence of environmental factors (Wexler et al., 2004). In this context, immunological response can play a role in the onset of disease.

Most CB₂ receptor functions are associated with immunological effects, based on their predominance over CB₁ receptors in immune tissues. Although several studies also point towards the presence of CB₂ receptors in normal CNS (Onaivi, 2006), it is likely that these receptors undergo an up-regulation in neuroinflammatory conditions in cells that show low levels of CB₂ receptors or are induced in cells that do not express CB₂ receptors in normal conditions (microglia) (Fernandez-Ruiz and Gonzales, 2005; Fernandez-Ruiz et al., 2007a). In response to brain injury, microglial cells are activated and recruited. The molecular steps involved in this activation process implement distinct cellular functions aimed at repairing damaged neural cells and eliminating toxins and pathogens from the area (Garden and Moller, 2006). The up-regulation of CB₂ receptors in microglia results in microglial proliferation, differentiation, and migration (Puffenbarger et al., 2000; Facchinetti et al., 2003; Carrier et al., 2004; Stella, 2004). CB₂ receptor activation in microglia reduces their ability to release detrimental factors including neurotoxic factors such as nitric oxide, proinflammatory cytokines, and reactive oxygen species (Fernandez-Ruiz et al., 2007a; 2008).

Parallel to CB₂ receptor increase in inflammatory conditions, CB₁ receptors and endocannabinoids also show equivalent responses (Fernandez-Ruiz and Gonzales, 2005; Fernandez-Ruiz et al., 2007a), suggesting that the activation of the ECS is an intrinsic response of the brain to maintain nerve cell homeostasis and to reduce injury in pathological conditions.

ALTERATIONS OF THE ECS IN HD

The first evidence of dysregulation of the ECS in HD was provided by Glass et al., who showed loss of cannabinoid CB₁ receptors in the SN, GP, and putamen in postmortem human brain with HD (Glass et al., 1993, 2000). The same authors also found that this loss of CB₁ receptors occurs in advance of other receptor losses such as those of the dopamine D₁ and D₂ receptors, and before the appearance of major symptoms (Glass et al., 2000), suggesting an involvement of these receptors in the pathogenesis and/or progression of neurodegeneration. In concordance with human data, CB₁ receptor messenger ribonucleic acid (mRNA) levels were decreased in the absence of neuronal loss in the lateral striatum, cortex, and hippocampus of transgenic mouse models of HD (Lastres-Becker et al., 2002a; Naver et al., 2003). In the HD94 transgenic mice also decreases in the number of specific binding sites and the activation of GTP-binding proteins by CB₁ receptor agonists were noticed in the basal ganglia (Lastres-Becker et al., 2002a). Loss of CB₁ receptors in the basal ganglia not only occurred in transgenic mice HD models, but also in rats after local intrastratial application of 3-nitropropionic acid (3-NP), a toxin that reproduces the mitochondrial complex II deficiency characteristic of HD patients (Lastres-Becker et al., 2002b). Furthermore, delaying the onset of HD symptoms by enriched environments has been shown to selectively slow down the loss of CB₁ receptors in the R6/1 transgenic mice model of HD (Glass et al., 2004); and abnormal sensitivity to CB₁ receptor stimulation, reported in the striatum of R6/2 transgenic HD mice, was shown to contribute to the hyperactivity of the GABA synapses, seen in this model (Centonze et al., 2005). With regard to endocannabinoid levels, a significant reduction in AEA was observed in the striatum of the 3-NP rat model of HD (Lastres-Becker et al., 2001).

Numerous studies have shown a neuroinflammatory contributing factor in the pathogenesis of HD. Palazuelos and colleagues showed increased CB₂ receptor levels in postmortem HD human brain tissue and in mice (Palazuelos et al., 2009). They also showed that deletion of CB₂ receptors in HD mice exacerbates disease progression. Recent work also reported that deletion of CB₂R in another slowly progressive HD model accelerates the onset of disease and exacerbates behavioral deficits (Bouchard et al., 2012). In the same study, treatment with CB₂ receptor selective agonists suppressed neurodegeneration, which was mediated by peripheral immune cells (Bouchard et al., 2012).

THERAPEUTIC POTENTIAL OF ENDOCANNABINOIDS IN HD

EFFECTS ON MOTOR SYMPTOMS

Although evidence from *in vitro* studies and experimental models provides rationale for endocannabinoid targeted therapy in HD, data on the symptomatic effects of CB₁ receptor agonists and antagonists in the basal ganglia circuits appear to be inconclusive.

The first pharmacological studies using classical cannabinoids in patients with HD failed to reduce hyperkinesia; the CB₁ receptor agonist nabilone was found to even increase choreatic movements (Muller-Vahl et al., 1999a,b). Studies in animal models of HD, however, demonstrated that direct agonists of CB₁ receptors, such as CP55,940, or inhibitors of the AEA uptake, such as AM404, are able to reduce hyperkinesia and lead to recovery from GABA-ergic deficits in rats with striatal lesions caused by local application of 3-NP (Lastres-Becker et al., 2002b). However, the relative contribution of vanilloid receptors and CB₁ receptors in these beneficial effects of AM404 needs to be evaluated, since the anti-hyperkinetic effect of AM404 was counteracted by capsazepine, a selective antagonist of TRPV1 (Lastres-Becker et al., 2002b; 2003b). Additionally, VDM11, another selective inhibitor of the AEA uptake, failed to ameliorate the ambulation of a rat model of HD (Lastres-Becker et al., 2003b), while the potent and selective AMT inhibitor UCM707 exhibited a notable anti-hyperkinetic activity in the 3-NP rat model of HD (De Lago et al., 2002, 2006).

EFFECTS ON PATHOGENESIS

Besides the potential of cannabinoids to ameliorate motor deterioration in HD, cannabinoids have also been used in attempts at delaying progressive neurodegeneration. Neuroprotective effects have been proposed for natural, synthetic, and endogenous cannabinoids *in vitro* and *in vivo* (Hampson et al., 1998; Nagayama et al., 1999; Sinor et al., 2000). Two mechanisms were implicated: (1) antioxidative properties of cannabinoids, proven through a receptor-independent mechanism *in vitro* (Hampson et al., 1998) and (2) reduction of excitotoxicity by inhibiting glutamate release (see also “Physiological role of the ECS within the CNS,” above).

These protective mechanisms of the ECS have been confirmed by several authors in experimental models of HD, although also here contradictions were found. For example, treatment with Δ^9 -THC after 3-NP administration significantly reduced GABA levels in the caudate-putamen of male Lewis rats (Lastres-Becker et al., 2004), while it increased malonate-induced striatal lesions (Lastres-Becker et al., 2003a).

In addition to their antioxidant and anti-excitotoxic properties, (endo)cannabinoids might also be protective in HD because of their anti-inflammatory actions. Δ^9 -THC acts as an immunosuppressive agent and induces alterations in the peripheral cytokine network, modulating the production of tumor necrosis factor α , interleukin 1, and interleukin 2 (Fischer-Stenger et al., 1993). (Endo)cannabinoids also exert a potential modulatory action on microglia via CB₂ receptors. These anti-inflammatory actions are of importance considering that cell death is accompanied by activation of microglia at the sites of neurodegeneration, which may be important in the initiation and/or progression of the neurodegenerative process (Pocock and Liddle, 2001).

Initially, inflammation tries to eliminate cell debris of neurons and is responsible for repair mechanisms, but chronic inflammation, as is the case in neurodegeneration, becomes harmful. This is due to toxicity caused by the release of different factors, such as nitric oxide, proinflammatory cytokines, and reactive oxygen species (ROS), all able to cause deterioration of neuronal homeostasis. Many studies have shown important anti-inflammatory effects of cannabinoid agonists, which are preferentially mediated by the activation of CB₂ receptors (Fernandez-Ruiz et al., 2007a; Stella, 2009). CB₂ receptor activation leads to decreased inflammatory responses (Munro et al., 1993; Ashton and Glass, 2007) and has been shown to have a protective role in neurodegenerative diseases (Arevalo-Martin et al., 2003; Pryce et al., 2003; Kim et al., 2006; Zhang et al., 2007; Garcia et al., 2011; Martin-Moreno et al., 2012). CB₂ receptors are therefore the key target for anti-inflammatory effects of cannabinoids. Studies have provided evidence about the CB₂ receptor up-regulation in the case of neuronal damage, for example by using postmortem brain samples from Alzheimer's disease patients, but also in HD and PD.

FUTURE PERSPECTIVES AND NEEDS

Although advances in defining the role of endocannabinoids in both normal and pathological conditions have provided some rationale for endocannabinoid target therapy in HD, experimental evidence has indicated that the effects of CB₁ receptor agonists or antagonists on motor symptoms are complex, among other considerations due to site specificity (Papa, 2008). Experimental evidence in models of HD has also indicated that the effects of drugs on motor symptoms depend on the disease severity and are probably dose and gender dependent (Sundram, 2006).

For the CB₂ receptor, activation has been shown to reduce proinflammatory events and enhance neuronal survival, thereby supporting the importance of this receptor as a potential therapeutic target in neuroinflammatory and neurodegenerative conditions (reviewed in Fernandez-Ruiz et al., 2007a).

RELEVANCE OF MOLECULAR IMAGING OF THE ECS IN HD

Today, *in vivo* molecular imaging opens new perspectives to further evaluate both in humans and in animals the neurobiological and potential clinical impact of the ECS in HD. Molecular imaging allows visualization, 3D localization, and quantification of molecular processes at the cellular level within intact living organisms that can be repeated over time in the same subjects (Massoud and Gambhir, 2003). Many molecular processes can be targeted, including receptor density and drug occupancy (Burns et al., 2007), transporter and enzyme activity, gene expression (Willmann et al., 2009), metabolite concentration, protein–protein interaction (Lake et al., 2012), transcriptional activity (Pouliot et al., 2011), signal transduction, and apoptosis (Wang et al., 2013). Functional imaging of brain cannabinoid

receptors using positron emission tomography (PET) and single photon emission computed tomography (SPECT) may lead to the identification of novel diagnostic biomarkers and guide dose—occupancy studies in drug development research. These techniques can also be used to examine the interaction between the ECS and other neurotransmitter systems both in control and pathological conditions.

IMAGING OF THE BRAIN CB₁ RECEPTOR

Radioligands that have been developed for imaging of brain CB₁ receptors to date may be broadly categorized as first and second generation compounds. First generation compounds were limited by their low specific binding and poor brain uptake. More recently developed compounds succeeded in combining a high binding affinity with more moderate lipophilicity, and have been used in preclinical and clinical investigations since 2006. The physicochemical and *in vivo* imaging properties of the first and second generation compounds are listed in Table 4.1.

Currently available CB₁ receptor radioligands for human CB₁ receptor imaging: “second generation compounds”

^[11C]JHU75528 or ^[11C]OMAR

In 2006, the Johns Hopkins PET group reported the synthesis of 4-cyano-1-(2,4-dichlorophenyl)-5-(4-^[11C]methoxyphenyl)-N-(pirrolidin-1-yl)-1*H*-pyrazole-3-carboxamide (^[11C]JHU75528 or ^[11C]OMAR), the first CB₁ receptor radioligand for quantitative PET studies (Horti et al., 2006; Fan et al., 2009). ^[11C]OMAR is structurally an analogue of SR141716 with comparable binding affinity for the CB₁ receptor and good CB₁/CB₂ selectivity. The lipophilicity of ^[11C]OMAR is lower than that of SR141716 as the hydrophobic methyl and chlorine substituents of SR141716 are replaced with cyano- and methoxy groups.

Extracellular electrophysiological recordings of rodent brain slices revealed that OMAR reverses the effects of the CB₁ receptor agonist WIN 55,212-2 on glutamate release in the striatal brain slices, pointing to functional antagonist properties (Fan et al., 2009). Radiosynthesis of ^[11C]OMAR is performed by ^[11C]methylation of the corresponding phenol precursor (Horti et al., 2006). In mice and baboon studies, ^[11C]OMAR showed promising results. When compared to the previously reported CB₁ receptor radioligands of the first generation, the target-to-non-target ratio of ^[11C]OMAR was substantially higher (Horti et al., 2006; Fan et al., 2009). ^[11C]OMAR also readily entered the brain to specifically and selectively label cerebral CB₁ receptors. The specific binding of ^[11C]OMAR *in vivo* can be saturated by pretreatment of non-labeled OMAR of SR141716 in a dose-dependent manner (Horti et al., 2006). Various central non-cannabinoid drugs did not reduce regional CB₁ receptor binding, indicating that ^[11C]OMAR does not bind to other central receptors such as D₁-, D₂-, D₃-, 5HT_{2A}-, 5HT_{1C/2C}-, opioid, and α4β2-nACh receptors. Small animal PET studies with ^[11C]OMAR showed 50% higher brain uptake in wild-type mice versus

Table 4.1 *In Vitro* and *In Vivo* Imaging Properties of the First and Second Generation of Radioligands for Imaging of the CB₁ Receptor

First Generation	Molecular Weight (MW)	CB ₁ Binding Affinity, K _i (nM)	Relative CB ₁ Binding Affinity, ^a K _{rel}	CB ₂ Binding Affinity, K _i (nM)	Lipophilicity, logD _{7.4}	Polar Surface Area (PSA)	Target-to-non-target Ratio		
							Mouse	Monkey	Human
[¹²³ I]AM251	555	0.6–1	0.3–2	2290	5.3	50	1.5; 2.2; 1.7 ^b	Low brain uptake	–
[¹²³ I]AM281	557	4.5	2.5	4200	3.7	59	≈1.9 ^b	≈1.4 ^c	0.21 ^d
[¹²⁴ I]AM281							–	–	0.37 ^d
[¹⁸ F]NIDA54	400	0.7	–	–	3.8 ^e	25	–	0.7	–
[¹⁸ F]PipISB	492	1.5 ^f	–	–	5.0	80	–	0.9	–
Second Generation	Molecular Weight (MW)	Radiochemical Yield	K _i nM/K _{rel}	Selectivity CB ₁ /CB ₂	Lipophilicity, logD _{7.4}	Polar Surface Area (PSA)	Target-to-non-target Ratio	Putamen Uptake, % SUV	Time to Reach Steady State
[¹¹ C]JHU75528 In baboon and human	470	16 ± 5%	11/0.3	250–480 ^g	3.3; 3.6 ^h	83	Putamen/Pons = 2.5 Putamen/Thalamus = 2.1	140–240	60–80 min
[¹¹ C]MePPEP In rhesus monkey	489	2.5 ± 1.1%	0.6 ^f	726	4.8	84	Putamen/Pons = 2.4 Putamen/Thalamus = 1.9	400–600	>120 min
[¹⁸ F]FMPEP-d2 In rhesus monkey and human	454	–	0.2	669	6.0	42	Putamen/Pons = 1.8	500–600	~90 min
[¹⁸ F]MK9470 In rhesus monkey and human	474	4.6 ± 0.3%	0.7	44–60	4.7	42	Putamen/Pons = 1.8 Putamen/Thalamus = 1.9	120–160	120–180 min

Note: The lowest CB₁ receptor density (non-target) is found in the pons and thalamus. The lipophilicity is expressed by the logD value at pH 7.4. The maximum permeability of CNS drugs is often seen if logD_{7.4} = 0–4.

^aPrecise inhibition assay conditions differ between laboratories. Therefore, K_{rel}, a ratio of the K_i for the test compound to that of SR141716 from the same laboratory and by the same method, provides a sense of comparative affinity

^bcerebellum/brainstem ratio

^ccerebellum baseline/cerebellum block with SR141716 ratio

^dbinding potential

^eexperimental data

^fin vitro functional binding activity (K_d)

^gK_i^{CB₂} = 2700, 5250 nM

^htwo different methods.

CB₁ knockout animals (Herance et al., 2011). [¹¹C]OMAR is converted to several hydrophilic radiometabolites in mice and baboon blood, but only a fraction of these radiometabolites penetrates the blood–brain barrier (BBB), i.e., ≈6% in mice, and likely correspond to [¹¹C]CO₂ (Horti et al., 2006). Kinetic analysis of baboon PET data demonstrated binding potential (BP) values of ≈1.4 in CB₁ receptor-rich regions at baseline, and a substantially lower BP value of 0.4 upon blocking. Steady-state was reached in baboon brain before the end of the 90-minute scan, which is a substantial advantage of this radioligand (Horti et al., 2006).

Human studies with [¹¹C]OMAR have been reported. [¹¹C]OMAR was used under an Investigational New Drug Application, approved by the Food and Drug Administration (FDA), to quantify brain CB₁ receptors in healthy subjects and patients with schizophrenia (Wong et al., 2010a). Human PET studies using the CB₁ receptor antagonist AVE1625 also showed the feasibility of [¹¹C]OMAR for the evaluation of drug occupancy studies (Wong et al., 2010b). To date, no studies using [¹¹C]OMAR in HD have been published.

[¹¹C]MePPEP and [¹⁸F]FMPEP-d2

The Eli Lilly, National Institutes of Health (NIH), and Karolinska University collaborative group developed two structurally related non-SR141716-based CB₁ receptor PET radioligands for human studies, [¹¹C]MePPEP and [¹⁸F]FMPEP-d2 (Terry et al., 2009, 2010b). Both compounds behave as CB₁ receptor inverse agonists.

The functional binding activity of MePPEP is very high ($K_i = 0.66$ nM) (Yasuno et al., 2008). Due to a high lipophilicity, [¹¹C]MePPEP is not very soluble in saline and requires formulation with Tween-80. The high lipophilicity is likely to be responsible for its low free fraction in monkey plasma (Yasuno et al., 2008).

Small animal PET studies in wild-type and CB₁ receptor knockout mice demonstrated that ≈65% of total brain uptake of [¹¹C]MePPEP represents specific binding (Terry et al., 2008). Blocking studies in the rodent brain also demonstrated that the CB₁ receptor inverse agonist SR141716 has a much higher *in vivo* potency to displace [¹¹C]MePPEP as compared to various other CB₁ receptor agonists. This is consistent with the existence of different binding sites for agonists and inverse agonists on the CB₁ receptor. In rhesus monkey studies, brain uptake of [¹¹C]MePPEP is high, but clearance is relatively slow, requiring 120 to 150 minutes of scanning (Yasuno et al., 2008).

When performing PET studies with [¹¹C]MePPEP in healthy subjects, also a high brain uptake, a slow washout from brain, and a distribution pattern consistent with that of CB₁ receptors *ex vivo* were observed (Terry et al., 2009). Quantification of [¹¹C]MePPEP in humans was also feasible using total volume of distribution (V_T) as an outcome measure. However, its precision and accuracy were highly influenced by the slow brain kinetics of [¹¹C]MePPEP and by the very low fraction of free radioligand in plasma. Further studies determined that CB₁ quantification was not limited by the measurements from brain, but rather by the measurements from plasma (Terry et al., 2009).

The abovementioned difficulties with the quantification of [^{11}C]MePPEP were the driving force behind the development of its derivative, [^{18}F]FMPEP-d2. The binding affinity of [^{18}F]FMPEP-d2 is comparable to that of [^{11}C]MePPEP, i.e., 0.2 nM (Terry et al., 2010b). To reduce the de-[^{18}F] fluorination commonly seen with [^{18}F]fluoromethoxy-labeled compounds and consequently the high uptake of [^{18}F]fluoride in the skull bone, two deuterium atoms were introduced into the molecule's structure. [^{18}F]FMPEP-d2 had high uptake in the monkey brain, with greater than 80% specific binding (Terry et al., 2010b). High brain uptake with [^{18}F]FMPEP-d2 was also observed in humans, in whom V_T was well identified within approximately 60 minutes. Retest variability of plasma measurements was good (16%); consequently, V_T had a good retest variability (14%), inter-subject variability (26%), and interclass correlation coefficient (0.89).

One limitation of [^{18}F]FMPEP-d2 is that also its plasma-free fraction in humans is low ($\approx 0.63\%$). Due to this nature, small differences in free fraction would be disproportionately large in percentage. Correcting V_T for free fraction, which is suggested by the authors to be a more correct quantification, added too much noise to the final outcome measurements and was therefore excluded. Nevertheless, the authors still recommended accounting in future studies for potential changes in free fraction, particularly with pharmacological challenges, as a proportional amount could be displaced from plasma proteins and may enter the brain.

Whole-body imaging studies using [^{11}C]MePPEP and [^{18}F]FMPEP-d2 demonstrated the radiation burden to be acceptable for multiple brain studies per year, i.e., $4.6 \pm 0.3 \mu\text{Sv/MBq}$ and $19.7 \pm 2.1 \mu\text{Sv/MBq}$, respectively. Brain uptake of both radioligands was $\approx 7\text{--}8\%$ of the total dose. [^{11}C]MePPEP undergoes exclusively hepatobiliary excretion, while [^{18}F]FMPEP-d2 is also excreted through the urinary tract (Terry et al., 2010a).

[^{18}F]MK9470

In the past decade, Merck researchers disclosed a series of acyclic, non-SR141716-based CB_1 receptor inverse agonists (Lin et al., 2006). Subsequent conformational analysis and receptor docking studies showed that the same binding area of CB_1 was targeted as for SR141716 (Lin et al., 2008). The pharmacological lead compound of the series, taranabant, demonstrated a substantially higher affinity ($K_i = 0.4 \text{ nM}$) than that of SR141716. The optimization of the taranabant structure for PET application led to a high CB_1 receptor affinity ($K_i = 0.7 \text{ nM}$) fluoroalkyl analogue MK9470 (Lin et al., 2006). The CB_1 receptor subtype selectivity of the compound MK9470 was about 60-fold over that of CB_2 receptors. *In vitro* affinity testing with a battery of over 100 known targets yielded no off-target activity below the micromolar level (Burns et al., 2007).

[^{18}F]MK9470 binds with relatively slow kinetics to the CB_1 receptor, and is readily displaced by unlabeled MK9470 in rats (Casteels et al., 2012), by CB_1 receptor antagonists such as the SR141716 analogue AM251, and by MK0364, an acyclic inverse agonist structurally analogous to MK9470 in monkey brain (Burns et al., 2007). Blocking studies in rats showed that $\approx 56\%$ of the radioligand

binding in brain was CB₁ receptor specific. The kinetics of [¹⁸F]MK9470 in rat brain can be modeled using a one-tissue compartment model with and without constrained radiometabolite input (Casteels et al., 2012).

A human biodistribution and radiation dosimetry study showed that brain uptake was about 5% of the injected activity and that [¹⁸F]MK9470 showed predominantly hepatobiliary excretion and an average effective dose of 22.9 microSv/MBq (Van Laere et al., 2008).

After intravenous injection in humans, [¹⁸F]MK9470 radioactivity in brain increased throughout the scanning period (120 minutes), even though radioligand concentration in arterial plasma decreased throughout the length of the scan (Burns et al., 2007). The highest uptake was observed in the striatum, frontal cortex, and posterior cingulate, whereas intermediate uptake was seen in the cerebellum and the lowest uptake was observed in the thalamus, pons, and hippocampus. Despite the favorable properties of [¹⁸F]MK9470 for imaging brain CB₁ receptors, including good brain uptake and low non-specific binding, its slow kinetics was a challenge for modeling acceptable outcome measures such as V_T within clinically applicable measurement times (Sanabria-Bohorquez et al., 2010). A reversible two-tissue compartment model using a global k₄ value was necessary to describe brain kinetics. Both V_T and V_{ND}k₃ were estimated satisfactorily and their test–retest variability was between 10 and 30%. The irreversible macroparameter K_i modeled the data well. The linear relationship between K_i and V_{ND}k₃ demonstrated that K_i also provides a reliable index of receptor binding. Fractional uptake ratios (FURs), which can be measured using a limited set of venous samples, were shown to be equivalent to K_i values. More simplified brain uptake measurements (standardized uptake value (SUV) and modified SUV) were reasonably well correlated to FUR. The authors concluded that in cases when plasma measurements are not statistically different, SUV and mSUV are sufficient outcome measures. Using this analytical method, we found that [¹⁸F]MK9470 had good precision (test–retest variability <7%) and inter-subject variability (16–35%) (see also Figure 4.4).

IMAGING OF THE BRAIN CB₂ RECEPTOR

Over the past few years substantial efforts have been invested in neuroimaging of microglial function in the brain, since a biomarker for neuroinflammation would be a useful tool in drug development, therapy follow-up, and disease progression. Neuroinflammation and microglial activation is a very complicated process. A lot of questions remain unanswered and *in vitro* or *ex vivo* studies only provide us some pieces of the puzzle. *In vivo* imaging studies with PET and SPECT of different neuroinflammatory targets, such as the CB₂ receptor, can help us better understand this complex process. Compared to CB₁ receptor radioligands, the development of radioligands for brain CB₂ receptor imaging is still in its pioneering phase. Only a limited number of publications are available at the moment and are focused on the development of PET ligands. Currently used CB₂ receptor

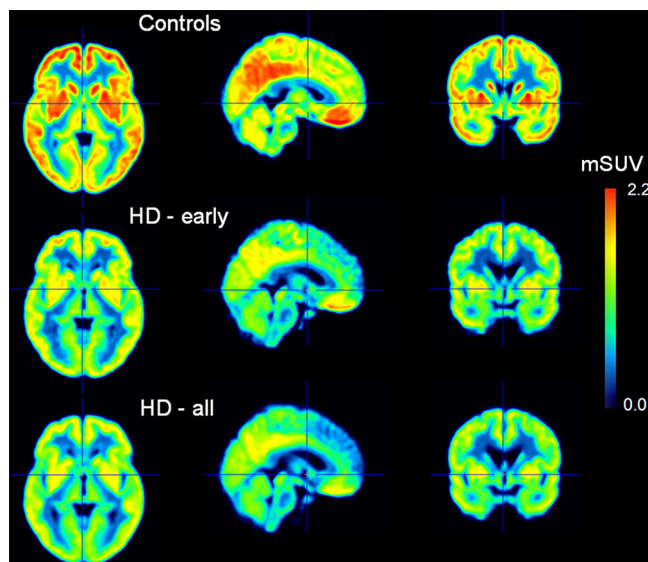


FIGURE 4.4

$[^{18}\text{F}]$ MK9470 and HD. Cross-sectional, partial volume-corrected, modified standardized uptake value (mSUV) parametric images, averaged for all controls ($n = 14$), early HD patients ($n = 7$), and total group of HD patients ($n = 19$).

radioligands can be categorized into four groups, depending on structure characteristics, i.e., derivatives of triaryl bis-sulfones, of indoles, of quinolines, and of thiazoles. The physicochemical and *in vivo* imaging properties of currently available CB_2 receptor radioligands are listed in Table 4.2.

Triaryl bis-sulfones

In 2005, a new class of CB_2 receptor inverse agonists, namely, the triaryl bis-sulfones, was reported (Lavey et al., 2005). The compound with the best combination of CB_2 receptor affinity ($K_i \text{ hCB}_2 = 0.4 \text{ nM}$) and selectivity for CB_2 receptor over CB_1 receptor ($K_i \text{ hCB}_1 / K_i \text{ hCB}_2 = 2262$) was Sch225336 (Shankar et al., 2005). Sch225336 showed immunomodulatory characteristics in both *in vitro* and *in vivo* experiments (Lunn et al., 2006). The Schering-Plough Research Institute managed to label Sch225336 with sulfur-35. Sulfur-35 is a long-lived ($T^{1/2} = 87 \text{ days}$) β -emitting isotope, and thus is not suitable for *in vivo* imaging, but useful for *in vitro* studies. Labeling with sulfur-35 has the advantage of achieving higher specific activities compared to tritium labeling, and thereby providing more sensitive detection of CB_2 receptors (Gonsiorek et al., 2006). Autoradiography studies incubating $[^{35}\text{S}]$ Sch225336 with human spleen resulted in labeling areas representing splenic white pulp (Gonsiorek et al., 2006).

Table 4.2 Physicochemical and Imaging Properties of CB₂ Receptor Radioligands

	^[11C] Sch225336	^[11C] X1	^[11C] GW405833	^[18F] FE-GW405833	^[11C] NE40	^[11C] A-836339	^[18F] X2
Molecular weight (MW)	540	395	447	356	372	310	398
LogD _{7.4}	2.2	4.6	2.5	2.8	3.9	3.4	4.4
Polar surface area (PSA)	132	39	43.7	43.7	80.4	42	77
K _i hCB2R (nM)	4.5	0.3	35	27.2	9.6	0.7	3.4
K _i hCB1R (nM)	78.5	3333	6450	10,000	>1000	>600	—
Brain uptake*	0.1	—	1.4	1.3	1.7	—	—
Brain washout**	1	—	14	1.9	4.3	—	—
Spleen uptake*	0.5	—	1.0	0.9	0.9	—	—
Spleen clearance**	5	—	2	3	1.3	—	—

*% ID 2 min post-injection—mice.

**% ID 2 min post injection/60 min post-injection ratio—mice.

In collaboration with Schering-Plough, Evens et al. reported labeling of Sch225336 with carbon-11 (Evens et al., 2008). Demethylation of [^{11}C]Sch225336 with boron tribromide provided two isomeric mono-methoxy derivatives, one of which was used for methylation with [^{11}C]methyl iodide providing [^{11}C]Sch225336. Despite its favorable LogD value of 2.15, [^{11}C]Sch225336 exhibited low brain uptake in mice. This is in accord with its high polar surface area (PSA) value and high molecular weight (MW = 540), which are above the conventional limits for passive BBB diffusion. Moreover, its brain uptake increased following the administration of cyclosporin A, which inhibits several BBB efflux transporters such as P-gp, indicating that [^{11}C]Sch225336 is an efflux transporter substrate. No specific binding of [^{11}C]Sch225336 to spleen tissue or blood cells was observed *in vivo* in mice, in contrast to the results with [^{35}S]Sch225336 (Gonsiorek et al., 2006). No further studies on [^{11}C]Sch225336 have been published.

In addition, the Chiba University PET center selected from a series of triaryl CB₂ radioligands [^{11}C]-X1 (Fujinaga et al., 2010). [^{11}C]-X1 exhibited a high binding affinity of 0.3 nM. [^{11}C]-X1 showed good BBB penetration in mice, but its slow brain washout suggests substantial non-specific binding that can be explained by its high lipophilicity.

Indole derivative

GW405833 is a well-known CB₂ receptor agonist with high affinity and selectivity (Valenzano et al., 2005). It has been used in several preclinical studies to investigate the anti-inflammatory properties and anti-nociceptive characteristics of CB₂ receptor agonists (LaBuda et al., 2005; Whiteside et al., 2005; Hu et al., 2009). The Leuven group labeled GW405833 with carbon-11 and also synthesized the [^{18}F]fluoroethyl derivative ([^{18}F]FE-GW405833) (Evens et al., 2011). Both [^{11}C]GW405833 and [^{18}F]FE-GW405833 showed moderate affinity for the CB₂ receptor and good BBB permeability. Unfortunately, [^{18}F]FE-GW405833 had much slower washout of radioactivity from the mouse brain than [^{11}C]GW405833, which was due to a large fraction of brain radiometabolites (50% at 30 minutes after injection), limiting its further development. None of these tracers showed spleen retention in mice or rats.

Taking advantage of the low expression of CB₂ receptors in healthy brain, this target can be used for developing a brain reporter gene system to further validate the above radioligands *in vivo* (Vandeputte et al., 2011). Both [^{11}C]GW405833 and [^{18}F]FE-GW405833 showed higher binding in the striatum where CB₂ receptor expression was induced by stereotactic injection of the vector in comparison with the contralateral sham-injected striatum. Binding was displaceable by IV injection of unlabeled GW405833, 20 minutes after tracer injection, thereby confirming reversibility of tracer binding. However, due to their relatively low binding affinity, both [^{11}C]GW405833 and [^{18}F]FE-GW405833 are unlikely candidates for further imaging in neuroinflammatory conditions. Also, [^{11}C]GW405833 showed slow washout and high non-specific

binding in healthy monkey brain (Vandeputte et al., 2011). GW842166X is a CB₂ receptor agonist that showed promising anti-hyperalgesia properties in animal pain models and that has entered human trials for the treatment of inflammatory pain (Giblin et al., 2009). It is thought that GW842166X crosses the BBB to perform its analgesic actions. Therefore, a PET study using carbon-11 labeled GW842166X was conducted (<http://clinicaltrials.gov>). However, no further data on the radiosynthesis or outcome of the study have been published to date.

Quinoline derivatives

Quinoline derivatives have been extensively studied as CB₂ receptor radioligands. Among them, JT3-907 is a well-characterized CB₂ receptor inverse agonist (Iwamura et al., 2001). The Leuven group synthesized 2-oxo-7-[¹¹C]methoxy-8-butyloxy-1,2-dihydroquinoline-3-carboxylic acid cyclohexylamide (NE40) and 2-oxo-7-[¹⁸F]fluoroethoxy-8-butyloxy-1,2-dihydroquinoline-3-carboxylic acid cyclohexylamide (Evens et al., 2009). By shortening the lipophilic carbon tail from a pentoxy to a butoxy group, a decrease of non-specific binding related to the lipophilicity of the tracer was envisaged. In competition binding studies, both compounds showed low nanomolar affinity for the CB₂ receptor. This was in line with the results of *in vivo* biodistribution studies in normal mice, where both tracers showed high spleen uptake and spleen retention. This spleen uptake was inhibited by pretreatment of mice with 1-(2,4-dichlorophenyl)-6-fluoro-N-piperidin-1-yl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide, a potent rodent CB₂ receptor inverse agonist (Mussinu et al., 2003), thereby strongly suggesting that retention in spleen is specific for CB₂ receptor binding.

In the rat model with local human (h)-CB₂ receptor expression, [¹¹C]NE40 demonstrated specific and reversible binding to hCB₂ receptors. [¹¹C]NE40 was successfully evaluated in a substantial number of preclinical safety studies (Evens et al., 2012). [¹¹C]NE40 is also the first CB₂ receptor PET radioligand that was selected for human PET studies. In healthy human brain, [¹¹C]NE40 exhibited rapid uptake and washout. Human findings demonstrated predominantly hepatobiliary excretion and an effective dose of 4.4 μSv/MBq in six healthy subjects (Ahmad et al., 2013). Apart from liver and intestines, [¹¹C]NE40 retention was observed in lymph nodes and spleen, indicating CB₂ receptor binding. Clinical PET studies using [¹¹C]NE40 in pathological conditions are ongoing.

In addition, Turkman and coworkers from the MD Anderson Cancer Center have also published a series of 2-oxoquinoline derivatives (Turkman et al., 2011) and reported one as a suitable candidate for PET studies (Turkman et al., 2012). Designed to be a metabolically stable fluorine-18 compound, [¹⁸F]-X2 (7-methoxy-8-butoxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid-(4-fluorobenzyl)amide-[¹⁸F]) had poor uptake in the spleen and only about 50% specific binding on CB₂ receptor positive tumor cells transfected in the mouse. Preclinical and clinical applications of this compound are hampered by its low solubility.

Thiozole derivatives

The Johns Hopkins University group recently synthesized [¹¹C]A-836339 [2,2,3,3-tetramethyl-cyclopropanecarboxylic acid [3-(2-methoxy-ethyl)-4,5-dimethyl-3H-thiazol-(2Z)ylidene]-amide], a selective CB₂ receptor agonist with high binding affinity, moderate lipophilicity, and an adequate PSA value for CNS application (Table 4.2) (Horti et al., 2010). [¹¹C]A-836339 shows in healthy CD1 mice specific binding in the spleen. It also exhibits good BBB permeability and subtle amount of specific binding in healthy mice brain. This is in agreement with the low expression of CB₂ receptors in this condition (Munro et al., 1993). Specific binding of [¹¹C]A-836339 was further studied in two animal models of neuroinflammation, a lypopolysaccharide (LPS)-induced mouse model and a transgenic amyloid mouse model of AD (APPswe/PS1dE9 mice). Pretreatment studies showed that ≈ 78 –84% of the brain radioactivity in LPS-treated mice was specific for the CB₂ receptor. Similar values of CB₂ receptor expression levels have been reported previously in this model (Mukhopadhyay et al., 2006). This high cerebral uptake of [¹¹C]A-836339 may, however, in part be associated with dysfunction of the BBB.

Brain distribution studies of [¹¹C]A-836339 in the AD mouse model showed that [¹¹C]A-836339 display *in vivo* ≈ 29 –33% of specific binding in various brain regions, which is consistent with the distribution of A β plaques in this model (Benito et al., 2003).

PET IMAGING OF CB₁ AND CB₂ RECEPTORS IN HD

We have used [¹⁸F]MK9470 to investigate CB₁ receptor availability *in vivo* in HD. Parametric images of partial volume-corrected receptor availability showed a widespread decrease of CB₁ in HD patients, compared with controls (Van Laere et al., 2010). The reductions of CB₁ availability ranged from $\approx 15\%$ in the cerebellum to $\approx 25\%$ in the frontal cortex. Interestingly, these reductions were observed irrespective of clinical disease stage, duration, CAG repeat, or patient age, but were related to disease burden in [(CAG)_n $\times$ age] in the prefrontal cortex. The mechanism by which HD promotes this loss of CB₁ receptors is suggested to be caused by interactions between mutant htt and nuclear transcription factors (Blazquez et al., 2011). Blazquez and coworkers showed in striatal cells that mutant htt down-regulates CB₁ receptors by controlling gene promoter activity via repressor element 1 silencing transcription factor. Our group found similar reductions in the brain uptake of [¹⁸F]MK9470 in a transgenic rat model of HD (Casteels et al., 2011). However, in the latter study, reductions were restricted to the basal ganglia. Rats, unilaterally lesioned with quinolinic acid in the left caudate-putamen to resemble the cell death characteristic of HD, demonstrated a disproportionately modest decrease in [¹⁸F]MK9470 on the ipsilateral side as compared to the lesion severity (Casteels et al., 2010). It appeared as if CB₁ receptors try to restore basal conditions, but that this action remains insufficient.

In the same study, an increase in [^{18}F]MK9470 binding was observed on the contralateral side and in the cerebellum, the latter of which corresponded to improved functional outcome (Casteels et al., 2010).

Whether *in vivo* CB₁ receptor measurements using [^{18}F]MK9470 may be a useful biomarker for HD is currently being further investigated in pre-manifest human carriers of the HD mutation using human PET and in R6/2 HD mice. Also, its change relative to CB₂ receptors using [^{11}C]NE40 needs to be determined in human HD patients.

GENERAL CONCLUSION

In this chapter, we have discussed the role of the endocannabinoid system in the pathophysiology of HD. We have described the potential of the available PET/SPECT radioligands for imaging the brain cannabinoid receptors, encompassing their successful development, some of their pitfalls, and the receptor quantification specifics.

Direct imaging of the ECS provides new insights into the basic operation of the normal brain, neurotransmitter feedback loops, and its role in HD, either by the modulatory aspects of the CB₁ receptor or by contribution of the CB₂ receptor in neuroinflammatory and neuroprotective responses.

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Endocannabinoids and amyotrophic lateral sclerosis

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AMYOTROPHIC LATERAL SCLEROSIS

Amyotrophic lateral sclerosis (ALS) (known as Lou Gehrig's disease in the USA), is a fatal neurodegenerative disorder caused by the selective and progressive injury and death of motor neurons in the spinal cord, brainstem, and motor cortex, ultimately affecting both upper and lower motor neurons (Ferraiuolo et al., 2011). ALS exists in two forms: (1) familial ALS (FALS), which represents only 5–10% of the patients and in which the cause of the disease is the existence of mutations in specific genes showing a Mendelian pattern of inheritance, generally in an autosomal dominant manner (Renton et al., 2014); and (2) sporadic ALS (SALS), which corresponds to most cases and in which the etiology is not really clear (Ahmed and Wicklund, 2011). On the one hand, the existence of two distinct etiologies, which sometimes translates to clinical and pathological aspects, suggests that ALS should be considered a syndrome rather than a single disease entity (Hardiman et al., 2011). On the other hand, SALS and FALS are frequently indistinguishable and share common characteristics: (1) all genes found mutated in FALS cases have been also found mutated in patients with diagnosis of SALS; (2) first-degree relatives of SALS patients have an increased risk of developing the disease; (3) inclusions for the most characteristic mutated proteins (see below) have been found not only in FALS cases but also in SALS patients; and (4) riluzole, the only approved treatment for ALS, has been found to be effective in both FALS and SALS cases (Andersen and Al-Chalabi, 2011). In general, the exact causes and the complete pathogenic process of ALS remain mostly unknown today, although some pathological mechanisms have been proposed, including processes that also operate in other chronic neurodegenerative disorders, such as oxidative stress, excitotoxicity, defects in glial–neuron crosstalk and

neuroinflammation, mitochondrial dysfunction, protein aggregation and deposition, and dysregulation of RNA transcription and processing (see below).

EPIDEMIOLOGICAL AND CLINICAL ASPECTS

The global incidence of ALS is approximately two to three cases per 100,000 subjects in the general population, with an average of 1:400 as an overall lifetime risk of suffering the disease (Hardiman et al., 2011), except in a few high incidence foci (Guam and Kii peninsula). This means that ALS is the third most common neurodegenerative disorder after Alzheimer's disease and Parkinson's disease. It is slightly more prevalent in men than in women (1.5 to 1). Given that life expectancy after diagnosis is approximately 3 years, the prevalence of the disease is only five to six cases per 100,000 subjects, significantly lower compared with other similar diseases of longer life expectancy (e.g., Parkinson's disease). The average for the onset of the disease is 64 years, but, contrary to other neurodegenerative disorders, the risk for developing ALS declines after 75 years, so ALS is the neurodegenerative disorder most common in subjects of middle age (Al-Chalabi and Hardiman, 2013). The disease is considered a rare disease and its impact in terms of social and health costs is high due to the disabling and dependent situation that patients rapidly reach.

Genetic studies have identified at least 15 genes associated with cases of FALS. The first mutations were found in the copper–zinc superoxide dismutase gene (*SOD-1*), which encodes for a key antioxidant enzyme, being pathological, in general, through a gain-of-neurotoxic function (Rosen et al., 1993). It is the ALS-1 subtype in the new classification of genetic cases and it corresponds to approximately 12% of FALS cases and 1% of SALS patients (Renton et al., 2014). Fifteen years passed until two new genes related to ALS were identified: (1) *TARDBP* (TAR-DNA binding protein 43; TDP-43; ALS-10), which is responsible of 4% of FALS cases and a smaller proportion of SALS (Buratti and Baralle, 2010; Lagier-Tourenne et al., 2010); and (2) *FUS* (fused in sarcoma; ALS-6), also present in 4% of FALS cases (Lagier-Tourenne et al., 2010). Both genes encode proteins involved in pre-mRNA splicing, transport, and/or stability, and both proteins share functional homology (Buratti and Baralle, 2010; Lagier-Tourenne et al., 2010), although it is not known whether their pathological condition depends on a gain-of-function in their RNA processing activity, or to a loss-of-function with their translocation to the cytoplasm in which they can be found in stress granules (Ferraiuolo et al., 2011). More recently, a GGGGCC hexanucleotide expansion in the non-coding region of the *C9orf72* (chromosome 9 open reading frame 72; not classified yet) gene has been also identified and related to the disease (DeJesús-Hernández et al., 2011; Renton et al., 2011; Cruts et al., 2013). It is considered the most frequent cause in FALS (approximately 40% of cases and up to 50% in Finland, which is the population that shows the highest incidence in Europe) and it is also present in 7% of SALS patients. *C9orf72* is also related to the RNA processing. Other recently identified important

Table 5.1 Distribution of ALS-related Genes in Five Categories Depending on Their Relation to ALS Pathogenesis

Pathogenic Mechanism	ALS-related Genes
Genes affecting RNA processing	ALS-4 (<i>senataxin</i> , <i>SETX</i>) ALS-6 (<i>FUS</i>) ALS-9 (<i>ANG</i>) ALS-10 (<i>TARDBP</i>) ALS-13 (<i>ataxin-2</i> , <i>ATXN2</i>) <i>C9orf72</i> (not classified yet)
Genes affecting vesicle trafficking	ALS-2 (<i>alsin</i> , <i>ALS</i>) ALS-8 (<i>VAMP-associated protein B</i> , <i>VAPB</i>) ALS-11 (<i>phosphatidylinositol 3,5-biphosphate 5-phosphatase</i> , <i>FIG4</i>) ALS-12 (<i>optineurin</i> , <i>OPTN</i>) ALS-14 (<i>VCP</i>)
Gene affecting oxidative stress	ALS-1 (<i>SOD-1</i>)
Genes affecting the ubiquitin-proteasome system (UPS) and autophagy	ALS-14 (<i>VCP</i>) ALS-15 (<i>ubiquilin-2</i> , <i>UBQLN2</i>)
Genes that presently have an unknown function	ALS-3 ALS-5 ALS-7

genes are *ANG* (angiogenin; ALS-9) and *VCP* (valosin-containing protein; ALS-14). It is important to remark that these new ALS-related genes have facilitated the identification of new pathogenic mechanisms that differ, in part, from the toxicity associated with mutations in *SOD-1*, leading to a novel molecular classification of ALS subtypes (Al-Chalabi and Hardiman, 2013), which differentiate five categories—these are summarized in Table 5.1. Additional mutations in *DAO* (*D-amino acid oxidase*), *DCTN1* (*Dynactin-2*), and *PFN1* (*Profilin-1*) genes have been also found in ALS patients (Bova and Kinney, 2013).

It is obvious that the intensive research conducted in the last few years has facilitated the identification of the genetic etiology of two-thirds of FALS cases and about 11% of SALS patients (Renton et al., 2014). However, as mentioned above, the current evidence indicates that not more than 10% of cases have a genetic origin, which implies that numerous factors, other than mutated causative genes, must be involved in 90% of patients. In some cases, this may have also a genetic basis with the contribution of several risk genes that increase the vulnerability to develop the disease, such as those encoding for the vascular endothelial growth factor, hereditary hemochromatosis protein paroxonase enzymes and others, as well as genetic variants of causative genes or mutations having an incomplete penetrance, which explains the small percentages of patients with a diagnosis of SALS having mutations in causative genes that have been indicated above (Ferraiuolo et al., 2011). However, it appears that the contribution of environmental factors, including their interaction with genetic factors, should also be

considered as a key element for explaining the etiology of SALS cases. Although this remains to be completely elucidated, a number of risk factors have been proposed and investigated, e.g., tobacco abuse, type of diet, urban versus rural life, exposure to electromagnetic radiation or toxic agents (pesticides, lead, and organic toxics), brain trauma events, family history of other neurodegenerative disorders, physical exercise (professional sport), age of menopause in women, and education, although the data were heterogeneous depending on the different cohorts investigated (Hardiman et al., 2011). Age is also another important risk factor but, contrary to other disorders in which aging is the major risk factor, the incidence of ALS declines at the age of 75 years (Al-Chalabi and Hardiman, 2013), so it does not have the same influence as in Alzheimer's disease and Parkinson's disease. On the other hand, the influence of some protective factors, e.g., cardiovascular risk factors (Körner et al., 2013), dietary supplements of vitamin D (Camu et al., 2014), vitamin E (Wang et al., 2011), or magnesium (Fondell et al., 2013), longer reproductive lifespan or prolonged estrogen exposure (de Jong et al., 2013), and treatment with non-steroidal anti-inflammatory drugs (Popat et al., 2007), has been also investigated.

Genetic testing is used for diagnosis in FALS cases but, given that most cases of ALS cases are sporadic, ALS diagnosis is frequently made on clinical examination grounds using well-standardized consensus criteria (RALSFRS: Revised ALS Functional Rating Scale) plus MRI imaging of the brain and the spinal cord (Hardiman et al., 2011). It is necessary, however, to exclude those conditions that can mimic ALS and that are present in other motor neuron disorders, e.g., primary lateral sclerosis (upper motor neuron signs only), progressive muscular atrophy (lower motor neuron signs only), and progressive bulbar palsy, or in mimic syndromes, e.g., multifocal motor neuropathy, hereditary spastic paraparesis, multiple system atrophy, spinobulbar muscular atrophy, and others (Hardiman et al., 2011). The time from symptom onset to definitive diagnosis is approximately 1 year. RALSFRS is also used for monitoring disease progression and is extensively used in clinical trial assessment (Hardiman et al., 2011).

The neurological evaluation of patients frequently reveals a combination of abnormalities in both upper and lower motor neurons. The damage to motor neurons produces muscle denervation and atrophy, resulting in a progressive muscle weakness and paralysis mainly affecting voluntary movements. This occurs predominantly in limbs in 65% of patients (spinal-onset disease), which has a better prognosis, but major symptoms are dysarthria or dysphagia in 30% of patients (bulbar-onset disease) and respiratory problems in 5% of cases (worst prognosis) (Hardiman et al., 2011). Weight loss and malnutrition are common features of ALS and are associated with poor prognosis too (Limousin et al., 2010). Patients frequently die from respiratory failure within 3 years after presentation of first symptoms. Only 10% of patients survive for more than 8 years.

Cognitive deficits are less common in ALS, but contrary to former ideas that established that patients do not develop any cognitive impairment at any time, alterations leading to dementia occur in 15% of patients and up to 25% of patients

develop executive deficits (Hardiman et al., 2011; Phukan et al., 2012). In fact, ALS overlaps with frontotemporal dementia (FTD) sharing different neuropathological characteristics, in particular in the cases of new ALS-related genes such as *TDP-43* (Neumann et al., 2006) and *C9orf72* (Ash et al., 2013), firmly establishing a genetic link between both disorders and leading to their association in the so-called ALS/FTD spectrum (Van Langenhove et al., 2012).

NEUROPATHOLOGICAL ASPECTS

As mentioned above, the entire spectrum of pathogenic mechanisms that collaborate to kill motor neurons has not been completely elucidated. However, several studies have confirmed the contribution, in a complex interplay, of multiple processes that also operate in other chronic neurodegenerative disorders. This includes oxidative stress, excitotoxicity, defects in glia–neuron crosstalk, protein degradation, aggregation, and deposition, mitochondrial dysfunction, impairment in endosomal trafficking, and, derived from the newly identified genes, dysregulation of RNA transcription and processing (Ferraiuolo et al., 2011). The involvement of these processes has been demonstrated in part in studies of patients, using biological samples (e.g., cerebrospinal fluid (CSF), postmortem tissues) or *in vivo* imaging techniques, but most of the information comes from studies conducted in experimental models of the disease. Modeling ALS in laboratory animals has been, frequently, a complicated task, given the numerous aspects related to the pathogenic mechanisms that remain to be completely elucidated. The strategy has consisted in the generation of transgenic models in mice, but also in other vertebrates (e.g., zebrafish) or invertebrates (e.g., *Drosophila*, *C. elegans*), overexpressing the wild-type or, in particular, the mutated forms of the different genes that have been related to the disease (Ludolph et al., 2010; Joyce et al., 2011; Wegorzewska and Baloh, 2011). In some cases (those related to a loss-of-function of the mutated protein, e.g., TDP-43), knockout models have been also developed for the study of motor neuron injury (Wu et al., 2012). As mentioned above, these models have facilitated studies addressed to identify the key events in ALS pathogenesis that are more directly related to the specific gene used for modeling, but they have also served for evaluating numerous neuroprotective compounds or other disease-modifying strategies. However, the limitations have been obvious. On the one hand, most of the ALS-related genes have been identified over the last few years, and only a few of them have already been used for modeling in laboratory animals. In fact, most of the information presently available has been obtained in *SOD-1* mutant mice, given that this gene was discovered in the 1990s and the experimental models developed a few years later. On the other hand, the genetic cases only account for 10% of patients, which represents a small percentage of all ALS patients in which the disease is frequently sporadic. Nevertheless, as will be addressed below, the identification of new genes is already facilitating the development of new models and helping to elucidate the entirety of pathological mechanisms involved in the disease, not only in FALS

cases but also in SALS patients (Ferraiuolo et al., 2011). *In vitro* strategies with cell cultures have been also used in the study of ALS. One of the most interesting tools is NSC-34 cells—a hybridoma cell line derived from the fusion of neuroblastoma cells with mice spinal cord cells (Cashman et al., 1992)—that are susceptible to be transfected with the different ALS-related genes (Muyderman et al., 2009).

Glutamate toxicity

Excitotoxic damage appears to be very active in ALS patients, as elevated levels of glutamate have been found in the CSF of specific groups of patients (Shaw et al., 1995). The mechanisms underlying this response have been investigated both in patients and in experimental models of ALS (e.g., SOD-1 mutant mice). Some studies have identified changes in the expression and function of glutamate transporters, in particular the excitatory amino acid transporter subtype 2 (EAAT2), which have been associated with the initial phases of the disease (Boston-Howes et al., 2006; Foran and Trotti, 2009). Other studies described that motor neurons are particularly vulnerable to AMPA-mediated stimulation (King et al., 2007), although it is not clear whether this is a cause contributing to the disease or a mere consequence of neuronal loss.

Other important evidence supporting the contribution of excitotoxicity in ALS comes from the recent identification of *DAO* as a new ALS-related gene (Mitchell et al., 2010). The enzyme encoded by this gene, DAAO, is responsible for the oxidative deamination of D-serine and other D-amino acids. A deficit in D-serine deamination due to a loss-of-function mutation in DAAO would elevate the levels of this D-amino acid and its action as a co-agonist of NMDA receptors together with glutamate, and then enhance the excitotoxic injury of motor neurons.

The importance of excitotoxicity in ALS is also supported by the fact that the only strategy that has shown any evidence of slower disease progression in patients has been obtained with an agent, riluzole, which, among its different actions, is able to reduce presynaptic glutamate release (Lacomblez et al., 1996). Paradoxically, other anti-excitotoxic agents, e.g., gabapentin, lamotrigine, topiramate, were not clinically active (Siciliano et al., 2010).

Glial activation and local neuroinflammation

High amounts of activated microglia and infiltrated lymphocytes have been found in those structures of the central nervous system (CNS) that are predominantly affected in ALS, in particular in cases of SALS, as revealed in several studies in patients (Henkel et al., 2004; Turner et al., 2004). Other studies conducted in FALS and SALS patients indicated that both the accumulation of activated microglia and the recruitment of peripheral cells to the CNS take place not only in areas of profound motor neuron degeneration, but also in areas of mild damage (Ince et al., 1996), and both events are detected early in ALS progression (Mantovani et al., 2009). In addition, CSF samples collected from ALS patients show the presence of different pro-inflammatory mediators, e.g., tumor necrosis factor α (TNF- α) (Kuhle et al., 2009). The presence of activated microglia and

pro-inflammatory mediators in ALS has been reproduced in experimental models of this disease (Ferraiuolo et al., 2011), which have enabled identification of chronic activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling by these mediators to be a key contributing factor to ALS disease progression and, accordingly, that inhibiting NF- κ B signaling may be a relevant therapeutic target in ALS (see below, and Bova and Kinney, 2013).

Another important aspect in relation to the inflammatory responses in ALS is the contribution of defects in astrocyte–neuron crosstalk. Primary cultures of astrocytes obtained from mSOD-1 transgenic mice secrete different inflammatory mediators (e.g., prostaglandin E2, leukotriene B4, nitric oxide) in basal conditions and after activation with TNF- α (Hensley et al., 2006). Interestingly, the co-incubation of motor neurons with these astrocytes (and also those obtained from FALS patients) was toxic for neurons and this toxicity could be attenuated by anti-inflammatory strategies (Di Giorgio et al., 2008; Marchetto et al., 2008). These responses were not found, or were significantly lower, when normal astrocytes were used, thus indicating that astrocytes containing mSOD-1 are particularly enabled to develop a pro-inflammatory state. By contrast, silencing of the *mSOD1* gene selectively in astrocytes caused a slower disease progression in *mSOD-1* transgenic mice (Yamanaka et al., 2008). Other authors have also indicated that the harmful effects exerted by astrocytes containing mSOD-1 on the homeostasis of motor neurons do not depend solely on their pro-inflammatory condition, as they also found a reduction in their trophic support to neurons (Ferraiuolo et al., 2011).

Oxidative stress

Oxidative stress seems to be a key pathological mechanism in ALS (Parakh et al., 2013), as revealed by the fact that CSF samples (and also serum and urine samples) from SALS and FALS patients show elevated levels of different markers of oxidative damage (Mitsumoto et al., 2008). Similar elevated levels of oxidative damage in proteins, lipids, and DNA have been also found in postmortem tissues from ALS patients (Ferraiuolo et al., 2011; Parakh et al., 2013). However, the most robust evidence about the relevance of oxidative stress in ALS has been obtained in those patients bearing mutations in the antioxidant enzyme SOD-1, which account for approximately 12% of FALS patients, and, in particular, from the transgenic models developed in mice and other species with these mutations. Thus, *mSOD-1* transgenic mice exhibit an abnormal oxidative metabolism with activation of several aberrant oxidative reactions (Barber and Shaw, 2010). These reactions would depend on mechanisms beyond the catalytic activity of SOD-1, and would presumably involve the microglial cells (Harraz et al., 2008), as the mutations found in this enzyme in FALS are all due to a loss-of-function. In addition, SOD-1 mutant mice also show a dysregulation of Nrf-2/ARE signaling (Kirby et al., 2005), which plays a key regulatory role in the antioxidant response. This dysregulation has been also found in ALS patients (Marden et al., 2007) and is susceptible to targeting for pharmacological correction (see below).

Defects in RNA transcription, processing, and stability

The involvement of defects in pre-RNA splicing, transport, and/or stability in ALS was evident after the identification of mutations in *TARDBP* and *FUS* in ALS (Liscic and Breljak, 2011). This has been recently extended with the identification of the GGGGCC hexanucleotide expansion in the *C9orf72* gene (Cruts et al., 2013), which accounts for more than 40% of genetic cases, as mentioned above. However, whether the mutation in the *C9orf72* gene leads to a gain- or a loss-of-function is still obscure, and the precise function of the protein encoded by the *C9orf72* gene also remains to be determined, although recent studies have indicated a possible role in RNA-mediated toxicity (Gendron et al., 2014). This information did exist for the proteins encoded by the other two genes. *TARDBP* encodes for a RNA–DNA binding protein called TDP-43. This protein is a major component of ubiquitinated inclusions found in neurons and glial cells in ALS patients, even in cases not related to mutations in the *TARDBP* gene (Neumann et al., 2006; Janssens and van Broeckhoven, 2013). In fact, the frequency of mutations in this gene is much lower than the occurrence of neuropathological changes in TDP-43 seen in autopsies (Renton et al., 2014). The only exception is those ALS cases related to mutations in *SOD-1* and *FUS* whose inclusions do not contain TDP-43 (Mackenzie et al., 2010). TDP-43 is predominantly located in the nucleus where it plays an important role in different aspects of RNA processing, e.g., transcriptional regulation, alternative splicing, and microRNA processing. This cellular location changes in cases of mutant TDP-43 as the protein is predominantly located in the cytoplasm, particularly in stress granules (Liu-Yesucevitz et al., 2010), losing the capability to regulate RNA processing (Janssens and van Broeckhoven, 2013). A similar situation occurs in the case of the *FUS* gene. It encodes also for a RNA–DNA binding protein that is also involved in transcriptional regulation, RNA and microRNA processing, and mRNA transport (Mackenzie et al., 2010). As in the case of TDP-43, its location is predominantly nuclear, but when mutated, FUS protein is sequestered in the stress granules in the cytoplasm (Dormann et al., 2010; Ito et al., 2011). Lastly, other less-studied ALS-related genes encode for proteins, e.g., the DNA–RNA helicase senataxin (ALS-4) and angiogenin (ALS-9), which have been also related to dysfunctions in RNA metabolism (Strong, 2010). However, the characteristics of these mutations and the exact function of these proteins in the context of ALS remain to be investigated.

Protein deposition and aggregation

Protein aggregates, in the form of ubiquitinated inclusions similar to those seen in other neurodegenerative disorders, have been found in ALS. They represent a key feature in ALS pathogenesis. As mentioned in the above section, recent studies have demonstrated that mutant TDP-43 is a major constituent of these ubiquitinated protein inclusions found in ALS (Neumann et al., 2006), providing a possible link between the genetic mutation and the cellular pathology. In fact, TDP-43-positive aggregates have been found mainly in genetic cases, but also in

SALS patients (Sreedharan et al., 2008). As mentioned above, TDP-43 is present normally in the nucleus where it develops its key functions, but ALS provokes an early cytoplasmic redistribution of this protein (Giordana et al., 2010). TDP-43 aggregates are also present in FTD, and thus they are representative of the so-called TDP-43 proteinopathies that include both disorders. SOD-1-positive inclusions have been also found in ALS, but restricted to genetic cases related to mutations in the *SOD-1* gene; only a few SALS cases are positive for mutant SOD-1 (Bosco et al., 2010). They are also representative of the transgenic mice expressing mutant SOD-1 (Shibata et al., 1994). Cytoplasmic inclusions containing mutated FUS protein have been also found in familial cases related to this gene (Groen et al., 2010).

The identification of these protein aggregates in cases of FALS and in experimental animals models that reproduce these genotypes, as well as the few cases of SALS that also present protein inclusions, has provided valuable information about the relation of mutated proteins with the pathogenesis in ALS, and also in FTD (Ferraiuolo et al., 2011). For example, the accumulation of intracellular protein aggregates elicits different cell responses that, despite having an initial cytoprotective function, can activate apoptotic responses when they become chronic, then contributing to cell degeneration and death. Thus, it can activate endoplasmic reticulum stress and this appears to be an early event in the progression of motor neuron injury (Atkin et al., 2006, 2008; Yamagishi et al., 2007), even appearing before muscle denervation (Saxena et al., 2009). Endoplasmic reticulum stress has been found in SOD-1 mutant mice and also in ALS patients, and has been reproduced in cultures of motor neurons, e.g., NSC-34 cells (Vijayalakshmi et al., 2011). Autophagy and its associated signaling pathways have been also found to be activated in response to the accumulation of aberrant proteins, but this response has been associated with a reduction in mutant SOD-1 aggregation (Hetz et al., 2009), therefore with a protective effect. In fact, there are a number of autophagy-related genes (e.g., *CHMP2B*, *VAPB*) whose mutations have been identified in rare cases of ALS (Parkinson et al., 2006; Chen et al., 2010; Cox et al., 2010). A last aspect that also relates ALS to characteristics of proteinopathy is the recent identification of *UBQLN2* as a new ALS-related gene in a five-generation family (Deng et al., 2011). This gene encodes a protein called ubiquilin-2, which is a member of a family of proteins (ubiquilins) that regulate the UPS. The *UBQLN2* gene is the ALS-15 subtype and has the singularity that the absence of male-to-male transmission suggests an X-linked pattern of inheritance, being the only case where ALS-related mutations are linked to sex chromosomes.

Mitochondrial dysfunction

Defects in mitochondrial function, as well as in mitochondrial turnover through axoplasmic transport, have also been implicated in ALS pathogenesis (Martin, 2011). The damage to mitochondria includes the formation of mutated protein aggregates in vacuoles in the mitochondrial intermembrane space (Wong et al., 1995), the oxidation of mitochondrial proteins and lipids (Mattiazzi et al., 2002; Wiedemann et al., 2002),

and the impairment of mitochondrial calcium-buffering activity (Damiano et al., 2006), all resulting in losses of mitochondrial function, dysregulation in energy metabolism, and activation of apoptotic machinery (e.g., caspase activation), and contributing to motor neuron degeneration (Martin, 2011).

Impairment in axonal transport

Given the long axonal processes of motor neurons, from which originates the high demand for turnover of essential axonal and presynaptic components (e.g., proteins, organelles), these neurons appear to be particularly vulnerable to impairments in processes of endosomal trafficking and axonal transport (Ferraiuolo et al., 2011). Possibly, this represents a cardinal element in the vulnerability of these neurons in ALS and a key element to explain the vulnerability of specific neuronal subpopulations in genetic cases in which mutated genes, although constitutively expressed, only affect motor neurons.

There is strong evidence, obtained in SOD-1 mutant mice, that both anterograde (from cell body to the neuromuscular synapse) and retrograde (from the synapse to the cell body) transport are impaired in ALS (Ferraiuolo et al., 2011). In addition, these defects have been found at early disease phases supporting the possibility that they play a key role in pathogenesis (Ferraiuolo et al., 2011). However, the mechanisms that underlie these defects remain still to be determined, although several recent observations provide preliminary information. For example, several studies have proposed that the reduction in mitochondrial transport may affect the transport of other components through the expected reduction in energy requirements (De Vos et al., 2007). Other studies conducted in SOD-1 mutant mice have indicated that the impairment in axonal transport is caused by alterations in the function of kinesin, a microtubule-dependent protein that plays a key role in axonal transport, alterations that would be caused by the elevated generation of TNF- α and other cytokines (Ackerley et al., 2004). In addition, the excitotoxic damage has been also found to disturb the function of neurofilaments in SOD-1 mutant mice (Ackerley et al., 2000), and some studies have proposed that this disturbance would be produced through phosphorylation of their constituent proteins by some protein kinases that are activated in experimental ALS. The alterations in the neurofilaments would necessarily impair the function of kinesin and dynein, the molecular motors involved in axonal transport (Ferraiuolo et al., 2011).

TREATMENTS APPROVED AND/OR UNDER INVESTIGATION

At present, the benzothiazole riluzole (Rilutek[®]) is the only licensed drug available for treatment of ALS patients (it was approved in 1995). Riluzole is an antiglutamatergic agent that blocks excitatory amino acid receptors in a non-competitive manner, inhibits glutamate release, and, in particular, inactivates voltage-dependent sodium channels located in motor neurons, thereby reducing their activity (Cheah et al., 2010; Bova and Kinney, 2013). However, its effects are limited, as it only extends the average survival of patients for 3–6 months, so there is an

urgent need for novel compounds (and also non-pharmacological strategies) active for the treatment of ALS, not only for symptom management and preservation of quality of life, but particularly for delaying/arresting the disease progression.

The recent advances in the genetic etiology of ALS developed over the last few years have provided new insights in relation to the contribution of these new mutated genes/proteins in the ALS pathogenesis, facilitating the identification of the ultimate molecular and cellular mechanisms that underlie motor neuron degeneration. These advances have also facilitated disease modeling and, in particular, the design and testing of novel targeted therapeutics. This is really an important goal in view of the absence of new approvals from the initial license of riluzole in 1995, and considering the intensive clinical research conducted during the last 15 years that has not validated any new therapeutic agent from a list of more than 30 compounds (e.g., creatine, celecoxib, minocycline, BDNF, IGF-1, and others) that had shown benefits in preclinical models (Glass, 2012). These continuous failures preclude the predictive value for drug testing of experimental models of ALS used so far, mostly the SOD-1 mutant mice, a problem that may be solved in the following years with the development of novel preclinical models based on the new ALS-related genes.

The studies described in the above section demonstrate that ALS can be considered as a multifactorial disease in which multiple pathological events cooperate to damage motor neurons. Accordingly, the best pharmacotherapies for ALS would be those that are based on a multidisciplinary setting, including pharmacotherapy but also other therapeutic and palliative strategies. In terms of pharmacotherapy, the use of drugs that act at different targets along the process of the disease is recommended for investigation. Several therapeutic interventions have been (or are presently being) investigated in preclinical models (preferentially in SOD-1 mutant mice), with antioxidant therapies being the most effective class of drugs to improve animal survival (Benatar, 2007). However, as mentioned above, these therapies have not shown benefits in ALS patients yet (Orrell et al., 2007). Another investigated target is the mitochondrial dysfunction (Bordet et al., 2007) with olexisome presently in a phase III clinical trial. Other investigated compounds, some of them recently in clinical testing in ALS patients, are talampanel, which acts by blocking AMPA receptors (Pascuzzi et al., 2010), and β -lactam antibiotics, which stimulate the function of the glutamate transporter EAA2 (Rothstein et al., 2005). One of these β -lactam antibiotics, ceftriaxone, showed a good tolerability in a phase II study (Berry et al., 2013) and it has been recently investigated in a phase III study (www.clinicaltrials.gov). Other interesting strategies are based on heat-shock protein co-inducers, and agents promoting autophagy and mitochondriogenesis, e.g., lithium, rapamycin (Siciliano et al., 2010; Habib and Mitsumoto, 2011). Additional candidates, some of which are in phase I clinical trials for safety and tolerability or in small phase II trials, include anti-Nogo-A antibodies, troponin activators, and histone deacetylase inhibitors (Bova and Kinney, 2013), whereas novel targets and therapeutics that are being investigated in preclinical models include: (1) anti-excitotoxicity, e.g., ephrin type-A receptor inhibitors (Van Hoecke et al., 2012); (2) oxidative stress, e.g., Nrf-2 enhancers (Neymotin et al., 2011; Vargas et al., 2012),

DJ-1 stabilizers (Yamashita et al., 2010); (3) cellular stress conditions, e.g., apoptotic signaling kinase-1 (ASK1) inhibitors (Nishitoh et al., 2008); (4) inflammation, e.g., NF- κ B inhibitors (Swarup et al., 2011); (5) defects in axonal transport, e.g., p38 α inhibitors (Dewil et al., 2007); and (6) activators of UPS, e.g., inhibitors of deubiquitinating enzymes (Bova and Kinney, 2013).

CANNABINOIDS AND ALS

Studies initiated in 2004 suggest cannabinoids to be a possible and promising disease-modifying therapy in ALS (Carter et al., 2010; Rossi et al., 2010). The promising perspectives that arose were based on the capability of cannabinoids to reduce microglial activation and neuroinflammation (an effect mediated by the activation of the CB₂ receptor and/or by modulating the signaling of peroxisome proliferator-activated receptor- γ (PPAR- γ)/NF- κ B), excitotoxicity (an effect that depends on targeting CB₁ receptors), and oxidative injury (an effect that is receptor independent and/or related to PPAR- γ /Nrf-2 signaling) (Fernández-Ruiz et al., 2010). Several preclinical studies with cannabinoids, all conducted in the G93A transgenic mouse that overexpresses a mutated form of SOD-1, have provided support for this proposed possibility (see below). In addition, cannabinoids may also control the toxicity of protein aggregates in ALS by actions based on their capability to enhance autophagy by inhibiting mTOR signaling (Salazar et al., 2009), although this possibility has not been investigated in ALS yet. A graphical summary of all pathogenic mechanisms that can be targeted by cannabinoids to preserve motor neurons and delay/arrest the progression of ALS is shown in Figure 5.1. The preclinical and small amount of clinical (only regarding the control of specific symptoms) evidence accumulated over the past 10 years will be reviewed and discussed in the following sections. However, as the efficacy of cannabinoid compounds in ALS may be based on the status of those endocannabinoid elements that are targeted by specific cannabinoids, we will review first the changes that the progression of ALS produces in endocannabinoid receptors and the enzymes in those CNS structures more affected in this disease, e.g., spinal cord, brainstem, and cortical areas.

ENDOCANNABINOID SIGNALING IN ALS

As mentioned above, the potential neuroprotective effects of cannabinoids should be likely correlated with the changes experienced by key endocannabinoid elements during the ALS pathogenesis. As in other disorders, these changes can be explained in two directions. On the one hand, the changes experienced by endocannabinoid receptors and enzymes may indicate endogenous protective responses or deficits that may help to adequately define the best pharmacological strategy in each case and in each disease phase. On the other hand, it is also possible that these changes, rather than being merely an adaptative response of the endocannabinoid signaling system to the progressive injury of motor neurons, may have an instrumental value

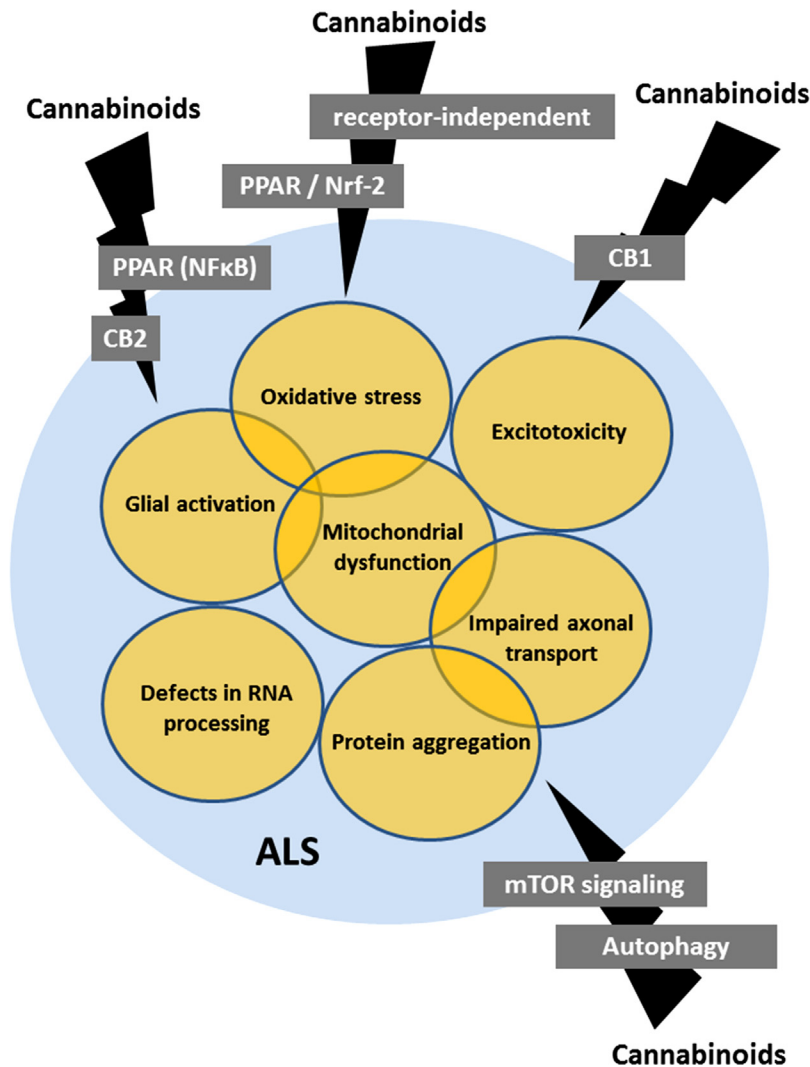


FIGURE 5.1

Schematic summary of specific mechanism by which cannabinoids may provide neuroprotection in ALS in relation with the cytotoxic mechanisms involved in the pathogenesis.

and be involved in the pathophysiology of ALS itself. Both possibilities have been found to arise in other neurodegenerative disorders (Fernández-Ruiz et al., 2007, 2010). Unfortunately, there is not much information available in the ALS domain about the changes occurring in these endocannabinoid elements in the affected CNS structures, so whatever conclusion is arrived at in this respect should be examined with caution. The only available data at present indicate that the levels of two major

endocannabinoids, anandamide and 2-arachidonoylglycerol, are elevated in the spinal cord of SOD-1 mutant mice (Witting et al., 2004; Bilsland et al., 2006). In addition, as in other disorders (Fernández-Ruiz et al., 2007, 2010), CB₂ receptors experience an important up-regulatory response in the spinal cord of SOD-1 mutant mice (Shoemaker et al., 2007; Moreno-Martet et al., 2014) and in ALS patients (Yiangou et al., 2006). Also as in other disorders, this up-regulation appears to predominantly occur in microglial elements recruited at lesioned sites (Yiangou et al., 2006). This is an important observation that should facilitate the beneficial effects derived from selectively targeting this receptor in the control of microglial toxicity for motor neurons, a key event in ALS pathogenesis (see details in “Glial activation and local neuroinflammation,” above). Another study demonstrated a down-regulation of CB₁ receptors in parallel with up-regulation of glutamate receptors in SOD-1 mutant mice at early presymptomatic phases (Zhao et al., 2008). This observation is also important as, in line with the idea of an involvement of the endocannabinoid system in the pathogenesis of ALS, it may predispose motor neurons to excitotoxic events, given the role that CB₁ receptors play in the control of glutamate homeostasis and the importance of excitotoxicity in ALS pathogenesis (see details in “Glutamate toxicity,” above). By contrast, our studies in the same mutant mice indicate that the expression of the CB₁ receptor in the spinal cord is not altered, although we analyzed the mice at an advanced phase in disease progression (Moreno-Martet et al., 2014). Our studies also indicated an increase in the expression of *N*-arachidonoyl-phosphatidyl-ethanolamine-phospholipase D (NAPE-PLD), the enzyme that synthesizes anandamide, in the spinal cord of SOD-1 mutant mice (only in males, not in females) but no changes in diacylglycerol lipase (DAGL), the enzyme that synthesizes 2-arachidonoylglycerol, and in fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), the two major degradative enzymes for the two major endocannabinoids (Moreno-Martet et al., 2014).

NSC-34 cells, a cell line frequently used as an *in vitro* model of ALS, have been also studied in relation to the presence of endocannabinoid receptors and enzymes (Moreno-Martet et al., 2012). In a non-differentiated state, they already express the enzymes involved in the synthesis (NAPE-PLD and DAGL) and degradation (FAAH and MAGL) of endocannabinoids, as well as CB₁ but not CB₂ receptors (Moreno-Martet et al., 2012). Interestingly, their differentiation, which makes these cells more sensitive to excitotoxic insults, was associated with higher expression of CB₁ receptors and the FAAH enzyme (Moreno-Martet et al., 2012), making this *in vitro* model a useful tool to investigate the beneficial effects of cannabinoids on the control of excitotoxic damage in ALS (see below).

TREATMENTS WITH CANNABINOIDS IN PRECLINICAL MODELS OF ALS

As mentioned above, the pharmacological evidence in support of the idea that cannabinoids may be a promising neuroprotective therapy in ALS has been obtained in SOD-1 (G93A) mutant mice. The first studies were conducted by

Abood and coworkers, who observed that the administration of the phytocannabinoid Δ^9 -THC delayed the onset of motor impairment and had a positive effect on the survival of these mice, which was significantly prolonged (Raman et al., 2004). These effects were evident when the cannabinoid was administered before the onset of ALS signs, but they were also found despite the treatment being initiated after the appearance of symptoms (Raman et al., 2004). With regard to the mechanisms that underlie these effects, the authors proposed a reduction in the oxidative stress and in the excitotoxic damage, as they also found that Δ^9 -THC was effective in the reduction of both cytotoxic events in an *in vitro* study using spinal cord neuronal cultures (Raman et al., 2004). Similar results were reported with another phytocannabinoid, cannabidiol, which, compared to Δ^9 -THC, is significantly less psychotropic (Weydt et al., 2005), with synthetic compounds that selectively activate the CB₂ receptor, e.g., AM-1241 (Kim et al., 2006; Shoemaker et al., 2007), and, to a lesser extent, with the non-selective cannabinoid agonist WIN 55,212-2 (Bilsland et al., 2006). Given that the cannabinoid compounds that are beneficial in ALS encompass a broad range of targets and mechanisms within the endocannabinoid system, and that the recently licensed cannabinoid-based medicine Sativex[®] has this type of broad-spectrum profile (Wright, 2007), we wanted to evaluate a similar combination of phytocannabinoids in SOD-1 mutant mice (Moreno-Martet et al., 2014). In our hands, the treatment of post-symptomatic SOD-1 mutant mice with a Sativex-like combination of phytocannabinoids preserved motor neurons in the spinal cord. Yet, this neuroprotective effect does not appear to be extended to the preservation of neuron-muscle synapse, as the beneficial effects found in neuronal survival did produce only a small delay in the onset of neurological symptoms but not an improvement in animal survival (Moreno-Martet et al., 2014). It is possible that different doses and/or ratios for the two major phytocannabinoids included in Sativex may be necessary to enhance the benefits of this combination in SOD-1 mutant mice; for example, a better response may be obtained with a combination having higher Δ^9 -THC and lower CBD. It is also possible that this may happen with combinations of Sativex with other approved (e.g., riluzole) or under-investigation agents. These possibilities will have to be examined in the coming years.

These pharmacological studies have been paralleled by experiments using mice deficient in specific endocannabinoid receptors or enzymes. Thus, elevation of endocannabinoid levels (mainly anandamide) obtained through the genetic ablation of FAAH enzyme also prevented the appearance of disease signs in the SOD-1 mutant mice (observed in animals having the SOD-1 mutation and the deficiency in FAAH in comparison with classic SOD-1 mutant mice), but it did not affect their survival (Bilsland et al., 2006). However, the genetic ablation of the CB₁ receptor had no effect on the onset of the disease in SOD-1 mutant mice, but it significantly extended lifespan (Bilsland et al., 2006). There are no data on the effects derived from the genetic ablation of CB₂ receptors in SOD-1 mutant mice, but the results of the pharmacological studies (Shoemaker et al., 2007) suggest that these may be a relevant target in ALS, so it is expected that SOD-1

mutant mice carrying a genetic deficiency in the CB₂ receptor gene should exhibit a significant worsening in their ALS phenotype.

Taken together, the different preclinical studies, using pharmacological treatments or genetically deficient mice, suggest that cannabinoids may have neuroprotective effects in ALS mediated by mechanisms other than the activation of CB₁ receptors. In part, these mechanisms may be related to cannabinoid receptor-independent antioxidant and anti-inflammatory properties of certain cannabinoids, e.g., depending on PPAR- γ receptor activation and/or modulation of transcription factors. This is supported by the fact that pioglitazone, an agonist of PPAR- γ receptors, was neuroprotective in the spinal cord of G93A mutant mice through effects that involved a reduction in both the activation of microglial cells and astrocytes and the expression of inducible nitric oxide synthase, NF- κ B, and cyclooxygenase-2 (Kiaei et al., 2005; Schütz et al., 2005). Other studies involved the induction of heat-shock proteins in neuroprotective effects following PPAR- γ receptor activation (Park et al., 2007). However, the data obtained with WIN 55,212-2 (Bilsland et al., 2006), which is not antioxidant, or in FAAH-deficient mice (Bilsland et al., 2006), suggest that other mechanisms, e.g., via CB₁ and/or CB₂ receptors, are also contributing. The role of CB₂ receptors is attractive given the important involvement of microglial cells in ALS (Turner et al., 2004; Sargsyan et al., 2005) and the abovementioned efficacy of CB₂ receptor agonists in the control of microglial toxicity in numerous neurodegenerative disorders (Fernández-Ruiz et al., 2007, 2010). With regard to CB₁ receptors, preliminary data obtained in our laboratory have indicated that non-selective cannabinoid agonists, e.g., WIN 55,212-2 and Δ^9 -THC, enhance cell survival against malonate-induced toxicity in cultured NSC-34 cells (unpublished results). As mentioned above, these cells express high amounts of CB₁ receptors, in particular when they are differentiated, so they may serve as a useful method to evaluate the effects of compounds targeting this receptor against excitotoxic conditions (Moreno-Martet et al., 2012).

TREATMENTS WITH CANNABINOIDS IN CLINICAL STUDIES WITH ALS PATIENTS

The possibility that certain cannabinoids may provide benefits in ALS has been also studied at the clinical level, although the number of clinical trials is still too reduced to obtain significant and reliable findings, thus stressing the urgent need for additional clinical investigation (Carter et al., 2010). First studies were exclusively observational (e.g., surveys) and based on ALS patients that self-medicated with cannabis for attenuating specific ALS-related symptoms, e.g., cramps, spasticity, drooling (Amtmann et al., 2004). However, they were then followed by a few, small, controlled clinical trials. For example, a randomized, double-blind, crossover trial conducted with oral Δ^9 -THC studied its effects on cramps (Weber et al., 2010). Cramps are an important symptom experienced by ALS patients during the course of the disease and frequently remain refractory for most of the

symptom-relieving medications used in ALS (e.g., lioresal, dantrolene, clonazepam, gabapentin). The study showed that Δ^9 -THC was well tolerated but without positive effects on cramp frequency and intensity (Weber et al., 2010). These results, however, should be interpreted with caution due to the small number of patients recruited (27) and the dose used (5 mg twice daily for 2 weeks) (Weber et al., 2010). Two additional studies indicated again good tolerability of Δ^9 -THC in ALS patients and a non-significant attenuation on cramps and fasciculations (Gelinas et al., 2002; Joerger et al., 2012), although a high inter-individual variability was found in Δ^9 -THC pharmacokinetics in one of these studies (Joerger et al., 2012). There are no clinical studies so far that have tried to demonstrate the potential of cannabinoids as disease-modifying therapies as largely supported by pre-clinical studies, so this hypothesis remains a major challenge for future research.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The studies reviewed here are all concordant with the view that cannabinoid-based medicines may serve as novel therapy able to delay/arrest neurodegeneration in ALS, due to their capability to normalize glutamate homeostasis, to reduce oxidative injury, and/or to attenuate local inflammatory events, and possibly also due to their capability to activate cellular responses (e.g., induction of autophagy) addressed to control the toxicity of protein aggregates. However, most of the studies that examined the neuroprotective potential of these compounds in ALS were conducted in animal or cellular models, whereas the few clinical trials that investigated cannabinoid-based medicines were focused on the alleviation of specific ALS-related symptoms, not on the control of disease progression. This latter aspect remains the major challenge for the future and it may be facilitated by the recent approval of the first cannabinoid-based medicines (e.g., Sativex) available for clinical use. This formulation, and the additional combination of phytocannabinoids, presents two important advantages: (1) its safety demonstrated in previous studies (Wright, 2007), and (2) its broad-range profile that appears to be adequate for a disease in which different cytotoxic mechanisms cooperate to damage motor neurons. Therefore, it is expected that an important amount of clinical research in subsequent years will allow these molecules to progress from the present preclinical evidence to real clinical exploitation.

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Endocannabinoids and epilepsy

6

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INTRODUCTION

Epilepsy is a common neurological condition that affects approximately 1% of the adult population and 2% of children (Hauser and Hesdorffer, 1990) and includes a diverse group of seizure disorders that vary in their region of origin, age of onset, pathophysiological manifestations, and underlying mechanisms. Approximately half of all seizure disorders fall into the category of acquired epilepsy (AE), whereby an initial brain insult such as stroke, status epilepticus (SE), or head trauma results in a permanent neuronal plasticity change, which underlies the pathophysiology of these conditions (Hauser and Hesdorffer, 1990). The phase during which the brain undergoes maladaptive alterations in neuronal function following an insult, and ultimately results in the occurrence of spontaneous recurrent seizures (SRSs), is termed epileptogenesis (DeLorenzo et al., 2005). Although the epilepsies as a group represent a complex subject, all of these disorders are associated with SRS discharges arising from the synchronous firing of a population of neurons due to a disruption in the balance between excitatory and inhibitory neuronal transmission (Lothman et al., 1991; Scharfman, 2007; Badawy et al., 2009a,b). In spite of a wide array of available anti-epileptic drug (AED) therapies, it is estimated that between 25 and 40% of newly diagnosed epilepsy patients will have seizures that are refractory to current treatments (Schmidt and Sillanpaa, 2012). The proceedings from a meeting of the International League Against Epilepsy/American Epilepsy Society set the framework for a number of future goals, one of which emphasized the need for the development of research strategies and model systems to elucidate novel therapeutic targets for seizure control (Wilcox et al., 2013).

The endocannabinoid system (ECS) plays an essential role in the brain through its regulation of many neuronal processes involved in both physiological and pathological conditions (Di Marzo et al., 1998; Alger, 2006; Mackie and Stella, 2006; Kano et al., 2009; Castillo et al., 2012). It is comprised of receptors that are acted upon by endogenous lipid ligands (endocannabinoids) and the enzymatic

machinery involved in their synthesis, uptake, and degradation. The brain cannabinoid type-1 (CB₁) receptor is a G_{i/o} protein-coupled receptor (GPCR) and has been identified as the primary mediator of the central effects of cannabinoids/endocannabinoids (Devane et al., 1988; Matsuda et al., 1990; Howlett, 1995). The CB₁ receptor is widely distributed throughout the brain and is one of the most abundant GPCRs in the CNS (Herkenham et al., 1991; Egertova and Elphick, 2000). Unlike classical neurotransmitters that are synthesized and maintained in vesicular storage, the endogenous cannabinoids, arachidonylethanolamine (AEA) and 2-arachidonylglycerol (2-AG) (Devane et al., 1992; Mechoulam et al., 1995), are synthesized “on demand” by the enzymes N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) and diacylglycerol lipases (DGL- α and DGL- β), respectively (Bisogno et al., 2003; Okamoto et al., 2004), in response to sustained neuronal depolarization and elevated intracellular Ca²⁺ levels (Kondo et al., 1998; Stella and Piomelli, 2001). Both AEA and 2-AG cross the synapse in a retrograde manner to act on presynaptic CB₁ receptors followed by rapid carrier-mediated reuptake (Di Marzo et al., 1994; Hillard et al., 1997; Piomelli et al., 1999) and enzyme degradation by fatty acid amide hydrolase (FAAH) (Deutsch et al., 2002) and monoacylglycerol lipase (MAGL) (Dinh et al., 2002), respectively. Activation of presynaptic CB₁ receptors results in responses that are mediated via a number of effector systems that include inhibition of adenylate cyclase-dependent cAMP accumulation and protein kinase A (PKA) activation, inhibition of voltage-gated Ca²⁺ channels, activation of G protein-coupled inwardly-rectifying K⁺ (GIRK) channels, and downstream activation of the mitogen-activated protein (MAP) kinase pathway (Howlett et al., 2004). The primary functional role of the brain ECS is the “on-demand” fine-tuning of synaptic transmission via regulation of presynaptic neurotransmitter release mechanisms. Following presynaptic release of neurotransmitters and subsequent postsynaptic membrane depolarization, endocannabinoids are synthesized, traverse back over the synapse to activate presynaptic CB₁ receptors, and inhibit further release of neurotransmitter, a process that has been termed either depolarization-induced suppression of inhibition (DSI) or depolarization-induced suppression of excitation (DSE) when occurring at inhibitory or excitatory synapses, respectively (Kano et al., 2009; Castillo et al., 2012).

Given that SRSs appear, and then cease, as a result of a transient dysregulation of synaptic transmission within either an isolated population (focal seizures) or broad region (generalized seizures) of neuronal networks, research efforts have focused on understanding the potential role that the ECS function/dysfunction has on epileptic seizure discharge. Additionally, although knowledge of the potential therapeutic benefits of *Cannabis sativa* can be dated as far back as 5000 years, it has only been in the last 25 years that an ever-developing understanding of the brain ECS at the molecular and cellular levels, through a plethora of research studies, has revealed potential therapeutic targets for the control of neuronal excitability (Mechoulam and Parker, 2013).

This chapter will attempt to concisely present the amassed research findings on the relationship between the brain ECS and the epileptic condition, and will be

organized into the following sections: (1) ECS regulation of excitatory neuronal synaptic transmission, (2) alterations in the ECS with seizures and epilepsy, and (3) therapeutic potential of modulating the ECS in seizures and epilepsy.

ECS REGULATES EXCITATORY NEURONAL SYNAPTIC TRANSMISSION

Following the discovery of the CB₁ receptor (Matsuda et al., 1990) and its endogenous ligands AEA and 2-AG (Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995), much research has been undertaken towards elucidating the mechanisms and the potential therapeutic role of targeting the ECS for the treatment of seizures and epilepsy (Pertwee, 2012; Hofmann and Frazier, 2013). In simplest terms, epileptic seizures are the result of an imbalance between excitatory glutamatergic and inhibitory GABAergic transmission resulting in synchronous hyperexcitable neuronal discharge (Lothman et al., 1991). Thus, it follows that control of seizure discharges would necessitate a need for the regulation of excitatory glutamatergic synaptic transmission. A paradox to the anticonvulsant properties of cannabinoids were the findings that CB₁ receptors are more abundantly expressed on axon terminals of inhibitory interneurons (Katona et al., 1999, 2000; Egertova and Elphick, 2000; Hajos et al., 2000), which preceded, in 2001, the first evidence for CB₁ receptor-dependent regulation of synaptic transmission via DSI of inhibitory postsynaptic potentials (IPSPs) (Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001, 2002). With the exception of findings in the cerebellum (Kreitzer and Regehr, 2001), there was little evidence at that time supporting the physiological existence of a CB₁ receptor-mediated regulation of excitatory glutamatergic synaptic transmission (i.e., DSE), although several studies using *in vitro* slice electrophysiology clearly demonstrated a role for cannabinoid-mediated suppression of glutamatergic synaptic transmission via a yet undetermined cannabinoid receptor (Szabo et al., 2000; Hajos et al., 2001; Hajos and Freund, 2002a,b). Thus, a physiological role for CB₁ receptor-dependent regulation of glutamatergic synaptic transmission had been the subject of rigorous debate, which has now been resolved by a number of pivotal studies over the last decade (Melis et al., 2004; Straiker and Mackie, 2005; Takahashi and Castillo, 2006; Katona et al., 2006; Kawamura et al., 2006; Kodirov et al., 2010; Xu et al., 2010; Peterfi et al., 2012). The endocannabinoid regulation of synaptic transmission is beyond the scope of this chapter, but several excellent reviews are available (e.g., Kano et al., 2009; Castillo et al., 2012).

The development of CB₁ receptor knockout (CB₁-KO) mice (Zimmer et al., 1999) allowed for the investigation of what role, or the lack thereof, the CB₁ receptor had in regulating different forms of synaptic transmission. Kano and colleagues demonstrated in hippocampal and cerebellar slice preparations from wild-type mice the presence of CB₁ receptor-dependent regulation of excitatory postsynaptic potentials (EPSPs), while tissue from CB₁-KOs was devoid of this activity

(Kawamura et al., 2006). Immunohistochemical staining for CB₁ receptors revealed weak signals that co-localized with the glutamatergic synaptic marker VGLUT1 in wild-type mice that were absent in CB₁-KO mice. Additionally, electron microscopy (EM) analysis for CB₁ receptors within the stratum radiatum of the hippocampus revealed positive gold particles at asymmetric excitatory synapses. From these findings, they determined that the number of CB₁ receptors on hippocampal excitatory terminals was 10–20 times lower than what was observed on inhibitory terminals. Chen and colleagues demonstrated in hippocampal slices from wild-type and CB₁-KO mice that select hippocampal innervations of CA1 pyramidal neurons by the excitatory Schaffer-collaterals (SC) originating from CA3 onto proximal dendrites generated a form of long-term depression (LTD) that was dependent on DSE, while excitatory perforant path inputs originating from the entorhinal cortex on to distal dendrites generated a form of LTD that was independent of CB₁ receptor activation (Xu et al., 2010). These findings demonstrate the spatial complexity of ECS-mediated control of synaptic transmission at the cellular level, whereby excitatory inputs onto the proximal (within the stratum radiatum) or distal (within the stratum lacunosum-moleculare) dendritic fields of an individual CA1 pyramidal neuron can undergo CB₁ receptor-dependent or -independent LTD, respectively. Utilizing electrophysiological, immunohistochemical, and *in situ* hybridization analysis, Katona and colleagues evaluated the role of the endocannabinoid 2-AG towards mediating LTD in hippocampus via excitatory afferents on to either glutamatergic pyramidal cells or specific GABAergic interneurons (Peterfi et al., 2012). Their results suggest that during high levels of glutamatergic transmission, such as during seizure discharge, the 2-AG-mediated LTD on to glutamatergic pyramidal cells from CB₁ receptor containing excitatory afferents has a lower threshold for induction when compared to that of LTD of GABAergic interneurons from CB₁ receptor containing inhibitory afferents. Such a scenario would allow for EC-mediated fine-tuning of suppression of glutamatergic transmission while allowing for a maintained GABAergic inhibitory tone, which may act as an intrinsic mechanism to prevent the transformation of neuronal transmission from normal activity to a pathological state such as with epileptic seizures.

Another series of eloquent studies utilizing conditional mutant mice has demonstrated that CB₁ receptors located on asymmetric glutamatergic terminals play an essential role in the brain as a defense mechanism against glutamate-induced excitotoxicity (Marsicano et al., 2003; Domenici et al., 2006; Monory et al., 2006; Ruehle et al., 2013; Steindel et al., 2013). Lutz and colleagues developed a mutant mouse strain designated as CB₁^{CaMKII α -Cre} that was devoid of CB₁ receptors selectively on forebrain principal neurons, while CB₁ receptor presence on inhibitory terminals remained intact (Marsicano et al., 2003). Following kainic acid (KA)-induced seizures, CB₁^{CaMKII α -Cre} mice displayed a decreased seizure threshold, increased mortality, increased hippocampal cell death, and a lack of sensitivity to the protective effects of endocannabinoid uptake blockers or pro-convulsant actions of CB₁ receptor antagonists. Additionally, following

KA-induced seizures Lutz et al. (Ibid.) demonstrated $CB_1^{CaMKII-Cre}$ animals lacked CB_1 receptor-dependent induction of protective intracellular cascades, which included activation of the extracellular signal-regulated kinase (ERK) pathway and increased transcriptional expression of the immediate early genes (IEGs) *c-fos*, *zif268*, and the neurotrophin brain-derived neurotrophic factor (BDNF).

A second paper from this group (Monory et al., 2006) extended their work with mutant mice with the development of two additional strains with conditional deletion of CB_1 receptors on terminals of forebrain GABAergic interneurons ($GABA-CB_1^{-/-}$) or on principal glutamatergic neurons regionally limited to the cortical forebrain areas ($Glu-CB_1^{-/-}$), while preserving CB_1 receptors in subcortical and diencephalic regions of the forebrain. The $GABA-CB_1^{-/-}$ mice displayed comparable KA-induced seizure scores to wild-type controls. In the $GABA-CB_1^{-/-}$ mice, CB_1 receptor antibody staining revealed that the highest level of localization of CB_1 receptors at asymmetric glutamatergic terminals within the hippocampus was indicated by a defined and intense band within the inner third molecular layer of the dentate gyrus. Diffuse staining signal was also observed throughout the strata molecularis, radiatum, and oriens. Double *in situ* hybridization analysis of CB_1 receptors with VGLUT1, a marker for glutamatergic terminals, demonstrated co-expression in mossy cell bodies within the hilar region of dentate gyrus and CA3 pyramidal cell bodies. The researchers concluded, by demonstrating a loss of CB_1 receptor-dependent suppression of EPSPs in $Glu-CB_1^{-/-}$ mice hippocampal slice, that virally induced and regionally select deletion of CB_1 receptors on glutamatergic terminals within the dentate gyrus of wild-type mice resulted in increased KA-induced seizure scores. Their findings indicate that CB_1 receptor-dependent regulation of glutamatergic afferents projecting from hilar mossy and CA3 pyramidal neurons on to dentate granule cell dendritic fields plays an essential role in the endocannabinoid-dependent protection against KA-induced seizures (Monory et al., 2006). A further discussion of the predominant role of CB_1 receptor-dependent regulation of glutamatergic transmission within the inner third molecular of the dentate gyrus in relation to findings in clinical and experimental epilepsy will be discussed later in this chapter.

Analysis of distribution and efficiency of CB_1 receptor agonist-stimulated [^{35} S] GTP γ S binding to measure CB_1 receptor–G protein coupling in the $Glu-CB_1$ -KO and $GABA-CB_1$ -KO animals revealed a comparable regional distribution to the above staining studies, and indicated that the functional efficiency of agonist-stimulated G protein signaling at CB_1 receptors localized to glutamatergic terminals was six-fold higher than that at CB_1 receptors on GABAergic terminals (Steindel et al., 2013). These findings are in agreement with an earlier study demonstrating variability in regional distribution and density of CB_1 receptor-stimulated G protein signaling and that the efficiency was inversely proportional to receptor density (Breivogel et al., 1997). Thus, although the level of CB_1 receptors on GABAergic terminals predominates over that on glutamatergic terminals, the increased efficiency of G protein signaling at the excitatory synapses would allow for a more sensitive

response to lower concentrations of agonists, which may explain the findings discussed above regarding differing sensitivities of CB₁ receptor-dependent induction of LTD at excitatory and inhibitory synapses (Peterfi et al., 2012). Utilization of the conditional CB₁-KO models has also elucidated a role for CB₁ receptor-dependent regulation of glutamatergic synaptic transmission towards suppressing cortical synchronous fast oscillations via a thalamocortical–striatonigral pathway, which would contribute to CB₁ receptor-dependent regulation of neuronal excitability and epileptic seizure discharge (Sales-Carbonell et al., 2013).

The above studies clearly establish a physiological role of the ECS towards the regulation of excitatory synaptic transmission via activation of CB₁ receptors on glutamatergic terminals. Furthermore, the evidence clearly demonstrates that this aspect of the ECS localized to excitatory synapses within corticolimbic brain regions and throughout network pathways involved in evoking high-frequency neuronal synchronous discharges is an essential defense mechanism in the brain that acts to protect against excessive glutamatergic excitatory transmission that occurs in pathophysiological states such as seizures and epilepsy. As an interesting side note, a recently discovered attribute of the select strain of the Amazonian rodent *Proechimys*, which is resistant to the development of SRSs following pilocarpine-induced SE, is that it has an overall higher level of hippocampal CB₁ receptor expression when compared to the expression patterns in the Wistar rat strain (Araujo et al., 2010).

ALTERATIONS IN THE ECS IN SEIZURES AND EPILEPSY

The discovery and cloning of the CB₁ and CB₂ receptors opened the gates for uncovering the components of the ECS (Niehaus et al., 2007; Piomelli, 2014). These discoveries were followed by the development of highly specific antibodies and pharmacological agents and ligands, which have allowed for ongoing research on roles that each component of the ECS has in both physiological and pathological processes. A number of studies, both experimental and clinical, have utilized these technologies to evaluate alterations in the brain ECS in association with seizures and epilepsy and allowed for a better understanding of the mechanisms that underlie the anticonvulsant/pro-convulsant properties of cannabinoid compounds.

REORGANIZATION OF CB₁ RECEPTOR EXPRESSION AND FUNCTION IN PILOCARPINE-INDUCED TEMPORAL LOBE EPILEPSY

Alterations in the ECS and brain CB₁ receptor expression and function have been demonstrated in a number of experimental models of seizures and epilepsy, which shed light on some of the mechanisms that underlie the regulatory role of the ECS in these pathologies. In rat or mouse, pilocarpine-induced SE is followed by a 2–4

week latency “seizure-free” phase of epileptogenesis during which time changes in neuronal plasticity culminate in a permanent state of altered neuronal hyperexcitability as evidenced by behavioral and electrographic SRSs, which are associated with many of the brain morphological and behavioral characteristics of human temporal lobe epilepsy (TLE) (Turski et al., 1983). Utilizing this model in the rat, DeLorenzo and colleagues were the first to demonstrate a CB₁ receptor-dependent role for the ECS towards the tonic regulation of epileptic seizure frequency and duration, whereby the administration of the cannabimimetic WIN 55,212-2 (WIN) suppressed epileptic seizures, while specific blockade of CB₁ receptors with SR141617A resulted in a pro-convulsant effect with an increase in seizure durations and frequencies reaching levels comparable to those seen in SE (Wallace et al., 2003) (Figure 6.1). Furthermore, levels of the endocannabinoid 2-AG were increased acutely following seizures, and epileptic animals displayed a long-lasting and permanent change in the distribution of hippocampal CB₁ receptor expression.

Additional work in this same model extended upon by Falenski et al. (2007) demonstrated a selective reorganization of hippocampal CB₁ receptor expression

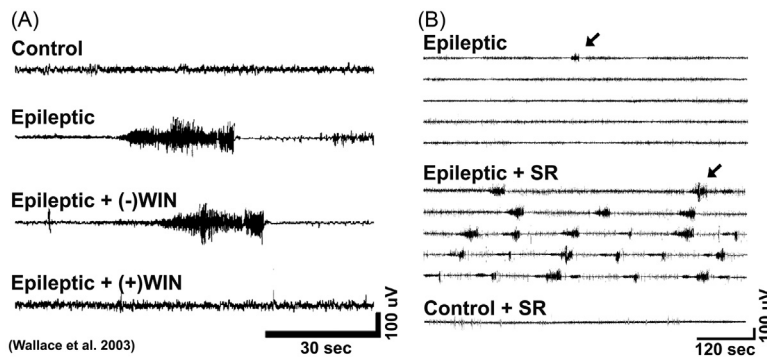
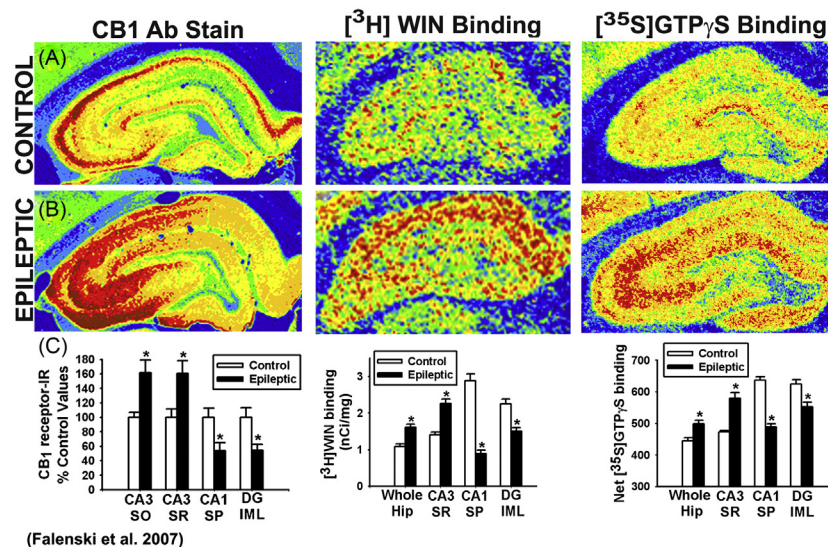


FIGURE 6.1

The effects of CB₁ receptor modulation on epileptiform activity in control and epileptic rats. (A) Representative EEG recordings of control, epileptic, *S*(-)-WIN55,212-, [(+)-WIN]-treated (5 mg/kg i.p.) epileptic animals and *R*(+)-WIN55,212-, [(+)-WIN]-treated (5 mg/kg i.p.) epileptic animals. + WIN treatment completely suppressed SRS activity in epileptic rats. (B) Antagonism of CB₁ receptors by SR141716A (10 mg/kg i.p.) in epileptic rats caused increased seizure frequency and produced status epilepticus in some animals. The data represent EEG and behavioral seizures observed over the 1-h recording period for epileptic and epileptic + SR conditions. These recordings represent continuous EEG recordings from an epileptic rat 60 min before and 60 min after treatment with SR141716A. Arrows represent individual seizures. The control + SR representative EEG recording demonstrates that treatment of control (non-epileptic) animals with SR141716A did not produce seizure activity.

Modified with permission from Wallace et al. (2003).

evidenced by loss in the dentate gyrus inner third molecular layer and stratum pyramidale of CA1–CA3, and a concomitant increase throughout the strata molecularis, radiatum, and oriens of the dentate gyrus and CA1–CA3 regions (Figures 6.2 and 6.3A). Furthermore, the redistribution of hippocampal CB₁ receptor expression levels in epileptic animals was linked to corresponding changes in hippocampal CB₁ receptor binding and G protein coupling (Figure 6.2). During the latency phase in this rat model of TLE, which lasts for 2–4 weeks following the initial pilocarpine-induced insult of SE, the process of epileptogenesis ensues during which time hippocampal CB₁ receptor expression is dramatically reduced within the first week, returning to near control levels by 2 weeks, and expressing the long-lasting pattern of reorganization by 4 weeks (Figure 6.2), at which time the animals start displaying SRSs (Falenski et al., 2009).



(Falenski et al. 2007)

FIGURE 6.2

Alterations in hippocampal CB₁ receptor expression, binding, and function in epileptic rats. (*Left panels*) Pseudo color enhanced staining for CB₁ receptor using an N-terminus antibody demonstrates a redistribution of hippocampal receptor levels with marked and significant increases within the stratum oriens (SO) and stratum radiatum (SR), while levels within the CA1 stratum pyramidale (SP) and inner molecular layer (IML) of the dentate gyrus (DG) were significantly reduced when compared to control. Redistribution of hippocampal CB₁ receptor expression in epileptic animals was mirrored by concomitant and significant changes in both [³H]-WIN receptor binding analysis (*middle panels*) and WIN-stimulated [³⁵S]-GTP-γS binding analysis (*right panels*).

Modified with permission from Falenski et al. (2007).

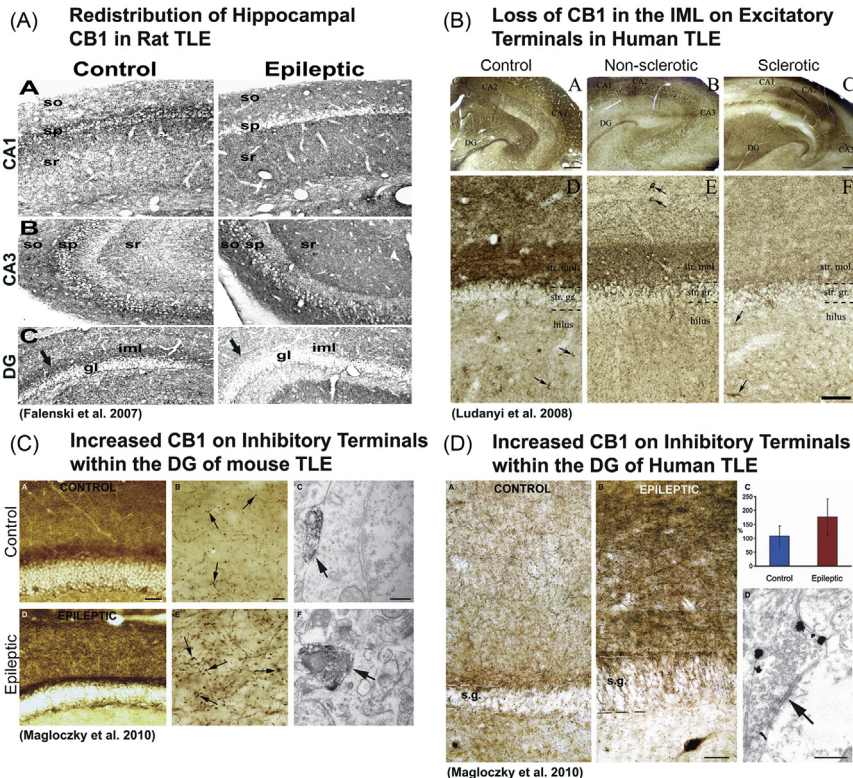
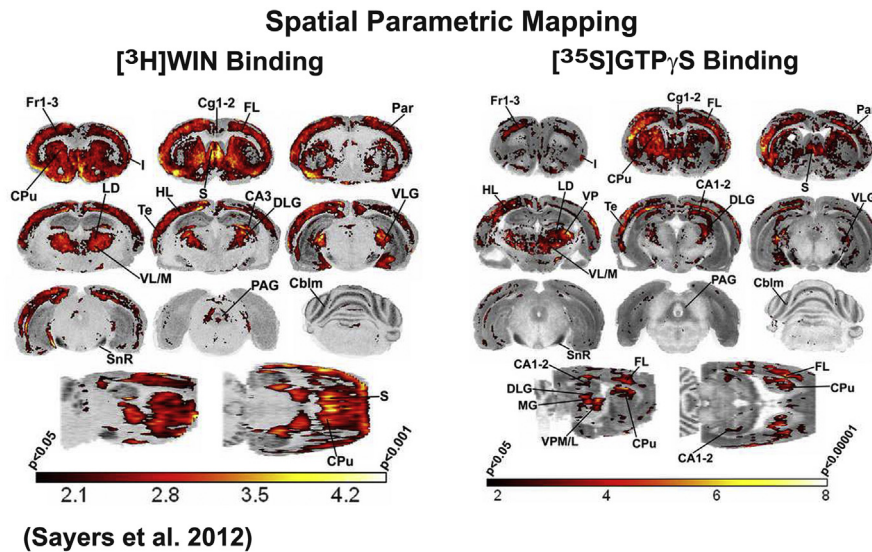


FIGURE 6.3

Alterations of CB₁ receptor expression in rodent and human temporal lobe epilepsy (TLE) hippocampus. (A) Immunohistochemical analysis using a C-terminus antibody to CB₁ receptor in TLE rat hippocampus showing a redistribution of CB₁ receptors with increases within the strata radiatum and oriens and dropout in staining within the CA1 stratum pyramidale and the inner third molecular layer of the dentate gyrus (arrows). (B) CB₁ receptor immunohistochemical staining with a C-terminus antibody showing a decrease within the IML in human sclerotic TLE hippocampus. The decrease in CB₁ receptors was confirmed to be occurring exclusively at asymmetric excitatory terminals by ultrastructural analysis (not shown). Scale bars, *upper* panels 500 μ m, *lower* panels 100 μ m. (C) Immunohistochemical analysis of TLE mouse dentate gyrus with an antibody specific for CB₁ receptor staining on symmetric inhibitory terminals showing marked increases within the stratum moleculare of the dentate gyrus DG with postsynaptic targets including both cell bodies and dendrites (arrows—*upper* and *lower* right panels, respectively). Scale bars, *left* panels 200 μ m, *middle* panels 50 μ m, *right* panels 1 μ m. (D) Immunohistochemical analysis of sclerotic human TLE dentate gyrus with an antibody specific for CB₁ receptor staining on symmetric inhibitory terminals showing marked increases within the stratum moleculare of the dentate gyrus. Confocal laser scanning analysis revealed a significant increase in CB₁ receptor-positive fibers in epileptic samples (*histogram*), which was confirmed by ultrastructural analysis to exclusively occur at symmetric inhibitory terminals (*lower-right* panel). Scale bars, *left* and *middle* panels 50 μ m, *lower-right* panel 0.5 μ m.

(A) Modified with permission from Falenski et al. (2007). (B) Modified with permission from Ludanyi et al. (2008). (C) and (D) Modified with permission from Magloczky et al. (2010).

**FIGURE 6.4**

Statistical parametric mapping (SPM) reveals significant increases in both [³H]WIN binding (*left panel*) and WIN-stimulated [³⁵S]GTPγS binding (*right panel*) in discrete forebrain regions of epileptic animals when compared to control ($n=6$ per group). Representative epileptic coronal, sagittal, and transverse images illustrate the regionally select increases in CB₁ receptor binding and WIN-stimulated [³⁵S]GTPγS binding as demonstrated by colored overlays (red to yellow) that correspond to levels of significance. Fr1-3, frontal cortex, areas 1–3; I, insular cortex; Cg1-2, cingulate gyrus, areas 1–2; CPu, caudate putamen; FL, forelimb cortex; S, septum; Par, parietal cortex; HL, hind limb cortex; LD, laterodorsal thalamic nucleus; VL/M, ventrolateral/medial thalamic nuclei; Te, temporal cortex, hippocampal area CA3; DLG, dorsal lateral geniculate nucleus; VLG, ventral lateral geniculate nucleus; MG, medial geniculate nucleus; VPM/L, ventral posterolateral/medial thalamic nuclei; SnR, substantia nigra; PAG, periaqueductal gray; Cblm, cerebellum.

Modified with permission from Sayers et al. (2012).

Additional work from the DeLorenzo lab using the rat pilocarpine model of TLE employed statistical parametric mapping (SPM), enabling the three-dimensional (3D) reconstruction of levels of whole brain CB₁ receptor binding and G protein coupling in epileptic rats (Figure 6.4). In addition to the earlier reorganization of CB₁ receptors observed within the hippocampus of epileptic animals, this work demonstrate long-lasting alterations in CB₁ receptor binding and G protein coupling throughout the forebrain with selective regional increases in striatum, cortex, and select nuclei of the thalamus (Sayers et al., 2012).

The work from these three studies demonstrates a temporal reorganization of brain CB₁ receptor expression and function alongside the development and maturation of SRS activity, which likely represents a compensatory mechanism evidenced by the CB₁ receptor-dependent regulatory role the ECS has towards suppressing excessive seizure discharge in pilocarpine-induced TLE. Work from Freund and colleagues utilizing the pilocarpine mouse model found that within 2 hours following SE, a pronounced decrease in CB₁ receptor staining was observed throughout the hippocampus and occurred on both symmetrical and asymmetrical terminals, while at days 1 and 3, levels for CB₁ receptors returned to control and then slightly increased, respectively (Karlocai et al., 2011). By 1 month following SE, patterns for hippocampal CB₁ receptor expression demonstrated an overall increase throughout the stratum moleculare and select increases around surviving sclerotic regions of CA1. The increases in CB₁ receptor stain were confirmed by EM to occur on both inhibitory and excitatory terminals, with symmetric terminals displaying an increase in the number of receptors per terminal. Utilizing an antibody that specifically labeled CB₁ receptors at inhibitory terminals, additional findings from this group demonstrated increases of CB₁ receptors within the dentate gyrus stratum moleculare including a marked increase in the inner third molecular layer (IML) (Figure 6.3C), and also preservation of interneuronal somatic staining in the CA1 and dentate gyrus regions (Magloczky et al., 2010).

Studies in the pilocarpine mouse model carried out by Bhaskaran and Smith (2010a) demonstrated an increased frequency of EPSPs that was sensitive to suppression by CB₁ receptor activation via decreasing release of glutamate from pre-synaptic terminals, while these observations were not present in control tissue. Western analysis revealed a significant increase in CB₁ receptor protein levels in the dentate gyrus of epileptic animals, which likely underlies the increased sensitivity of the enhanced EPSPs to CB₁ receptor agonists. In light of the above studies, several papers from Houser and colleagues demonstrated in pilocarpine-TLE mice that cholecystokinin/CB₁ receptor-positive terminals innervating CA1 and the IML of the dentate gyrus were markedly reduced while increased innervations were observed on glutamatergic spines throughout the strata radiatum and oriens (Wyeth et al., 2010, 2012).

OTHER EXPERIMENTAL FINDINGS

In a model of febrile-induced seizures (HT) in P10 rat pups, Soltesz and colleagues utilized hippocampal slice electrophysiology to demonstrate that HT resulted in a long-lasting (5 weeks post-HT) increase in CB₁ receptor-dependent DSI in CA1, as well as in the emergence of a novel CB₁ receptor-dependent DSI in the dentate gyrus that was not detected in control slices (Chen et al., 2003). In association with the enhanced DSI findings, immunoblot, light, and EM analysis revealed that CB₁ receptor protein levels were significantly increased throughout the hippocampus and shown to be selectively up-regulated throughout the

molecular layers on cholecystokinin (CCK)-positive terminals, which were the result of increased CB₁ receptor levels and not newly sprouted processes. In rats, [Bojnik et al. \(2012\)](#) demonstrated an increase in anandamide-stimulated GTP γ S binding in hippocampal and cortical membranes occurring three hours following KA-induced seizures, with corresponding increases in mRNA levels for CB₁ receptor and CRIP1a; CRIP1a is the cannabinoid receptor interacting protein that has been shown to modulate CB₁ receptor activity ([Niehaus et al., 2007](#)). In a paper by Friedman and colleagues, juvenile rat pups (second to third week postnatal) demonstrated a dose-dependent effect of WIN against KA-induced seizures in that 0.5 and 1.0 mg/kg WIN showed protection both behaviorally and morphologically 3 days post-insult, while a higher dose of 5 mg/kg WIN displayed seizure scores and hippocampal neuronal cell loss comparable to findings in control KA animals (no WIN) ([Rudenko et al., 2012](#)). In agreement with the above studies, CB₁ receptor staining was elevated in hippocampal stratum radiatum, stratum oriens, and the IML of the dentate gyrus in the control KA seizure group, while it was unchanged in animals that received 0.5 and 1.0 mg/kg WIN and decreased/down-regulated in the animals administered 5.0 mg/kg WIN, with or without KA (control no seizure), suggesting that juvenile rats may have an increased sensitivity to CB₁ receptor agonist-induced receptor desensitization/down-regulation.

In a genetic model of absence seizures in the rat (WAG/Rij), it is suggested that an enhanced GABAergic tone in the ventrobasal thalamic nuclei (VBTN) contributes to the generation of spike-wave discharges (SWDs) within the thalamic–cortical–thalamic network, which underlie absence seizures. *In situ* hybridization and immunoblot analysis revealed significant decreases in CB₁ receptor mRNA and protein levels in the reticular thalamic and the ventrobasal thalamic nuclei regions in symptomatic WAG/Rij rats, which may contribute to a decrease in DSI resulting in enhanced GABAergic tone in these regions ([van Rijn et al., 2010](#)).

Homer proteins are localized at the postsynaptic density where they act as a scaffolding network that couples to and regulates selected target proteins involved in many levels of synaptic plasticity, including protein components involved in endocannabinoid production. A small alternatively spliced isoform of the Homer family, Homer 1a (H1a), acts to modulate postsynaptic function through uncoupling/deregulating of target proteins from the Homer scaffolding network, and has been shown to have increased expression following a number of neuronal processes that include long-term potentiation (LTP) and increased levels of BDNF ([Worley et al., 2007](#)). Thayer and colleagues have demonstrated that transfection of an expression construct for H1a in hippocampal neuronal cultures acts to decrease metabotropic-induced suppression of excitation (MSE) and enhance DSE, both of which were dependent on CB₁ receptor activation and likely attributed to H1a acting to decrease or increase 2-AG synthesis, respectively ([Roloff et al., 2010](#)). To test this effect, under more physiological conditions, they showed that addition of BDNF to hippocampal cultures resulted in a 32-fold increase in H1a mRNA expression and a significant suppression and enhancement of MSE and DSE, respectively. A second study in the same hippocampal culture

preparation demonstrated that epileptiform seizures induced by bicuculline + 4-aminopyridine (4-AP) resulted in a group I mGluR-dependent increase in expression of H1a and decrease in MSE. Furthermore, 4 hours of bicuculline + 4-AP-induced epileptiform activity occluded mGluR5 activation-induced IP3-sensitive Ca^{2+} mobilization. They speculate that H1a regulation of CB₁ receptor-mediated MSE and DSE may contribute to neuronal plasticity changes and protective mechanisms, respectively (Li et al., 2012). In support of the above findings, increased H1a expression following pilocarpine-induced status epilepticus in the rat occurs and is thought to act as a countermeasure against neuronal hyperexcitability (Cavarsan et al., 2012).

ALTERATIONS IN THE ECS IN HUMAN TEMPORAL LOBE EPILEPSY

In clinical epilepsy research, PET scan imaging studies, evaluation of surgically resected or postmortem hippocampal tissue, and analysis of cerebrospinal fluid (CSF) indicate a number of alterations in the ECS in association with the pathophysiological state of TLE in humans. Using the [¹⁸F]-MK9470-specific marker for CB₁ receptors, PET scan imaging in patients with mesial TLE with hippocampal sclerosis revealed an increase in CB₁ receptor availability in the ipsilateral hemisphere of the TLE zone, as well as a decrease in the superior insular cortex both ipsilateral and contralateral to the epileptic focus when compared to controls. The increase in CB₁ receptors was directly correlated to the number of seizures and inversely correlated to seizure latency in the month prior to PET imaging (Goffin et al., 2011). Analysis of CSF from patients with newly diagnosed TLE who had not started AED therapy showed that levels for the endocannabinoid anandamide were significantly elevated compared to controls, while no differences in levels for 2-AG were found (Romigi et al., 2010).

Utilizing quantitative polymerase chain reaction (qPCR), and immunohistochemical and EM analysis of human surgically resected or postmortem tissue from controls and patients with intractable TLE with either sclerotic or non-sclerotic hippocampal pathology, Katona and colleagues demonstrated a significant overall decrease in CB₁ receptor mRNA and protein levels in both sclerotic and non-sclerotic hippocampal tissue, with the exception of a modest increase in receptor staining in stratum oriens of the CA2–CA3 subfields in sclerotic samples (Ludanyi et al., 2008). Staining analysis revealed a marked decrease in CB₁ receptors within the IML of the dentate gyrus (Figure 6.3B) and ultrastructural EM analysis revealed that these losses in receptor levels within the IML occurred exclusively on glutamatergic terminals, while no change in receptor levels was observed on symmetric GABAergic terminals. Additionally, mRNA levels for the cannabinoid receptor interacting protein (CIRIP1a) and the 2-AG synthesizing enzyme DGL- α were significantly decreased in sclerotic tissue, while levels for DGL- β , and for

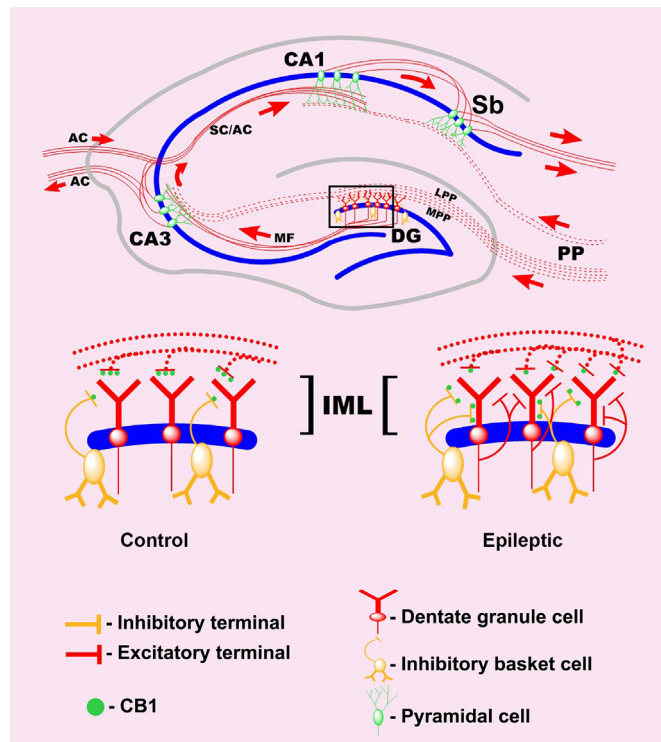
NAPE-PLD and FAAH (anandamide synthesizing and degrading enzymes, respectively), and MAGL (2-AG degrading enzyme) showed no change from controls.

Freund and colleagues utilized an antibody that exclusively stained for CB₁ receptor on GABAergic terminals and compared expression patterns at the light and EM levels in human hippocampal tissue from control and patients with intractable TLE (Magloczky et al., 2010). In comparison to the loss of CB₁ receptors at glutamatergic terminals in the above paper, the results from this study demonstrated a significant increase in CB₁ receptor staining on inhibitory GABAergic terminals within the stratum molecularis of the dentate gyrus as well as in the interneuronal somata throughout the CA1 region and dentate gyrus (Figure 6.3D).

In agreement with findings in experimental models of seizure and epilepsy, the human data presented above demonstrate alterations in a number of components of the ECS as well as a reorganization of the hippocampal CB₁ receptor at inhibitory and excitatory terminals. Such a redistribution of receptors in human TLE hippocampus would result in a shift in the CB₁ receptor-dependent regulation of synaptic transmission that would increase at GABAergic (DSI) and decrease at glutamatergic (DSE) terminals, a scenario that would be expected to contribute to epileptic seizure initiation.

DO ALTERATIONS IN CB₁ RECEPTORS WITHIN THE IML OF THE DENTATE GYRUS CONTRIBUTE TO TLE?

In the hippocampus, the presence of an excitatory network termed “trisynaptic pathway” involves incoming excitatory projections (perforant path) from the entorhinal cortex onto the dentate granule cells, which then project terminals to innervate the CA3 pyramidal cells that send axons onto the CA1 pyramidal cells via the Schaffer collaterals. Ultimately, the CA1 pyramidal cells project to the subiculum, whereupon the main hippocampal output returns to the entorhinal cortex completing the loop (Figure 6.5). The hippocampus is a limbic structure that is primed to generate seizure discharges largely due to intrinsic properties of CA3 pyramidal neurons that have a low threshold for excitatory discharges due to their extensive interconnectivity with neighboring CA3 cells. Regulation of this intrinsic seizure-prone excitatory hippocampal circuit is, in part, mediated by dentate granule cells (McNamara, 1999). Observed in temporal lobe epilepsy, in both humans and animal models, is a rewiring of the hippocampal network as evidenced by a process termed “mossy fiber sprouting” (MFS), whereby dentate granule cell mossy fiber terminals are redirected back onto neighboring granule cell dendrites within the IML of the dentate gyrus (Figure 6.5) (Sutula et al., 1988; Represa et al., 1993; Okazaki et al., 1995). There has been substantial debate as to the functionality of MFS having either a cause or effect role in the generation of epileptic seizures, although recent data suggest the former.

**FIGURE 6.5**

Diagrams of hippocampal network and working model of altered endocannabinoid-mediated regulation of synaptic transmission within the IML of epileptic DG. (*Upper diagram*) The tri-synaptic hippocampal circuit begins with input from the entorhinal cortex (EC) onto the dentate gyrus (DG) and CA3 pyramidal cells via the perforant path (PP) consisting of both lateral (LPP) and medial (MPP) projections. Mossy fibers project from DG cells onto CA3 pyramidal cells, which then send projections via the Schaffer collateral (SC) and associated commissural (AC) pathways onto the ipsilateral and contralateral CA1 pyramidal cells, respectively. CA1 cells can also receive input from the PP and send their axonal projections to the subiculum (Sb), which then send the primary hippocampal output back to the EC. (*Lower diagrams*) Diagrams of proposed working model of alterations within the IML of the DG in TLE. Both experimental and clinical TLE are associated with loss of vulnerable hilar cells and a rewiring of DG mossy fibers projecting back onto DG cell dendrites within the IML. Additionally, TLE is associated with increased innervations of the IML by inhibitory symmetric terminals possibly arising from GABAergic basket cells within the DG. A shift of CB₁ receptor levels from excitatory asymmetric terminals to inhibitory symmetric terminals occurs in both experimental and clinical TLE. Such a change of CB₁ receptor distribution on presynaptic terminals within the IML would be expected to decrease DSE and increase DSI, resulting in an overall increase in excitatory transmission at this point within the epileptic hippocampal network.

Houser et al. (2012) have demonstrated with EM analysis of MFS in human TLE a dramatic increase in complexity of projecting terminals at asymmetric excitatory synapses, which present with ultrastructural characteristics indicative of functional synapses. Additionally, in TLE mice, induced expression of the activity-related markers Fos and phosphorylated-ERK in the dentate gyrus following spontaneous seizures indicates that the maladaptive MFS within the IML is functional and, thus, likely has a contributory role in the generation of epileptic seizures. As reviewed in the previous section, conditional knockout mice (GABA-CB₁^{-/-}) that expressed CB₁ receptor only on glutamatergic terminals that form asymmetric excitatory synapses demonstrated that the highest level of hippocampal expression was within the IML of the dentate gyrus (Monory et al., 2006). Findings reviewed above in human TLE hippocampus demonstrate a dramatic loss of CB₁ receptors on asymmetric excitatory terminals within the IML of the dentate gyrus (Figure 6.3B) (Ludanyi et al., 2008). In agreement with these clinical findings, a long-term dropout in CB₁ receptor expression within the IML has also been observed in the rat pilocarpine model of TLE, while receptor expression is increased in the strata radiatum and oriens (Figure 6.3A) (Falenski et al., 2007). In contrast, CB₁ receptor levels on GABAergic symmetric terminals were observed to be increased within the IML of the dentate gyrus in both human tissue and a mouse model of TLE (Figure 6.3C and D) (Magloczky et al., 2010). Thus, in the TLE hippocampus, a rewiring of dentate gyrus cell mossy fiber axon terminals within the IML may contribute to a state of hyperexcitability by undermining a regulatory circuit responsible for governing the intrinsic seizure-prone state of the hippocampal/CA3 network, while CB₁ receptor-dependent regulation of synaptic transmission is redistributed from glutamatergic terminals (decreased DSE) and increased at GABAergic terminals (increased DSI) within the IML of the epileptic dentate gyrus (Figure 6.5).

Although all of the neuroplasticity changes associated with epileptogenesis and the development of SRSs are most likely not exclusively mediated by alterations in the ECS alone, the combination of MFS, increased excitatory transmission, and the maladaptive shifting of CB₁ receptor-dependent regulation of synaptic transmission within IML of the dentate gyrus represents a “perfect storm” scenario, which may possibly contribute to a pathophysiological state that would favor initiation of SRS discharge. This scenario would support a pro-convulsant effect of CB₁ receptor agonism, which is in disagreement with a considerable amount of experimental findings demonstrating CB₁ receptor-dependent anticonvulsant effects. A resolution to this discrepancy could be the up-regulation of CB₁ receptors observed throughout the CA1–CA3 strata radiatum and oriens regions, which may act to suppress/block spreading seizure discharges originating upstream in the hippocampal circuit. Further studies to evaluate the functionality of CB₁ receptor-dependent regulation/dysregulation of synaptic transmission within the IML of the epileptic hippocampus are warranted.

THERAPEUTIC POTENTIAL OF MODULATING THE ECS IN SEIZURES AND EPILEPSY

THE PHYTOCANNABINOIDS

The use of *Cannabis sativa* medicinally can be historically dated as far back as the second millennium BC (Mechoulam and Parker, 2013). The first formal publication on the therapeutic potential of *Cannabis*, which included its anticonvulsant properties, was presented by William B. O'Shaughnessy in 1842 (O'Shaughnessy, 1842). A major breakthrough in *Cannabis* research was the isolation and synthesis of the major psychoactive constituent Δ^9 -tetrahydrocannabinol (Δ^9 -THC) by Mechoulam and Gaoni in the 1960s, which was the starting point in a new era of cannabinoid research towards elucidating the biological effects of the constituent compounds and ultimately revealing the existence of the ECS (Mechoulam and Gaoni, 1965). Of the 421 constituents found in *Cannabis sativa* (Turner et al., 1980), 80 fall within the classification of phytocannabinoids, of which Δ^9 -THC is primarily responsible for the central psychotropic effects via activation of CB₁ receptors (Izzo et al., 2009). Earlier studies found that Δ^9 -THC possessed anticonvulsant properties characteristic of certain classical AEDs as indicated by its effectiveness against both acute and epileptic seizures (Lemberger, 1980). The anticonvulsant activity of Δ^9 -THC has been demonstrated in the maximal electro shock (MES), audiogenic, and pentylenetetrazole (PTZ) seizure tests, although adverse effects of psychotoxicity and CNS excitation (Consroe and Wolkin, 1977; Karler and Turkanis, 1981), as well as the neuronal hyperexcitability following withdrawal (Karler and Turkanis, 1980), limit the clinical efficacy of this cannabinoid in seizure disorders. Additionally, contrary to the suppressive effects of Δ^9 -THC in some models of seizure, proconvulsant effects were observed in rabbits (Martin and Consroe, 1976) and beagles (see commentary: Feeney, 1977). In light of the above experimental findings with Δ^9 -THC, several clinical reports have presented seizure exacerbation in patients with focal epilepsy following withdrawal from self-medicating with cannabinoids (Hegde et al., 2012) and an incidence of first-ever seizures in multiple sclerosis patients undergoing a trial with long-term administration of cannabinoid-based therapy for control of spasticity (Wade et al., 2006).

Another major phytocannabinoid present in *Cannabis sativa* is the non-psychotropic constituent cannabidiol (CBD), which has shown substantial promise for its therapeutic potential in control of seizures and epilepsy. Earlier experimental studies in seizure models indicated that CBD demonstrated anticonvulsant activity against tonic-clonic-type seizures and enhanced the effect of the classical AED phenytoin in an audiogenic seizure model, while having a negative effect when co-administered with the AEDs clonazepam, chlorthalidone, and trimethadione (Consroe and Wolkin, 1977). Karler and Turkanis (1981) carried out a comparative evaluation of CBD, Δ^9 -THC, phenytoin, and ethosuximide in both MES and PTZ models of seizure and found that CBD demonstrated effective

suppression of seizures, lacked neurotoxicity and the development of tolerance, and had potential therapeutic efficacy comparable to that of classical AEDs in controlling seizures in grand mal, cortical focal, and complex partial epilepsies. In light of these earlier findings, the only clinical trial for CBD as an antiepileptic agent was performed in 1980 by Mechoulam and colleagues in epileptic patients who had maintained little control of their disease with classical AEDs. The authors demonstrated a positive response to prolonged (4½ months) CBD treatment, with 50% of patients being seizure free and 38% having partial improvement (Cunha et al., 1980); none of the patients or healthy volunteers receiving CBD showed any adverse effect from the treatment regimen.

In recent years, a number of studies utilizing both *in vivo* and *in vitro* models of seizure and epilepsy have further confirmed a therapeutic potential for CBD (Wallace et al., 2001; Jones et al., 2010, 2012; Shirazi-zand et al., 2013) in addition to the non-psychotropic phytocannabinoids cannabidivarin (CBDV) (Hill et al., 2012a, 2013) and Δ^9 -tetrahydrocannabivarin (THCV) (Hill et al., 2010) as anticonvulsant agents, and have determined that their mechanism of action is unlikely via CB₁ receptor modulation, but may involve mechanisms regulating Ca²⁺ (Jones et al., 2010; Shirazi-zand et al., 2013). Analysis of CBD's many pharmacological effects over the years has shed light on a number of underlying mechanisms that may contribute to its seizure suppressive properties and include antioxidant effects, and inhibition of AEA degradation and reuptake, as well as adenosine reuptake, regulation of mitochondrial-dependent Ca²⁺ homeostasis and inhibition of T-type Ca²⁺ channels, and direct modulation/activation of transient receptor potential (TRP) ion channels, G protein-coupled receptors 55 (GRP55), and serotonin 5HT_{1A} receptors (Hill et al., 2012b; Mechoulam et al., 2007). A paper presenting findings from a survey of parents of children with treatment-resistant epilepsies who had failed to respond, on average, to 12 different AEDs and chose to self-medicate with CBD-enriched cannabis-based medicines (CBMs), found that of 19 families that satisfied the study criteria, two (11%) were seizure free, eight (42%) had an 80% reduction of their seizures, and six (32%) had a 20–60% suppression of seizures as a result of implementing CBD-enriched CBM therapy (Porter and Jacobson, 2013). Although this somewhat subjective survey shows promise with CBD-based therapies in this population of refractory epilepsy, in conclusion the authors voice concern over possible risks to patients resulting from the lack of regulation and quality control in the production and distribution of available CBMs, and the need for objective clinical trials to thoroughly assess the therapeutic efficacy of CBD or other CBMs in seizure and epilepsy (Porter and Jacobson, 2013). This latter concern is further substantiated by Gloss and Vickrey (2012) that ran an extensive database and literature search of clinical studies, case reports, and meeting proceedings, as well as consulting directly with drug manufacturers on the use of CBMs for the treatment of epilepsy, which concluded that at this time no reliable determinations can be made on the use of CBMs for the clinical treatment of seizures and epilepsy, underscoring the need for properly designed clinical trials with sufficient power.

TARGETING CB₁ RECEPTORS IN SEIZURES AND EPILEPSY

There have been an ever-growing number of studies in experimental models of seizures and epilepsy demonstrating the anticonvulsant properties of cannabinoids (Lemberger, 1980; Karler and Turkanis, 1981), and was not until the discovery of the CB₁ receptor, its endogenous ligands, and the enzymes involved in their synthesis, uptake, and degradation, that subsequent development of highly specific pharmacological agents to target the ECS allowed for elucidating the anticonvulsant potential of CB₁ receptor modulation. Wallace et al. (2001) were the first to demonstrate a CB₁ receptor-dependent anticonvulsant effect of Δ^9 -THC and WIN in the mouse MES seizure model of partial seizures with secondary generalization as evidenced by their anti-seizure actions being blocked with the antagonist SR141617. Additional work from this group in the MES model demonstrated anticonvulsant activity of anandamide and its metabolically stable analogue O-1812, as well as a significant decrease in MES-induced seizure threshold with SR141617 alone, indicating a role for the ECS towards regulating seizure discharge through a CB₁ receptor-dependent pathway (Wallace et al., 2002). An earlier study in the mouse MES model demonstrated that the endogenous fatty acid ethanolamide *N*-palmitoylethanolamide (PEA) was protective in this model of partial seizures (Lambert et al., 2001), which may be acting as an anticonvulsant indirectly through CB₁ receptors (Cittraro et al., 2013b) and will be discussed in more detail below. In the maximal dentate activation rat model of limbic partial complex seizures (Stringer and Lothman, 1990), where an electrical stimulus to the dentate gyrus results in the onset of an after-discharge response, Sardo and colleagues showed that pre-administration of WIN resulted in an increase in latency to onset and a decrease in duration of after discharge (Rizzo et al., 2009). The suppressive effect of WIN could be blocked by AM251, indicating a CB₁ receptor-dependent mechanism, while AM251 administered alone had no effect on the parameters after discharge, suggesting the lack of an endogenous endocannabinoid tone in regulation of the MDA-induced response.

In the rat pilocarpine model of TLE, DeLorenzo and colleagues demonstrated that both Δ^9 -THC and WIN administration suppressed epileptic seizure activity while antagonism of CB₁ receptors with SR141617 resulted in increased durations and frequency of seizures reaching levels comparable to those found with clinical SE (Wallace et al., 2003) (Figure 6.1). These findings not only demonstrate the anticonvulsant properties of exogenous cannabinoids in this model of TLE, but also reveal a possible compensatory shift in CB₁ receptor-dependent endogenous tone that may be attributed to a redistribution of hippocampal CB₁ receptors observed in a number of studies discussed earlier in this chapter (Wallace et al., 2003; Falenski et al., 2007, 2009; Ludanyi et al., 2008; Magloczky et al., 2010; Karlocai et al., 2011; Sayers et al., 2012). A study by Bhaskaran and Smith (2010a) carried out an extensive electrophysiological analysis of excitatory synaptic transmission in hippocampal slices from control and pilocarpine-induced TLE mice, and demonstrated CB₁ receptor-dependent suppression of EPSP frequency in epilepsy slices that was

attributed to a suppression of miniature EPSPs indicating the presynaptic regulation of excitatory neurotransmitter release. Additionally, CB₁ receptor expression levels in the dentate gyrus region were shown to be elevated in epilepsy tissue, which was associated with a decrease in dentate gyrus stimulated-induced after-discharge threshold that could be blocked by anandamide and WIN in a CB₁ receptor-dependent fashion. In an amygdala kindling model in the rat, WIN (500 µg/rat, i.c.v.) was shown to significantly suppress after-discharge and seizure durations and increase latency of stage 4 kindled seizures (Naderi et al., 2012), while findings from Wendt et al. (2011) in a mouse kindling model of TLE indicated that WIN and the FAAH enzyme inhibitor URB597 had no anticonvulsant activity in the fully kindled state. The discrepancy between the outcomes of these two studies may result from differences in species, kindling paradigm, or route of administration. An additional finding from the above study in the mouse kindling model of TLE was that daily administration of WIN early on during the kindling (acquisition) phase of this model resulted in an increase in threshold for stimulus-induced seizure and a decrease in seizure severity when compared to kindled controls (no WIN) (Wendt et al., 2011), which is in agreement with findings from the 1970s and 1980s showing that a number of cannabinoids and levonantradol were ineffective at suppressing fully kindled seizures in rats and cats, but showed some promise for blocking the development of the kindling effect and also in the possible adjunctive therapy with classical AEDs (Wada et al., 1975; Corcoran et al., 1978; Ehlers et al., 1981).

In absence epilepsy, a hallmark characteristic of the EEG is SWDs, which are driven by a pathophysiological synchronization of activity within the thalamocortical circuit, which involves the ventrobasal thalamic nuclei, somatosensory cortex, and the reticular thalamic nucleus (Blumenfeld, 2005). In the WAG/Rij genetic rat model of absence epilepsy, which expresses spontaneous absence-like seizures, Ngomba and colleagues demonstrated significant decreases in CB₁ receptor protein levels within the reticular thalamic nucleus (RTN) and VBTN of symptomatic WAG/Rij rats, and administration of WIN was anticonvulsant via a CB₁ receptor-dependent mechanism (van Rijn et al., 2010). They propose the decrease in CB₁ receptor expression is in GABAergic interneurons localized within the RTN that project their terminals into the VBTN, and this loss in endocannabinoid-mediated presynaptic regulation of these inhibitory inputs contributes to an increased GABAergic tone within the VBTN, which has been shown to underlie SWD initiation by McCormick and Bal (1997). De Sarro and colleagues (LoVerme et al., 2005) have presented findings from two papers in the WAG/Rij model which indicate a role for PEA and endogenous activation of CB₁ receptors towards suppression of SWDs. In symptomatic WAG/Rij rats, SWD activity was suppressed by administration of AEA or PEA, this latter functioning through activation of the nuclear receptor peroxisome proliferator activated receptor- α (PPAR- α) or inhibition of FAAH resulting in increased AEA levels (Lambert and Di Marzo, 1999). The anticonvulsant effect of PEA could be blocked by antagonists for either CB₁ (SR141617) or PPAR- α (GW6471) receptors, while the AEA suppression of SWDs was only sensitive to CB₁ receptor

antagonism (Citraro et al., 2013b). These findings demonstrate that PEA can act to suppress SWDs in WAG/Rij rats, which likely occurs through either direct activation of PPAR- α or indirectly through CB₁ receptor-dependent mechanisms. A second study in the WAG/Rij model from this group demonstrated that specifically localized stereotactic administration of the CB₁ receptor agonists WIN or AEA within select targets throughout the thalamocortical circuit resulted in suppression of SWDs, while injection of the antagonist SR141617 exacerbated SWD activity only when administered into the ventroposteromedial thalamic nuclei (Citraro et al., 2013a). The above findings in the WAG/Rij rat model of absence epilepsy allow for a better understanding of how maladaptive alterations in the ECS may underlie SWD generation and may lead to future research studies to develop potential therapeutic strategies of targeting the ECS in this condition.

In addition to the findings of CB₁ receptor-dependent anticonvulsant properties of cannabinoids in the mouse MES model reviewed above, a number of studies have demonstrated the seizure suppressing property of CB₁ receptor activation in other acute seizure models. In PTZ induced seizures, a model for generalized absence and myoclonic seizures (White, 1997; Loscher, 2011), earlier studies found that the anticonvulsant potential of Δ^9 -THC was limited as a result of psychotoxicity and lethality (Lemberger, 1980; Karler and Turkanis, 1981). In more recent years, a number of studies have employed acute seizure models to demonstrate CB₁ receptor-dependent anticonvulsant properties of select agonists, which include WIN, ACEA, and AEA in the PTZ model (Shafaroodi et al., 2004; Gholizadeh et al., 2007; Bahremand et al., 2009; Naderi et al., 2011, 2012; Andres-Mach et al., 2012; Vilela et al., 2013), ACEA in penicillin-induced focal seizures (Kozan et al., 2009; Cakil et al., 2011; Arslan et al., 2013), and WIN in KA-induced seizures (Rudenko et al., 2012). More in-depth discussions of the use of these models to evaluate CB₁ receptor modulation of seizure activity in regard to CB₁ receptor agonists as adjunctive AED therapy, and also the interaction of the ECS with other neuronal systems towards seizure, will be addressed further in this chapter.

Primary hippocampal neuronal cultures exposed to low-Mg²⁺ conditions result in the initiation of high-frequency and unremitting burst activity similar to that in clinical SE, and replacement of Mg²⁺ in the culture media following 3 hours of low-Mg²⁺-induced SE results in the development of a permanent hyperexcitable state in the hippocampal cultures evidenced by the presence of spontaneous recurrent epileptiform discharges (SREDs) (Sombati and DeLorenzo, 1995). WIN has been demonstrated to have anticonvulsant activity by suppressing both low-Mg²⁺-induced SE and SREDs in a CB₁ receptor-dependent manner (Blair et al., 2006). Additionally, the endocannabinoids 2-AG and methanandamide blocked low-Mg²⁺-induced SE-like activity in this hippocampal culture model in a concentration-dependent manner (Deshpande et al., 2007b). Furthermore, in the low-Mg²⁺-induced SE model, WIN maintained anticonvulsant potency while the conventional AED agent lorazepam developed pharmacoresistance to its anticonvulsant activity (Deshpande et al., 2007a). The presence of a CB₁ receptor-dependent endogenous tone towards the regulation of SRED activity following low-Mg²⁺ treatment was evident in that

antagonism of the CB₁ receptor with either SR141617 or AM251 resulted in exacerbation of seizure activity, reaching high-frequency SE-like activity (Deshpande et al., 2007c). Low-Mg²⁺-induced SE in this preparation has been shown to result in a loss of Ca²⁺ homeostasis (Pal et al., 1999), which is required for the permanent expression of SRED activity in hippocampal cultures (DeLorenzo et al., 1998). Thayer and colleagues have demonstrated in a similar hippocampal culture preparation that CB₁ receptor agonists suppress glutamatergic-driven Ca²⁺ spikes through an inhibitory G protein-dependent blockade of presynaptic glutamate release (Shen et al., 1996). In view of these findings, it is likely that a primary mechanism underlying the anticonvulsant properties of CB₁ receptor activation in low-Mg²⁺-induced SE and SREDs in hippocampal cultures, as well as the tonic CB₁ receptor-dependent regulation of epileptiform activity, involves suppression of glutamatergic-driven Ca²⁺ spikes.

CB₁ RECEPTOR AGONISTS IN ADJUNCTIVE AED THERAPY

The above review of research findings, which support a role for the anticonvulsant properties of CB₁ receptor activation, are very convincing and underscore the function of the ECS towards the regulation of both physiological and pathophysiological neuronal synaptic transmission. Although there appears to be a great potential for cannabinoids as anticonvulsant agents, their therapeutic application for seizure control is limited by both the presence of psychotropic/psychotoxic effects and the development of pharmacological tolerance. Thus, a number of studies have researched the application of cannabinoids in adjunctive therapy strategies for seizure control. Two studies utilizing both the PTZ and MES mouse models by Czuczwar and colleagues have generated a number of papers on the adjunctive effect of cannabinoids with classical and second-generation AEDs (Luszczki et al., 2006, 2010). In two papers using the mouse MES model of partial seizures, the highly selective CB₁ receptor agonist arachidonyl-2'-chloroethylamide (ACEA) plus phenymethylsulfonyl fluoride (PMSF) was evaluated alone or in combination with the AEDs valproate, carbamazepine, lamotrigine, oxcarbazepine, phenobarbital, phenytoin, and topiramate for protection against MES-induced seizures as well as for memory (step-through passive-avoidance task), muscle strength (grip test), motor impairment (chimney test), and brain and free plasma levels of AEDs. At the subeffective dose of ACEA (2.5 mg/kg) + PMSF (30 mg/kg), no changes in MES-induced seizure parameters were observed, while the combination of ACEA + PMSF with the AEDs resulted in an enhancement of both valproate and phenobarbital as indicated by a significant decrease in the ED₅₀ dose required for anticonvulsant activity without changes in adverse effects (strength and memory tests). Analysis of brain and free blood plasma levels of the AEDs revealed an increase in valproate and no change in phenobarbital, demonstrating a pharmacokinetic and pharmacodynamic effect, respectively, with the co-administration of ACEA + PMSF. Thus, the CB₁ receptor agonist ACEA may prove to be an effective adjunctive therapy in combination with phenobarbital for the treatment of clinical partial seizures.

Two additional studies in the MES model from this group evaluated the combinatorial effect of the cannabimimetic WIN on the anticonvulsant efficacy of both classical and second-generation AEDs (Luszczki et al., 2011b, 2013). In a dose–response effect of WIN alone in MES-induced seizures, 15 mg/kg showed some protection while the doses of 2.5, 5, and 10 mg/kg were subeffective; thus, the three lower doses in combination with the four classical AEDs phenytoin, phenobarbital, valproate, and carbamazepine were evaluated for protection against MES-induced seizures as well as for memory, muscle strength, motor impairment, and brain and free plasma levels of AEDs. At the 10 and 5 mg/kg doses, WIN significantly enhanced the anticonvulsant properties (decrease in ED₅₀) of all AEDs, and valproate and carbamazepine, respectively, while having no effect on total brain levels of anti-seizure drugs. Yet, any combination of each AED with either the 5 or 10 mg/kg doses of WIN was not devoid of an increase in acute adverse effects as indicated by alterations in one or more tests (i.e., decrease in muscle strength and memory, or increase in motor impairment), thus occluding any potential for the clinical efficacy of WIN in combination with these AEDs in seizure control (Luszczki et al., 2011b). Conversely, in combination with the second-generation AEDs lamotrigine, oxcarbazepine, pregabalin, and topiramate, WIN 5 mg/kg significantly enhanced the anticonvulsant effects of all AEDs with the exception of oxcarbazepine, without altering total drug brain levels or acute adverse effects. Thus, the researchers conclude that at the lower dose of 5 mg/kg, WIN may have potential benefits in combination with select second-generation AEDs to enhance anticonvulsant properties without increasing the risk of acute adverse effects (Luszczki et al., 2013).

In the PTZ mouse model of generalized absence and myclonic seizures, two studies from the above group demonstrated similar findings that ACEA significantly enhanced the anticonvulsant activity of phenobarbital with no adverse effects (Andres-Mach et al., 2012), while WIN increased anticonvulsant efficacy of classical AEDs but showed no clinical potential due to adverse effects (Luszczki et al., 2011a). Naderi et al. (2008) evaluated the effects of the CB₁ receptor agonist WIN, antagonist AM251, the endocannabinoid uptake inhibitor AM404, and the FAAH inhibitor URB597 on the anticonvulsant efficacy of diazepam (DZ) in the mouse MES model. Following determination of the ED₅₀ for protection against MES seizures (equal to 0.43 mg/kg and 1.49 mg/kg for DZ and WIN alone, respectively), the authors demonstrated that a ratio of 3:1 (DZ:WIN) resulted in a synergistic enhancement of anticonvulsant activity, while the ratios of 1:1 and 1:3 resulted in an additive effect only. AM251 and AM404 showed no effects on MES seizures either alone or in combination with DZ. Administration of URB597 alone resulted in significant protection against MES seizures, while it resulted in antagonizing anticonvulsant efficacy when combined with DZ. The researchers conclude that the synergistic increase in protection against MES seizures with the 3:1 ratio of DZ and WIN may prove to be a potential therapeutic strategy and that the antagonistic effects of FAAH inhibition on DZ anticonvulsant efficacy was likely attributed to an increased endocannabinoid tone at GABAergic synapses.

TARGETING ENDOCANNABINOID DEGRADATION FOR SEIZURE CONTROL

Another strategy for exploiting the role of the ECS in the control of the neuronal hyperexcitability associated with seizures and epilepsy is the targeting of degradation mechanisms of AEA and 2-AG to increase endocannabinergic tone within the synapse. In the brain, the actions of the two endocannabinoids, following their on-demand synthesis and release, are rapidly terminated by the degradation enzymes FAAH and MAGL (Piomelli, 2014). Bahr and colleagues have presented findings from three studies using synthetic inhibitors specifically targeted to either FAAH or to both FAAH and MAGL in both *in vitro* organotypic hippocampal slice culture and *in vivo* rat models of KA-induced excitotoxicity and seizures (Karanian et al., 2007; Naidoo et al., 2011, 2012). The first two studies evaluated the irreversible and reversible FAAH inhibitors AM374 and AM5206, respectively. Both compounds demonstrated select and potent inhibition of FAAH as indicated by a 2.5 to 4.8-fold increase in brain AEA levels, activation of the MAPK/ERK signaling pathway, or select inhibition of FAAH over MAGL in a fluorometric assay. In KA-treated rats, both AM374 and AM5206 administration resulted in a significant decrease in seizure scores. Western immunoblot and histochemical analysis demonstrated that both AM374 and AM5206 also blocked KA-induced alterations in markers for neuronal degeneration and hippocampal pyramidal cell loss in rat and hippocampal slice cultures, respectively. The CB₁ antagonist AM251 blocked the protective effects of AM374 in the rat, with levels of pyramidal cell loss and alterations in neuronal degeneration markers equal to that of KA-treated alone controls, demonstrating a CB₁ receptor-dependent mechanism involved in the protection afforded FAAH inhibition (Karanian et al., 2007; Naidoo et al., 2011). A third study from this group compared the protective properties of the hydrolase inhibitor AM6701, which demonstrated equipotent inhibition of both FAAH and MAGL, with those of the compound AM6702, which demonstrated 44-fold higher selectivity for inhibition of FAAH over MAGL (Naidoo et al., 2012). Utilizing the *in vivo* and *in vitro* models (rat and hippocampal slice culture) and the same experimental strategy of the above studies, both compounds were protective against KA-induced seizures, hippocampal pyramidal cell loss, and excitotoxicity-induced changes in neuronal degeneration markers, although the dual inhibition of both FAAH and MAGL by AM6701 resulted in a higher overall protection than that achieved with the selective FAAH inhibitor AM6702. Additionally, behavioral tests demonstrated that dual inhibition of FAAH and MAGL afforded better protection than selective inhibition of FAAH against KA-induced decreases in performance of balance and coordination.

In acute PTZ-induced seizures in rat and mouse, Naderi et al. (2011, 2012) showed that inhibition of either FAAH or MAGL can confer protection against chemoconvulsant-induced seizures. Interestingly, the FAAH inhibitor URB597 showed protection in the PTZ-treated mouse when systemically administered via the intraperitoneal route, while i.c.v. administration in the rat yielded no protective effects suggesting a possible route of administration or species dependency for protection.

Contrary to the above studies demonstrating a protective/anticonvulsant effect of FAAH inhibition, Cravatt and colleagues (Clement et al., 2003) developed a genetic knockout mouse strain devoid of FAAH [FAAH(−/−)] and with over 10-fold higher levels of brain AEA, and found that these mice demonstrated increased seizure severity following KA or bicuculline. Pre-administration of exogenous AEA in FAAH(−/−) mice resulted in a further exacerbation of seizure activity and increased hippocampal cell loss following KA or bicuculline, which was shown to be CB₁ receptor dependent in bicuculline-treated animals. Several possibilities may explain the conflicting results of the above study with those demonstrating anticonvulsant properties of FAAH inhibition, which may include a compensatory shift of CB-dependent mechanisms favoring regulation of GABAergic synaptic transmission or a possible increase in AEA availability at the transient receptor potential vanilloid type 1 (TRPV1) receptors, which may evoke pro-convulsant actions in FAAH(−/−) mice, the latter of which will be further discussed below. Thus, targeting the endocannabinoid degradation machinery to enhance the anticonvulsant/protective effects of the ECS may prove an effective therapeutic strategy for the control of seizures and epilepsy. Yet, this approach should be considered carefully in that inhibition of both FAAH and MAGL is capable of increasing endocannabinergic tone to levels resulting in CB₁ receptor-dependent behavioral effects comparable to those of exogenously administered cannabinoids such as Δ^9 -THC (Long et al., 2009; Wise et al., 2012).

CB₁ RECEPTOR ANTAGONISM AS A PROPHYLACTIC TREATMENT FOR SEIZURES AND EPILEPSY

To this point in this chapter, the findings discussed primarily support a role for CB₁ receptor activation towards anticonvulsant activity, while the opposite is observed for antagonism of this pathway, as indicated by exacerbation of seizure discharges. However, there is evidence that supports a paradoxical strategy for targeting the ECS during the early stages of pathological alterations in neuronal physiology, whereby antagonism of CB₁ receptor may prevent the development of the epileptic phenotype (Armstrong et al., 2009). Soltesz and colleagues carried out electrophysiological analysis of hippocampal slices from rat pups that underwent hyperthermia-induced seizures (febrile seizures) and found an enhancement of DSI that resulted from an increase in the number of CB₁ receptors on terminals of CCK+ GABAergic interneurons (Chen et al., 2003). This maladaptive plasticity of the ECS with febrile seizures was persistent in that both the potentiation of DSI and increased CB₁ receptors on inhibitory terminals were still present 5 weeks following the hyperthermic insult. From these findings, the authors hypothesized that the CB₁ receptor-dependent enhancement of DSI may contribute to the neuronal hyperexcitability following febrile-induced seizures. A single administration of receptor agonist SR141617 either prior to or within 2 minutes following the hyperthermia treatment prevented the enhancement of DSI, up-regulation of CB₁

receptor on GABAergic terminals, and the persistent neuronal hyperexcitability observed in this model of febrile seizures (Chen et al., 2007). Utilizing another brain insult model of long-lasting neuronal hyperexcitability, this same group demonstrated that a single administration of SR141617 within 2 minutes following lateral fluid percussion-induced traumatic brain injury (TBI) in P20 rats prevented the persistent increase in seizure susceptibility associated with this model of brain insult (Echegoyen et al., 2009).

In the DHPG (type I metabotropic receptor agonist) model of epileptiform activity in hippocampal slices, Karr et al. (2010) demonstrated that co-incubation of SR141617 with DHPG blocked the induction of LTD/decrease release probability as well as a shift to epileptiform activity. They propose that DHPG results in a CB₁ receptor-dependent suppression of synaptic transmission and changes in membrane excitability leading ultimately to network synchronization. SR141617 had no effect in hippocampal slices with established DHPG-induced epileptiform activity, suggesting that the time window for the CB₁ receptor-dependent contribution to, or prophylactic blockade of, neuronal hyperexcitability takes place during the early acquisition phase in this model.

Dudek et al. (2010) evaluated the effect of SR141617 administration in an adult rat model of KA-induced TLE and found that CB₁ receptor antagonism immediately following the first KA-induced seizure had no effect on the development of TLE. They concluded that the discrepancy between their findings and those of the Soltesz study may be due to differences of developmental stage, degree of neuronal injury/death, or model differences.

The above studies suggest that the efficacy of utilizing CB₁ receptor antagonist in a prophylactic manner to inhibit maladaptive changes in neuronal plasticity leading to a hyperexcitable state shows promise, and may be dependent on several factors that include timing of administration, developmental stage, or the mode of neuronal insult, which may result in either an increased excitatory/excitotoxic synaptic transmission (DSE) or alter inhibitory mechanisms (DSI) driving the pathophysiological network synchronization.

Another strategy for implementing antagonism of CB₁ receptors for the control of seizures is supported by experimental findings that have been shown in the opiate (Crain and Shen, 1995; Wang et al., 2005) and endocannabinoid (Paquette et al., 2007) systems, wherein ultra-low doses of antagonists at G protein-coupled receptors can both enhance pharmacological efficacy and block the development of pharmacological tolerance to agonist exposure. The underlying mechanism of this phenomenon is believed to involve the antagonists blocking a small subpopulation of stimulatory G_s protein-coupled receptors that have been shown to mediate the paradoxical (stimulatory) effects with both opioid (Wang et al., 2005) and CB₁ receptors (Glass and Felder, 1997; Felder et al., 1998; Hampson et al., 2000; Chen et al., 2010). Thus, this strategy has been investigated in the mouse models of PTZ-induced myoclonic (PTZ i.v. infusion) and grand mal (PTZ systemic administration) seizures, whereby ultra-low doses (pico/nanogram/kg ranges) of the CB₁ receptor antagonist AM251 resulted in an increase in the anticonvulsant potency of

the CB₁ receptor agonist ACEA 100–100,000 fold (Gholizadeh et al., 2007). The therapeutic approach of augmenting the anticonvulsant efficacy of CB₁ receptor agonists via adjunctive treatment with ultra-low doses of antagonists may allow for the development of novel therapeutic strategies that can benefit from maintaining CB₁ receptor-dependent seizure control with lower dose regimens of cannabinoids without the unwanted psychotropic effects or the development of tolerance.

ECS INTERACTION WITH OTHER BRAIN SYSTEMS IN SEIZURE AND EPILEPSY

In addition to control of glutamatergic and GABAergic transmission, the ECS interacts at multiple levels with other neuronal signaling systems, which include the nitroergic, opioidergic, noradrenergic, dopaminergic, serotonergic, and cholinergic (Carney et al., 2009; Kano et al., 2009; Welch, 2009; Carvalho et al., 2010; Howlett et al., 2010; Castillo et al., 2012; Ohno-Shosaku et al., 2012). Additionally, many of these signaling systems/transmitters can modulate endocannabinergic tone via binding to their respective G_{q/11} protein-coupled receptors (Katona and Freund, 2012) and the CB₁ receptor is capable of interacting with an array of associated proteins (Howlett et al., 2010), as well as undergoing heterodimerization with other GPCRs (Rozenfeld et al., 2012) to increase the range of endocannabinoid-mediated physiological/pathophysiological responses. Thus, a number of studies have investigated the role of cross-system interactions with the ECS in regard to regulation/dysregulation of neuronal hyperexcitability and seizure discharge. In the mouse model of PTZ-induced seizure, increase in release of nitric oxide (NO) with L-arginine administration enhanced the anticonvulsant efficacy of the CB₁ receptor agonist ACEA, while inhibition of the nitric oxide synthesizing enzyme (NOS) with either N^G-nitro-L-arginine methyl ester (L-NAME) or 7-nitroindazole (7-NI) resulted in a decrease in anticonvulsant efficacy of ACEA. Additionally, inhibition of NOS enhanced the pro-convulsant effect of the CB₁ receptor antagonist AM251 (Bahremand et al., 2009). Thus, activation of the NO pathway results in a synergistic interaction with the ECS in control of seizure discharge. Using the same experimental design and seizure model, this same group demonstrated that co-administration of the α -2-adrenoreceptor agonist clonidine (0.1 and 0.5 mg/kg), which alone displayed marginal anticonvulsant properties at higher doses (1.0 and 5.0 mg/kg), and the antagonist yohimbine, resulted in suppressing and enhancing the anticonvulsant effect of ACEA, respectively. Additionally, low doses of clonidine resulted in increasing the pro-convulsant effect of CB₁ receptor antagonism with AM251 (Shafaroodi et al., 2013).

In the rat model of PTZ-induced seizures, Naderi et al. (2011) demonstrated that i.c.v. administration of either WIN or the GABA_A agonist isoguvacine (IGN) alone produced anticonvulsant effects, while their combined administration resulted in no anticonvulsant activity. The FAAH inhibitor URB597 alone had no effect on PTZ-induced seizures, but acted to block the anticonvulsant effect

of IGN. The researchers hypothesized that IGN may be canceling out an inhibitory feedback mechanism (self-inhibition) of GABAergic interneurons that requires CB₁ receptor-dependent induction of DSI. This inhibitory feedback mechanism of GABAergic interneurons has been shown to occur in layer V of the rat neocortex, where they project on to the dendrites of glutamatergic pyramidal neurons and regulate the intrinsic excitability of this network (Bacci et al., 2004).

In a hippocampal culture preparation that displays high-frequency spike neuronal discharge activity following withdrawal from the cannabimimetic WIN (1 μ M, 24 h), Deshpande et al. (2011) demonstrated that a profound agonist-induced down-regulation of CB₁ receptors was associated with a suppression of GABAergic transmission as a result of decreased GABA_A receptor channel number, and probably contributed to the observed increase in neuronal excitability in this preparation.

Messer and Levine (2012) utilized a mouse hippocampal slice preparation to demonstrate that the anticonvulsant efficacy of CB₁ receptor agonism was dependent on the conditions used to evoke epileptiform activity, wherein WIN was effective at suppressing low-Mg⁺⁺-induced seizures while ineffective with low-Mg⁺⁺/high K⁺-evoked seizures. The presynaptic GABA_B receptor antagonist baclofen partially restored WIN's ability to suppress seizures in the low-Mg⁺⁺/high K⁺ conditions, which suggested that an interaction between presynaptic GPCRs may contribute to their observed results. They conclude that such opposing interactions between CB₁ and GABA_B receptors towards the control of excitatory synaptic transmission may underlie the paradoxical effects that cannabinoids produce in different models of epilepsy.

An increasing amount of evidence supporting an interaction or “cross-talk” between the opioid and endocannabinoid systems has been generated, which is especially true in regard to systems involved in cognition, reward, dependence, and tolerance (Fattore et al., 2004; Robledo et al., 2008). Additionally, synergistic or antagonistic interactions between the opioid and endocannabinoid systems may be targeted in the development of more efficacious pharmacotherapy in pain management (Welch, 2009) or drug addiction and relapse (Fattore et al., 2007), respectively. The findings from several studies suggest that such approaches may also hold true in the control of seizures. In PTZ-induced clonic seizures in the mouse, the CB₁ receptor agonist ACPA has anticonvulsant activity, whereas the opioid agonist morphine demonstrates biphasic activity being anticonvulsant at low doses (0.5–1.0 mg/kg) and pro-convulsant at higher doses (30 mg/kg). The “cross-talk” between these systems was evident in that CB₁ (AM251) and opioid (naltrexone) receptor antagonists blocked the anticonvulsant activity of low-dose morphine and ACPA, respectively, while the combination of the two agonists was synergistic towards seizure control. Interestingly, the pro-convulsant effect of high-dose morphine (30 mg/kg) was blocked by CB₁ receptor antagonism, suggesting an additional level of interaction between the opioid and endocannabinoid systems in seizure control (Shafaroodi et al., 2004). A more recent study from the same group took the strategy of using an ultra-low dose of receptor antagonists, discussed in the section above, to further evaluate interactions between these two systems in the

mouse PTZ seizure model. Co-administration of an ultra-low dose of the opioid antagonist naltrexone (1–500 pg/kg) significantly potentiated the anticonvulsant effect of the CB₁ receptor agonist ACEA, as indicated by a 10–100-fold increase in efficacy. The synergy between naltrexone and ACEA was still dependent on CB₁ receptor activation in that the addition of AM251 blocked the anticonvulsant effect (Bahreman et al., 2008). Thus, the above studies suggest that drawing on the interactions between the opioid and endocannabinoid systems may allow for the development of novel therapeutic strategies in seizure control.

The endovanilloid and endocannabinoid systems cross paths in that the endocannabinoid AEA is a full agonist at the TRPV1 channel, a non-selective cation channel that has been termed the “capsaicin receptor” due to its responsiveness to the constituent that is present in hot chili peppers, and that has been characterized to regulate nociception on peptidergic sensory neurons induced by either thermal or chemical stimuli. Additionally, TRPV1 has been demonstrated to reside in the CNS within cortical, cerebellar, olfactory, midbrain, and hindbrain regions (Starowicz et al., 2007).

Two studies a potential role for TRPV1 activation in ECS-mediated regulation/dysregulation of acute and epileptic seizure discharge. Bhaskaran and Smith (2010b) carried out electrophysiological studies in dentate granule cells of hippocampal slices from TLE mice and showed that application of the TRPV1 agonist capsaicin significantly increased EPSP frequency, while having no effect in control slices. Additionally, in TLE slices, AEA was either pro-convulsant or anticonvulsant against increased EPSP frequency when co-incubated with specific antagonists for either CB₁ receptor or TRPV1, respectively, and immunoblot analysis revealed a significant increase in TRPV1 levels in the epileptic dentate gyrus. In the mouse model of acute PTZ-induced seizures, Manna and Umathe (2012) demonstrated that i.c.v. administered AEA showed biphasic properties being anticonvulsant at low doses (10, 20, or 40 µg/mouse) and pro-convulsant at high doses (80 or 100 µg/mouse), while TRPV1 antagonism with capsazepine enhanced the seizure suppressive effect of AEA even at the high doses. Dose-dependent increases in endogenous AEA tone with either AM404 or URB597 demonstrated comparable biphasic effects on PTZ seizures to exogenous AEA, as well as enhancement in their protective effects with TRPV1 antagonism at all doses studied. The authors concluded by demonstrating that either TRPV1 agonism (capsaicin) or antagonism (capsazepine) alone resulted in exacerbation of or protection against PTZ-induced seizures, respectively.

Thus, the above studies indicate that either high doses of exogenous AEA or increases of endogenous AEA to levels that would “spill over” to activate the TRPV1 channel would produce pro-convulsant effects, and may explain some of the paradoxical effects of cannabinoids in different seizure models. Interestingly, the increase in seizure susceptibility observed in the abovementioned study in FAAH knockout mice with a greater than 10-fold increase in brain AEA levels (Clement et al., 2003) could be the result of TRPV1 channel activation. In light of these findings, the approach of enhancing endocannabinoid tone, especially that of AEA, as a strategy for suppressing excessive neuronal excitability should be carefully considered.

CANNABINOID-BASED MEDICINES: IMPLICATIONS FOR THEIR PROLONGED USE IN CLINICAL SEIZURES AND EPILEPSY

Evidence put forth in the previous sections supports that the ECS performs as an essential regulator of neuronal synaptic transmission and functions as an unending defense mechanism against excitotoxicity in the brain primarily via activation of CB₁ receptors on asymmetric excitatory terminals. Additionally, over the last decade, the findings from many experimental studies employing a variety of *in vivo* and *in vitro* models of seizures and epilepsy clearly indicate that either exogenous cannabinoids or increasing endogenous endocannabinoid tone can suppress seizure activity through activation of presynaptic CB₁ receptors. Although anecdotal data have suggested beneficial effects in the use of CBMs for their anti-convulsant properties in human seizures and epilepsies, to date there is limited scientific evidence suggesting clinical efficacy for this approach. A telephone survey of 136 patients under management by a Canadian tertiary care epilepsy center obtained data on individual levels of marijuana use in association with the state of their clinical condition, as well as each patient's perception and knowledge of the possible risks/benefits of self-medicating with marijuana (Gross et al., 2004). Although 41% of the surveyed patients had some knowledge of a potential risk/benefit of marijuana use with epilepsy, there were 28 (21%) epileptic patients who were active marijuana users, of which 68% and 54% perceived that self-medication improved epileptic seizure severity and frequency, respectively. The overall findings suggest that marijuana use by epileptics is higher than that of the general population, and is independent of factors such as age, gender, and employment status. In regard to the state of their clinical condition, patients with longer duration of disease and increased seizure frequency were more likely to self-medicate with marijuana, which, although inconclusive, may result from either a tendency to search out alternative treatments for their condition or a possible causal relationship between level of marijuana use and increased seizure frequency (Gross et al., 2004).

A more recent review of cannabinoid use in clinical epilepsy carried out an extensive search of six databases and found that no reliable conclusions can be made regarding the clinical efficacy of cannabinoid use for the management of epilepsy (Gloss and Vickrey, 2012), although a follow-up commentary generally agreed with their findings, but suggested that the non-psychotropic cannabinoid CBD may prove to have some beneficial effects in the treatment of seizure disorders (Miller, 2013).

The subject of CBMs for the treatment of epilepsy and other ailments has gained substantial momentum and positive attention over the last decade, which has led to a number of states within the USA approving their use. Interestingly, there have been isolated case reports that suggest the contrary following prolonged administration of CBMs. In one paper presenting two separate cases of patients with a history of cannabis use, one epileptic patient who was self-medicating with *Cannabis* was admitted to the epilepsy monitoring unit and

experienced an increase in seizure frequency upon withdrawal from *Cannabis* (Hegde et al., 2012). The second case involved an undiagnosed patient who had experienced amnesic episodes with loss of consciousness and unexplained injuries over a 2-year period and had a 40-year history of smoking six to eight marijuana cigarettes per day; upon admission to the epilepsy monitoring unit (EMU) and cessation of cannabis use, within 24 hours the patient developed SE consisting of five seizures in a 12-hour period, which were brought under control with anticonvulsant therapy. The patient stated that his experience in the EMU was similar to the amnesic episodes experienced over the previous 2 years (Hegde et al., 2012). In a clinical trial evaluating long-term use of a CBM (Sativex®) for the treatment of spasticity associated with multiple sclerosis, treatment resulted in subjective symptomatic relief in a majority of patients, although of the 137 patients in the study, four experienced first-ever seizures (Wade et al., 2006). The researchers concluded that this treatment regimen shows promise for symptomatic relief in multiple sclerosis, but further evaluation of the effects of long-term CBM on seizure threshold is warranted.

Prolonged exposure to cannabinoids results in the development of tolerance as indicated by a progressive decrease in their pharmacological efficacy (Martin et al., 2004; Gonzalez et al., 2005), which, in the CNS, is primarily attributed to CB₁ receptor desensitization or down-regulation (Sim-Selley, 2003). The development of tolerance in humans, following chronic and heavy cannabis smoking or ingesting high doses of cannabinoids, has been demonstrated both behaviorally (Gorelick et al., 2013) and at the cellular level with a down-regulation of CB₁ receptors (Villares, 2007; Hirvonen et al., 2012; Ceccarini et al., 2013). Additionally, upon cessation of using cannabinoids, heavy users who have become dependent on cannabis as defined in the DSM-IV (American Psychiatric Association, 2000) experience a withdrawal syndrome further confirming an adaptation of the ECS in humans with chronic cannabinoid exposure. With the current availability of many strains of *Cannabis sativa* and cannabinoid-containing edibles that contain potent levels of Δ^9 -THC, there has been a substantial increase in the number of adolescents and adults being treated for cannabis dependence and withdrawal in the last 10–15 years (Budney and Hughes, 2006). The concern in regard to the current chapter is that unmanaged self-treatment with CBMs by epileptics may lead to the development of tolerance as a result of CB₁ receptor adaptation, a scenario that may result in exacerbation of the epileptic condition upon withdrawal. Experimental findings in an *in vitro* model of epileptiform discharges in hippocampal neuronal cultures demonstrated an acute CB₁ receptor-dependent anticonvulsant effect of WIN (Blair et al., 2006), while prolonged exposure resulted in increase in seizure frequency, which was directly correlated to concentration of WIN and level of agonist-induced CB₁ receptor down-regulation (Blair et al., 2009). Findings from both *in vivo* and *in vitro* preparations of epileptiform activity have demonstrated that antagonism of CB₁ receptors with SR141617 results in increased severity of seizure discharge, further confirming the essential role that an intact CB₁

receptor pathway has in the management of seizure discharge in the epileptic phenotype (Blair et al., 2009; Deshpande et al., 2007c, 2011; Wallace et al., 2002, 2003). This effect has also been observed in an isolated clinical case in a patient with a history of generalized idiopathic epilepsy who had been seizure free for 20 years, and experienced the recurrence of partial seizures following the initiation of rimonabant therapy for the treatment of obesity; following termination of rimonabant treatment, the seizure activity ceased (Braakman et al., 2009). The development of novel therapeutic strategies aimed at modulating the ECS shows much potential in the management of seizures and epilepsy, as well as treating other neurological disorders, although in light of the above findings, consideration should be made regarding the effects of prolonged cannabinoid exposure and subsequent adaptations of the ECS, which may result in undermining its intrinsic defense properties in the brain.

CONCLUSION AND FUTURE DIRECTIONS

The complexity and sensitivity of the brain ECS underlies one of its essential functions to maintain an on-demand fine-tuning of brain-network communication, and is evident in its ability to regulate neuronal transmission both temporally and spatially throughout the CNS. It is within these attributes that the ECS plays a fundamental role in mediating neuronal processes, which underlie learning and memory, anxiety, depression, addiction, appetite and feeding, pain, neuronal excitability, and protection (Kano et al., 2009). Thus, in a pathophysiological state whereby neuronal mechanisms become compromised and hyperexcitability ensues, the ECS is triggered to intervene resulting in the on-demand synthesis and release of lipid-derived transmitters that act to rein in overzealous neuronal discharges much like an operator's response to a runaway stagecoach. In this sense, the ECS functions in a suppressive manner and could be thought of as the "emergency brakes" of the brain. The intent of this chapter in the previous sections was to briefly summarize the enormous amount of progress made in experimental research over the last two decades towards understanding the brain ECS and more specifically present findings that clearly demonstrate maladaptive changes that occur with this system in association with seizures and epilepsy, and how these alterations may be contributing in either a compensatory or causative manner towards the expression of these neuropathological conditions. To this end, much experimental work has focused on targeting select aspects of the brain ECS as a means for developing novel therapeutic strategies for the treatment of seizure disorders.

The findings reviewed in the previous sections from a multitude of experimental *in vivo* and *in vitro* models of seizure and epilepsy provide clear evidence that activation of presynaptic CB₁ receptor suppresses epileptiform activity. The actions of many experimental cannabimimetics, as well as the primary natural constituent of *Cannabis sativa* Δ^9 -THC, produce both their anticonvulsant and psychotropic/

psychotoxic effects via a CB₁ receptor-dependent mechanism. Thus, there has been a tremendous amount of research focused on the therapeutic potential for exogenous CB₁ receptor agonists for the treatment of seizure disorders and other neurological maladies. Drawbacks for the exclusive use of exogenously administered cannabinoid compounds as long-term anticonvulsant agents include their ubiquitous and non-specific CB₁ receptor agonism throughout the CNS and psychotropic/psychotoxic effects, and the development of pharmacological tolerance. A potential therapeutic strategy for exploiting the anticonvulsant activity of cannabinoids has been in their application in adjunctive therapy with classical AEDs. These experimental findings indicate that this combined therapeutic approach may allow for a significant lowering of the effective doses needed of each agent for seizure control, thus resulting in the potential benefit of decreasing dose-dependent adverse effects.

Another adjunctive approach has been investigated by taking advantage of the “cross-talk” between the ECS and the opioid system for seizure control, whereby initial experimental findings demonstrate that the combined pharmacological interventions of these two systems can result in a greater than 100-fold increase in efficacy for each individual agent. The above adjunctive approaches may hold greater promise for utilizing CB₁ receptor agonists for the treatment of seizures and epilepsy. Contrary to a CB₁ receptor agonism approach to seizure suppression, other evidence suggests that ECS-mediated regulation of synaptic transmission immediately following a brain insult may contribute to the development of maladaptive plasticity changes that ultimately result in recurrent seizure discharge. To this end, a single administration of CB₁ receptor antagonists immediately following a brain insult has been shown to act in a prophylactic manner by blocking the development of the pathophysiology that underlies the epileptic phenotype (Chen et al., 2007; Echegoyen et al., 2009).

A more plausible approach to targeting the ECS for seizure control is underscored by the unquestionable evidence from many experimental studies that in the neuropathophysiological state of recurrent seizure activity, there is an increase in tone of the endogenous “defensive” response of on-demand synthesis and release of the endocannabinoids AEA or 2-AG, which act to restrict further seizure discharge via their actions at presynaptic CB₁ receptors. Thus, the use of highly specific pharmacological inhibitors of select metabolic enzymes (FAAH and MAGL) or reuptake mechanisms (Piomelli, 2014), would act to prolong the intrinsic response of the ECS to seizure discharge. Targeting these specific aspects of the ECS may allow for enhancing the “defensive” response to hyper-synchronous neuronal discharges that are specifically confined both temporally and spatially, and may be effective in treating a wide spectrum of epilepsy disorders wherein seizure activities arise from regionally diverse origins throughout the complex neuronal-circuitry pathways within the brain.

Evidence suggesting a role for the ECS in regulation of seizure discharge through CB₁ receptor-independent pathways is supported by findings demonstrating anticonvulsant properties of the non-psychotropic phytocannabinoids CBD, CBDV, and THCV, which are devoid of activity at CB₁ receptors. From this

group, CBD has been the most extensively studied in seizure and epilepsy research and has been evaluated in a clinical trial, which indicated its promising therapeutic potential as an anticonvulsant (Cunha et al., 1980). Additionally, some anecdotal findings indicate that select populations of epileptic patients may benefit from using CBMs enriched in CBD for symptomatic control of their disease (Porter and Jacobson, 2013). The cellular mechanism(s) involved in the seizure suppressive properties of this group of phytocannabinoids is still unclear; however, a number of physiological effects of CBD have been elucidated, which may eventually contribute to a better understanding of their anticonvulsant effects and interactions they have with the ECS. Although CBD shows virtually no activity at CB₁ receptors, there is evidence that it modulates a number of other associated components of the ECS, which allows us to segue into a brief discussion of potential future discoveries of ECS-mediated control of neuronal excitability. CBD has been shown to regulate a number of systems in association with endocannabinoid signaling, which include the TRP family of protein channels, GRP55 and 5HT_{1A} receptors (Hill et al., 2012b; Mechoulam et al., 2007). Recent discoveries have given support to the possible existence of non-CB₁/CB₂ receptors as indicated by the assembly of a list of potential candidate proteins that may act as a “CB₃” receptor. Future research endeavors will more than likely elucidate additional ECS-associated systems, which may allow for a more thorough understanding of its regulation of both physiological and pathophysiological neuronal processes. Further discussion on this subject is beyond the scope of this chapter, although a consortium of international investigators has published an extensive review on potential cannabinoid receptors and ligands “beyond” CB₁ and CB₂ receptors (Pertwee et al., 2010).

This chapter would not be complete without briefly mentioning the involvement of the brain ECS in regulating the physiological processes of another “non-neuronal” cell type with a gargantuan presence within the CNS called glial cells. Experimental studies have presented evidence that establishes a presence, in some cases activity dependent, of CB₁, CB₂, and CB-like receptors on microglia, astrocytes, and astrocytoma brain cells, and that activation of these receptors is involved in controlling pro-inflammatory processes within these glial cell types (Stella, 2010). Inflammatory processes are thought to play an essential role in the process of epileptogenesis following an initial brain injury, which may allow a time window for implementation of prophylactic anti-inflammatory therapeutics to block the development of the pathophysiology that ultimately results in the epileptic phenotype (Vezzani et al., 2013). Thus, targeting ECS-mediated regulation of pro-inflammatory responses of glial cells immediately following a brain insult may allow for a novel therapeutic strategy towards the prevention of recurrent seizure disorders. Experimental findings have demonstrated a role for CB₁ receptor-dependent regulation of astroglial cells on the formation of working memory, suggesting the involvement of a CB₁ receptor–glial cell pathway in the synaptically-driven process of LTP (Han et al., 2012). More pertinent to this chapter were findings in an *in vitro* model of 4-AP-induced epileptiform activity in hippocampal slice cultures that demonstrated that activation

of CB₁ receptor on astrocytes was shown to result in a Ca²⁺-dependent increase in epileptiform activity, which was most likely attributed to increased release of glutamate (Coiret et al., 2012). Thus, endocannabinoid-mediated control of glial cell function during pathophysiological conditions in the brain may open avenues for the development of novel therapeutic strategies for the treatment and possibly prevention of the epileptic condition.

The medicinal use of *Cannabis* to modulate the ECS to treat seizures has been occurring, without any knowledge of its existence, for many hundreds of years, being first noted in the 15th century in Baghdad to treat the epileptic son of a caliphate counselor (Mechoulam, 1986). Since its discovery over the past 20 years, the importance of the ECS's essential role in mediating a multitude of physiological processes throughout many organ systems is made evident by the ever-increasing number of experimental publications within the scientific literature. As a result of exceptional studies in basic neuroscience research, great strides have been made towards a better understanding of the complex nature of the way in which the brain ECS can be rapidly summoned for the on-demand synthesis and release of the ECs AEA and 2-AG to regulate both physiological and pathophysiological neuronal activity. Substantial evidence from studies in both *in vivo* and *in vitro* models of seizure and epilepsy clearly demonstrates that the ECS acts, in both a phasic and tonic manner, to control hyperexcitable neuronal activity primarily via CB₁ receptor activation, and alterations occur within this endogenous system likely to support compensatory mechanisms. Basic scientific research studies that target different components of the brain ECS with specific pharmacological agents have shown great promise towards the development of novel therapeutic strategies for the control and possible prevention of epileptic seizure disorders.

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Endocannabinoids and migraine

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ENDOCANNABINOID SYSTEM AND MIGRAINE

Cannabis has been used for recreational and medicinal purposes throughout the world for many centuries. While best known for its psychotropic effects, cannabis has long been known to have analgesic, immunomodulatory, and anti-inflammatory effects. The use of cannabis as a symptomatic and prophylactic treatment of migraine was highly regarded in the 19th century. Patient self-report surveys on marijuana have been positive for a variety of symptoms including headache (Schnelle et al., 1999). El-Mallakh described three long-term daily marijuana users who developed headache after cessation of marijuana, matching clinical experience of headache being a symptom of withdrawal (El-Mallakh, 1987). Robbins and colleagues described a man with cluster headache who was able to abort an attack in 5 minutes with marijuana inhalation (Robbins et al., 1999).

Cannabinoids may offer significant “side benefits” beyond analgesia. These include anti-emetic effects, well established with Δ^9 -tetrahydrocannabinol (THC) (Pertwee, 2012). In 1915, Sir William Osler, the acknowledged father of modern medicine, proposed the treatment of migraine with *Cannabis indica* (Osler and McCrae, 1915), and the following year Dr. Dixon, Professor of Pharmacology at King’s College and the University of Cambridge, reported the therapeutic effects of smoked cannabis for headache treatment (Ratnam, 1916). The botanical origin of cannabis has been debated to be as far east as China, but most experts suspect it to be in Central Asia, possibly in the Pamir (Camp, 1936). Use of marijuana has long been known to cause an increase in relaxation and euphoria along with, at times, memory impairment. Marijuana can be associated paradoxically with anxiety and dysphoria in some people and this relates to a biphasic effect of cannabinoids. The reduction in anxiety is likely beneficial in the headache patient. The adverse effect on working memory is presumably due to a very high density of CB₁ receptors in the hippocampus. Marijuana is known to contain over 60 cannabinoids, which made early isolation of THC, thought to be the only mood altering constituent,

more difficult (Gaoni and Mechoulam, 1964). Cannabidiol, another plant cannabinoid, has been shown to have potent anti-inflammatory effects among other actions and this could theoretically be important in reducing headache (Mechoulam et al., 2009). In recent years, a variety of “herbal” preparations that are now illegal has been sold on the black market under the brand names “K2” and “Spice.” These products contain synthetic agents that have CB₁ receptor agonist activity, such as cannabicyclohexanol. This group of psychoactive designer drugs is also known as synthetic cannabis, the active ingredients of which have only been poorly studied and should not be used for headache relief (McGeeney, 2013).

MIGRAINE PATHOGENESIS

Migraine is a neurological syndrome characterized by altered bodily perceptions, with increased sensory sensitivity to light, sound, and head movement, severe headaches, and nausea. Although the pathophysiology of migraine is to a great extent still elusive, the activation of the trigeminovascular system followed by neurogenic inflammation in the dura mater is widely recognized as one of the main mechanisms underlying migraine attacks (Moskowitz, 1993). Evidence also suggests that the *primum movens* of migraine attacks is represented by the interaction of internal or external triggers with dysfunctional brainstem centers involved in regulating pain sensation (Goadsby, 2002; Knight, 2005). Dysfunction of brainstem centers is associated with activation of the trigeminovascular system and with dilatation of cerebral blood vessels (Myers, 1999). The dilated blood vessels activate the trigeminal sensory nerve fibers mechanically, therefore inducing the release of glutamate, substance P, calcitonin gene-related peptide (CGRP), and other inflammatory neuropeptides from the sensory nerve terminals (Moskowitz et al., 1989; Moskowitz, 1993). These inflammatory chemicals irritate and further dilate blood vessels, thus inducing more release from the sensory neurons and an increase of pain impulses that are transmitted to the nucleus trigeminalis caudalis (NTC). NTC in turn relays pain signals to higher brain centers including the thalamus and cortex.

Nitric oxide (NO) has been proposed to play a crucial role in the activation of the trigeminovascular system by activating perivascular sensory afferent nerve fibers (via 5-hydroxytryptamine (serotonin) 2B/2C (5-HT_{2B/2C}) receptors), in the meninges, thereby contributing to the release of vasoactive neuropeptides (Messlinger et al., 2000; Strecker et al., 2002). NO may also cause additional NO formation, vasodilatation, and neurogenic inflammation, which may all further contribute to the development of migraine pain.

Various components of the immune system have been examined in relation to headache (Bruno et al., 2007). While great strides have been made in advancing our understanding of neuroimmunology, the complexities of the system make its specific role in headache pathology unclear. CGRP triggers the secretion of

cytokines via stimulation of CGRP receptors found on T-cells (Bruno et al., 2007). Cytokines are involved in inflammation, in modulation of the pain threshold, and also in trigeminal nerve fiber sensitization. In small trials, cytokines have been proven to precipitate headache (Rozen and Swidan, 2007). In addition, recent studies have shown that glial cells, previously thought to serve only a supportive role, are now known to directly influence the microenvironment of trigeminal ganglion neurons through gap junctions and paracrine signaling (Bruno et al., 2007). Following trigeminal activation, CGRP secreted from neuronal cell bodies activates adjacent glial cells to release NO and inflammatory cytokines, which, in turn, initiate inflammatory events in the trigeminal ganglia that lead to peripheral sensitization. The neuronal–glial signaling is thought to be an important process, ultimately leading to the initiation of migraine. The glial modulation of neurons through immune mediators is an unexplored area for new migraine medications.

CORTICAL SPREADING DEPRESSION

In 10% of migraineurs, painful attacks are preceded or associated with aura symptoms. The aura consists of fully reversible symptoms that precede or accompany the headache. The aura is commonly described as changes in the visual field. Visual images change, and there can be loss of focus, spots of darkness, and zigzag flashing lights. It often begins with a hazy spot close to the center of vision and can form into a star shape that further develops into a shape known as fortification (semicircular with angles). This scintillating vision consists of luminous, bright, flickering colors of the spectrum, like a prism catching light. It can be combined with a scotoma (an area of vision that appears to be obstructed or missing). The visual image fades as the headache begins. The headache is intense, throbbing, and usually contralateral to the visual field changes. Cortical spreading depression (CSD) refers to a phenomenon that manifests as a self-propagating wave of neuronal hyperexcitability followed by a transient depression (Leao, 1944). CSD is accompanied by characteristic ionic, metabolic, and hemodynamic changes and may play an essential role in some neurological disorders including migraine with aura (Somjen, 2001; Sanchez-Del-Rio et al., 2006). The hypothesis that the aura is the human equivalent of CSD has been well established (Goadsby, 2007). Propagation of a CSD-like wave in human neocortical tissues generates aura symptoms in migrainous patients (Hadjikhani et al., 2001). Furthermore, it was proposed that CSD might also trigger the rest of the migraine attacks including pain (Moskowitz, 1993).

ENDOCANNABINIDS AND MIGRAINE

CLINICAL DATA

Based on experimental evidence of the antinociceptive action of endocannabinoids and their role in the modulation of trigeminovascular system activation, it

was hypothesized that the endocannabinoid system may be dysfunctional in migraine patients, thus suggesting the potential use of cannabinoids in the treatment of migraine and in cluster attacks refractory to the usual drugs prescribed (Sarchielli et al., 2007; Cupini et al., 2008; Russo, 2008). Additionally, it should be noted that the human gene encoding the CB₁ receptor—*cnr1*—has been mapped to chromosome 6q14–15 (91.8–96.1 cM), which is situated within the region that has showed linkage with migraine (71–101 cM on chr6) (Nyholt et al., 2005). A recent study aimed to evaluate the relationship between alterations of *cnr1* gene and headache in migraineurs has reported a significant haplotypic association between *cnr1* gene and headaches as regards three highly predictive symptoms for migraine: nausea, photophobia, and disability. The results show that the risk haplotype results in attenuated CB₁ receptor expression or function, therefore making the carriers more vulnerable to migraine and also causing an alteration in the peripheral trigeminovascular activation (Juhász et al., 2009).

Previous studies have shown that female patients suffering from episodic migraine have increased interictal CB₁ receptor binding especially in brain regions that exert top-down influences to modulate pain, supporting the idea that endocannabinoid deficiency may play a role in the pathophysiology of migraine (Van der Schueren et al., 2012). Reduced levels of anandamide (AEA) and increased levels of *N*-palmitoylethanolamine (PEA), an endocannabinoid-like compound that does not bind to cannabinoid receptors, were found in the cerebrospinal fluid of patients with chronic migraine (Sarchielli et al., 2007). It was suggested that the reduced levels of AEA may be associated with increased activation of the trigeminovascular system, which, in turn, may lead to increased CGRP and NO production (Akerman et al., 2004a), thus contributing to facilitate/maintain central sensitization in chronic head pain.

Perrotta et al. (2012) have demonstrated, in migraineurs evolved into chronic medication-overuse headache (MOH), an acute reduction of the activity of the enzyme fatty acid amide hydrolase (FAAH). This reduction of activity was associated with reduction of the facilitation in pain processing immediately (10 days) after withdrawal treatment. The authors have suggested that decrease of FAAH activity could be the consequence of a mechanism devoted to acutely reduce the degradation of endocannabinoids. In a previous study, Cupini et al. (2008) reported a reduction in a specific AEA membrane transporter (EMT) and FAAH levels in platelets of subjects with chronic migraine and MOH, as compared to either controls or episodic migraine group. FAAH and EMT activities observed in both chronic migraine and MOH patients did not seem to be related to differences in gender, having been observed in both sexes. Female migraineurs show increased FAAH and EMT activities, a finding that is consistent with a lowered endocannabinoid tone and perhaps a reduced concentration of AEA in blood (Cupini et al., 2006). Finally, 2-arachidonoylglycerol (2-AG) and AEA levels were significantly lower in MOH patients and chronic migraine patients than in the control subjects, without significant differences between the two patient groups. Serotonin levels were also strongly reduced in the two

patient groups and were correlated with 2-AG levels, with higher values for MOH patients (Rossi et al., 2008).

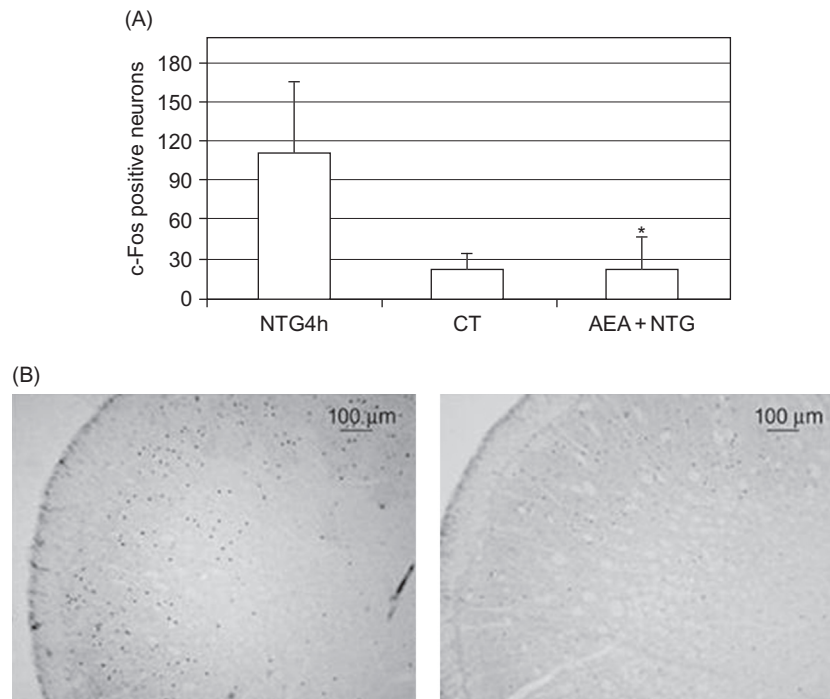
EXPERIMENTAL DATA

Experimental evidence shows that the antinociceptive action of endocannabinoids (eCBs), related to the modulation of trigeminovascular system activation (Akerman et al., 2007) and consequently to the inhibition of trigeminal nerve activation, may be helpful in evaluating new targets for the treatment of migraine. eCBs exert a critical control over cerebrovascular tone, by interacting with serotonergic transmission, NO production, and CGRP release (Pertwee, 2001). CB₁ receptors have been detected in the periaqueductal gray (PAG) matter, rostral ventromedial medulla, and NTC, which are potential migraine generators and pain modulators (Mailleux and Vanderhaeghen, 1992; Moldrich and Wenger, 2000).

Studies with neurovascular models of migraine

Model of Nitroglycerin

Systemic administration of nitroglycerin (NTG), an NO donor, has been used extensively as an animal model of migraine pain since NTG consistently provokes spontaneous-like migraine attacks in migraine sufferers and induces hyperalgesia in the rat through the activation of spinal and brain structures involved in nociception (Buzzi et al., 2003; Tassorelli et al., 2006). CB₁ receptors have been identified also in many of the NTG-activated areas located in the brainstem, hypothalamus, and midbrain (Mailleux and Vanderhaeghen, 1992; Moldrich and Wenger, 2000; Van Sickle et al., 2005). In a previous study, we reported significant changes in the activity of enzymes that catabolize AEA and 2-AG, and FAAH and a cytosolic monoacylglycerol lipase (MAGL), respectively, in the brainstem and hypothalamus of rats following NTG administration. In particular, in the mesencephalon NTG increased the activities of both AEA and 2-AG, thus suggesting a reduction in the endogenous levels of both enzymes. On the other hand, only FAAH was found to increase in the hypothalamus and in the medullary area that contains the NTC. In the same areas, an up-regulation of CB₁ receptor binding sites was also observed. These findings seem to suggest that AEA, rather than 2-AG, is the endocannabinoid more likely implicated in the modulation of pain originating in the cephalic area. It is noteworthy that these changes were paralleled by a reduction in NTG-induced hyperalgesia and NTG-induced c-Fos expression in the NTC (Greco et al., 2010) (Figure 7.1). In addition, URB937, an FAAH inhibitor specific for peripheral tissues, causes analgesia in animal models of pain (Clapper et al., 2010). We evaluated whether URB937 administration may alter nociceptive responses in this animal model of migraine (Greco et al., 2012). Rats received systemic NTG and URB937 before being evaluated via the tail flick test or via the formalin test. The findings showed that URB937 did inhibit NTG-induced hyperalgesia at the formalin test with only a minimal influence on the hyperalgesia at the tail flick,

**FIGURE 7.1**

c-Fos expression in the nucleus trigeminalis caudalis and its modulation by anandamide. (A): number of c-Fos-positive neurons in the group of animals treated with nitroglycerin (NTG4 h), with vehicle (CT), or with anandamide and nitroglycerin (AEA + NTG). * $p < 0.05$ vs. NTG4 h. (B) micrographs of representative sections of the nucleus trigeminalis caudalis of rats treated with nitroglycerin (left) or pretreated with AEA (right) before receiving nitroglycerin.

Data from Greco et al. (2009).

suggesting that availability of AEA, probably at the meningeal level, is effective in reducing migraine pain (Greco et al., 2012).

CB₁ receptors are localized on fibers in the spinal trigeminal tract and spinal NTC (Tsou et al., 1998), and therefore their activation in trigeminal neurons following pretreatment with AEA might inhibit CGRP release from central terminals of primary afferent fibers, thus reducing NTG-induced c-Fos expression. Alternatively, AEA may inhibit c-Fos expression via activation of CB₂ receptors. Selective activation of CB₂ receptors indeed suppresses spinal c-Fos protein expression and pain behavior in a rat model of inflammation, following carrageenan injection in the paw (Nackley et al., 2003). In addition, when considering that NTG promotes the activity of nuclear

factor-kappa B (*NF- κ B*)—a gene implicated in neuroinflammation—and inflammation in dura mater and NTC of rats with a time course consistent with migraine attacks in susceptible individuals (Reuter et al., 2002; Greco et al., 2005), it is conceivable that AEA might inhibit expression of proteins through a potential inhibition of *NF- κ B* inactivation (Sancho et al., 2003). In addition, in a recent study (unpublished data) we have found that AM1241, a CB₂ receptor agonist, significantly reduces the nociceptive behavior of the rats made hyperalgesic by NTG administration in the second phase of the formalin test, therefore suggesting a role for CB₂ receptors in this animal model of migraine. Accordingly, activation of CB₂ receptors reduces spinal Fos protein expression and pain behavior in a rat model of inflammation (Nackley et al., 2003). This finding is in agreement with the analgesic effect of CB₂ receptor stimulation observed in the model of carrageenan-induced inflammation (Quartilho et al., 2003; Nackley et al., 2003, 2004).

Studies with Electrical Dural Stimulation and Cutaneous Facial Receptive Field Activation of the Ophthalmic Division of the Trigeminal Nerve

Previously, responses to both electrical dural stimulation and cutaneous facial receptive field activation of the ophthalmic division of the trigeminal nerve and the effect of cannabinoid agonists and antagonists were studied (Akerman et al., 2007). The data show that AEA inhibits neurons of the trigeminovascular system only after transient inhibition of transient receptor potential vanilloid-1 (TRPV1) receptors, therefore suggesting a minor role for TRPV1 receptor in the modulation of the trigeminovascular system (Akerman et al., 2004a). The inhibition of trigeminal firing in the trigeminocervical complex is indeed reversed by the administration of a specific CB₁ receptor antagonist, which demonstrates conclusively that the central effects of eCBs are CB₁ receptor mediated (Akerman et al., 2007). Therefore, manipulation of CB₁ receptors may involve the responses of trigeminal neurons with A- and C-fiber inputs from the dura mater. This may be a direct effect on neurons in the NTC itself, or in discrete areas of the brain that innervate these neurons.

Recently, it was reported that activation of CB₁ receptors in the ventrolateral periaqueductal gray (vlPAG) attenuated dural-evoked A-fiber neurons (maximally by 19%) and basal spontaneous activity (maximally by 33%) in the rat trigeminocervical complex, but had no effect on cutaneous facial receptive field responses (Akerman et al., 2013). This inhibitory vlPAG-mediated modulation was inhibited by specific CB₁ receptor antagonism, given locally, and with a 5-HT_{1B/1D} receptor antagonist, given either locally in the vlPAG or systemically. These findings demonstrate that brainstem endocannabinoids provide descending modulation of both basal trigeminovascular neuronal tone and A-fiber dural-nociceptive responses, which differs from the way the brainstem

modulates spinal nociceptive transmission. Furthermore, these data demonstrate a novel interaction between serotonergic and endocannabinoid systems in the processing of somatosensory nociceptive information, suggesting that some of the therapeutic action of triptans may be via endocannabinoid-containing neurons in the vlPAG (Akerman et al., 2013). The brainstem is heavily populated with CB₁ receptors and descending modulation of trigeminovascular nociceptive transmission through midbrain nuclei (PAG and rostroventral medulla) is likely to be responsible for the quick antinociceptive effect on headache, as often voiced by marijuana users (Akerman et al., 2011).

Studies with vascular models of migraine

The CB₁ receptor seems to be involved in the NO/CGRP relationship that is likely to underline headache attacks. In fact, AEA is able to inhibit neurogenic dural vasodilatation, as well as CGRP-induced and NO-induced dural vessel dilation in the intravital microscopy model, although some of the blood pressure changes caused by AEA are mediated by an as-yet-unknown non-cannabinoid receptor, as suggested by the observation that AM251, a CB₁ receptor antagonist, is unable to reverse these effects (Akerman et al., 2004b).

It has been shown that AEA activating the TRPV1 receptor on trigeminal ganglion neurons may promote the release of CGRP (Tognetto et al., 2001). AEA is able to cause a dose-dependent (1, 3, and 5 mg kg⁻¹) increase in dural vessel diameter, while capsazepine (3 mg kg⁻¹), a TRPV1 receptor antagonist, and CGRP(8-37) (300 µg kg⁻¹), a CGRP receptor antagonist, attenuate AEA-induced dural vessel dilation. In addition, in rats pretreated with AM251 (3 mg kg⁻¹), the dilation induced by AEA (5 mg kg⁻¹) is unaltered, while it results attenuated by a specific TRPV1 receptor antagonist (Akerman et al., 2004a), thus confirming that AEA exerts its vasodilator effect through a mechanism dependent on TRPV1 activation, but which does not involve CB₁ receptors. This apparent discrepancy might depend on the high AEA concentrations used, as it is known that AEA activates CB₁ or TRPV1 receptors in a concentration-dependent manner (Pertwee and Ross, 2002; Van Sickle et al., 2005).

Effect of Cannabinoid Receptor Activation on Cortical Spreading Depression

The effects of THC as well as synthetic cannabinoid CB₁ and CB₂ receptor agonists on CSD in rat neocortical slices were investigated by Kazemi et al. (2012). THC (1–20 µM) dose-dependently suppressed cortical spreading depression (CSD) amplitude, duration, and propagation velocity. The CB₁ receptor agonist WIN 55,212-2 mesylate (1–10 µM) also significantly suppressed the field excitatory postsynaptic potentials (fEPSPs) and long-term potentiation of CSD. However, the cannabinoid CB₂ receptor agonist JWH133 (1–20 µM) did not affect CSD.

POTENTIAL SITES AND MECHANISMS OF ACTION OF ECs

The trigeminovascular system has long been implicated as integral to the pain, inflammation, and secondary vascular effects of migraine. This system receives inputs from other higher brain centers, such as hypothalamus, thalamus, and PAG (Bartsch et al., 2004). Therefore, activation of CB₁ receptors in these sites may influence trigeminovascular neuronal firing. CB₁ receptors are present in small neurons that express the high affinity catalytic receptor for the neurotrophin TrkA (Friedel et al., 1997), and in neurons that express substance P or CGRP in the dorsal root ganglia (Hohmann and Herkenham, 1999b; Pertwee, 2001), from where they are transported to central and peripheral terminals (Hohmann and Herkenham, 1999a). Thus, bidirectional transport of CB₁ receptors raises the question as to whether the modulation of pain by cannabinoids is centrally or peripherally mediated. The mechanisms by which cannabinoids produce their anti-migraine effects are not fully known; however, several studies have proposed different hypotheses. Figure 7.2 summarizes the potential mechanisms related to the effects of eCBs on migraine.

EFFECTS ON THE SEROTONIN SYSTEM

In 1985, Volfe et al. reported that THC inhibits the release of serotonin from the blood of migraine sufferers during an attack (but not at other times). However, a more profound understanding of cannabis and its action in the brain has only recently been reached with the discovery of AEA in the human brain (Devane

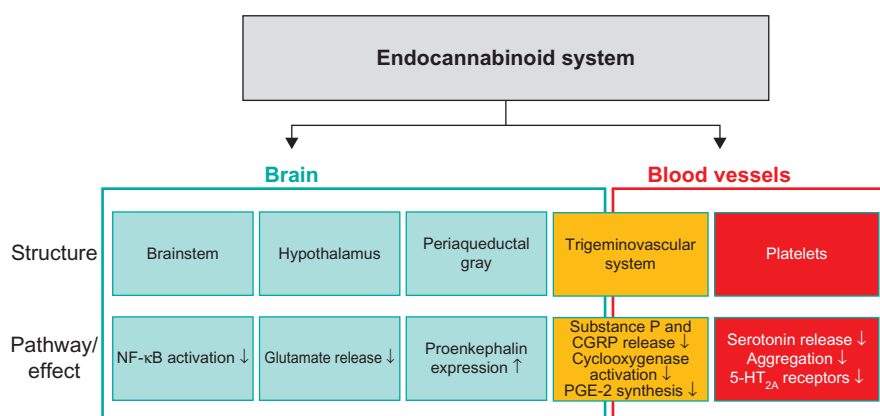


FIGURE 7.2

Schematic drawing of the possible sites and mechanisms of action of the endocannabinoid system in the pathophysiology of migraine at the cerebral, neurovascular, and vascular levels.

From Greco et al. (2010).

et al., 1992). Other researches shed light on possible mechanisms of therapeutic action of the cannabinoids on migraine, suggesting the inhibitory effect of AEA and other cannabinoid agonists on rat serotonin type 3 (5-HT₃) receptors (Fan, 1995). AEA has been shown to potentiate 5-HT_{1A} receptors, while oleamide, another endogenous brain lipid structurally related to AEA, has been shown to enhance 5-HT₂ receptor function *in vivo* (Kimura et al., 1998). Both these effects envisage a potential therapeutic effect, though never tested, for eCBs in the acute/chronic treatment of migraine.

EFFECTS ON THE NMDA/GLUTAMATE SYSTEM

A trigeminovascular system has long been implicated as integral to the pain, inflammation, and secondary vascular effects of migraine, linked through the NMDA/glutamate system (Storer and Goadsby, 1999). Cannabinoid agonists inhibit voltage-gated calcium channels, and activate potassium channels to produce presynaptic inhibition of glutamate release (Shen et al., 1996). NMDA receptor antagonism was felt to be effective in eliminating hyperalgesia associated with migraine (Nicolodi and Sicuteri, 1995), as well as a “secondary hyperalgesia” with exaggerated responses to noxious stimuli in areas adjacent to the pain. THC and phytocannabinoids also act as neuroprotective antioxidants against glutamate neurotoxicity and cell death mediated via NMDA, AMPA, and kainate receptors (Hampson et al., 1998).

EFFECTS ON INFLAMMATORY MOLECULES

Although the pathophysiology of migraine is to a great extent still elusive, the activation of the trigeminovascular system followed by neurogenic inflammation in the dura mater is widely recognized as one of the main mechanisms underlying migraine attacks (Moskowitz, 1993). The anti-inflammatory contributions of THC are also extensive, including inhibition of prostaglandin E₂ (PGE-2) synthesis (Burstein et al., 1973), decreased platelet aggregation (Schaefer et al., 1979), and stimulation of lipoxygenase (Fimiani et al., 1999). THC has 20 times the anti-inflammatory potency of aspirin and twice that of hydrocortisone but in contrast to all non-steroidal anti-inflammatory drugs (NSAIDs), demonstrates no cyclo-oxygenase (COX) inhibition at physiological concentrations (Russo and Guy, 2006). Endocannabinoids are also rapidly generated in response to pro-inflammatory stimulation of immune cells, and they might operate a negative feedback control over the pro-inflammatory response, possibly by negatively regulating the activation of transcription factors involved in the inflammatory response (Berdyshev et al., 2001). Previous studies have shown that, in murine macrophages and splenocytes, cannabinoids and the endocannabinoid 2-AG may either activate or inhibit NF- κ B activity via CB₁ receptor and protein kinase A-dependent mechanisms (Daaka et al., 1997).

LIMITATIONS

While the antinociceptive actions of cannabinoids are well established, their potential therapeutic use continues to be limited by their side effects profile. Clearly, the development and use of novel cannabinoid compounds for the relief of pain in humans will hinge on the ability to dissociate psychotropic effects from therapeutic ones. In addition, changing of synaptic plasticity by activation of CB₁ receptors may affect signal processing as well as learning and memory in different regions of the brain (Kazemi et al., 2012). These side effects should be taken into consideration in further development of new cannabinoid derivatives.

NEW POTENTIAL THERAPEUTIC APPROACHES

INHIBITORS OF CATABOLISM

Enhancing endocannabinoid tone has been proposed as an alternative means of activating CB₁ receptors without concomitant overt psychotropic effects associated with potent synthetic CB₁ receptor agonists. Enhancing endocannabinoid tone via FAAH or MAGL inhibition elicits anti-inflammatory effects in several animal models (Holt et al., 2005; Jayamanne et al., 2006; Booker et al., 2012). *In vitro* studies suggest that endocannabinoids elicit anti-inflammatory effects comparable to those of exogenous cannabinoids. Increasing AEA tone, either directly or via inhibition of its degradation or uptake, has been demonstrated to reduce the levels of pro-inflammatory cytokines and inflammatory mediators such as TNF- α , IL-1 β , and NO, and to enhance the release of the anti-inflammatory cytokine IL-10 *in vitro* (Puffenberger et al., 2000; Correa et al., 2009, 2010). Selective and potent inhibitors of FAAH have been developed and tested *in vivo* in several animal models of disease so far. Three FAAH blockers in particular seem promising for future clinical development: URB597 (Mor et al., 2004), arachidonoylserotonin (N-arachidonoyl-serotonin, AA-5-HT) (Bisogno et al., 1998), and SA73 (Sanofi-Aventis) (Zhang et al., 2007). In particular, URB597 is an irreversible FAAH inhibitor, still at the preclinical stage, with anxiety, depression, and pain as the most likely therapeutic targets (Piomelli et al., 2006). It has potent analgesic activity in models of neuropathic pain when administered orally, and it has proved efficacious following systemic administration in models of inflammatory pain (Jayamanne et al., 2006) and inflammation (Holt et al., 2005).

CB₂ RECEPTOR AGONISTS

Recently, CB₂ receptor agonists have been proposed as a valid alternative to CB₁ receptor agonists in pain modulation, either because of their mainly peripheral distribution, so they do not cause adverse central effects, or because of their capability

to inhibit signs of acute nociceptive, inflammatory, and neuropathic pain in preclinical studies (Malan et al., 2003; Li and Zhang, 2012; Murineddu et al., 2013).

Although there is a large body of evidence supporting the potential utility of selective cannabinoid CB₂ receptor agonists for the treatment of pain (Guindon and Hohmann, 2008), the mechanism and site of action responsible for CB₂-mediated analgesia remain unexplained.

CONCLUSIONS

In several studies, the antinociceptive effect of cannabinoids has been unequivocally demonstrated in models of inflammatory and neuropathic pain, although some controversies exist on the localization of these pain-protective effects. Migraine has numerous relationships with eCBs and a deficiency in the endocannabinoid system has been hypothesized to underlie the pathophysiology of migraine. However, biochemical studies providing a scientific basis for the potential efficacy of (endo)cannabinoids in migraine are, so far, limited.

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Role of the
endocannabinoids
in psychological
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Cannabinoids and schizophrenia

8

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INTRODUCTION

The discovery of the endocannabinoid system (ECS) has stimulated a growing body of psychiatric research focusing on the role of this system in major psychiatric disorders like schizophrenia. Initially, epidemiological studies revealed that frequent and in particular early cannabis use at least doubles the risk for psychotic symptoms and schizophrenia. This was confirmed by respective replication studies. In parallel, research has focused on endocannabinoids (eCBs), their synthesizing and metabolizing enzymatic apparatus, and the central nervous distribution of the best known cannabinoid receptors (CB₁R and CB₂R) and their relation to psychotic symptoms in schizophrenia. In addition, animal models related to aspects of schizophrenia have been developed that target the ECS. Based on these findings, a neurobiological role of the ECS involving the disruption of endogenous cannabinoid signaling and functioning in schizophrenia and a mechanism for the deleterious influence of cannabis use in schizophrenia have been suggested. The proposed model was the starting point for psychopharmacological studies raising evidence for further studies to explore this therapeutic potential.

ENDOCANNABINOID SYSTEM AND SCHIZOPHRENIA

Over recent years there has been substantial progress in our understanding of the neurobiological fundamentals of schizophrenia and related psychoses. Initially, the dopamine hypothesis represented our first valid model for an improved understanding of schizophrenia, based on the observation that antipsychotic drugs influenced catecholamine metabolism and blocked dopamine receptors (Carlsson and Lindqvist, 1963). This idea developed into the hypothesis of a hyperactive dopamine system in schizophrenia patients (van Rossum, 1966).

In the meantime it became apparent that the pathophysiology of the disease is much more complex. On the one hand, hyperactivation of dopamine D₂ receptors in mesolimbic pathways was primarily associated with positive symptoms, whereas the negative symptoms were thought to result from a hypoactivity of the dopamine system in mesocortical projections (Davis et al., 1991). On the other hand, it became obvious that dysfunctions of other neurotransmitter systems and their interactions also play an important role in the manifestation of schizophrenia. Among these systems are the glutamatergic (Kim et al., 1980), the GABAergic (Garbutt and van Kammen, 1983), the serotonergic (Meltzer, 1989), and the endocannabinoid system (Emrich et al., 1997).

Various observations indicate that the ECS reacts adaptively on neurotransmitter anomalies and has a protective effect in the development of schizophrenic psychosis (Leweke, 2012). Today, the best investigated parts of the ECS are those involving the eCB (eCBs) with anandamide and 2-arachidonoyl-sn-glycerol (2-AG) as the most well known synthesizing and degrading enzymes respectively (Table 8.1), as well as cannabinoid receptors (at least CB₁R and CB₂R). In particular, anandamide seems to play an important protective role in schizophrenia. Individuals in at-risk mental states (initial prodromal syndrome) showed significantly elevated anandamide levels in cerebrospinal fluid (CSF). Those individuals with higher CSF anandamide levels showed a lower risk for transition to acute psychosis (Koethe et al., 2009). Antipsychotic-naïve patients with a first-episode acute schizophreniform psychosis

Table 8.1 Summary of the Enzymes involved in Formation and Hydrolysis of the Endocannabinoids Anandamide and 2-Arachidonoyl-sn-Glycerol (2-AG)

	Anandamide	2-Arachidonoyl-sn-Glycerol (2-AG)
Synthesizing enzymes	• N-acyltransferase (NAT) • N-acylphosphatidylethanol-selective phospholipase D (NAPE-PLD) • α/β-hydrolase 4 (ABDH 4) • Phosphodiesterase • Phospholipase A₂ (PLA₂) • Lyso-phospholipase D (lyso-PLD)	• Phospholipase C-β (PLC-β) • Diacylglycerol lipase-α (DGL-α) • Phospholipase A₁ (PLA₁) • Lyso-phospholipase C (lyso-PLC)
Degrading enzymes	• Fatty acid amide hydrolase (FAAH 1; FAAH 2) • N-acylethanolamine-hydrolyzing acid amidase (NAAA) • Cyclooxygenase 2 (COX-2) • Lipoxygenase 12 and 15 • Cytochrome p450	• Monoacylglycerol lipase (MAGL) • α/β-hydrolase 6 and 12 (ABDH 6; ABDH 12) • Cyclooxygenase 2 (COX-2) • Lipoxygenase 12 and 15 • Cytochrome p450

The enzymes of the most important biosynthesis and inactivation pathways are shown in bold. Alternative enzymes that have been discussed in recent years are also listed.

also showed significantly elevated anandamide levels in CSF compared to healthy controls. These were inversely correlated with the expression of psychotic symptoms (Leweke et al., 1999a ; Giuffrida et al., 2004).

Interestingly, antipsychotic treatment seems to affect anandamide levels. While first-episode patients briefly treated with dopamine $D_{2/3}$ receptor antagonists, such as first generation antipsychotics or amisulpride, showed anandamide levels comparable to those of healthy controls, patients treated with second generation antipsychotics with additional antagonistic effects at serotonin 5-HT_{2A} receptors still had significantly elevated levels of anandamide in CSF (Giuffrida et al., 2004). Nevertheless, these levels did not reach the concentrations in antipsychotic-naïve patients. Since treatment with $D_{2/3}$ receptor antagonistic antipsychotics is associated with anandamide concentrations close to normal levels, it has been assumed that the ECS modulates the dopaminergic system. This hypothesis is supported by animal studies. A microdialysis study (Giuffrida et al., 1999) demonstrated that both neuronal activity of the dorsal striatum and local injection of a D_2 receptor agonist stimulated anandamide release, an effect that was prevented by administration of a D_2 receptor antagonist. The effects of D_2 receptor agonists on locomotor activity and stereotypes of rats can be enhanced by pretreatment with a CB_1R antagonist (Giuffrida et al., 1999) and reduced by the inhibition of the anandamide transport (Beltramo et al., 2000; Martin et al., 2008). These findings indicate that the ECS operates as a negative feedback system that counteracts dopamine release (Figure 8.1).

Besides changes of the anandamide levels, alterations of the CB_1R densities in schizophrenic patients were also reported. Two postmortem autoradiography

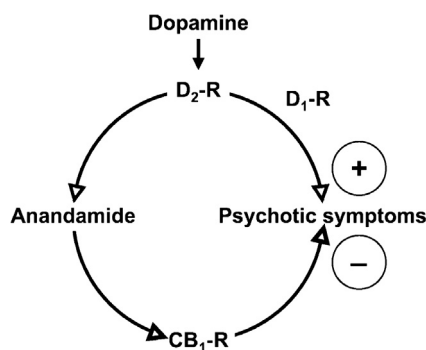


FIGURE 8.1

Model of the proposed negative feedback mechanism based on dopamine–endocannabinoid interaction in schizophrenia. A severe activation of dopamine receptors seems to result in psychotic symptoms in schizophrenia patients. In parallel, this activation is accompanied by increased anandamide release that in turn seems to alleviate psychotic symptoms.

Adapted from Leweke and Koethe (2008) with permission.

studies found an increase of CB₁R density in the anterior cingulate cortex and dorsolateral prefrontal cortex (Dean et al., 2001; Zavitsanou et al., 2004). In contrast, immunohistochemical analysis revealed no evidence for an increased or decreased CB₁R density in the anterior cingulate cortex of schizophrenic patients compared to healthy controls (Koethe et al., 2007). Another postmortem study observed significantly reduced amounts of CB₁R mRNA and protein (Eggan et al., 2008).

One reason for these inconsistent results is without doubt that those postmortem analyses can only capture the end of a long lasting disease with various personal histories (e.g., kind and duration of treatment, life circumstances).

In the meantime, positron emission tomography (PET) studies has allowed for analysis of CB₁R availabilities at different time points of the disease. The first two PET studies using the selective, high-affinity CB₁R radioligands [¹⁸F]MK-9470 or [¹¹C]OMAR demonstrated an elevated receptor availability across several brain regions in schizophrenic patients that can be interpreted as an increased CB₁R density (Wong et al., 2010; Ceccarini et al., 2013a). This effect was more pronounced in first-onset antipsychotic-naïve compared to treated patients (Ceccarini et al., 2013a). Furthermore, different antipsychotic monotherapies affected significantly and differently the CB₁R binding in distinct brain regions. In particular, the mesocortical up-regulation of CB₁R in drug-free patients was reduced by administration of antipsychotics, but in a treatment-specific manner (Ceccarini et al., 2013a). This therapeutic influence on CB₁Rs is in line with the above-described effects of antipsychotics on anandamide levels in CSF and supports the hypothesis of an indirect interaction between the ECS and the dopamine system. The increased dopaminergic activity in antipsychotic-naïve patients seems to evoke an up-regulation of the ECS, which in turn attenuates dopamine release. In response to a reduced dopamine activity owing to administration of D₂ receptor antagonists, the ECS appears to be down-regulated as well.

CANNABIS AND SCHIZOPHRENIA

Since both eCBs and cannabinoid receptors are involved in schizophrenia it is obvious that exogenous cannabinoids in the form of phytocannabinoids may play a role in the development of the disease. Cannabis consumption is discussed as a risk factor for the development of a schizophreniform disorder. The question whether the “cannabis psychosis” can be considered a nosological entity on its own is currently negated in literature. So far, there is no convincing definition that differentiates “cannabis psychosis” from other schizophrenia-spectrum diseases. In fact, it is more likely that cannabis use acts as an additional stress factor in predisposed vulnerable persons in line with the postulated vulnerability/stress model of schizophrenia (Leweke et al., 2004).

Epidemiological data particularly substantiate the close relationship between cannabis consumption and schizophrenic psychoses. A number of studies

analyzing large cohorts found that already moderate cannabis consumption (more than 20 times in a lifetime) is attended by an increased risk of developing psychotic symptoms (Andreasson et al., 1987; van Os et al., 2002; Zammit et al., 2002; Fergusson et al., 2003; Henquet et al., 2005) and an earlier onset of schizophrenia (Large et al., 2011; Di Forti et al., 2014; Donoghue et al., 2014). Nevertheless, the risk enhancement seems to be related to the amount of cannabis used (Zammit et al., 2002; Moore et al., 2007). In particular, the frequent use of high potency cannabis preparations seems to be associated with psychosis (Di Forti et al., 2009) and an earlier onset of the disease (Di Forti et al., 2014). In addition, the age of onset seems to be relevant. Arseneault et al. (2002) demonstrated that adolescent cannabis users exhibited significantly more psychotic symptoms than controls (non-users) at the age of 26 years. Regression analysis showed that adolescents who had consumed cannabis at the age of 15 years had a four times higher risk of developing psychotic symptoms. These results were specific inasmuch as cannabis use during adolescence did not predict depressive outcomes in adulthood.

Most experimental approaches make use of pure cannabinoids like the phytocannabinoid Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the main psychotomimetic cannabinoid compound of *Cannabis sativa*. Noteworthy, although often reported synonymously, the effects of “recreational” use of cannabis (marijuana or hashish) and the experimental intravenous, *per os*, or pulmonary administration of Δ^9 -THC have to be differentiated (Koethe et al., 2009). In healthy humans, administration of cannabis preparations or single cannabinoids such as Δ^9 -THC can induce complex qualitative changes, which in relation to psychopathology, perception, and cognitive parameters resemble those of schizophrenic patients (Leweke et al., 1999b, 2000; D’Souza et al., 2004; Koethe et al., 2006; Bhattacharyya et al., 2009; Fusar-Poli et al., 2009; Mason et al., 2009). In addition, highly psychosis-prone individuals experienced more pronounced psychotic-like symptoms after acute cannabis use than control subjects (Mason et al., 2009). In consideration of the idea that cannabis use serves as an additional stress factor in predisposed vulnerable persons, it is interesting that the psychotomimetic effects of cannabis or Δ^9 -THC are also influenced by the functional polymorphism (V158M) in the catechol-*o*-methyltransferase gene (*COMT*). Carriers of the Val/Val genotype were most sensitive to cannabis exposure and reported an increase in hallucinations (Henquet et al., 2009).

In schizophrenic patients, however, positive and negative symptoms, perceptual changes, and cognitive deficits as well as extrapyramidal side effects of antipsychotic drugs are transiently exacerbated by Δ^9 -THC (D’Souza et al., 2005). Another study showed that patients with a clinical diagnosis of a psychotic disorder are more sensitive to the psychotomimetic effects of cannabis (Henquet et al., 2010) and show a significant increase in hallucinations (mainly auditory). In addition, patients were more sensitive to the mood-enhancing effects of cannabis as a significant decrease in negative affects was observed.

A CB₁R PET study with the radioligand [¹⁸F]FMPEP-d2 (Hirvonen et al., 2012) showed that chronic cannabis consumption caused reversible and regionally

selective reduction of CB₁R availability. A decreased PET signal was therefore interpreted as a reduction of CB₁R density. The signal reduction was correlated with years of cannabis smoking. After approximately 4 weeks of abstinence from cannabis, receptor availability returned to normal levels. Another CB₁R PET study using the radioligand [¹⁸F]MK9470 also detected a regional specific decrease of receptor availability, although neither study observed exactly the same regional pattern (Ceccarini et al., 2013b). The human PET results are in line with animal studies that reported a regional selective reduction of CB₁R density after chronic Δ^9 -THC administration (Rodriguez De Fonseca et al., 1994; Romero et al., 1995, 1997, 1998; Breivogel et al., 1999). Such a receptor density reduction is deemed a basic principle of tolerance development towards Δ^9 -THC.

Cannabis consumption also influences anandamide levels in CSF (Leweke et al., 2007). First-episode, antipsychotic-naïve, schizophrenic low-frequency cannabis users exhibited 10-fold higher CSF anandamide levels than in schizophrenic high-frequency users and healthy controls (both high and low frequency users; Figure 8.2). These results indicate that cannabis counteracts the protective up-regulation of the ECS in schizophrenic patients. A more recent study examined the levels of eCBs and related lipids in the CSF of subjects with a different level of cannabis consumption, and suggested altered anandamide signaling related to cannabis use (Morgan et al., 2013). Anandamide levels were significantly reduced in the CSF of heavy compared to light cannabis users, whereby the levels of 2-AG, oleoylethanolamide, and palmitoylethanolamide were comparable. Furthermore, anandamide levels were also negatively correlated with drug-free psychotic symptoms. It has not yet been clarified if cannabis consumption directly affects anandamide levels or if the effect is indirectly induced, e.g., by CB₁R internalization.

CANNABIDIOL AND SCHIZOPHRENIA

Due to the likely protective effects of anandamide, a new innovative therapeutic concept for the treatment of schizophrenia and related psychoses might be based on the strengthening of the ECS, in particular of anandamide-related transmission. One promising candidate for such modulation of anandamide is the non-psychotomimetic ingredient of the cannabis plant: cannabidiol. The exact mechanism of action of cannabidiol is not yet fully determined. However, there is evidence that it exhibits its main antipsychotic effect by inhibition of anandamide degradation, i.e., by inhibition of anandamide's main metabolizing enzyme, fatty acid amide hydrolase (FAAH; McKinney and Cravatt, 2005). The ability of cannabidiol to inhibit FAAH has been demonstrated in cell cultures and membrane preparations (Watanabe et al., 1996; Bisogno et al., 2001; Leweke et al., 2012).

In healthy volunteers, cannabidiol counteracts the psychoactive effects of Δ^9 -THC. It reduced positive symptoms, anxiety, and perceptual changes induced

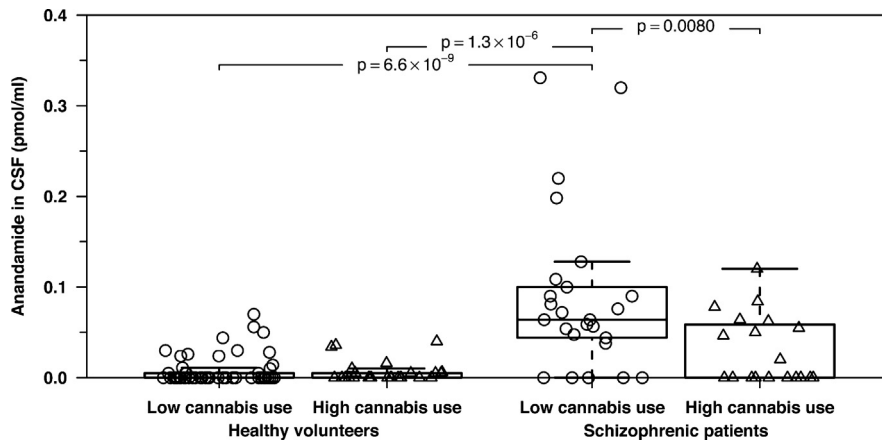


FIGURE 8.2

Box–whisker plots (interquartile range from the first to third quartile; whiskers extend to the smallest and largest values excluding outliers) of anandamide in cerebrospinal fluid (CSF) of healthy volunteers and schizophrenia patients depending on the frequency of cannabis consumption (low-frequency use: ≤ 5 times; high-frequency use: >20 and <50 times in life). In healthy volunteers (left panel) anandamide levels were not influenced by the frequency of lifetime cannabis use (box plot with circles: low frequency, $n = 55$; box plot with triangles: high frequency, $n = 26$). On the other hand, anandamide levels were significantly elevated in acute antipsychotic-naïve schizophrenia patients with ≤ 5 times of cannabis consumption in life (right panel, box plot with circles, $n = 25$) compared to patients with high-frequency cannabis use (right panel, box plot with triangles, $n = 19$) and to healthy controls. For statistical analysis a Kruskal–Wallis rank sum test was performed followed by Wilcoxon rank sum tests. Due to Bonferroni corrections, p -values ≤ 0.0083 were considered statistically significant.

From *Leweke et al. (2007)* with permission.

by Δ^9 -THC (Zuardi et al., 1982; Leweke et al., 2000; Bhattacharyya et al., 2010). Thus, the ratio of cannabidiol and Δ^9 -THC in different cannabis preparations might influence the effects. This was shown by a web-based cross-sectional study with 1877 participants (Schubart et al., 2011). Based on the preferred cannabis type, subjects were divided into two groups: low and high cannabidiol content cannabis users. In addition, positive, negative, and depressive psychiatric experiences were assessed by the self-rating instrument CAPE (Community Assessment of Psychic Experiences). Subjects who preferred cannabis with high cannabidiol content reported significantly fewer positive symptoms than those using cannabis preparations with low cannabidiol concentrations. These findings are in line with a study analyzing the association between the cannabidiol content in the hair of cannabis users and psychotic symptoms (Morgan and Curran, 2008). Subjects who had evidence of only Δ^9 -THC in their hair showed higher scores of hallucinations and delusions than those with Δ^9 -THC and cannabidiol or no

cannabinoids at all in the hair. Interestingly the same group speculated that this effect can only be attributed to recreational users with higher levels of Δ^9 -THC in the hair and not to daily cannabis users (Morgan et al., 2012). In addition, subjects with cannabidiol in the hair performed better in a recognition memory task irrespective of their degree of cannabis use.

In 1995, the antipsychotic effect of cannabidiol was first reported in an individual treatment attempt (Zuardi et al., 1995). The clinical improvement after daily cannabidiol treatment could not be increased by administration of an antipsychotic. Several years later cannabidiol was administered to three treatment resistant patients (Zuardi et al., 2006). One of the patients showed a slight improvement of positive and negative symptoms. Although cannabidiol treatment was not effective in the two other patients, no side effects were observed at all. Independently, the first controlled, randomized, double-blind clinical trial was concluded (Leweke et al., 2012) in which the efficacy of cannabidiol was compared to that of amisulpride, a standard atypical antipsychotic. Both drugs significantly reduced positive, negative, and general symptoms. The efficacy of cannabidiol was comparable to that of amisulpride but cannabidiol possessed a superior, placebo-like side effect profile. In addition, there was a strong indication that the improvement of psychotic symptoms was significantly related to an increase in serum levels of anandamide. Together with the above-mentioned preclinical data, these results indicate that cannabidiol might strengthen the postulated feedback mechanism depicted in Figure 8.1. Thus, the antipsychotic effects of cannabidiol seem to result from enhancement of anandamide levels based on FAAH inhibition.

CONCLUSIONS

There is a strong relationship between the ECS, cannabis use, and schizophrenia. On the one hand, the ECS has to be regarded as part of the pathophysiology of schizophrenia, while on the other hand, cannabis use may contribute to weaken this system, a system that most likely plays a protective role in the neurobiology of this disease. Targeting this mechanism may become a viable new approach to the treatment of schizophrenia.

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Cannabinoids and bipolar disorder

9

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INTRODUCTION

Bipolar affective disorder is characterized by recurrent episodes of mood disturbances, namely, depression and mania. Major depression is a mood episode consisting of sad mood, loss of interest, poor concentration, and feelings of guilt. Alternatively, mania is characterized by expansive mood, decreased sleep, increased energy, and impulsive behavior (American Psychiatric Association, 2013). Major subtypes of the disorder are bipolar I and bipolar II. Individuals with bipolar I disorder experience full mania, which is commonly accompanied by symptoms of psychosis. Bipolar II disorder is characterized by a different presentation, which includes episodes of major depression and hypomania, or milder symptoms of mania. Hypomania is usually not associated with a marked decrease in functioning. About 3% of the worldwide population meets criteria for a bipolar disorder, with equal distribution between men and women as well as racial and ethnic groups (Merikangas et al., 2007). The main focuses of bipolar disorder treatment are amelioration of acute affective symptoms and prevention of episodic relapse. Common treatment interventions include second generation antipsychotics, anticonvulsants, lithium, and psychotherapy (Geddes and Miklowitz, 2013).

PREVALENCE OF CANNABIS USE IN BIPOLAR DISORDER

Substance use disorders are notably common among individuals diagnosed with bipolar affective disorder. Lifetime prevalence rates of up to 60% are found in

epidemiological samples (Regier et al., 1990). This frequency of substance use disorder comorbidity in bipolar disorder is the highest among major psychiatric diagnoses. Compared to the general population, patients with bipolar disorder have a two to three-fold increase in the rate of cannabis use disorders (CUDs) (Stinson et al., 2006; Koskinen et al., 2010). In fact, cannabis is often shown to be the most commonly misused drug in bipolar disorder patients (Cerullo and Strakowski, 2007).

The National Epidemiologic Survey on Alcohol and Related Conditions (NESARC) (Grant et al., 2005) demonstrated the substantial magnitude of substance use among subjects with mental illnesses. With over 43,000 adults interviewed, the NESARC is the largest epidemiological survey on mental illness and substance abuse. The majority of individuals endorsing any cannabis use were diagnosed with bipolar disorder. Rates of frequent (weekly) cannabis use, less than weekly cannabis use, and CUDs among individuals with mental illness were 8.4%, 9.6%, and 9.4%, respectively, compared to 0.6%, 1.1%, and 0.4% among individuals without any mental illness, a statistically significant difference. Individuals with mental illness represented 72% of all cannabis users and were responsible for 83% of cannabis consumption by the entire sample (Lev-Ran et al., 2013b). Furthermore, individuals with bipolar disorder with comorbid CUDs had an exceptionally high prevalence of additional substance use. Prevalence of nicotine, alcohol, and non-cannabis drug use disorders were greater than 67%, 66%, and 71%, respectively (Lev-Ran et al., 2013a).

Cannabis use is also extremely prevalent in clinical samples. Cross-sectional evaluations of patients with a diagnosis of bipolar disorder consecutively admitted to psychiatric clinics show frequencies of CUDs ranging from 20 to 40% (Winokur et al., 1998; Chengappa et al., 2000; Cassidy et al., 2001; Agrawal et al., 2011). In one study with 471 bipolar disorder cases and 1761 controls, individuals with bipolar disorder were 6.8 times more likely to report lifetime histories of cannabis use as compared to controls (Agrawal et al., 2011). Complementary findings are evident in research on individuals seeking treatment for cannabis use. Analysis of the Danish substance abuse treatment register during a 5-year period (1996–2001) showed that 40.7% of subjects seeking treatment for cannabis use ($n = 3114$) had been treated for a psychiatric condition (Arendt et al., 2007). In addition, treatment-seeking subjects were 4.9 times more likely to be diagnosed with bipolar disorder than a control group drawn from the general population with no documented treatment for cannabis use ($n = 15,570$).

Elevated prevalence rates of comorbid CUDs can be found in patients with bipolar disorder even from early stages of the illness. The University of Cincinnati First-Episode Mania Study followed 144 subjects with a DSM-IV diagnosis of bipolar I disorder who presented with a first episode of manic or mixed mood for at least 4 months (Strakowski et al., 1998). Forty-six percent of the sample met criteria for CUDs during their lifetimes (Strakowski et al., 2007). The McLean-Harvard First-Episode Mania Study reached similar results. All subjects were diagnosed with bipolar I disorder and presented with a first manic or mixed mood episode (Tohen et al., 2003). Patients were followed for up to 24 months after initial hospital discharge. Based on data from the first 112 subjects, 33% of

the sample met criteria for substance use disorders at baseline (Baethge et al., 2005). Prevalence increased to 39% by 2-year follow-up. CUDs were the second most prevalent substance-related comorbidity, after alcohol-related disorders.

In the past several years, a growing body of data has accumulated indicating that cannabis use is associated with an increased risk of psychotic disorders (Moore et al., 2007). Data from large-scale studies suggest cannabis use is also associated with an increased incidence of bipolar disorder. Data derived from the Netherlands Mental Health Survey and Incidence Study (NEMESIS), a large prospective survey of adults, point to a large effect of cannabis exposure on outcome measures in bipolar disorder (van Laar et al., 2007). After adjustments for possible confounding factors such as age, education, parental psychiatric history, and lifetime alcohol use disorders, baseline cannabis use was associated with an almost five-fold increased risk of first incidence of bipolar disorder. A trend towards a dose response between frequency of use and increased risk of incidence was also observed. However, conflicting results are found in the literature. Manrique-Garcia et al. (2012) analyzed data derived from a very large Swedish conscript study. Data originated from examinations of 50,087 men for compulsory military training in 1969 and 1970 and were linked with inpatient registries. The authors found no association between cannabis use and later diagnosis of bipolar disorder, even among heavy users.

To date, researchers have evaluated the effects of cannabis on various aspects of bipolar disorder, primarily focusing on course of illness and treatment outcome. The goal of this chapter is to review the different potential impacts of cannabis use on several aspects of bipolar disorder, including clinical course, functioning and neurocognitive impairments, and diagnostic and treatment challenges.

CLINICAL CORRELATES OF CANNABIS USE IN BIPOLAR DISORDER

AGE AT ONSET

Age at onset (AAO), defined as the first affective episode of either polarity meeting DSM-IV criteria, appears to have a tri-modal distribution in bipolar disorder (Bellivier et al., 2003). A number of reports indicated that an early AAO of bipolar disorder has a deleterious impact on clinical presentation. Individuals with early AAO of bipolar disorder have been reported to have an increased frequency of exhibiting psychotic symptoms (McGlashan, 1988; Schürhoff et al., 2000; Schulze et al., 2002; Yildiz and Sachs, 2003), more frequently recurring and severe manic and mixed mood states (Angst, 1986; Schürhoff et al., 2000; Hamshere et al., 2009), greater lifetime comorbid Axis I and II disorders (Bashir et al., 1987; Chen and Dilsaver, 1995; Schürhoff et al., 2000; Goldberg and Garno, 2009), and a greater likelihood of a family history positive for affective disorders (Hamshere et al., 2009).

A number of studies have demonstrated a significant relationship between cannabis use and AAO of bipolar disorder (Table 9.1). Data from the NESARC study, previously described in this chapter, showed a significantly earlier AAO among cannabis users (Lev-Ran et al., 2013a). The AAO of a mood episode was approximately 19 years of age for bipolar patients who used cannabis compared to 25 years in bipolar patients with no history of cannabis use. Moreover, patients with CUDs experienced significantly more depressive, manic, and/or hypomanic episodes per year. Additional studies using clinical samples have reported similar findings, demonstrating an association between cannabis use and an earlier AAO ranging from 3.1 to 8.9 years (Ongür et al., 2009; De Hert et al., 2011; Aas et al., 2013). Retrospective data from two large datasets from France and the USA also show an association between earlier AAO of bipolar disorder and CUDs (Etain et al., 2012). AAO was slightly earlier for patients with a CUDs in the study by Braga et al. (2012), but the association was not significant.

Lagerberg et al. (2014) further corroborated these findings. Using data collected from all major hospitals in Oslo, Norway, from 2003 to 2011, the authors analyzed the correlations between amount of cannabis use and AAO. Increased levels of cannabis use predicted a significantly earlier AAO. In a sample of 324 patients diagnosed with bipolar I, II, or not otherwise specified (NOS) disorder, the AAO was 23.2 (± 9.7) years for patients with no or negligible use of cannabis, 20.5 (± 7.3) years for patients who used cannabis more than 10 times in 1 month, and 18.6 (± 5.0) for patients with a lifetime diagnosis of cannabis abuse or dependence.

The sequence of onset of a CUD and bipolar disorder can also have distinct effects on the course of both conditions. Strakowski et al. (2007) used data from the previously mentioned University of Cincinnati First-Episode Mania study. One-hundred and forty-four patients with CUDs were separated into two groups according to the order of sequence of onset: the Bipolar First group, consisting of patients for whom bipolar disorder preceded or first occurred concurrently with the onset of cannabis abuse, and the Cannabis First group, for whom the onset of a CUD preceded the onset of bipolar disorder. AAO of bipolar disorder was significantly later for subjects in the Cannabis First group. The authors suggested that the use of cannabis might precipitate the onset of bipolar disorder in patients with low vulnerability for the illness.

Agrawal and colleagues (2011) observed similar results in a sample of 471 individuals with bipolar disorder and 1761 controls. The authors noted that patients who started using cannabis after their onset of a manic, hypomanic, or depressive episode had an earlier AAO of bipolar disorder (17.5 years) as compared to those who started cannabis use before the onset of bipolar disorder (21.5 years).

Finally, in a naturalistic sample of 151 bipolar I and II disorder patients, subjects with an onset of bipolar disorder prior to the onset of substance use had an earlier AAO of bipolar disorder when compared to patients whose onset of substance use disorder occurred first (Lagerberg et al., 2011). However, cannabis use was associated with earlier AAO as compared to patients with alcohol-related

Table 9.1 Studies Assessing Age at Onset of Bipolar Disorder and Cannabis Use

Reference	Sample	Finding	Statistics
Aas et al., 2013	587 BD cases (75 cannabis abuse)	Cannabis abuse AAO: 19.6 ± 5.8 years No cannabis abuse AAO: 25.0 ± 10.5 years	$z = -4.17$, $p < 0.001$
Agrawal et al., 2011	471 BD cases 1761 controls	Primary BD AAO: 17.5 years Secondary BD AAO: 21.5 years	Not reported
Braga et al., 2012	200 BDI cases (50 CUD history; 150 no CUD history)	CUD AAO: 21.3 ± 7.2 years No CUD AAO: 22.5 ± 8.4 years	$t = 1.02$, $df = 198$, $p = 0.32$
De Hert et al., 2011	90 BD cases with psychotic features	Cannabis use associated with reduced AAO by 8.9 years	$p < 0.0001$
Etain et al., 2012	1194 BD cases (480 French patients; 714 US patients)	Early AAO associated with lifetime cannabis misuse	France: OR = 2.60; 95% CI, 1.51–4.48 USA: OR = 1.75; 95% CI, 1.02–3.01
Lagerberg et al., 2011	151 BD cases ^a	Primary BD AAO: 21.8 ± 9.0 years Secondary BD AAO: 25.9 ± 9.8 Cannabis use AAO: 19.5 ± 5.4 years Alcohol use AAO: 27.9 ± 11.8 years No use AAO: 22.5 ± 9.1 years	$p = 0.02$ $p = 0.005$ (cannabis use vs. alcohol use)
Lagerberg et al., 2014	324 BD cases	No/low cannabis use AAO: 23.2 ± 9.7 years Intermediate cannabis use AAO: 20.5 ± 7.3 years Lifetime CUD AAO: 18.6 ± 5.0 years	$p = 0.02$ (lifetime CUD vs. no/low use) $p = 0.09$ (intermediate use vs. no/low use) $p = 0.6$ (lifetime CUD vs. intermediate use)
Lev-Ran et al., 2013a	1905 BD cases	CUD AAO mania: 19.5 ± 0.81 years No CUD AAO mania: 25.1 ± 0.37 years CUD AAO depression: 18.5 ± 0.95 years No CUD AAO depression: 24.4 ± 0.37 years	$p < 0.0001$ $p < 0.0001$

(Continued)

Table 9.1 Studies Assessing Age at Onset of Bipolar Disorder and Cannabis Use
Continued

Reference	Sample	Finding	Statistics
Ongür et al., 2009	92 BD cases with psychotic features 61 schizoaffective disorder cases 80 schizophrenia cases	Lifetime CUD associated with reduced AAO of psychosis by 3.1 years ^b	$\beta = -3.11$, $t_{198} = -3.54$; $p < 0.001$
Strakowski et al., 2007	69 cases with BD and CUDs 75 BD only cases	Primary BD AAO: 16 ± 6 years Secondary BD AAO: 23 ± 6 years No CUD AAO: 18 ± 10 years	$p = 0.002$ (secondary BD vs. both other groups)

BD cases include individuals diagnosed with bipolar I disorder, bipolar II disorder, and/or bipolar disorder NOS.

AAO, age at onset of bipolar disorder (unless otherwise noted).

Primary BD, individuals with an onset of BD that precedes the onset of substance use.

Secondary BD, individuals with an onset of BD that follows the onset of substance use.

CUD, cannabis use disorder.

Significant *p*-values are typed in bold.

^aCases included individuals who had lifetime histories of alcohol use and cannabis use.

^bNo significant difference between different diagnoses.

disorders and non-users. The latter finding was in contrast to both previous studies' results. The authors speculated that this discrepancy could be due to the high rates of family history of mood and psychotic disorders in their sample of patients with CUDs as compared to the samples used in other studies.

COURSE OF ILLNESS

Mood and psychotic symptoms

The use of cannabis has been associated with increased severity of manic symptoms, number of mood episodes, and greater overall morbidity in individuals with bipolar disorder. Several large-scale studies using national registries and epidemiological samples in Europe have examined this relationship both in the general population and clinical samples (Henquet et al., 2006; van Rossum et al., 2009). Henquet and colleagues (2006) prospectively evaluated 4815 participants recruited from the general population in the Netherlands, inquiring about drug use, as well as mood and psychotic symptoms. Participants who used cannabis at baseline were more than twice as likely to report manic symptoms at the first- and third-year follow-up than those who did not, even after controlling for clinical and demographic characteristics (odds ratio = 2.11, 95% confidence interval: 1.06, 4.20). Interestingly, participants who used cannabis and denied all manic or psychotic symptoms at baseline were more likely to experience manic symptoms at follow-up (adjusted odds ratio = 2.70, 95% confidence interval: 1.54, 4.75).

It should be noted that this study specifically evaluated patients presenting with subthreshold manic and psychotic symptoms, requiring only 2 days of duration to be rated as present. As a result, manic symptoms may be overrepresented.

Individuals with bipolar disorder and histories of CUDs included in the NESARC study declared a significantly greater median number of manic, hypomanic, and depressive episodes per year (1.8) than did individuals without CUDs (0.7) ($p = 0.004$) (Lev-Ran et al., 2013a). There was also a trend level association between CUDs and greater likelihood of rapid cycling ($p = 0.06$). Individuals with or without CUDs reported similar quality of life and equivalent exposure to treatment. It should be pointed out that the NESARC data were collected from epidemiological samples. Compared to typical clinical samples, the number of individuals diagnosed with bipolar disorder receiving psychiatric treatment was notably low. Approximately half of bipolar subjects in the total sample had never been treated.

Similar results were observed in clinical samples. Van Rossum et al. (2009) analyzed data collected prospectively during the first year of the EMBLEM study and compared 436 patients who used cannabis to 2990 who did not. All patients were recruited during a manic or mixed mood episode and assessed at multiple time points for 12 months. Participants who used cannabis were less likely to be compliant with their medication regimen. However, based on Clinical Global Impression (CGI) scales adapted for bipolar disorder (Spearing et al., 1997), participants who used cannabis presented with more severe manic symptoms and bipolar illness overall, even after controlling for clinical and demographic characteristics, including medication compliance. These findings support those of the previously mentioned study by Strakowski and colleagues (2007). In their bipolar sample experiencing a first episode of mania, patients with histories of cannabis use exhibited symptoms of mania for a significantly longer period of time and experienced more rapid cycling than those with no history of cannabis use. However, not all studies agree on the correlation between cannabis use and increased cycling. As noted earlier, Agrawal and colleagues (2011) found no difference in frequency of mood episodes when comparing bipolar patients with CUDs to those without. CUDs were associated with increased likelihood of mixed episodes, a predictor of poor outcome in bipolar disorder (Keller et al., 1986).

Studies have examined the association of cannabis use with other markers of severity in bipolar disorder in addition to mood symptoms (van Rossum et al., 2009; Agrawal et al., 2011; Aas et al., 2013; Etain et al., 2013; Lev-Ran et al., 2013a). As noted, the strength of association between cannabis use and psychosis has been replicated in multiple studies. In addition, the presence of psychosis is considered an indication of a more severe mood episode in the course of affective disorders. In the EMBLEM study, participants who used cannabis were rated as more severely psychotic based on the presentation of hallucinations or delusions (van Rossum et al., 2009). However, other studies found no (Agrawal et al., 2011) or minor (Henquet et al., 2006) correlation between cannabis use and increased psychosis.

Sequence of onsets

Although many of the studies reviewed point to an association between cannabis use and the course of bipolar disorder, they are all limited by cross-sectional designs, which preclude conclusions regarding causality. Two prospective studies attempted to determine the temporal association between cannabis use and mood symptoms. In the previously mentioned study by Strakowski et al. (2007), patients with a first episode of mania whose bipolar disorder preceded or first occurred concurrently with the onset of cannabis abuse exhibited symptoms of mania for a significantly longer period of time than patients for whom the onset of a CUD preceded the onset of bipolar disorder. Both groups experienced more rapid cycling than did subjects with no history of cannabis use. The timing of substance use disorders and bipolar disorder was also evaluated in 166 patients from the McLean-Harvard First-Episode Project who were followed for 4.7 years (Baethge et al., 2005). In order to analyze the temporal association between cannabis use and mood symptoms, the longitudinal course was divided into 3-month intervals. Cannabis use during the preceding or concurrent quarter was strongly associated with manic or hypomanic symptoms, but not depressive symptoms. Therefore, cannabis misuse by patients suffering from a first episode of mania predicts poorer resolution of symptoms. In addition, continued use is associated with higher rates of mood relapse, particularly of manic episodes.

Suicide

Bipolar disorder is associated with markedly increased suicide rates, and suicide prevention strategies are a major concern in treatment planning. The association between cannabis use and suicide is unclear. Some studies indicate that bipolar patients who abused cannabis were significantly more likely to have attempted suicide (Agrawal et al., 2011; Aas et al., 2013). Notwithstanding, other large sample studies, such as the NESARC, failed to show any direct correlation between history of cannabis use and suicide (Lev-Ran et al., 2013a). Past research has demonstrated a strong correlation between impulsivity and bipolar disorder (Swann et al., 2009; Ekinici et al., 2011; Perroud et al., 2011). Etain and colleagues (2013) studied a sample of 385 euthymic bipolar patients and 185 healthy controls to determine whether the presence of higher levels of trait impulsiveness could be used to identify clinical characteristics, including suicidal behavior. Although impulsivity was associated with a lifetime history of cannabis misuse in bipolar patients and greater clinical severity, they did not find an association between impulsivity and suicide attempts.

Comorbid disorders

In both the EMBLEM and NESARC studies, bipolar patients who used cannabis were more likely to be diagnosed with additional co-occurring disorders, particularly substance use disorders. In the EMBLEM study (van Rossum et al., 2009), bipolar patients who used cannabis also declared more alcohol use, or other substance use disorders. In the NESARC study (Lev-Ran et al., 2013a), bipolar patients with a CUD had a 71.9% prevalence of another substance use

disorder compared to 19% in those without a CUD. In addition, bipolar patients with a CUD were also twice as likely to meet criteria for personality disorders, including antisocial, dependent, histrionic, and obsessive-compulsive personality disorder.

Psychosocial functioning

Impairment in life functioning has been gaining more attention as an important area of research in bipolar disorder. Bipolar disorder is associated with more psychosocial dysfunctioning than is found in the general population even during clinical remission. Analyses of the EMBLEM data indicated that bipolar subjects who used cannabis engaged in fewer relationships and reported less life satisfaction than non-users (van Rossum et al., 2009). Agrawal and colleagues (2011) demonstrated that bipolar patients with CUDs were 1.8 times more likely to report a disability in life functioning due to bipolar disorder than those without, even after relevant covariates were controlled for, such as co-occurring psychopathology and other substance use disorders. Furthermore, individuals whose cannabis use preceded the onset of a bipolar disorder mood episode were 1.75 times more likely to report a bipolar disorder-related disability as compared to those whose cannabis use began at the same time as, or after the onset of, a first mood episode.

CANNABIS USE AND NEUROCOGNITION IN BIPOLAR DISORDER

Bipolar disorders were traditionally distinguished from schizophrenia by the course of illness, which included distinct mood episodes and periods of functional recovery. However, more recent data using modern diagnostic criteria indicate that up to 30% of those suffering from bipolar disorder experience only limited inter-episode recovery (American Psychiatric Association, 2000). Neurocognitive deficits similar in profile, but less severe than those associated with schizophrenia have been identified in the course of bipolar disorder (Burdick et al., 2011) and are correlated with functional impairment (Green, 2006; Kurtz and Gerraty, 2009). Furthermore, these deficits are present even when patients are not suffering from acute mood episodes (Robinson et al., 2006; Burdick et al., 2011). Cognitive deficits have also been associated with cannabis use (Grant et al., 2003; Gonzalez, 2007; Crean et al., 2011; Schreiner and Dunn, 2012), but only a few studies have investigated the impact of comorbid cannabis use on cognitive ability in patients diagnosed with bipolar disorder (Ringen et al., 2010; Braga et al., 2012).

CANNABIS AND COGNITION

Extensive research has focused on the neurocognitive effects of cannabis use (Grant et al., 2003; Crean et al., 2011; Meier et al., 2012; Schreiner and Dunn,

2012). These studies can primarily be divided into studies of acute effects [see review by Crean et al. (2011)] and long-term effects [see review by Schreiner and Dunn (2012)]. Many of these studies report conflicting results across the major domains of cognitive function, which may be due to methodological issues, such as the use of cross-sectional designs relying on retrospective data and including samples using other drugs that may confound results. In addition, some studies have included subjects classified simply as cannabis users while others focus on those meeting DSM criteria for abuse or dependence. Overall, studies have demonstrated an association between cannabis use and a mild deficit in memory performance and some aspects of executive functioning (Gonzalez, 2007; Crean et al., 2011). Also, the effect of cannabis use on cognition in itself may be complex, impacting brain development at critical periods rather than having a stable effect.

A negative effect of acute cannabis intoxication on neurocognition has been observed across studies. Schreiner and Dunn (2012) conducted two meta-analyses to evaluate the long-term effects of cannabis use on neurocognition, the first of which updated a previous meta-analysis by Grant et al. (2003) on the non-acute effects of cannabis. This analysis encompassed 1000 cannabis-using participants and 839 controls. In contrast to these findings, cannabis users performed worse than comparison groups on global neurocognitive performance and all other domains except motor and simple reaction time. The second meta-analysis specifically examined the long-term effects by assessing studies including participants who were abstinent for at least 25 days. This analysis revealed no difference between cannabis users and controls. This suggests that the reported deleterious effect of cannabis on cognition appears to resolve within a month of discontinued use. Of note, Verdejo-Garcia and colleagues (2007) compared male cannabis users abstinent for 25 days to healthy controls and cocaine users on repeat testing of the Iowa Gambling Task (IGT) (Bechara et al., 1994), a measure of emotion-based decision-making. Cannabis users did not perform worse than the healthy controls, but demonstrated a delay in learning between the first and second trials of the test. Furthermore, Crean et al. (2011) found that attention and concentration deficits have been observed primarily for infrequent users, pointing to a habituating effect in regular users.

Meier and colleagues (2012) analyzed data drawn from a large birth cohort of 1037 subjects. In their analysis, participants who began using cannabis prior to age 18 demonstrated lowered IQ scores and neurocognitive performance in all domains of cognitive function at age 38. No change was observed for participants who began using cannabis after 18 years of age. A dose response was also observed; endorsing cannabis use at more time points correlated with a greater reduction of IQ score. Cannabis use was also associated with collateral report of memory and attention problems, which the authors argued reflects an impact in the real world, beyond test performance. Moreover, the results were consistent across socioeconomic groups (Moffitt et al., 2013).

NEUROCOGNITIVE DEFICITS IN BIPOLAR DISORDER

Two meta-analyses provide a comprehensive overview of neurocognitive functioning in bipolar disorder. Not surprisingly, the effects of acute mood episodes were significant with regard to neurocognitive performance. Kurtz and Gerraty (2009) compared the performance of subjects tested during an acute mood episode to that of subjects exhibiting a full remission of mood symptoms. Acute mood states of either polarity were associated with exacerbated verbal learning impairment. Depression was also associated with significantly more impaired semantic fluency than during the euthymic phase. However, when compared to individuals with no psychiatric diagnoses, even euthymic bipolar disorder groups have exhibited significant deficits across most domains, namely, memory, executive functioning, attention, and processing speed (Robinson et al., 2006). The most pronounced deficits were in verbal learning and delayed memory, as well as executive functioning (Robinson et al., 2006; Torres et al., 2007; Arts et al., 2008; Bora et al., 2009).

The presence of substantial deficits during the euthymic phase suggests that neurocognitive impairment is a trait, rather than state-based aspect, of bipolar disorder. It is possible that cognitive difficulties are related to residual or subthreshold mood symptoms, or are secondary to psychotropic medications, but this has not yet been systematically studied. Regardless, patients face challenges in remembering information and efficiently engaging in tasks requiring problem-solving and abstract reasoning. These findings frequently translate clinically into patients whose mood symptoms have seemingly improved, but are still reporting difficulties with poor memory and concentration, and problems in their day-to-day functioning.

CANNABIS AND COGNITION IN BIPOLAR DISORDER

In a study of cannabis use in outpatient participants suffering from mood and psychotic disorders, Ringen et al. (2010) compared bipolar I, II, and NOS patients, 18 of whom used cannabis and 111 who did not, on measures of motor speed, attention, and working memory, executive functioning, verbal learning, and memory. The groups performed similarly on most measures, but participants who used cannabis actually performed significantly better on a test of semantic fluency. A limitation of the study was that data regarding clinical state or symptom levels were not reported for the subset of patients diagnosed with bipolar disorder, nor were the diagnoses of abuse or dependence versus simple use.

A subsequent study by Braga et al. (2012) demonstrated improved performance associated with cannabis use in patients diagnosed with bipolar disorder. The authors compared 50 patients with bipolar I disorder diagnoses and histories of cannabis use disorder (CUD+) to 150 patients with no cannabis use disorder history (CUD−). The groups did not differ regarding mood symptoms and most

relevant demographic variables. Men were more likely to abuse cannabis than were women. The CUD+ group performed better on tests of attention and working memory, as well as set-shifting, an aspect of executive functioning. This study is noteworthy as it only included patients diagnosed with bipolar I disorder. The CUD+ group was more likely to endorse a history of psychotic symptoms, suggesting a more severe course of illness in bipolar patients with histories of CUDs.

The finding of improved neurocognitive performance associated with cannabis use by bipolar patients seems counterintuitive, particularly since cannabis use negatively affects verbal memory and executive functions, the domains most impaired in bipolar disorder (Burdick et al., 2007). This seemingly beneficial association between cannabis use and cognition in bipolar patients has also been replicated in some studies focused on patients with schizophrenia (DeRosse et al., 2010; Yücel et al., 2012). There are several potential explanations for this. First, the study by Braga and colleagues (2012) assessed patients with lifetime histories of CUDs instead of current use. In addition, Ringen and colleagues (2010) included all patients with cannabis use within 6 months of the recruitment, but provided no data regarding last use. As noted, studies of long-term effects of cannabis use point to full cognitive recovery within a week. As a result, any effect of cannabis on neurocognition may have resolved by the time of testing. It is also possible that cannabis use may reflect better premorbid function; seeking out and negotiating with others to procure cannabis arguably requires both social and cognitive skills (DeRosse et al., 2010; Ringen et al., 2010; Braga et al., 2012). It may be the case that cannabis-using samples reflect a subgroup of individuals with better cognitive performance prior to onset of the bipolar disorder than those who never use cannabis. However, it should be noted that in the study by Braga et al. (2012), individuals with or without histories of cannabis use did not differ significantly on estimates of premorbid IQ. As the two studies reviewed used differing inclusion criteria, further investigation is warranted and any conclusion at this point is preliminary.

Although no direct effect of cannabis use on cognition in bipolar disorder has been identified, several considerations for treatment may be noted. As short-term effects have been documented, weekly use or greater can result in persistently compromised memory and executive functioning skills, such as planning and decision-making. Patients who use cannabis, perhaps to self-medicate for psychiatric symptoms such as anxiety, should be educated about the potential negative aspects of regular use. In addition, Cahill and colleagues (2010) noted that psychotherapy necessarily requires cognitive skills on the part of the patient, particularly the ability to draw on past experiences and weigh risks and benefits when making decisions. The use of cannabis may interfere with a patient's cognitive ability to engage in psychotherapy to its maximum effect. Similarly, patients may have more difficulty engaging in motivational interviewing-based therapy as a result of the deficits associated with bipolar disorder.

IMPACT ON DIAGNOSIS AND TREATMENT

DIAGNOSIS

Optimal treatment for individuals with comorbid bipolar disorder and CUDs requires appropriate assessment and diagnoses. Due to the high rates of comorbidity, a comprehensive evaluation of individuals with bipolar disorder should include a systematic assessment of CUDs (Lev-Ran et al., 2013a). This would not only provide clinicians with relevant diagnostic information, but also inform treatment planning. For this reason, it is vital that clinicians accurately identify and diagnose a CUD when present. Unfortunately, it has been shown that accurate diagnosis of a CUD in patients with bipolar disorder can prove to be difficult.

In a study of 80 adolescent participants who presented with some symptoms of mania as measured by elevated scores on the Young Mania Rating Scale (YMRS) (Young et al., 1978), Black et al. (2012) found low diagnostic agreement among skilled interviewers and diagnosticians for CUDs. The researchers studied both the CUD diagnoses obtained during the initial assessments and the best-estimate consensus diagnoses determined by a group including a child psychiatrist, an addiction psychiatrist, study nurses, coordinators, and therapists. The initial diagnoses were based on the use of a Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children—Present and Lifetime (KSADS-PL) (Birmaher et al., 2009). The consensus diagnoses were based on the KSADS-PL in addition to five other assessment tools. Diagnostic agreement between the initial diagnoses and the best-estimate consensus diagnoses was not significantly better than chance. The researchers suggested poor reliability may be due to limitations in patient reporting during interviews, which are likely to include patient concerns about confidentiality. Another potential reason may be the patient's impaired insight into their behaviors due to a manic mood episode.

TREATMENT

Despite the documented association between cannabis use and poor prognosis in individuals with bipolar disorder, few research studies have looked at the impact of cannabis use on bipolar disorder treatment outcome. The few studies that have examined this connection have indicated that cannabis users fare worse than non-users in terms of treatment outcomes, including compliance and prognosis (van Rossum et al., 2009). Data regarding specific treatment strategies for bipolar disorder patients with co-occurring CUDs are limited. Kemp and colleagues (2009) conducted a 6-month, double-blind, placebo-controlled comparison randomized controlled trial (RCT) of lithium monotherapy and lithium plus valproic acid in 149 patients with bipolar disorder and comorbid substance use disorders (SUDs). In the first phase of the study, all of the participants received treatment with lithium and valproic acid. During this phase, 79% of patients discontinued prematurely, mostly

due to non-adherence or poor response to the treatment. Among the 15 subjects diagnosed with comorbid CUDs who completed the protocol, 53% no longer met criteria for a CUD at the end of the first phase of the study. Furthermore, [Geller and colleagues \(1998\)](#) conducted a 6-week placebo-controlled RCT of lithium monotherapy with 25 adolescents with bipolar disorders who were primarily addicted to cannabis plus alcohol (56%), alcohol only (28%), and cannabis only (8%). A significant decrease on urine drug assays for both substances was noted. Finally, a few case series point to a potential positive effect of divalproex for bipolar disorder with comorbid SUDs, but no specific data are available for subjects with CUDs ([Brady et al., 1995](#); [Albanese et al., 2000](#); [Hertzman, 2000](#)).

Neurobiological studies, as well as animal model studies and anecdotal reports, indicate that substances that activate specific cannabinoid receptors in the endocannabinoid system in the brain might elicit anti-manic or antidepressant-like effects ([Gruber et al., 1996](#); [Grinspoon and Bakalar, 1998](#); [Hill and Gorzalka, 2009](#)). The endocannabinoid system is diffusely distributed in the brain and is associated with modulation of several vital functions, such as pain, appetite, memory, and mood. It is also the system through which cannabis exerts its psychoactive effects. In a study of rats' performances on the Porsolt forced swim test, [Hill and Gorzalka \(2005\)](#) found that pharmacological stimulation of the main cannabinoid receptor, cannabinoid type 1 (CB₁) receptor, had an antidepressant effect in the rats. Furthermore, pharmacological and genetic blockade of the CB₁ receptor induced a phenotypic state in rats comparable to melancholic depression with symptoms of reduced food intake, heightened anxiety, increased arousal and wakefulness, deficits in extinction of aversive memories, and hypersensitivity to stress. Based on these findings, the authors suggested that symptoms of melancholic depression may be caused by an endocannabinoid deficiency. Therefore, activation of the endocannabinoid system might serve as a pharmacological option for treatment-resistant depression.

At least two constituents of cannabis activate the endocannabinoid system, Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD) ([Ashton and Moore, 2011](#)). When used in small to moderate doses, THC produces effects commonly associated with cannabis intoxication, such as euphoria, anxiety reduction, sedation, and muscle relaxation. However, in high doses or rapid administration, THC can induce transient psychotic and anxiety symptoms, detachment, and cognitive impairment ([D'Souza et al., 2004](#); [Morrison et al., 2009](#)). Conversely, CBD antagonizes the intoxicant and psychomimetic actions of THC ([Zuardi et al., 2010](#)). CBD may have potential antipsychotic, anticonvulsive, antidepressant, and anxiolytic properties ([Mechoulam et al., 2007](#); [Zuardi et al., 2010](#)), which are similar to the characteristics of psychotropic medications used in the treatment of bipolar disorder ([Ashton et al., 2005](#)). One study has examined the efficacy of CBD in the treatment of bipolar disorder. [Zuardi and colleagues \(2010\)](#) conducted a single-blind case series with two patients with bipolar disorder in a manic episode. CBD monotherapy was not associated with symptom improvement as measured by scores on the YMRS and

the Brief Psychiatric Rating Scale (Overall and Gorham, 1962). Therefore, as of now, no available data can substantiate the potential utility of endocannabinoid modulators for the treatment of bipolar disorder.

CONCLUSIONS

In recent years, focus on the impact of cannabis use in bipolar affective disorder has greatly increased. The growth in this area of research is relevant given the high prevalence rates of bipolar disorder with comorbid cannabis use. Research on substance use disorders, and cannabis in particular, struggles with several limitations. Most studies rely on participant self-report for measures of amount and frequency of cannabis use. Self-measures are markedly vulnerable to recall bias, which may potentially compromise the validity of a study. However, it should be noted that widely used self-report measures, such as the Timeline Follow-Back (TLFB), have shown high agreement with biological measures (Hjorthøj et al., 2012). Moreover, due to ethical and legal reasons, most research studies do not offer cannabinoid substances to study subjects, and have to rely on subjects' reports of personal use. THC levels can vary widely in cannabis that can be purchased on the street. Apart from doing challenge studies in which the levels of THC can be standardized, it would be virtually impossible to control for the potential varying effects of different types of cannabis.

Regarding research on cannabis use and bipolar disorder, the majority of available studies are cross-sectional, which preclude any conclusions regarding causality. In addition, the inclusion criteria for participants tend to vary greatly between studies. As mentioned earlier, some studies include participants with subthreshold symptoms of either CUDs or bipolar disorder, whereas others only include those who meet DSM criteria for the diagnoses. This could potentially cause target symptoms to either be under- or overrepresented in a study sample. Lastly, the potential effects of cannabis use in bipolar disorder may be the result of a complex interaction. The few prospective studies available suggest that exposure to cannabis in different phases of life can have distinct effects. This was elegantly highlighted by the large birth cohort study conducted by Meier et al. (2012). As described previously, cannabis was associated with a small but significant decline in IQ, but the effects were only observed among subjects who started using prior to age 18. It is plausible that only studies with targeted samples will be able to provide conclusive answers regarding the impact of cannabis use on bipolar disorder.

With these limitations in mind, a review of the literature suggests potential implications of cannabis use among subjects diagnosed with bipolar disorder. The strongest findings point to an association between cannabis use and an earlier AAO, increased severity and frequency of mood episodes, particularly mania, and increased numbers of comorbid Axis I and II disorders, as well as more severe

deficits in psychosocial functioning. The associations between cannabis use and increased likelihood of suicide attempts and psychosis are less conclusive. Taken together, findings indicate that cannabis use may be associated with worse prognostic outcomes in patients with both disorders.

Clinicians treating patients with bipolar disorder should pay careful attention to address comorbid cannabis use. Diagnosing a CUD can be challenging, even when professionals use structured diagnostic instruments. Clinicians should thoroughly probe for negative effects of cannabis use, not only on the patient's general functioning, but also on the course of their mood disorder. As [Strakowski et al. \(2007\)](#) noted, cannabis use and mood disorders can interact in multiple ways. Cannabis use may potentially trigger mood episodes, prolong the time to remission, or actually serve as a means for potential self-medication. In addition, cannabis use appears to be a predictor of treatment non-compliance. Psychoeducation should be stressed more highly for this group.

In addition to the potential negative impact of cannabis use on the course of bipolar disorder, advances in neurobiological research have facilitated greater understanding of the role of the endocannabinoid system in bipolar disorder and how cannabinoid modulators may serve as a treatment for the illness. To date, a few animal studies and anecdotal reports have suggested potential therapeutic effects. It should be noted that at this time, no definitive evidence suggests cannabis may be beneficial in any way. Medical research with illicit drugs is often difficult, particularly hampered by legal and ethical constraints. As several states have recently legalized the use of cannabis for medical and in some cases even recreational use, it may become easier for research studies to be conducted in the future in order to determine any potential therapeutic effect of cannabinoids.

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Cannabinoids and the Tourette syndrome

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TOURETTE SYNDROME

DEFINITION AND CLINIC

Tourette syndrome (TS) is a childhood-onset, neuropsychiatric disorder characterized by motor and vocal tics. According to DSM-5, the following diagnostic criteria have to be fulfilled to make the diagnosis of TS (307.23, ICD-10 F95.2): (1) both multiple motor and one or more vocal tics have been present at some time during the illness, (2) tics may wax and wane in frequency, (3) tics have persisted for more than 1 year since first tic onset, (4) onset is before age 18 years, and (5) tics are not caused by another medical condition or a substance.

Tics are sudden, rapid, recurrent, non-rhythmic motor movements or vocalizations. Most patients report that their tics are preceded by a premonitory urge. Some patients feel that this premonitory feeling is even more troublesome than the tics. Furthermore, tics are temporarily suppressible. Tics wax and wane spontaneously in frequency, number, intensity, and complexity. In addition, tics can be influenced by environmental factors: most patients feel that stress, anxiety, discomfort, and anger increase the tics, while relaxation and concentration lead to a temporary tic reduction. Finally, onset, course, and intensity of tics strongly depend on age. The typical age of onset is between age 6 and 8 years. Tics reach their peak severity most often between age 10 and 14. In later adolescence and adulthood in most patients, tics decrease spontaneously. Overall, in the vast majority of patients, TS has a favorable prognosis with significant improvement in adulthood. However, there are still a substantial number of adult patients who feel a significant impairment of quality of life because of their tics. Particularly in patients suffering from severe and complex tics (including copro- and echophenomena), the prognosis is poor with persistence of several tics into adulthood (Robertson, 2000; Leckman, 2002).

More than 80% of all patients with TS suffer not only from tics, but also from psychiatric comorbidities. The most common comorbidities are attention-deficit-hyperactivity disorder (ADHD) (in children in about 50%) and obsessive compulsive

behavior (OCB) (between 40 and 80%). Several patients suffer from mild to moderate OCB, but do not fulfill diagnostic criteria for obsessive compulsive disorder (OCD) according to DSM-5. Other common comorbidities are self-injurious behavior, depression, anxiety, impulsivity, rage attacks, sleeping problems, and learning disorders. Most patients suffer not only from one, but two or even more comorbidities. Therefore, in most patients, TS presents as a multifaceted and complex neuropsychiatric disorder that significantly impairs quality of life in a substantial number of patients (Robertson, 2000; Müller-Vahl et al., 2010).

EPIDEMIOLOGY, GENETICS, AND PATHOLOGY

Not only chronic tic disorders in general (3–4%) but also TS (0.7–1%) are common disorders. For unknown reasons, TS is more common in males than in females (4:1) (Knight et al., 2012).

Without doubt, there is a significant genetic contribution to TS. However, until today, no single specific genetic locus has been identified. There is substantial evidence that TS is a complex, genetically heterogeneous disorder. It can be assumed that multiple variations in multiple genes contribute to the risk for TS. Results obtained from twin studies indicate that not only genetic, but also environmental factors contribute to the development of TS. There is some evidence that several perinatal factors may increase the risk for TS including older paternal age, severe maternal psychosocial stress during pregnancy, maternal smoking during pregnancy, delivery complications, and low Apgar score (Hoekstra et al., 2013; Paschou, 2013). It is matter of debate whether specific infections, recent exposure to pharyngeal group A streptococcus, or other immunopathogenic mechanisms increase the risk for TS (Martino et al., 2009). Several lines of evidence support a role of cortico-striato-thalamo-cortical circuitry in the pathology of TS (see Figure 10.1).

However, the specific pathophysiological abnormality has not yet been defined. Results from neuroimaging studies suggest an involvement of the striatum (with a reduction in the volume of the caudate), larger volumes in the corpus callosum, and cortical thinning in sensorimotor, prefrontal, and cingulate cortices. Results in children differ from those in adults indicating that TS is a neurodevelopmental disorder (Felling and Singer, 2011; Ganos et al., 2013). Furthermore, there is evidence for an involvement of the dopaminergic system. This hypothesis is mainly based on the fact that dopamine receptor antagonists are the most effective drugs for the treatment of tics. Results from a limited number of neuroimaging studies suggest an alteration of the tonic–phasic presynaptic neurotransmitter release system. However, there is increasing evidence that there is an imbalance not only in the dopaminergic system, but also in several other neurotransmitter systems including the glutamatergic, GABAergic, serotonergic, histaminergic, and cannabinoid receptor systems (Martino and Leckman, 2013).

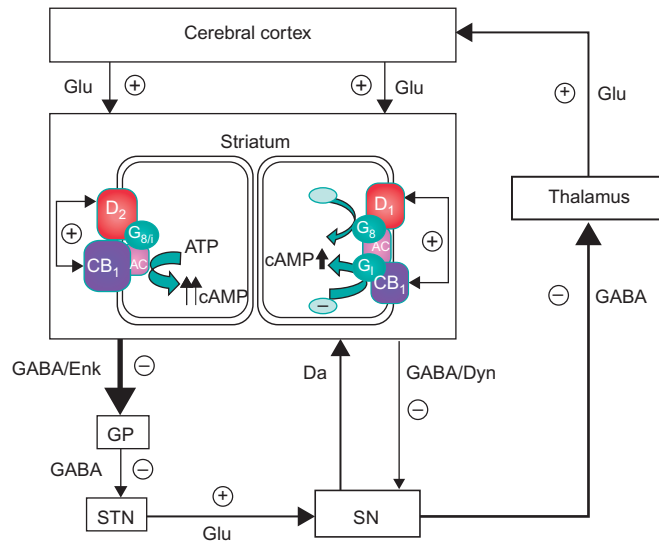
**FIGURE 10.1**

Diagram of the basal ganglia motor circuit. A simplified diagram showing the main connections between brain regions involved in TS, i.e., the basal ganglia motor circuit. The presence of CB₁ receptors on striato-nigral GABAergic projection neurons (which also express dopamine D₁ receptors) allows the cannabinoids to modulate the afferent glutamatergic inputs into the striatum. In addition, their presence on striatopallidal GABAergic projection neurons (which also express dopamine D₂ receptors) allows the modulation of the efferent inhibitory outputs from the medium-spiny projection neurons. ATP: adenosine triphosphate; cAMP: cyclic adenosine monophosphate; Dyn: dynorphins; Enk: enkephalins; Glu: glutamate; GP: globus pallidus; STN: subthalamic nucleus; SN: substantia nigra.

THERAPY

Different therapeutic methods can be used to manage the symptomatology of TS. However, there is no therapy known to be effective not only in the treatment of tics, but also in improving associated behavioral disorders. In patients who suffer from both severe tics and significant psychiatric comorbidities, therefore, different treatment strategies have to be used. All known treatment options are mere symptomatic treatments that improve, but do not cure the tics. For the treatment of tics, behavioral therapy (habit reversal training, exposure and response prevention training) and pharmacological therapy are recommended as first-line treatments (Roessner et al., 2011; Verdellen et al., 2011). In severely affected, adult, treatment-resistant patients, in addition, deep brain stimulation can be used (Müller-Vahl et al., 2011). Antipsychotics (dopamine receptor blocking drugs) are considered the most effective agents in the treatment of tics. However, because these drugs often have significant adverse effects, pharmacotherapy with

antipsychotics is recommended only in those patients who are significantly impaired and/or suffer from severe tics. Until today, several different drugs have been suggested for the treatment of tics including antipsychotics, noradrenergic agents, dopamine receptor agonists, benzodiazepines, monoamine transporter inhibitors such as tetrabenazine, nicotine, botulinum toxin, GABA_B receptor agonists such as baclofen, the antiepileptic drug topiramate, and cannabinoids. However, there is still a lack of well-designed and well-powered studies on the treatment of tics, and, therefore, recommendations heavily depend on the experts' experiences and preferences as well as local availability of the drugs rather than scientific evidence. By European experts the following drugs are recommended and most often used, respectively: risperidone, aripiprazole, sulpiride, and tiapride (Roessner et al., 2011). In contrast, American experts gave quite different recommendations compared to the ESSTS (European Society for the Study of Tourette Syndrome) guidelines (Roessner et al., 2011): guanfacine and tetrabenazine as first choice treatment followed by fluphenazine, risperidone, and other atypical antipsychotics, clonazepam, topiramate, and botulinum toxin (Jankovic and Kurlan, 2011).

For the treatment of associated OCD, behavioral therapy and/or selective serotonin-reuptake inhibitors (SSRI) are recommended. Psychostimulants such as methylphenidate are the treatment of choice in patients suffering from comorbid ADHD. In patients with severe and complex symptoms, combined treatment with several drugs is often inevitable.

ALTERNATIVE TREATMENTS

Although the therapeutic spectrum for the treatment of TS has expanded during the last few years, there is still a substantial number of patients who are unsatisfied with available treatment strategies either due to less efficacy or to significant adverse effects. In addition, some patients—including a large number of those patients who are extremely severely affected—are treatment resistant to all established treatment strategies. Therefore, many patients with TS seek complementary or alternative medicine (CAM) including nutritional supplements and diets, massage, chiropractic manipulations, meditation and yoga, acupuncture, hypnosis, homeopathy, and chiropractic. According to two American surveys, between 64 and 87% of all patients with TS use at least one alternative medicine (Mantel et al., 2004; Kompoliti et al., 2009). Most importantly for treating physicians, 80% of these patients did not inform their doctor before initiating CAM (Kompoliti et al., 2009).

In addition, it has been known for many years that several patients use legal and illegal drugs such as nicotine, alcohol, and marijuana as a self-medication for their tics (Müller-Vahl et al., 1997a,b). Since many patients report that drinking alcohol leads to a tic reduction, it has been speculated that some patients use alcohol as a self-medication, which may cause alcohol abuse (Müller-Vahl et al., 1997b). While there are no reports about cannabis addiction in patients with TS, there is evidence that the risk for alcohol dependency is increased in this group of patients (Pauls et al., 1988).

Table 10.1 Dosage of Cannabinoids in the Treatment of 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 per day	30 mg per day
Nabiximols	1 spray dose	1 spray dose every 3–5 days	individually	6–10 spray doses per day	12 spray doses per day (maximum licensed daily dosage)

MEDICINAL USE OF CANNABINOID-BASED MEDICINES

Cannabis sativa L. has been used for medicinal purposes in many cultures for hundreds of years, for example for the treatment of pain, spasms, asthma, insomnia, depression, and loss of appetite. In modern medicine, cannabinoid-based medicines (CBM) have once again gained importance, after the determination of the exact chemical structure of Δ^9 -tetrahydrocannabinol (THC), the most psychoactive ingredient of *Cannabis sativa* L., and, in addition, the identification of a cannabinoid receptor system with different receptor types (CB₁, CB₂) and specific ligands—endocannabinoids such as anandamide (arachidonylethanolamide) and 2-arachidonoylglycerol (2-AG).

Today, in many countries the cannabinoid THC (dronabinol, nabilone) and the cannabis extract nabiximols—containing THC:cannabidiol (CBD) 1:1—are approved for clinical use for the treatment of nausea and vomiting associated with cancer chemotherapy, anorexia in HIV/AIDS, and spasticity in multiple sclerosis (see Table 10.1). However, there is substantial evidence that cannabinoids are also effective in the treatment of other conditions such as neuropathic pain, spasms, and movement disorders (Grotenhermen and Müller-Vahl, 2012).

EFFECTS OF *CANNABIS SATIVA* L. IN PATIENTS WITH TOURETTE SYNDROME

CASE REPORTS

In 1988, Sandyk and Awerbuch from the University of Arizona, USA, reported on three patients with TS and suggested for the first time that smoking marijuana might ameliorate different symptoms of this disease (Sandyk and Awerbuch, 1988). They reported on a 15-year-old boy who experienced a general relaxation, reduced urge to tic, reduction of motor tics of 50%, and improvement of self-injurious behavior

when smoking one to two cigarettes of marijuana per day. The second patient was a 17-year-old man who suffered from severe motor and vocal tics. Smoking marijuana resulted in a generalized relaxation, tic reduction of about 60–70%, and improvement of attention. The third patient (a 39-year-old man) experienced a reduction of several symptoms when smoking only one-half to one cigarette of marijuana per day including relaxation, reduction of motor tics, and improvement of hypersexuality. In all these patients, prior treatment with different drugs including haloperidol, clonidine, naltrexone, imipramine, and benzodiazepines, respectively, was unsatisfactory due to adverse effects or lack of benefit.

Five years later in 1993, Hemming and Yellowlees from the Far West Mental Health Service in Australia, reported on a 36-year-old man who retired at age 33 because of his TS (Hemming and Yellowlees, 1993). He suffered from motor and vocal tics including coprolalia. Treatment with haloperidol and pimozide did not improve the tics significantly. At the age of 35, for the first time he took one “cone” of marijuana per night. A cone is a cone-shaped container that can be attached to a “bong” or water pipe to smoke cannabis. Within 1 week, he was “completely symptom free.” He reported that he had no tics during the following year when taking a constant dose of only one cone of marijuana every night.

RESULTS FROM A PROSPECTIVE SURVEY

In 1998, Müller-Vahl et al. performed a survey to further investigate the effect of *Cannabis sativa* L. on tics and psychiatric comorbidities in a larger group of patients (Müller-Vahl et al., 1998). They used a standardized questionnaire and asked 64 consecutive patients with TS (55 males and nine females, mean age 30.3 years, range 15–64 years) attending the movement disorder clinic at the Hannover Medical School, Germany. All patients were interviewed about use of marijuana (including amount, frequency, and duration) and the influence both on tics and on behavioral disorders. Seventeen out of 64 patients (27%) reported use of marijuana (two smoked marijuana regularly for more than 1 year, 15 reported occasional use), while 14 out of 17 patients (82%) felt that use of marijuana improved different symptoms. Nine patients reported moderate or marked reduction of tics, four patients felt a complete remission of motor and vocal tics, four patients felt an amelioration of premonitory urges, and two patients each reported a remission of OCB and improvement of ADHD, respectively. Beneficial effects lasted 3–24 hours according to the patients’ reports. None of the patients reported about serious adverse effects or a deterioration of symptoms during or after smoking marijuana.

CONTROLLED TRIALS

There is only a very small number of open and controlled studies available investigating the effects of CBM in TS. In all these studies, however, neither *Cannabis sativa* L. nor cannabis extracts containing different cannabinoids were used, but only pure THC. The main reason why pure THC and not other substances have

been used so far is that in many countries use of marijuana for medical purposes is still not allowed. In Germany, where most of the available studies have been performed, until recently only pure THC (in capsules or as an oil) could be prescribed and could be used in clinical trials. Today, in many countries, also the cannabis extract nabiximols is available, a plant extract from *Cannabis sativa* L. containing THC and CBD at a 1:1 ratio.

EFFECTS OF Δ^9 -TETRAHYDROCANNABINOL IN PATIENTS WITH TOURETTE SYNDROME

UNCONTROLLED SINGLE-CASE STUDIES

In 1999, Müller-Vahl et al. reported for the first time on successful treatment with a single dose of 10 mg oral Δ^9 -tetrahydrocannabinol (THC) in a 25-year-old male patient with TS (Müller-Vahl et al., 1999). The patient's tics had started at age 6. In addition, he suffered from several comorbidities including ADHD, OCB, anxiety disorder, lack of impulse control, and self-injurious behavior. At age 19, he started smoking marijuana (2–3 g per day) and felt a marked reduction of both tics and associated behavioral disorders. Therefore, he stopped less effective medical treatment with pimozide. After he had stopped smoking marijuana for 3 days, he was treated once with a single dose of 10 mg oral THC. In this prospective single-case study, for the first time valid and reliable rating scales were used to assess the clinical effect of THC in TS. His tics improved by 80%, as measured by using the total tic severity score of the Tourette's Syndrome Global Scale (TSGS) (Harcherik et al., 1984). Accordingly, the patient felt a tic improvement of 70%. In addition, he reported an improvement of attention, impulse control, OCB, and premonitory urges. Using different neuropsychological tests, improved signal detection, sustained attention, and reaction time could be demonstrated after THC treatment. The improvement began 30 minutes after treatment and lasted for about 7 hours. No adverse effects occurred.

In another prospective open uncontrolled case study, a 24-year-old female was described (Müller-Vahl et al., 2002a). Motor and vocal tics had started at age 9. During the following years, the patient had been treated with more than eight different drugs including several antipsychotics. However, pharmacotherapy was either ineffective or caused significant adverse effects including dyskinesia, galactorrhea, and amenorrhea. At age 23, her tics further deteriorated and she suffered from extreme vocal tics with very loud shouting. Therefore, risperidone (up to 8 mg/day), amisulpride (up to 1200 mg/day), and THC (up to 10 mg/day) were tried either alone or in combination. According to both self (Leckman et al., 1988) and examiner rating scales (Leckman et al., 1988; Shapiro et al., 1988) for tics, the combination of amisulpride and THC was found superior compared to monotherapy with THC or amisulpride. In addition, the patient felt a reduction of the premonitory urge. She was followed for more than 6 months without

showing significant deterioration. The only adverse effect was minimal galactorrhea caused by amisulpride. From this case study, it is suggested THC may augment anti-tic effects of dopamine receptor blocking drugs. This hypothesis is in accordance with preliminary findings in animal studies, demonstrating that haloperidol-induced hypokinesia significantly increases after co-administration of THC (Moss et al., 1984). It, therefore, has been suggested that combined treatment with cannabinoids and antipsychotics might be of therapeutic value in hyperkinetic movement disorders such as TS (Moss et al., 1989).

In another case report, a 42-year-old patient was described who suffered from vocal tics including coprolalia, multiple motor tics (including head, arm, and leg jerking as well as repetitive standing up and down), and ruminating obsessive thoughts (Brunnauer et al., 2011). Different drugs (including antipsychotics, alpha-2-agonists, clonazepam, and tetrabenazine) were ineffective and, therefore, treatment with THC (up to 15 mg/day) was started resulting in a decrease of his tics by 75%, according to the Yale Global Tic Severity Scale (YGTSS) (Leckman et al., 1989). Since the patient worked as a truck driver, his driving ability was assessed using computerized tests according to the German guidelines for road and traffic safety during THC treatment compared to “off”-treatment. Although the patient passed the tests under both conditions, his concentration as well as visual perception were much better during THC treatment compared to “off”-treatment. The authors, therefore, suggested that at least in patients with TS, treatment with cannabinoids may have beneficial effects on psychomotor functions related to driving performance.

Accordingly, in a 28-year-old male suffering from ADHD (without tics), it has been reported that his driving-related performance significantly improved after oral intake of THC (Strohbeck-Kühner et al., 2007). The authors concluded that “...in persons with ADHD, THC may have atypical and even performance-enhancing effects.”

To the best of our knowledge, there is only one single-case report available describing the successful treatment of a 15-year-old boy with treatment refractory TS (Hasan et al., 2010). In healthy cannabis users there is evidence that high consumption of cannabis has negative long-term consequences on cognitive performance and may induce psychosis. This risk seems to be much higher in children and adolescents before puberty (Moore et al., 2007; Meier et al., 2012). Therefore, in patients in this age group, it has been suggested to weigh up the advisability of (long-term) treatment with cannabinoids very carefully (Grotenhermen and Müller-Vahl, 2012). However, it is unclear whether these results obtained from healthy cannabis users can be transferred directly to patients suffering from TS. On the one hand, it has been speculated that in TS there might be changes in the endogenous cannabinoid receptor system, and on the other hand, doses used for treatment of tics in most patients are much lower compared to doses used by healthy cannabis users. Finally, effects (such as “high feeling”) desired by healthy users are regarded as adverse effects in the context of treatment.

In the above-mentioned case report (Hasan et al., 2010), a 15-year-old boy is described who suffered from treatment-resistant severe motor and vocal tics including self-injurious tics, ADHD, and mild OCB. Due to these symptoms he was dismissed from school. Treatment with tiapride, sulpiride, risperidone, aripiprazole, olanzapine, and haloperidol (alone or in combination with up to three different antipsychotics) failed to improve his tics. Co-medications with an SSRI and clonazepam were ineffective. Methylphenidate and atomoxetine improved ADHD symptoms but increased his tics. Because the patient reported that inner tension and tic severity decreased when smoking marijuana illegally, treatment with THC (up to 15 mg/day) was initiated (in combination with aripiprazole (30 mg/day) and risperidone (3 mg/day). After 7 weeks, tics improved by nearly 50% (according to the YGTSS) accompanied by a significant improvement in quality of life (according to the Gilles de la Tourette syndrome-quality of life scale, GTS-QOL) (Cavanna et al., 2008). In addition, for the first time, treatment with methylphenidate (30 mg/day) was tolerated without tic exacerbation, resulting in an improvement of ADHD symptoms. No significant adverse effects occurred. Using transcranial magnetic stimulation (TMS), intracortical inhibition was found to be increased during THC treatment. The authors, therefore, suggested that THC might counteract deficits of intracortical inhibition in patients with TS and comorbid ADHD by modulating the release of several neurotransmitters including dopamine and F-aminobutyric acid. From this case study, it is further suggested that the combination of an antipsychotic agent and THC might be effective even in those patients who are treatment resistant to monotherapy with dopamine receptor antagonists.

RANDOMIZED CONTROLLED TRIALS USING THC

To date, there are only two preliminary controlled trials available investigating the efficacy and safety of orally administered THC in TS. In a randomized, double-blind, placebo-controlled, crossover trial 12 adult patients (11 men, one woman, mean age = 34 ± 13 (SD) years, range 18–66 years) were treated once with a single dose of 5, 7.5, or 10 mg THC (dosages were chosen according to patients' body weight, sex, age, and prior use of marijuana) (Müller-Vahl et al., 2002b). Patients were randomly assigned a single dose of oral THC first or a single dose of visually identical placebo first on 2 days separated by a 4-week washout phase. After intake of THC, the patients felt a significant tic improvement compared to placebo ($p = 0.015$) according to the Tourette Syndrome Symptom List (TSSL) (Leckman et al., 1988). They also experienced an improvement of OCB ($p = 0.041$) (according to TSSL). The examiner rating scale TSGS demonstrated a significant improvement ($p = 0.015$) for the subscore "complex motor tics." Data became more robust when including only those patients who were treated with 7.5 or 10.0 mg THC ($n = 8$), suggesting that higher dosages are more effective. All in all, 10/12 patients (83%) experienced a "global improvement" after intake of THC (mean of $+35\% \pm 28.0$, range 20–90%), but only 3/12 (25%) after placebo (mean of $+7\% \pm 13.7$, range 10–40%). Five patients experienced transient mild adverse

effects after intake of THC including headache, nausea, dizziness, hot flush, tiredness, poor powers of concentration, and cheerfulness. One patient reported dizziness, anxiety, trembling, sensitivity to noise and light, dry mouth, and ataxia lasting for about half an hour. It can be assumed that most of these adverse effects were caused because dosages were not uptitrated. In clinical practice, treatment with THC will routinely be started with low doses of 2.5 to 5.0 mg/day, but not with relatively high doses of 7.5 or 10 mg THC/day—especially in patients who had never used cannabinoids before. None of these adverse events required specific treatment. No serious adverse events occurred.

To evaluate the influence of THC on a broad range of psychological symptoms, the Symptom Checklist 90-R (SCL-90-R) (Derogatis et al., 1973) was used. According to the SCL-90-R, THC had no influence on depression, somatization, interpersonal sensitivity, anxiety, anger-hostility, paranoid ideation, and psychoticism, but there was evidence for a deterioration of OCB. However, limitations of the SCL-90-R measuring OCB are well known. Furthermore, no detrimental effects of THC could be detected on short-term verbal and visual memory, recognition, verbal learning, intelligence, information processing, vigilance, reaction time, sustained attention, and divided attention (Müller-Vahl et al., 2001).

In a follow-up study, a randomized, double-blind, parallel group, placebo-controlled design was used to investigate efficacy and safety of THC over 6 weeks in 24 adult patients with TS (19 men, five women, mean age = 33 + 11 (SD) years, range 18–68 years) (Müller-Vahl et al., 2003b). To reduce adverse effects, a very low starting dose of 2.5 mg THC/day was chosen. The dosage was uptitrated slowly by 2.5 mg every 4 days until a maximum dose of 10 mg/day was reached or adverse events occurred. If the dosage was not tolerated, patients were allowed to reduce the dose to a minimal dose of 5.0 mg/day. The study consisted of six visits (visit 1 = baseline, visits 2–4 = during treatment, visit 5 = immediately after treatment was stopped, visit 6 = follow-up 6 weeks after withdrawal). Several of the rating scales used demonstrated superiority of THC over placebo at visit 4 (treatment days 30–31): (1) the Tourette's Syndrome Clinical Global Impression Scale (TS-CGI) (Leckman et al., 1988) ($p < 0.05$), (2) the examiner tic rating scale STSS ($p = 0.033$); (3) the examiner tic rating scale YGTSS for the subscore "motor global scale" ($p = 0.040$); and (4) the examiner Rush videotape-based rating scale (Goetz et al., 1999) ($p = 0.030$). Using the TS-CGI, in addition, at visit 3 (treatment days 20–22) a significant difference ($p < 0.05$) was found between the THC and placebo group. Furthermore, ANOVA demonstrated a trend towards an overall significant difference ($p = 0.079$). The tic self-rating TSSL demonstrated a significant difference ($p < 0.05$) 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 ($p = 0.037$). At visits 2 (treatment day 9), 3, and 4, respectively, several other measurements demonstrated a trend towards a significant difference ($p < 0.1$) for global tic scores or tic subscore. There were no group differences at baseline (before treatment) and visits 5 and 6 (after treatment was stopped).

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 et al., 2001), (2) Benton-Visual-Retention-Test (BVRT) (Benton, 1945), (3) Divided Attention (TAP) (Zimmermann and Fimm, 1989), and (4) multiple choice vocabulary test (Mehrfachwahl-Wortschatztest, MWT-B) (Merz et al., 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 span, and divided attention. Measuring immediate verbal memory span, there was even a trend towards a significant improvement during and after treatment ($p = 0.082$). Furthermore, no significant influence on OCB, anxiety, depression, and “the current emotional state” was found (Müller-Vahl et al., 2003a).

THE NEUROCHEMISTRY OF TOURETTE SYNDROME: WHICH ROLE DOES THE CB₁ RECEPTOR SYSTEM PLAY?

The neurochemistry of TS is still unclear. Although most evidence supports a major role of the dopaminergic system, several other neurotransmitter systems have been suggested to be involved in the pathophysiology of TS. Comparable to the “dopamine hypothesis” of TS—based on the clinical experience that dopamine receptor blocking drugs reduce tics—a “cannabinoid hypothesis” has been suggested, based on the beneficial effect of CBM in the treatment of tics (Müller-Vahl et al., 1998; Müller-Vahl, 2013).

This hypothesis is supported by the fact that the highest densities of CB₁ receptors were found in the cerebellum, the substantia nigra pars reticulata, the globus pallidus, and the hippocampus (Herkenham et al., 1990). This characteristic regional pattern suggests an important role of the CB₁ receptor system in movement control. Endocannabinoids may have a major role in the regulation of synaptic neurotransmission, including the activity of the excitatory neurotransmitter glutamate, but also of the inhibitory transmitter GABA, and several monoamines such as dopamine, serotonin, noradrenaline, acetylcholine, and neuropeptides (Pertwee, 2008). There are several lines of evidence suggesting a complex interaction between the CB₁ receptor and the dopaminergic system within the nigro-striatal and the meso-cortico-limbic systems (Fitzgerald et al., 2012).

It, therefore, can be speculated that beneficial clinical effects of CBM are caused by a modulation of the dopaminergic transmission. However, one might also hypothesize that interactions between the CB₁ receptor and other neurotransmitter systems are of importance. Finally, it has even been suggested that TS might be caused primarily by changes within the endogenous central cannabinoid receptor system (Müller-Vahl et al., 1998).

To date there is only one study available using the CB₁ antagonist [123I]AM281 (N-(morpholin-4-yl)-1-(2,4-dichlorophenyl)-5-(4-[123I]iodophenyl)-4-methyl-1H-pyrazole-3-carboxamide) and single photon emission computed tomography (SPECT) to investigate central cannabinoid CB₁ receptors in six patients with TS before and after THC treatment (Berding et al., 2004). In this study, for the first time, in patients with TS specific binding of [123I]AM281 to CB₁ receptors could be detected using SPECT. Mean binding did not change after oral treatment with THC (1.25–10.0 mg/day). However, in the only patient with a marked tic reduction after THC treatment, specific binding clearly declined. As no control group was included in this study, no statement can be made about possible changes in CB₁ receptor binding sites in TS.

Furthermore, in one study the central cannabinoid receptor (*CNR1*) gene encoding the CNR1 receptor was considered as a candidate gene for TS and systematically screened by single strand conformation polymorphism (SSCP) analysis and sequencing. When investigating a group of 40 patients with TS plus 81 healthy controls as well as two subsequent cohorts (patients with TS: $n = 56$ and 64 , respectively; controls: $n = 55$ and 66 , respectively) no evidence was found suggesting that TS is caused by genetic variations of the *CNR1* gene (Gadzicki et al., 2004).

ADVERSE EFFECTS

It can be assumed that adverse effects of CBM in patients with TS do not differ from adverse effects described in other groups of patients. In general, cannabinoids are considered as well tolerated. The American Institute of Medicine declared that “Marijuana is not a completely benign substance. It is a powerful drug with a variety of effects. However, except for the harms associated with smoking, the adverse effects of marijuana use are within the range of effects tolerated for other medications” (Joy et al., 1999).

According to a systematic review on the drug safety of CBM, the most frequently reported non-serious adverse effects are dizziness followed by drowsiness, disorientation, impaired concentration, and impaired balance (Wang et al., 2008). Most commonly reported psychological effects are relaxation, euphoria, dysphoria, unpleasant feelings, heightened sensory, altered time perception, anxiety, panic (but also reduction of anxiety), impairment of memory, and reductions in psychomotor and cognitive performance. In the majority of cases, psychological effects can be prevented by slow and individual titration. Most patients

develop tolerance to these adverse effects (Grotenhermen and Müller-Vahl, 2012). In the context of treatment, patients usually assess psychological effects such as euphoria and heightened sensory as unwanted adverse effects leading to dose reduction. In adults, it has been demonstrated that regular cannabis use at high doses neither causes irreversible long-term effects on cognitive performance nor increases the risk of psychosis (Moore et al., 2007; Meier et al., 2012).

Dry mouth is the most commonly reported physical effect followed by tachycardia, orthostatic hypotension, reduced lacrimation, muscle relaxation, and increased appetite. In the context of controlled medical administration, withdrawal symptoms of THC and nabiximols are hardly ever a problem. To date, no case of abuse and diversion of nabiximols has been reported (Robson, 2011).

In TS, available studies suggest that cannabinoids do not impair neuropsychological performance (Müller-Vahl et al., 2001, 2003a), but rather can improve concentration and visual perception (Brunnauer et al., 2011). There is some evidence that CBM containing several cannabinoids such as cannabis extracts and herbal cannabis are more effective and better tolerated than pure THC (Robson, 2011). In TS, however, there are no studies available comparing different CAM directly with respect to efficacy and safety.

In general, CBM should not be used in patients suffering from a psychotic illness, hepatitis, and significant cardiac disorder as well as in pregnant and breast-feeding women. CBM should be used with caution in patients with a history of substance abuse. Since in young (healthy) people, frequent use of high doses of cannabis before puberty is associated with increased rates of psychotic symptoms, depression, and anxiety (Moore et al., 2007). In children CBM should be regarded as a last resort. However, it is unclear whether effects in healthy cannabis users can be directly transferred to patients suffering from TS, particularly against the background of a possible involvement of the CB₁ receptor system in the pathogenesis of TS. A summary of the main outcomes in studies that evaluated CBM in TS is given in Table 10.2.

PRACTICAL ASPECTS FOR THE TREATMENT OF PATIENTS WITH TS WITH CBM

Based on the only two studies investigating the effect of THC in TS (Müller-Vahl et al., 2002b, 2003b), in 2009 a Cochrane review on “Cannabinoids for Tourette’s Syndrome” was published (Curtis et al., 2009). The authors stated that “Cannabinoid medication might be useful in the treatment of the symptoms in patients with Tourette’s syndrome,” but finally concluded: “There is not enough evidence to support the use of cannabinoids in treating tics and obsessive compulsive behaviour in people with Tourette’s syndrome” and ended with the sentence: “Longer trials with larger numbers of patients are necessary to establish the long term efficacy and safety of cannabinoids in treating the symptoms of GTS.”

Table 10.2 Studies in Tourette Syndrome Using Cannabinoid-based Medicines

Reference	Number (Sex) of Patients	Age	Substance	Study Design	Main Outcome
Sandyk and Awerbuch, 1988	3 (male)	15, 17, 39	<i>Cannabis sativa</i> L.	Case report	Tic reduction; premonitory urge; self-injurious behaviour; general relaxation; improvement of attention and hypersexuality
Hemming and Yellowlees, 1993	1 (male)	36	<i>Cannabis sativa</i> L.	Case report	Symptom free
Müller-Vahl et al., 1999	1 (male)	25	THC	Case report	Tic reduction; improvement of premonitory urges; improvement of attention, impulse control, and OCB
Müller-Vahl et al., 2002a	1 (female)	24	THC (in combination with amisulpride)	Case report	Tic reduction; improvement of premonitory urges
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	Tic reduction; 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	<i>Cannabis sativa</i> L.	Case series (standardized interview)	Tic reduction or remission; improvement 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., 2002b	24 (19 male, 5 female)	18–68	THC	Randomized, double-blind, parallel group, placebo-controlled trial	Tic reduction; global improvement

Despite the weak evidence and the lack of well-designed and well-powered studies for nearly all available treatment strategies for tics, as well as less efficacy and significant adverse effects of several substances, nonetheless CBM such as THC are recommended for the treatment of TS in adult patients by many experts, when first-line treatments fail to improve tics (Roessner et al., 2011; Pringsheim et al., 2012). Thus, in treatment-resistant adult patients, therapy with CBM should be taken into consideration.

Depending on national laws, there are different possibilities for medically supervised treatment with CBM. On the one hand, commercial preparations of dronabinol (THC), the synthetic THC derivative nabilone, and the cannabis extract nabiximols are available in many countries. In addition, dronabinol is available as a prescription drug, mixed specially for the patient. On the other hand, in some countries, medically supervised treatment with herbal cannabis is possible. Based on available studies and our own clinical experience, treatment with CBM should be started at a low dose of 2.5 or 5 mg THC/day and should be slowly uptitrated to a daily dose of 10–20 mg according to efficacy and tolerability. Depending on the route of intake, the individual duration of effect, and patients' desire for treatment, CBM should be used once or several times daily.

Although in a small number of patients we have some clinical experience with the use of nabiximols in the treatment of TS, no case studies have been published so far. There are also no studies available using medicinal cannabis containing standardized levels of THC and CBD, although in many countries medicinal cannabis can be prescribed or can be used after patients have received a certificate of exemption.

From our experience, most clinical effects of *Cannabis sativa* L. seem to be related to THC. Therefore, in most patients with TS, similar effects can be expected after treatment with *Cannabis sativa* L., cannabis extracts (such as nabiximols), and pure THC. However, during the last few years we have treated a small number of patients who experienced stronger effects or less adverse effects when using marijuana (illegally) or medicinal cannabis (legally) compared to pure THC. This clinical experience in patients with TS is in accordance with reports from other groups of patients (Hazekamp et al., 2013).

Longer trials including a larger number of patients are urgently needed to further investigate efficacy and safety of CBM in the treatment of TS. As in other groups of patients, it would be of interest to compare the effect of pure THC to those of 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 obtained from a limited number of case reports and two small controlled trials consistently provide evidence for beneficial effects of CBM in the

treatment of tics in patients with TS. In addition, there is some evidence that CBM may also improve associated behavioral problems such as OCB, attention deficits, impulsivity, and self-injurious behavior in this group of patients. Neuropsychological tests demonstrated that THC has no detrimental effects on memory, reaction time, concentration, and attention, but on the contrary may even improve memory, concentration, and visual perception. It can be assumed that beneficial effects of CBM in TS 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 longer trials including a larger number of patients are urgently needed to further investigate the effect of cannabinoids in the treatment of TS, even today CBM should be taken into consideration when treatment with other substances fails to improve tics.

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The role of endocannabinoid function in posttraumatic stress disorder: Modulating the risk phenotype and rendering effects of trauma

11

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INTRODUCTION

Posttraumatic stress disorder (PTSD) is a devastating wound of war (Tanielian and Jaycox, 2008) and a debilitating consequence of trauma in civilian settings (Santiago et al., 2013). The disorder is characterized by four groups of symptoms, including persistent and intrusive recollections of the event, avoidance of situations that trigger memories of the experience, negative alterations in cognitions and mood, and alterations in arousal. In the latest *Diagnostic and Statistical Manual* (DSM-5), the latter category encompasses aggressive and self-destructive behavior (APA, 2013). In addition, a dissociative subtype of PTSD is characterized by depersonalization or disruptions in the perception of self and environment (Lanius et al., 2010). Finally, PTSD is often comorbid with depressive, anxiety, substance use, and other disorders (Kessler et al., 1996). Comorbidity is associated with greater severity of symptoms, more psychosocial and medical problems, and treatment complications (McCarthy and Petrakis, 2010).

As the *sine qua non* of PTSD is exposure to a traumatic event, the quest to identify brain mechanisms involved in its pathophysiology has focused on neural systems with the capacity to translate acute trauma into symptoms of the disorder. The endogenous cannabinoid (eCB) system has emerged as a key candidate. Moreover, it is widely understood that the particular effects of acute trauma vary, depending in part on a context described by premorbid characteristics of temperament. The eCB system is also implicated in three such dimensions of risk for PTSD.

In this chapter, we will summarize evidence that eCB function (1) differentiates between individuals with and without PTSD; (2) contributes to variation in traits considered risk factors for PTSD; (3) modulates the response to trauma and is itself altered by chronic stress; and (4) affects a dimension of risk shared by PTSD and cannabis use, which frequently co-occur. We will also (5) present evidence that eCB-targeting drugs may be therapeutic in some individuals with PTSD, and (6) discuss implications of these findings for research, treatment, and policy.

OVERVIEW OF THE ENDOCANNABINOID SYSTEM

The discovery of delta-9-tetrahydrocannabinol (Δ^9 -THC) from marijuana in 1964 (Gaoni and Mechoulam, 1964) and the cloning of the cannabinoid type 1 receptor¹ (CB₁; Matsuda et al., 1990) are landmark achievements in the study of the eCB system. Together these discoveries led the characterization of the system (Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995) and developed our understanding of it as a major neuromodulatory system. Endocannabinoids such as 2-arachidonoyl glycerol (2-AG) and anandamide, acting as retrograde neurotransmitters, are synthesized and released from the postsynaptic terminal of the neuron. They bind to CB₁ receptors at the presynaptic terminal, inhibiting the release of other neurotransmitters such as an excitatory amino acid glutamate and an inhibitory gamma aminobutyric acid (GABA) (Degroot and Nomikos, 2007). Anandamide is degraded by fatty acid amide hydrolase (FAAH), located primarily in the postsynaptic terminal, and 2-AG is degraded in the presynaptic terminal by monoacylglycerol lipase (MGL) (Tsou et al., 1998; Dinh et al., 2002).

The eCB system modulates the activity of a variety of neural pathways, including those affecting processes related to avoidance and withdrawal, such as fear and the stress response (Mechoulam and Parker, 2013), and those related to approach (i.e., reward) (Fattore et al., 2010). Consistent with this, anandamide, 2-AG, FAAH, MGL, and CB₁ receptors are highly expressed in brain regions critical to these functions, such as prefrontal cortex (PFC), amygdala, ventral tegmental area, striatum, hypothalamus, thalamus, and hippocampus (Herkenham et al., 1991; Breivogel and Sim-Selley, 2009; Fattore et al., 2010; McLaughlin and Gobbi, 2012). In addition, eCB signaling is implicated in impulsivity and control, possibly reflecting its modulatory action on glutamate and dopamine signaling in the PFC and striatum (Pattij and Vanderschuren, 2008). The eCB system is affected by genetic variation in humans (Hillard et al., 2012) and is altered by a variety of experiences, including early life adversity (Lee and Gorzalka, 2012; Viveros et al., 2012) and exposure to cannabis (Chadwick et al., 2013; Fratta and Fattore, 2013).

¹In contrast to CB₁ receptors, which are found primarily in the brain, CB₂ receptors are found in the immune and hematopoietic systems (Pacher et al., 2006). In this chapter, we will focus exclusively on the CB₁ receptor.

The eCB system also modulates activity of the hypothalamic–pituitary–adrenocortical (HPA) axis, which plays a crucial role in the response to stress. Most relevant to PTSD, both eCBs and stress hormone glucocorticoids modulate aversive memory after stress exposure (Suzuki et al., 2004; Steiner and Wotjak, 2008; Riebe and Wotjak, 2011; Atsak et al., 2012; Akirav, 2013; de Bitencourt et al., 2013). Taken together, the evidence suggests that the HPA axis and eCB system act in concert to regulate stress responses.

Many compounds have been used to investigate effects of eCB function on emotional memory and anxiety in preclinical studies. Initially, studies evaluated effects of the exogenous CB₁ receptor agonist, Δ^9 -THC; more recently, synthetic agonists, including HU210, CP55,940, and WIN 55,212-2, have been used. Conversely, the effects of blocking CB₁ receptors have been studied using CB₁ receptor antagonists such as SR141716A (rimonabant), AM251, and AM281. In addition, eCB reuptake inhibitors (e.g., AM404, UCM707, VDM11, OMDM1, and OMDM2) and inhibitors of the anandamide-degrading enzyme FAAH (e.g., URB597 and URB592), which increase endogenous anandamide levels in the brain, have been used.

Advances in transgenic mouse technology have provided the ability to knock out FAAH and CB₁ genes in mice, and the resulting lines of knockout (KO) mice have been useful in probing eCB function. While CB₁ KO mice lack CB₁ receptors (Haller et al., 2004), FAAH KO mice exhibit highly elevated levels of anandamide but not 2-AG (Cravatt et al., 2001). Although the development of compensatory mechanisms and abnormal activity of other neurotransmitter systems due to the permanent deletion of specific genes complicate the interpretation of findings, KO mouse lines have been useful in studying the role of the eCB system in fear and anxiety.

In humans, pharmacologic challenges together with neuroimaging and behavioral metrics of their effects have been used to explore eCB function. Also, researchers have exploited the genetic diversity that characterizes the eCB system in humans, focusing on single nucleotide polymorphisms (SNPs) in or near *CNR1*, the gene for the CB₁ receptor, and the gene for the enzyme FAAH. As we will describe, a number of studies have evaluated relationships between phenotypes relevant to PTSD and this polymorphic variation.

ENDOCANNABINOID FUNCTION IN PTSD

A first step in establishing that a variable is associated with a disorder is the demonstration that it distinguishes between individuals who meet diagnostic criteria for that disorder and those who do not (Sher and Trull, 1996). A study using PET imaging found abnormal CB₁ receptor-mediated anandamide signaling in individuals with PTSD as compared to trauma-exposed and healthy controls (Neumeister et al., 2013), suggesting that compromised eCB signaling is a risk factor for and/or a consequence of PTSD. A second study using a peripheral measure of eCB function focused on PTSD in a population-based cohort selected for

physical proximity to the World Trade Center at the time of the 9/11 attacks (Hill et al., 2013). In this study, reduced levels of circulating 2-AG, which are known to be elevated in response to stress, were observed in individuals with PTSD, as compared to individuals who had been similarly proximal to Ground Zero but who did not develop PTSD. This suggests that deficient eCB signaling is a component of the glucocorticoid dysregulation that characterizes PTSD.

Two studies have examined polymorphic variation in eCB genes in relation to PTSD diagnosis. In the first, one allelic variant in the *CNR1* gene was associated with PTSD; that association did not replicate in a second, independent sample (Lu et al., 2008). A second study examined a variant in the *FAAH* gene in Vietnam veterans with PTSD who had suffered penetrating traumatic brain injury (Pardini et al., 2012). This variant modulated risk in veterans with intact ventromedial PFC (vmPFC) but not in those with vmPFC lesions. As this region modulates negative affects via its effects on perceived fear (Diekhof et al., 2011), the findings suggest that in the presence of an intact vmPFC, genetic variation in eCB signaling affects risk for PTSD through its effects on perceived fear, while vmPFC lesions disrupt fear perception and thereby increase risk independently of FAAH function. A second FAAH variant was associated with more severe re-experiencing of trauma and more negative childhood experiences.

While these findings are consistent with other work implicating eCB function in PTSD, methodological issues limit the interpretation of findings from such association studies (Tabor et al., 2002). In particular, in this area, the small number of studies and the fact that they focused on various different SNPs is limiting. Moreover, while the effects of SNPs associated with the *FAAH* gene on eCB signaling are understood, the functional effects of polymorphisms in *CNR1* are not (Hillard et al., 2012), complicating the interpretation of genetic association studies. More generally, etiologic research that compares individuals who do and do not meet criteria for a particular disorder suffers from a number of limitations, including heterogeneity within diagnostic categories. An emerging dimensional view of psychopathology provides an alternative to the “distinct etiology” approach, one that addresses the problem of heterogeneity.

ROLE OF THE ENDOCANNABINOID SYSTEM IN MODULATING RISK FOR PTSD

In the dimensional view, mental disorders reflect extreme ends of continuously distributed dimensions, variation in which is the result of a complex interaction of multiple genetic and environmental influences (Clark, 2005; Widiger and Samuel, 2005). While this approach does not rule out categorical distinctions that are clinically useful, it considers that there are no discrete breaks in the distribution of these traits that would provide an absolute distinction between normal variation and clinical disorders (Krueger et al., 2005). In the dimensional view, specific

etiologic agents—such as trauma—determine distinct characteristics of disorders in a milieu shaped by core dimensions (Brown and Barlow, 2005).

Substantial evidence implicates three such dimensions in PTSD, namely, negative emotionality, reward sensitivity, and control. Negative emotionality has long been considered a hallmark of PTSD (Jakšić et al., 2012), while reward deficits have garnered attention recently (Stein and Paulus, 2009). Finally, externalizing behaviors, which are commonly associated with PTSD (APA, 2013), are widely considered to reflect deficits in control (Patrick et al., 2013).

These broad dimensions relate closely to those that affect variation in behavior more broadly (Clark, 2005). While negative affectivity reflects a system of withdrawal, positive affectivity reflects a system of approach, of which reward is a central component (Berridge et al., 2009). Constraint, or control, is not an affective system but rather plays a regulatory role (Clark, 2005; Rothbart et al., 2011; Sharma et al., 2014). Individual differences in the core processes underlying these dimensions are associated with neural systems that have been largely conserved by evolution (Patrick et al., 2002) and affect risk for psychopathology (Clark, 2005).

While we will frame our discussion of eCB involvement in risk for PTSD in terms of these three dimensions of temperament, it is important to note that each dimension is associated with multiple, related constructs. For example, fear and anxiety each bear a conceptual relationship to an avoidance or withdrawal system but are distinct constructs, rooted in separate but interacting brain systems (Perkins et al., 2007; McNaughton, 2011; Sylvers et al., 2011; Riebe et al., 2012). Interindividual variation in fear and in anxiety are affected by different sets of genetic factors (Kendler et al., 2003) and relate differently to risk for, and symptoms of, mental disorders, including PTSD (Krueger, 1999; Watson, 2005). Whereas an exaggerated fear response is a hallmark symptom of PTSD (Shvil et al., 2013), it has been consistently reported that PTSD affiliates primarily with an *anxious-misery factor* defined primarily by mood disorders and shows no affinity with a *fear factor* defined by panic and phobic disorders (Cox et al., 2002; Slade and Watson, 2006; Miller et al., 2008). Similarly, the approach system comprises dissociable components including but not limited to positive affectivity (Berridge et al., 2009), and control has been articulated into multiple dimensions (de Wit, 2009). While a comprehensive discussion of these distinctions is beyond the scope of this chapter, highly articulated phenotypes facilitate efforts to identify associations with eCB and other neural function.

NEGATIVE EMOTIONALITY: A KEY ROLE FOR ENDOCANNABINOID SIGNALING IN PTSD

Negative emotionality refers to the tendency to experience heightened negative affect and to perceive the world as threatening and stressful (Watson and Clark, 1984). Individuals who score high on measures of negative emotionality are

susceptible to frequent and intense aversive emotions (e.g., anxiety, anger) and report elevated distress even in the absence of external stressors (Watson and Clark, 1984; Tellegen and Waller, 2008). Whereas a robust avoidance response is necessary for survival, an exaggerated or dysregulated avoidance response is problematic. A high level of negative emotionality, an affective component of the avoidance response, is a key symptom of many psychiatric disorders (Jakšić et al., 2012). Genetic factors contribute substantially to individual differences in negative emotionality (Tellegen et al., 1988; Blonigen et al., 2008) and may exert their influence through effects on neural structures that modulate negative emotions. The neural circuitry underlying negative emotion includes subcortical limbic structures such as the amygdala, hippocampus, ventral striatum, and thalamus, as well as cortical structures such as the anterior cingulate cortex and the PFC, and CB₁ receptors are expressed in many of these brain regions (McLaughlin and Gobbi, 2012).

Nearly two decades ago, based on the ubiquitous relationships between distress disorders and negative affectivity, it was proposed that these disorders share a common underlying diathesis involving negative emotionality (Clark et al., 1994). Many studies have now confirmed that negative emotionality—or the related dimension of neuroticism (Tellegen and Waller, 2008)—is associated with all of the distress disorders including PTSD (Kotov et al., 2010). Indeed, in a study of Gulf War veterans, the association between negative emotionality and PTSD was among the strongest of all the anxiety disorders, second only to that between negative emotionality and generalized anxiety disorder (Gamez et al., 2007). Further, a recent study of Vietnam veterans found that negative temperament shares the most variance with overall severity of PTSD, as compared to other dimensions of temperament (Wolf et al., 2011).

The strongest evidence implicating negative emotionality in combat-related PTSD comes from prospective studies. A meta-analysis found associations between negative emotionality and PTSD when data from cross-sectional and prospective studies were combined and when data from prospective studies were examined separately (Rubin et al., 2008). Similarly, in combat-exposed National Guard soldiers recently returned from Iraq, high levels of negative emotionality, measured one month before deployment, were associated with more severe post-deployment PTSD symptoms (Meis et al., 2010). Finally, in a study of non-combat-related PTSD, neuroticism score at baseline significantly affected relative risk for PTSD in response to trauma 10 years later (Breslau and Schultz, 2012).

Human psychopharmacologic neuroimaging studies and a small number of candidate-gene studies implicate eCB in negative affect. Phan et al. (2008) reported that Δ^9 -THC, the anxiolytic effects of which are mediated by selective agonism of CB₁ receptors localized in the basolateral amygdala (BLA), reduced amygdala reactivity to social signals of threat in recreational cannabis users. Important to medication development efforts, Δ^9 -THC increased subjective reports of *drug high* and *feel drug* but did not affect subjective arousal or anxiety. In another study, an acute oral dose of Δ^9 -THC was found to attenuate subgenual

anterior cingulate cortex reactivity during induction of negative affect in recreational marijuana users (Rabinak et al., 2012). This region is implicated in emotion processing generally and negative affect in particular (Pechtel and Pizzagalli, 2011). A third study found that amygdala reactivity in response to angry and fearful facial expressions was inversely related to level of cannabis smoked in the past week (Cornelius et al., 2010). Taken together, these findings demonstrate that cannabinoids inhibit activity in brain regions involved in the response to threat, possibly via their enhancing effects on eCB signaling.

In a study comparing the effects of Δ^9 -THC (10 mg), cannabidiol (CBD; 600 mg), and placebo, CBD decreased activation in response to intensely fearful faces in the amygdala and the anterior and posterior cingulate cortex, regions critical in mediating responses to anxiogenic stimuli (Fusar-Poli et al., 2009). CBD also attenuated the skin conductance response (SCR), a measure of autonomic arousal, and there was a trend for reduced subjective anxiety following CBD relative to placebo. In contrast with the findings for CBD and with the findings described above, Δ^9 -THC had no effect on amygdalar or cingulate responses to the above-mentioned faces; indeed, Δ^9 -THC increased subjective anxiety as well as the amplitude of the SCR to those faces.

Broadly consistent with the findings of pharmacologic studies, individuals with the *FAAH* 385A variant, which is associated with decreased *FAAH* availability and presumably with enhanced eCB signaling (Hillard et al., 2012), exhibited reduced threat-related amygdala reactivity, as compared to C385 homozygotes (Hariri et al., 2009). Further, the relationship between amygdala reactivity and trait anxiety was diminished in those carriers. An SNP in the *CNR1* region, the functional effects of which are not yet clear (Hillard et al., 2012), has been associated with levels of negative affect and craving experienced during withdrawal from marijuana (Haughey et al., 2008). Taken together, these findings are consistent with the idea that factors facilitating eCB signaling in humans, such as exogenous cannabinoids or polymorphic variation, tend to attenuate behavioral and brain measures of negative affect and threat reactivity.

Using primarily pharmacologic means to manipulate eCB function, animal models have produced the strongest evidence implicating eCB function in anxiety (see Table 11.1). These studies have demonstrated consistently that the effects of cannabinoid drugs on anxiety symptoms are bidirectional. That is, low doses are anxiolytic, while high doses are anxiogenic. This may reflect the fact that, depending on dose, cannabinoids can inhibit either excitatory amino acid glutamate or inhibitory amino acid GABA neurotransmission. Moreover, as cannabinoid receptors are highly expressed in multiple brain regions, cannabinoids may have differential effects on various brain regions involved in fear and anxiety.

In a study comparing the effects of 12 days of systemic administration of a low (5 μ g/kg) and a high dose (100 μ g/kg) of the potent CB₁ receptor agonist HU210, the high dose increased anxiety in rodents, as indicated by a reduction in time spent in the center zone and an increase in fecal boli in open field testing (Hill and Gorzalka, 2006). This suggests that chronic exposure to a high dose of a

Table 11.1 Effects of Endocannabinoid Drugs on Anxiety Behavior in Rodents

Paradigm	Species	Intervention ^a	Anxiety Behavior	Reference
Elevated plus maze	Mouse	Nabilone (10–100 µg/kg, ip)	Anxiolytic	Onaivi et al., 1990
		Cannabidiol (1–10 mg/kg, ip)	Anxiolytic	
		THC (10–20 mg/kg, ip)	Anxiogenic	
	Mouse	WIN 55,212-2 (0.3–10 mg/kg, ip)	Anxiolytic	Patel and Hillard, 2006
		CP55,940 (0.001–0.3 mg/kg, ip)	Anxiolytic	
		AM404 (0.3–10 mg/kg, ip)	Anxiolytic	
		URB597 (0.03–0.3 mg/kg ip)	Anxiolytic	
		FAAH knockout	Anxiolytic	
	Mouse	CB ₁ knockout	Anxiogenic	Terzian et al., 2011
	Mouse	CP55,940 (1 µg/kg, ip)	Anxiolytic	Rey et al., 2012
		CP55,940 (50 µg/kg, ip)	Anxiogenic	
	Mouse	ACPA (0.0125–0.05 mg/kg, ip)	Anxiolytic	Chegini et al., 2013
	Rat	THC (1–10 mg/kg, ip)	Anxiogenic	Onaivi et al., 1990
	Rat	HU210 (5–100 µg/kg, ip)	No effect	Hill and Gorzalka, 2006
	Rat	ACPA (1.25–5 ng intra-CeA)	Anxiolytic	Zarrindast et al., 2008
	Rat	THC (10 µg intra-PFC and 5 µg intra-vHP)	Anxiolytic	Rubino et al., 2008
		THC (1 µg intra-BLA)	Anxiogenic	
	Rat	URB597 (0.1–0.3 mg/kg, ip)	Anxiolytic	Cippitelli et al., 2011
	Rat	AM251 (10–200 µg, icv)	Anxiogenic	Kupferschmidt et al., 2012
	Rat	ACEA (5.0 pmol intra-PL)	Anxiolytic	Fogaca et al., 2012
Zero-maze	Rat	URB597 (0.05–0.1 mg/kg, ip)	Anxiolytic	Kathuria et al., 2003
		URB532 (0.1–10 mg/kg, ip)	Anxiolytic	
Ultrasonic test	Rat	URB597 (0.05–0.1 mg/kg, ip)	Anxiolytic	Kathuria et al., 2003
		URB532 (0.1–10 mg/kg, ip)	Anxiolytic	
	Rat	SR141716A (0.3–20 mg/kg, ip)	Anxiogenic	Varga et al., 2012
Light-dark box	Rat	URB597 (0.3 mg/kg, ip)	Anxiolytic	Butler et al., 2012
		THC (0.3 mg/kg, ip)	Anxiolytic	
Open field	Rat	HU210 (100 µg/kg, ip)	Anxiogenic	Hill et al., 2006
		Anandamide (0.1 mg/kg, ip)	Anxiolytic	
Social investigation	Mouse	CB ₁ knockout	Anxiogenic	Litvin et al., 2013
		AM251 (5 mg/kg, ip)	Anxiogenic	

ip = intra-peritoneal; *THC* = delta-9-tetrahydrocannabinol; *FAAH* = fatty acid amide hydrolase; *CB₁* = cannabinoid receptor type 1; *ACPA* = arachidonylcyclopropylamide; *intra-CeA* = intra-central nucleus of amygdala; *intra-PFC* = intra-prefrontal cortex; *intra-vHP* = intra-ventral hippocampus; *intra-BLA* = intra-basolateral amygdala; *icv* = intra-cerebroventricular; *ACEA* = arachidonyl-2'-chloroethylamide; *intra-PL* = intra-prelimbic medial prefrontal cortex.

^a*CB₁* agonists = arachidonylcyclopropylamide, HU210, WIN 55,212-2, CP55,940; *CB₁* antagonists = AM251, SR141716 (rimonabant), SR147778, SR14176A; *FAAH* inhibitors = URB532, URB597; uptake inhibitor = A404; endocannabinoid = anandamide; exogenous cannabinoid = THC; phytocannabinoid = cannabidiol; synthetic cannabinoid = nabilone.

cannabinoid agonist increases emotionality and sensitizes the stress response. In another study, microinjection of Δ^9 -THC into the PFC (10 μ g) and ventral hippocampus (5 μ g) decreased anxiety in the elevated plus-maze, while higher doses induced anxiety behavior (Rubino et al., 2008). In contrast, a low dose of Δ^9 -THC (1 μ g) in the BLA increased anxiety behavior, whereas higher doses were ineffective. Thus, the effects of cannabinoids are dose dependent and brain-region specific, such that mild activation of CB₁ receptors in the PFC and ventral hippocampus is anxiolytic while activation in the BLA is anxiogenic.

Few studies have examined the association between genetic variation in eCB genes and measures of trait anxiety. In humans, polymorphic variation in the CNR1 region has been associated with neuroticism (Juhász et al., 2009) and, in the presence of certain genetically determined serotonergic variants, with trait anxiety (Lazary et al., 2009). As noted above, however, the functional effects of CNR1 variants on eCB function are not known (Hillard et al., 2012). In animals, CB₁ KO mice exhibited greater anxiety behavior (i.e., reduced exploration of the open arms of the plus-maze apparatus) when compared with wild-type mice while general locomotor activity did not differ between the two strains (Haller et al., 2002). Further suggesting a relationship between genetic variation in eCB and trait fear, Choi et al. (2012) found that CB₁ mRNA levels in the PFC were positively correlated with fear behavior in mice selectively bred for high and low fear.

AN EMERGING FOCUS ON REWARD DYSFUNCTION IN PTSD

The capacity to determine the affective or motivational significance of ongoing events is essential for survival, and it is not surprising that neural mechanisms have evolved to permit rapid evaluations of the reward value of stimuli encountered in the environment (Yeung and Sanfey, 2004). The substrates of this reward system are well articulated (Hyman et al. 2006; Volkow et al., 2012), and include areas such as the ventral tegmental area, striatum, amygdala, and PFC, in which eCB function plays a crucial role (Fattore et al., 2010). For example, diminished sensitivity to reward in Fischer as compared to Wistar rats has been linked to reduced eCB signaling in the Fischer strain (Brand et al., 2012), and CB₁ KO mice exhibit reduced sensitivity to reward (Sanchis-Segura et al., 2004). In particular, CB₁ receptors play a crucial role in the rewarding effects of cannabinoids (Tanda et al., 1997, 2000).

While negative emotionality has long been considered a hallmark of PTSD, altered reward function has more recently become a focus. Considering the symptom of emotional numbing in PTSD, Litz and Gray (2002) proposed that the disorder reflects an imbalance between fear and reward. Similarly, Stein and Paulus (2009) argued that PTSD involves excessive fear and diminished reward, and Robinson and Shergill (2011) conceived of the disorder as a dysregulation of approach-reward and fear-avoidance processing. Most recently, a model of

dysregulated systems of reward and negative affect in addiction (Koob and LeMoal, 2008) has been generalized to PTSD (Logrip et al., 2012). As the extended amygdala, which relies heavily on eCB function, is positioned at the interface of the systems that mediate reward and negative emotionality, it is of particular significance to these models (Logrip et al., 2012).

Consistent with these formulations, a persistent inability to experience positive emotions (i.e., diminished reward) is now considered a symptom in the diagnosis of PTSD (APA, 2013). In particular, reward deficits may characterize an internalizing subtype of PTSD associated with depression, social avoidance, and anhedonia (Miller, 2003), and a growing number of laboratory studies have identified reward deficits in PTSD using neural and behavioral measures (Sailer et al., 2008; Elman et al., 2009; Vythilingam et al., 2009; Admon et al., 2012).

Genetic variation contributes substantially to individual differences in positive emotionality, an affective component of the approach system (Tellegen et al., 1988; Blonigen et al., 2008). Whereas it is not yet known how genes assert their influence on this system, a small number of imaging studies implicate genetic variation in eCB function. In the earliest of these, four SNPs in the *CNR1* region modulated striatal response to happy but not disgust faces. Although limited by a small sample size, the results of the study implicate *CNR1* in the rewarding effects of positive social interaction (Chakrabarti et al., 2006). Another study demonstrated an association between an allelic variant in *CNR1* and weaker bilateral amygdala, putamen, and pallidum activity in response to masked happy faces (Domschke et al., 2008).

Filbey et al. (2010) evaluated the brain response to marijuana cues in 3-day abstinent marijuana smokers. The smokers were grouped according to allelic variants in *CNR1* and *FAAH* SNPs previously associated with cannabis dependence (Agrawal and Lynskey, 2009; López-Moreno et al., 2012). A *CNR1* variant was associated with an enhanced response to marijuana cues in reward-related areas, including the orbitofrontal cortex, anterior cingulate gyrus, and inferior frontal gyrus; and a *FAAH* variant was associated with an increased response in the nucleus accumbens.

Most recently, in a monetary incentive delay task, Δ^9 -THC attenuated brain activity during reward feedback as compared to placebo (van Hell et al., 2012). In the same task, striatal hyperactivity during anticipatory stages of reward was observed in boys who were frequent but abstinent users of cannabis, as compared to non-using boys (Jager et al., 2013), an effect that was most pronounced on neutral (i.e., non-rewarding) trials. The authors propose that striatal hyperactivity in adolescent cannabis users reflects an overly sensitive reward circuit and an attendant difficulty in disengaging the motivational circuit when no reward can be obtained. Cannabis users in another study showed attenuated brain responses to anticipated monetary rewards in reward-related areas, suggesting that chronic cannabis use reduces the sensitivity of some regions of the reward system to rewarding stimuli, perhaps via down-regulation of CB₁ receptors (van Hell et al., 2010). In the latter two studies, however, it is unclear if the findings reflect the effects of chronic use or predisposing factors.

Finally, three imaging studies evaluated effects of cannabinoids on both negative affect and reward. In the first, reduced threat-related amygdala reactivity and greater reward-related ventral striatal reactivity were demonstrated in carriers of an *FAAH* variant associated with enhanced eCB signaling (Hariri et al., 2009). In the second study, following acute Δ^9 -THC, performance accuracy was decreased for stimuli with negative but not positive emotional content (Bossong et al., 2013). Moreover, Δ^9 -THC interacted with emotional content to affect activity in a network comprising the amygdala, orbital frontal gyrus, hippocampus, parietal gyrus, PFC, and regions in the occipital cortex. Activity was reduced for negative and increased for positive stimuli, suggesting that Δ^9 -THC reduces the negative bias in emotional processing. In the third study, heavy marijuana smokers who reported using marijuana in the past week exhibited decreased anterior cingulate gyrus and amygdala activity while viewing masked angry and happy faces as compared to non-smokers, who showed relative increases in activation within these regions during the viewing of masked faces (Gruber et al., 2009).

PTSD AND EXTERNALIZING: ENDOCANNABINOID FUNCTION AND CONTROL

The ability to evaluate and adjust behavior by means of inhibitory intervention is crucial for the maintenance and control of cognitive and motor events. Control is a dispositional dimension that describes individual differences in this adaptive function. It involves the ability to modulate responses to internal states and environmental stimuli (Hopwood et al., 2011) and is manifest in behavioral tendencies toward restraint as compared to impulsiveness. Individual differences in this dimension are substantial and are affected by genetic as well as shared environmental factors (Tellegen et al., 1988). Control can be articulated into multiple facets, such as impulsive choice, temporal discounting, and response inhibition, and the neural substrates of its various aspects are distinct but overlapping (van der Molen, 2000; de Wit, 2009; Basar et al., 2010). These substrates encompass the PFC, striatum, nucleus accumbens, and limbic regions, and include areas in which CB₁ receptors are highly expressed. It has been proposed that the eCB system affects control through its modulatory action on glutamate and dopamine signaling in the PFC and striatum (Pattij and Vanderschuren, 2008).

PTSD is often associated with externalizing behaviors, including substance use, high-risk sexual practices, risky driving, and aggression (Friedman et al., 2011). Reflecting this, externalizing behavior has been added as a diagnostic criterion for PTSD (APA, 2013). In particular, deficits in control that co-occur with high levels of negative emotionality characterize an externalizing subtype of PTSD associated with risk taking and aggression (Miller, 2003).

Consistent with a role for eCB function in impulsive behavior (see Chapter 14), epidemiologic studies have demonstrated that marijuana use is

associated with a number of risky behaviors including antisocial (Brook et al., 2011), violent (Walton et al., 2009), and high-risk sexual behavior (Kingree et al., 2000). Whereas these associations may reflect various factors, laboratory studies have confirmed that Δ^9 -THC increases the occurrence of risk-taking behavior, and that this effect varies with dose and across different aspects of control (Lane et al., 2005; Pattij and Vanderschuren, 2008). For example, Δ^9 -THC increases impulsive responding on stop-signal tasks and the Stroop Color-Word Interference Test, but it does not affect performance on either go/no-go or delay or probability discounting tasks (McDonald et al., 2003; Metrik et al., 2012).

Similarly divergent effects of cannabinoids on facets of control have been demonstrated in animals. In one study, using two rat models of impulsivity, the 5-choice serial reaction time task (5-CSRTT; impulsive action) and the delayed reward task (DRT; impulsive choice), the effects of CB₁ receptor activation on amphetamine-induced impulsivity were evaluated (Wiskerke et al. 2011). In the 5-CSRTT, pretreatment with the CB₁ receptor antagonist/inverse agonist SR141716A or the neutral CB₁ receptor antagonist O-2050 improved baseline inhibitory control and attenuated amphetamine-induced inhibitory control deficits, suggesting that CB₁ receptor activation by endogenously released cannabinoids mediates impulsive action. In contrast, direct CB₁ receptor activation by Δ^9 -THC did not affect control. In the DRT, both CB₁ receptor antagonists prevented amphetamine-induced reductions in impulsive decision making, suggesting that CB₁ receptor activity decreases this form of impulsivity.

Only two studies have examined eCB polymorphic variation in relation to control. Ehlers et al. (2007) found that impulsivity was associated with variation in the region of *CNR1*, the functional effects of which are not yet known, while a different *CNR1* SNP has been found to moderate the relationship between impulsivity and marijuana problems (Bidwell et al., 2013).

Most relevant to externalizing behavior associated with PTSD, recent work suggests that negative emotions promote impulsive action, disrupting control and biasing behavioral decisions in favor of those that lead to immediate reward (Baumeister and Scher, 1988; Cyders and Smith, 2008; Gipson et al., 2012). Mood-based impulsivity is associated with trait negative affect and predicts externalizing behavior (Settles et al., 2012), including that associated with PTSD (Harmon et al., 2012; Weiss et al., 2012; Wright et al., 2012). While no studies have addressed eCB function in negative urgency, neural structures hypothesized to play a role, such as the amygdala and the anterior cingulate (Cyders and Smith, 2008), rely heavily on eCB function.

While externalizing behavior is associated with undercontrol, a dissociative subtype of PTSD may reflect overcontrol. In addition to the core symptoms, this subtype is characterized by depersonalization and/or derealization, including feelings of detachment, unreality, distance, or distortion (Wolf et al., 2012; APA, 2013; Stein et al., 2013). Lanius et al. (2010) propose a model of emotion dysregulation in which re-experiencing and hyperarousal reflect emotional undermodulation, mediated by failure of prefrontal inhibition of limbic regions, including the

amygdala, which is heavily reliant on eCB signaling. In contrast, the dissociative subtype involves hyperinhibition of the same limbic regions by the PFC. In addition, an altered sense of time is the most common dissociative symptom (Ursano and Fullerton, 1999), and predicts PTSD after a traumatic event (Bryant et al., 2011). Further implicating eCB function in this dissociative subtype, the cerebellum, in which CB₁ receptors are highly dense (Takahashi and Linden, 2000), subserves critical aspects of time perception (Coull et al., 2011).

In summary, variation in three fundamental dimensions of temperament—negative emotionality, reward sensitivity, and control—affects the likelihood of PTSD following acute trauma and shapes the constellation of symptoms in trauma-exposed individuals. Further, substantial research implicates eCB function in these dimensions of risk and suggests that compromised eCB signaling may constitute a risk phenotype for PTSD.

EXPERIENCE-DEPENDENT CHANGES IN PTSD: ENDOCANNABINOIDS AND SYNAPTIC PLASTICITY

When PTSD was first added to the *Diagnostic and Statistical Manual* (DSM-III) it was unique in that the diagnosis required exposure to a traumatic event (APA, 1980). Although no longer distinctive in this regard, exposure to a traumatic event remains fundamental to the diagnosis (APA, 2013). Moreover, substantial evidence demonstrates that previous trauma, particularly when it occurs early in development, increases risk for PTSD following a traumatic event occurring later in life. Accordingly, in the quest to identify brain mechanisms relevant to PTSD, investigators have considered the capacity of neural systems to translate traumatic experience into symptoms of PTSD, focusing on the neurobiological basis of phenotypes such as fear memory expression and the response to social reward. In addition, they have focused on neural systems that are themselves modified by trauma in such a way as to fundamentally alter the stress response. In both regards, the eCB system has emerged as a key candidate.

RENDERING ACUTE TRAUMA INTO SYMPTOMS: ANIMAL MODELS OF FEAR

Emotional memories after exposure to traumatic events tend to be remembered well, frequently retrieved, and not extinguished quickly. This is essential for survival; however, this adaptive process may have become pathological in PTSD, which is characterized by exaggerated fear behavior and impaired fear extinction (Shvil et al., 2013).

Acquisition and reconsolidation of fear memory following stress exposure have been extensively studied with animal models. Classical fear conditioning

involves the pairing of a previously neutral conditioned stimulus (CS; a discrete cue or context) with an unconditioned stimulus (US; a mild footshock), which induces rapid fear learning. Re-exposure to the CS without the US can lead to the expression of fear responses. Using common behavioral metrics of fear such as freezing and fear-potentiated startle, a single mild footshock can induce a fear memory that lasts weeks or even months in animals (Maren, 2008; Pamplona et al., 2008).

Fear extinction involves exposing animals to fear-eliciting cues or context (i.e., a CS) without the previously paired unconditioned, or aversive, stimulus (US), and is considered new learning that contributes to a decrease in fear responses (Myers and Davis, 2002; Quirk et al., 2006). With repeated presentation of the CS in the absence of the US, conditioned fear responses gradually decrease (Quirk and Mueller, 2008). Multiple brain regions are implicated in this process, including the amygdala, hippocampus, and medial PFC (Quirk et al., 2006; Sotres-Bayon et al., 2006). As impaired extinction of fear memory is considered a model of one of the core symptoms of PTSD (Milad et al., 2006), classical fear conditioning and extinction paradigms have been used extensively in animal work on the disorder.

The eCB system has become a major focus of research in fear extinction (Davis et al., 2006; Chhatwal and Ressler, 2007; Varvel et al., 2009) (see Table 11.2). This work suggests that cannabinoid receptor activation facilitates fear extinction (Marsicano et al., 2002; Kamprath et al., 2006; Pamplona et al., 2006) and impairs fear memory retrieval (Niyuhire et al., 2007; Atsak et al., 2012). For example, administration of a low dose of the CB₁ receptor agonist WIN 55,212-2 before extinction training facilitates extinction of contextual fear memory up to 30 days after conditioning (Pamplona et al., 2006). These effects are blocked by pretreatment with SR147778, a selective cannabinoid antagonist. In contrast, in another study, a high dose of WIN 55,212-2 had no effects on extinction of fear-potentiated startle (Chhatwal et al., 2005). While the CB₁ receptor antagonist rimonabant disrupted extinction in both conditioned freezing and passive avoidance tasks (Niyuhire et al., 2007), extinction rate in an operant conditioning task for reward was not affected. Thus, the CB₁ receptor plays a vital role in the extinction of fear memories but may not be essential for extinction of learned responses in appetitively motivated tasks.

In contrast to the acute administration of cannabinoid agonists, chronic administration of WIN 55,212-2 (10 mg/kg for 7 days) has been found to impair fear extinction in rats (Lin et al., 2008). Following chronic administration, rats were subjected to a standard fear conditioning procedure, and retention of memory was measured with a potentiated startle paradigm. WIN 55,212-2-pretreated rats exhibited substantially less extinction to cue-alone presentation, and the reduction in fear-potentiated startle typically seen with infusion of CB₁ receptor agonists into the medial PFC was absent in WIN 55,212-2-pretreated rats. This suggests that long-term exposure to cannabinoids may impair fear extinction and limit the therapeutic efficacy of these drugs.

Table 11.2 Effects of Endocannabinoid Drugs on Fear Extinction in Rodents

Paradigm	Species	Intervention ^a	Fear Extinction	Reference
Auditory fear conditioning	Mouse	CB ₁ knockout	Impaired	Marsicano et al., 2002
		SR141716A (3 mg/kg, sc)	Impaired	
	Mouse	CB ₁ knockout	Impaired	Cannich et al., 2004
	Mouse	CB ₁ knockout	Impaired	
	Mouse	SR141716A (3 mg/kg, ip)	Impaired	Niyuhire et al., 2007
	Mouse	CB ₁ knockout	Impaired	
	Mouse	CB ₁ knockout	Impaired	Dubreucq et al., 2010
		SR141716A (3 mg/kg, sc)	Impaired	
Contextual fear conditioning	Mouse	CB ₁ knockout	Impaired	Kamprath et al., 2011
		AM251 (1 ng, bilateral, intra-CeA)	Impaired	
	Mouse	CB ₁ knockout	Impaired	Terzian et al., 2011
	Mouse	SR141716A (10 mg/kg, ip)	Impaired	
	Rat	SR141716A (1 mg/kg, ip)	Impaired	Pamplona et al., 2006
		WIN 55,212-2 (0.25 mg/kg, ip)	Facilitated	
	Rat	SR141716A (1 mg/kg, ip)	Impaired	Pamplona et al., 2008
		WIN 55,212-2 (0.25 mg/kg, ip)	Facilitated	
		AM404 (10 mg/kg, ip)	Facilitated	
	Rat	AM404 (1 mg/ml, icv)	Facilitated	
		Cannabidiol (2 mg/ml, icv)		Bitencourt et al., 2008
	Rat	Anandamide (0.17 ng, intra-CA1)	Facilitated	
	Rat	Cannabidiol (3–30 mg/kg, ip)	Facilitated	Stern et al., 2012
	Rat	ACEA (0.1 mg/kg, ip)	Facilitated	
				Reich et al., 2013b

(Continued)

Table 11.2 Effects of Endocannabinoid Drugs on Fear Extinction in Rodents *Continued*

Paradigm	Species	Intervention ^a	Fear Extinction	Reference
Fear-potentiated startle	Rat	SR141716A (1.5–5 mg/kg, ip)	Impaired	Chhatwal et al., 2005
		AM404 (10 mg/kg, ip)	Facilitated	
		WIN 55,212-2 (5 mg/kg, ip)	No effect	
	Rat	AM251 (2 µg, intra-IL)	Impaired	Lin et al., 2009
		WIN 55,212-2 (0.05 µg, intra-IL)	Facilitated	
		AM404 (0.2 µg, intra-IL)	Facilitated	
Inhibitory avoidance	Rat	URB597 (0.3 µg, intra-IL)	Facilitated	Abush and Akirav, 2010
		AM251 (6 ng, intra-CA1)	Facilitated	
		WIN 55,212-2 (5 µg, intra-CA1)	Facilitated	
		AM404 (200 ng, intra-CA1)	Facilitated	
Passive avoidance	Rat	WIN 55,212-2 (0.5 mg/kg, ip)	Facilitated	Segev et al., 2014
	Mouse	SR141716A (3 mg/kg, ip)	Impaired	Niyuhire et al., 2007
		SR141716A (5 µg, intra-CA1)	Impaired	
Aversive Barnes maze conditioning	Mouse	SR141716A (3 mg/kg, ip)	Impaired	Niyuhire et al., 2007

CB₁ = cannabinoid receptor type 1; sc = subcutaneous; ip = intra-peritoneal; intra-CeA = intra-central nucleus of amygdala; icv = intra-cerebroventricular; intra-CA1 = intra-dorsal hippocampus; ACEA = arachidonyl-2'-chloroethylamide.

^aCB₁ agonists = arachidonylcyclopropylamide, HU210, WIN 55,212-2, CP55,940; CB₁ antagonists = AM251, SR141716 (rimonabant), SR147778, SR14176A; FAAH inhibitors = URB532, URB597; uptake inhibitor = A404; endocannabinoid = anandamide; exogenous cannabinoid = THC; phytocannabinoid = cannabidiol; synthetic cannabinoid = nabilone.

Studies of the effects of cannabinoid compounds on fear memory reconsolidation have produced conflicting results. For example, administration of cannabinoid receptor agonists after training has facilitated memory consolidation in inhibitory avoidance tasks (Campolongo et al., 2009; Hauer et al., 2011), while cannabinoid agonists given shortly after exposure to intense stress have prevented the impairment in avoidance extinction induced by intense stress (Ganon-Elazar and Akirav, 2009, 2012, 2013). While these results consistently demonstrate that the eCB system plays a crucial role in fear memory and extinction, they also reveal complexities. The effects of cannabinoid compounds on fear memory consolidation and extinction appear to depend on dose, route of administration, timing and duration of exposure, and testing paradigm.

CB₁ receptor KO mice show impaired short-term and long-term extinction in auditory fear conditioning tests, but no effects on memory acquisition and consolidation have been observed (Marsicano et al., 2002). In one study, administration of CB₁ receptor antagonist SR141716A to wild-type mice mimicked the phenotype of impaired extinction, implicating CB₁ in fear memory extinction. CB₁ KO mice are impaired not only in extinction of the fear response to a tone after conditioning, but also in habituation of the fear response to a tone after sensitization to inescapable footshock (Kamprath et al., 2006). This suggests that there are two distinct CB₁ receptor-dependent systems for short- and long-term fear adaptation, respectively.

Together, conditioning and extinction of fear memory model an important mechanism for coping with traumatic events. Indeed, the fear extinction protocol used in animal studies resembles exposure therapy used in the treatment of PTSD (Davis et al., 2006). That is, exposure-based therapy attempts to extinguish previously learned trauma-related thoughts, feelings, and behaviors (Foa, 2006; Schnurr et al., 2007). Thus, identifying factors that facilitate extinction in animal models has important clinical implications. In particular, drugs that facilitate fear extinction in animals may improve outcome when combined with exposure therapy.

The eCB system plays an important role in synaptic plasticity, crucial in learning and memory, affecting activity-dependent changes in synaptic strength. CB₁ receptor activation generally reduces fear memory expression and impairs fear memory retrieval (Niyuhire et al., 2007; Atsak et al., 2012) (see Table 11.3). For instance, in one study, activation of the CB₁ receptors in the dorsolateral periaqueductal gray (dlPAG) by microinjection of anandamide or anandamide transport inhibitor (AM404) reduced freezing and cardiovascular responses in the aversively conditioned context (Resstel et al., 2008). These effects were blocked by pretreatment with AM251, a CB₁ receptor antagonist. Since the dlPAG is involved in defensive responses and conditioned fear, facilitation of eCB function in this region may attenuate expression of contextual fear responses. Another study found that chronic unpredictable stress enhanced hippocampal-dependent episodic fear memories (Reich et al., 2013a). These stress-induced fear responses were attenuated by CB₁ receptor agonists, suggesting that compromised eCB signaling following stress contributes to exaggerated fear responses. In turn, this suggests that abnormal fear memory and fear response in PTSD in part reflect compromised eCB function.

Table 11.3 Effects of Endocannabinoid Drugs on Fear Expression in Rodents

Paradigm	Species	Intervention ^a	Fear Expression	Reference
Olfactory fear conditioning	Rat	AM251 (50 ng, intra-mPFC)	Decreased	Laviolette and Grace, 2006
Auditory fear conditioning	Rat	AM251 (3 mg/kg, ip)	No effect	Arenos et al., 2006
	Mouse	AM251 (1 ng, intra-CeA)	Increased	Kamprath et al., 2011
Contextual fear conditioning	Rat	SR141716A (1 mg/kg, ip)	No effect	Pamplona et al., 2006
		SR147778 (1 mg/kg, ip)	No effect	
	Mouse	AM251 (1 mg/kg, ip)	No effect	Mikics et al., 2006
		WIN 55,212-2 (1 mg/kg, ip)	Increased	
	Rat	AEA (5 pmol, intra-dIPAG)	Decreased	Resstel et al., 2008
		AM404 (50 pmol, intra-dIPAG)	Decreased	
	Rat	AM404 (50 pmol, intra-vmPFC)	Decreased	Lisboa et al., 2010
	Rat	SR141716A (0.5 µl, intra-dIPAG)	Decreased	Olango et al., 2012
	Rat	URB597 (0.3 mg/kg, ip)	Decreased	Butler et al., 2012
Inhibitory avoidance	Rat	AM251 (5.5 ng, intra-HP)	Decreased	de Oliveira Alvares et al., 2005
	Rat	WIN 55,212-2 (5 µg, intra-CA1)	Decreased	Abush and Akirav, 2010
	Rat	WIN 55,212-2 (0.5 mg/kg, ip)	Decreased	Ganon-Elazar and Akirav, 2012
Passive avoidance	Mouse	SR141716A (1 mg/kg, ip)	No effect	Mazzola et al., 2003
Trace fear conditioning	Mouse	AM251 (5 mg/kg, ip)	Enhanced	Reich et al., 2008
	Rat	ACEA (0.1 mg/kg, ip)	Decreased	Reich et al., 2013a
Fear-potentiated startle	Rat	AM404 (10 mg/kg, ip)	Decreased	Chhatwal et al., 2005

intra-mPFC = intra-medial prefrontal cortex; *ip* = intra-peritoneal; *intra-CeA* = intra-central nucleus of amygdala; *AEA* = anandamide; *intra-dIPAG* = intra-dorsolateral periaqueductal gray; *intra-vmPFC* = intra-ventromedial prefrontal cortex; *intra-HP* = intra-hippocampal; *intra-CA1* = intra-dorsal hippocampus; *ACEA* = arachidonyl-2'-chloroethylamide.

^aCB₁ agonists = arachidonylcyclopropylamide, HU210, WIN 55,212-2, CP55,940; CB₁ antagonists = AM251, SR141716 (rimonabant), SR147778, SR14176A; FAAH inhibitors = URB532, URB597; uptake inhibitor = A404; endocannabinoid = anandamide; exogenous cannabinoid = THC; phytocannabinoid = cannabidiol; synthetic cannabinoid = nabilone.

LONG-TERM ENDOCANNABINOID ADAPTATIONS: THE LEGACY OF EARLY LIFE ADVERSITY

Adaptation in the face of physical or psychological stress is crucial for survival. Efficient mechanisms have evolved to serve this purpose, recruiting various systems to limit or avoid exposure to stressors (Radley et al., 2011). In the past decade, research has increased our understanding of long-term adaptations in these neuronal circuits following chronic exposure to external insults, such as early life adversity (Radley et al., 2011) and addictive drugs (Fratta and Fattore, 2013). These changes affect the ability of an organism to respond to new challenges and contribute to increased risk for stress-related disorders such as PTSD. A variety of molecular mechanisms have been investigated, and plasticity in the eCB system has been implicated (Lee and Gorzalka, 2012; Viveros et al., 2012; Fratta and Fattore, 2013).

Animal studies have found that exposure to various stressors alters eCB levels in the brain, implicating eCBs in the extinction of aversive memories and in adaptation to aversive situations more generally. For instance, presentation of the CS during fear extinction results in elevated levels of eCBs in the BLA, a region known to control extinction of fear memories (Marsicano et al., 2002). Since CB₁ receptors modulate GABA-mediated inhibitory currents in the BLA, this may explain the facilitation of fear extinction induced by eCB system activation. Moreover, social isolation stress during adolescence alters eCB gene expression in the PFC, substantia nigra, and ventral tegmental area of rats (Robinson et al., 2010), suggesting that childhood adversity, a risk factor for stress-related disorders, disrupts eCB function. Conversely, early environmental enrichment increases CB₁ receptor expression in the hypothalamus and the BLA of mice (El Rawas et al., 2011), perhaps attenuating stress responses and protecting against PTSD.

Finally, in humans, CB₁ mRNA levels in the dorsolateral PFC have been found to decrease gradually from birth to 50 years of age, and in the same study, CB₁ receptor levels in the PFC of major depression patients were found to be higher than those of age-matched controls (Choi et al., 2012). This suggests that CB₁ receptor levels are developmentally regulated and that disruption of the normal developmental pattern, such as that caused by early life stress, may contribute to major depressive and other stress-related disorders in adulthood.

PTSD AND CANNABIS USE: ENDOCANNABINOID FUNCTION IN AN INTERDEPENDENT TRAJECTORY

Epidemiologic studies reveal that 44% of adults in the US report having smoked marijuana in their lifetime (National Institute on Drug Abuse, 2013), while 3.9 and 8.3% of the population meet criteria for abuse and dependence, respectively (Haberstick et al., 2014). Moreover, large epidemiological surveys reveal higher rates of cannabis use and cannabis use disorder (CUD) among individuals with mental

illness as compared to the general population (Regier et al., 1990; Kessler et al., 1996; Lev-Ran et al., 2013). While the association between PTSD and substance use disorder (SUD) is widely recognized (Najt et al., 2011), few studies have examined the prevalence of comorbid PTSD and CUD specifically. A recent analysis (Cougler et al., 2011) of data from a large epidemiologic survey of adults from the US (Kessler et al., 2004) revealed that PTSD diagnoses were associated with increased odds of lifetime history of cannabis use as well as past year daily cannabis use. Two conceptualizations of the functional relationship between PTSD and substance use are particularly relevant to the role of eCB function in the co-occurrence of PTSD and CUD. It has been proposed that the two share a common diathesis, perhaps comprising a high level of negative emotionality, and that individuals use cannabis to cope with negative emotion generally and symptoms of PTSD in particular.

A SHARED DIATHESIS

In terms of the emerging dimensional view of mental disorders, comorbidity arises from elevations on core processes common to co-occurring disorders (Krueger, 1999; Watson, 2005; Krueger and Markon, 2006). That is, comorbidity reflects diatheses, or liabilities, shared by two disorders, variance in which is affected by genetic as well as environmental factors. A great deal of evidence suggests that such a diathesis may explain in part the comorbidity between PTSD and cannabis use and, further, implicates eCB function in that diathesis.

Multivariate genetic analysis of twin data provides a powerful means of estimating the extent to which genetic and environmental factors that contribute to variance in one phenotype also contribute to individual differences in a second phenotype (Carey, 2003). Studies using these techniques have produced strong evidence for a shared diathesis between PTSD and dependence on various substances, including nicotine (Koenen et al., 2005; Scherrer et al., 2008) and alcohol (Xian et al., 2000; McLeod et al., 2001; Scherrer et al., 2008; Sartor et al., 2011). While no study has addressed a shared diathesis for PTSD and cannabis use specifically, a common liability has been demonstrated for PTSD and illicit drug dependence, the largest portion of which was accounted for by cannabis dependence (Xian et al., 2000).

Genetic association studies indirectly implicate eCB function in a shared diathesis for PTSD and cannabis use. Polymorphic variation in CNR1 and FAAH has been associated with PTSD and related dimensions, and has also been implicated in SUD generally and CUD in particular (Agrawal and Lynskey, 2009; Benyamina et al., 2011; López-Moreno et al., 2012). Other data implicate compromised eCB function in the development of substance use (Parolaro and Rubino, 2008; Pava and Woodward, 2012), particularly in the switch from abuse to dependence (Serrano and Parsons, 2011). Thus, evidence converges to suggest that compromised eCB function, arising from genetic as well as environmental sources, affects risk for both PTSD and cannabis use, perhaps through its effects on negative emotionality.

SELF-MEDICATING WITH CANNABIS

The self-medication hypothesis, the most commonly cited explanation for the association between PTSD and substance use (Keane and Kaloupek, 1997; Jacobsen et al., 2001; Stewart and Conrod, 2003), posits that alcohol and other substances are used to regulate, escape, or avoid undesirable affective states (Khantzian, 1997).² It has been widely used to explain the relationship between PTSD and cannabis use (Bonn-Miller et al., 2014) and provides a framework for understanding the role of eCB signaling in this comorbidity.

Consistent with the self-medication hypothesis, anecdotal reports by military veterans suggest that cannabis reduces negative affect and ameliorates symptoms of PTSD (Bremner et al., 1996; Hillard et al., 2012; Passie et al., 2012). Empirical studies confirm that the acute subjective effects of cannabis typically include negatively reinforcing effects such as reduced anxiety and stress relief, as well as positively rewarding effects such as euphoria and an enhanced sense of relaxation and well-being (Zuurman et al., 2009; Marco and Laviola, 2012). Importantly, however, both anecdotal and empirical reports also reveal anxiogenic and other aversive effects.

The findings of a small set of studies that investigated coping motives for marijuana use in PTSD are also consistent with the self-medication hypothesis. In a sample of cannabis-dependent military veterans, PTSD symptom severity was associated with use of cannabis to cope, cannabis use problems, severity of cannabis withdrawal, and experiences of craving related to compulsivity and emotionality (Bonn-Miller et al., 2007; Boden et al., 2013), while difficulties in emotion regulation appear to fully mediate the association between symptom severity and coping motives (Bonn-Miller et al., 2011). Similarly, Bujarski et al. (2012) found that past 2-week posttraumatic stress symptoms predicted coping motives for use in a sample of adolescents. Finally, in one study, the association between PTSD symptoms and future drug problems was best explained by a PTSD-specific self-medication mechanism (Haller and Chassin, 2012).

It has also been proposed that cannabis is used by individuals with PTSD to reduce aggressive behavior (Barrett et al., 2011). However, while this motive for use has been documented in depressed individuals with prior convictions of violence (Arendt et al., 2007), a parallel study has not been done for PTSD. Moreover, whereas low doses of cannabis reduce aggression in rats, high doses, or administration in a stressful situation, exacerbate aggression (Mechoulam, 2002). Similarly, cannabis may attenuate or increase aggressive responding in humans, depending on context (Cherek and Dougherty, 1995).

²Although some have argued against the self-medication hypothesis (Lembke, 2012), that debate is outside the scope of this chapter. Moreover, it is increasingly recognized that the functional relationship between PTSD and substance use is complex, reflecting the interplay of many factors, the relative importance of which changes over time.

To summarize, CUD is often comorbid with PTSD. This may reflect, in part, a diathesis shared between the two disorders (and more broadly, between PTSD and SUD). Considerable evidence suggests that compromised eCB signaling is a part of that diathesis, and that the alleviating effects of smoked cannabis reflect normalization of eCB function in individuals with PTSD. Whereas this suggests that eCB-targeting drugs hold promise for PTSD, the evidence also strikes a cautionary note, revealing complexities that will complicate this effort.

THE ENDOCANNABINOID SYSTEM IN THE TREATMENT OF PTSD: A CAUTIONARY TALE

Despite important progress, there is no universally effective treatment for PTSD. Substantial evidence suggests that eCB signaling is deficient in PTSD, thereby recommending the eCB system as a therapeutic target for that disorder (Clapper et al., 2009; Passie et al., 2012; Neumeister, 2013; Trezza and Campolongo, 2013). The eCB system is also a target for treatment of anxiety disorders (Crippa et al., 2011), emotion dysregulation (Marco and Laviola, 2012), depression (Mangieri and Piomelli, 2007), and CUD (Clapper et al., 2009), all of which are associated with PTSD. Moreover, as preclinical evidence suggests that the eCB system regulates fear memory processing and extinction, enhancing eCB function may ameliorate core deficits in PTSD. However, multiple challenges confront the effort to develop eCB-targeting therapies.

Although cannabis is consumed mainly for its euphoriant properties, which are often accompanied by decreased anxiety and increased sociability, dysphoric reactions, anxiety, panic, and psychosis have also been reported (Patel and Hillard, 2006; Crippa et al., 2009; Moreira and Wotjak, 2010; Ashton and Moore, 2011). The same bidirectional profile has been described in rodents following cannabinoid agonist administration, with low doses being anxiolytic and high doses anxiogenic (Moreira and Lutz, 2008; Viveros et al., 2005). Other factors such as route of administration (Akirav, 2011) and brain region (Martin et al., 1999; Rubino et al., 2008) also moderate the drug effect. Baseline impulsivity modulates the effects of cannabis on measures of inhibitory control (Wiskerke et al., 2011) and social context influences its effects on aggression (Cherek and Dougherty, 1995). More generally, the effects of cannabis vary across a wide range of peripheral and central domains (Zuurman et al., 2009).

Undesirable effects of CB₁ receptor agonists, including anxiety at high doses, diminish the argument for their use, as does their abuse potential and the possibility of rapid receptor desensitization, which may decrease efficacy of the drugs. Indirect cannabinoid agonists that inhibit uptake or degradation of eCB, such as anandamide and 2-AG, may be more promising in the treatment of fear and anxiety (Bitencourt et al., 2008; Pamplona et al., 2008; Trezza and Campolongo, 2013). Supporting this, recent studies have demonstrated therapeutic potential of indirect agonists in fear memory extinction.

Cannabidiol (CBD) is a non-psychoactive phytocannabinoid, which appears to have a remarkably safe profile for use in humans. As CBD indirectly enhances eCB signaling by preventing the degradation of anandamide, it may provide an alternative to the use of direct CB₁ receptor agonists. CBD facilitates extinction of contextual fear memory in rodents (Bitencourt et al., 2008), an effect that is blocked by the CB₁ receptor antagonist SR141716A. In another study, systemic administration of CBD (10 mg/kg) immediately after memory retrieval disrupted reconsolidation of fear memory in rats (Stern et al., 2012). These effects were blocked when rats were pretreated with the CB₁ receptor antagonist AM251. Thus, CBD may disrupt reconsolidation of fear memory and facilitate fear memory extinction by facilitating activation of CB₁ receptors, and therefore may be a useful compound in the effort to target dysfunctional cognitive and emotional processes associated with stress exposure. Similarly, AM404, which prevents degradation of anandamide in the brain by inhibiting the enzyme FAAH, has been found to have anxiolytic properties based on various metrics, including behavior in an elevated plus-maze and separation-induced ultrasonic vocalizations (Bortolato et al., 2006), fear potentiated startle (Lin et al., 2006), and contextual fear memory (Bitencourt et al., 2008).

Further, fear memory reconsolidation and extinction may have distinct temporal and biochemical signatures. For example, in one study, the CB₁ receptor antagonist SR141716A blocked fear memory extinction but not memory reconsolidation (Suzuki et al., 2004). The cognitive enhancer D-cycloserine, which facilitates extinction of fear memory in animals through mechanisms that are not directly related to eCB function, may also be effective in humans (Walker et al., 2002; Ressler et al., 2004). The findings of a growing number of animal studies suggest that compounds that enhance central eCB function may have therapeutic potential similar to that of D-cycloserine. Thus, drugs that either block fear memory reconsolidation or facilitate fear extinction may have therapeutic potential for treatment of PTSD.

The effort to isolate therapeutic from undesirable effects of cannabinoids must also consider interindividual differences in response to these drugs (Pacher et al., 2006; Bosier et al., 2010). This variability likely reflects the effects of genetic differences in eCB (and other neural) function, as well as effects of environmental factors such as early life adversity and adolescent exposure to cannabis and other substances. Identifying factors that predict which individuals will benefit from the anxiolytic properties of such drugs will be critical.

Further complicating the effort to develop eCB-targeting pharmacotherapies, PTSD is a heterogeneous disorder. Substantial evidence supports the validity of a typology that distinguishes between an internalizing form of the disorder, characterized by high negative emotionality and a reward deficit, and an externalizing form in which negative emotionality is coupled with a deficit in control (Miller, 2003). Moreover, as described above, a dissociative subtype of PTSD has received attention in the past decade (Lanius et al., 2010; Friedman et al., 2011; Wolf et al., 2012; Stein et al., 2013). It is likely that eCB-targeting drugs will be differently efficacious in subtypes of the disorder.

A concern of particular relevance in the search for eCB drugs to treat PTSD is its comorbidity with SUD generally and with CUD in particular. It is widely agreed that treatment of PTSD, as well as that for SUD, is less effective when the disorders co-occur in the same individual (McCarthy and Petrakis, 2010). While there is some evidence that eCB-targeting drugs may also be useful in treating SUD (Clapper et al., 2009), their efficacy when the disorders are comorbid must be specifically addressed. For example, the eCB uptake inhibitor LY2183240 reduces fear-potentiated startle while increasing alcohol-seeking behavior in mice selectively bred for high alcohol preference (Powers et al., 2010), raising concerns that some eCB-enhancing drugs may not be useful for patients with comorbid PTSD and SUD.

Long-term exposure to drugs of abuse, including cannabis, causes persistent neuroadaptations. In turn, these adaptations lead to the recruitment of stress response systems, a salient feature in the progression to drug dependence (Koob and Le Moal, 2008). It has been proposed that disorders such as PTSD, which are characterized by exaggerated stress responses, predispose individuals to this increase in negative affect. This increases the likelihood of addictive disorders and may perpetuate and worsen addictive disorders once established (Logrip et al., 2012). Thus, compensatory adaptations of eCB function in response to eCB-targeting drugs—even when they are therapeutic initially—will need to be evaluated. Moreover, as eCB function is critical to multiple regulatory systems, including but not limited to the stress and reward responses, understanding potential adaptations across systems will be critical.

Finally, positive effects of cannabis are associated with heavy use, abuse, and dependence in adolescents and young adults (Grant et al., 2005; Scherrer et al., 2009). Individuals who have positive reactions to marijuana may be more likely to use it to cope with negative affect and other symptoms of PTSD, and more likely to use the drug heavily and become dependent. Moreover, sensitivity to reward predicts risky alcohol use among individuals who are high in negative emotionality (Allen and Gabbay, 2013). While this concern is particularly salient in view of the movement to utilize medical marijuana in the treatment of PTSD, the abuse liability of all potential cannabinoid therapeutics will need to be evaluated, with a particular focus on individuals at high risk for abuse and dependence.

FUTURE DIRECTIONS

In this chapter, we have presented findings from human and animal research that suggest compromised eCB function contributes to risk for PTSD via its effects on negative emotionality, reward function, and control. Further, reflecting its involvement in the stress response, the eCB system plays a fundamental role in rendering the effects of acute trauma, in both the short and longer term. However, many remaining questions require empirical analysis, and the results of this analysis should be utilized in a national discussion about the use of medical marijuana in the treatment of PTSD and more broadly about rapidly changing marijuana policy and law.

ETIOLOGIC RESEARCH

There is an imperfect correspondence between clinical diagnoses and dysregulated biological systems (Sanislow et al., 2010; Patrick et al., 2013). PTSD involves disruptions in various core processes, including those affected by eCB function, and eCB function is also compromised in disorders other than PTSD. This suggests that a fruitful approach to investigating eCB dysfunction in PTSD will be to focus directly on its contribution to its core dimensions such as negative emotionality and reward. The Research Domain Criteria (RDoC) framework provides a tool that promotes this approach (Morris and Cuthbert, 2012). By design, RDoC uncouples research efforts from diagnostic categories and refocuses attention on fundamental mechanisms of psychopathology, with the eventual goal of understanding how abnormalities in these mechanisms drive clinical symptoms. As negative and positive valence systems are two of the overarching domains in the RDoC, the framework is well suited to the study of eCB involvement in fundamental processes in PTSD. This approach promises to avoid problems of heterogeneity and promote the identification of articulated targets for eCB therapy.

THERAPEUTIC IMPLICATIONS

As we have outlined, many challenges confront the effort to develop eCB-targeting medications for PTSD. In addition to these challenges, an improved understanding of the pathophysiological role of the eCB system, as well as a more articulated characterization of the functional deficits in PTSD, will facilitate clinically successful treatment strategies. However, in addition to medication development, the evidence outlined in this chapter has vital implications for behavioral, cognitive, and social interventions. For example, animal studies have demonstrated that early environmental enrichment enhances the number of CB₁ receptors and appears to afford protection against the behavioral, neural, and molecular effects of trauma and drugs of abuse. This encourages ongoing efforts to develop or identify behavioral interventions that inoculate against the effects of environmental insults. Characterizing eCB mechanisms that mediate this effect may provide neural metrics that can be used in refining such interventions and assessing their success.

The evidence outlined in this chapter has important implications for efforts to promote the use of marijuana to treat PTSD in military veterans and others (Hamilton, 2013; Bonn-Miller et al., 2014). We have outlined complexities and potential harms associated with the use of smoked marijuana, which include adverse consequences such as increased anxiety and risk for dependence, particularly in individuals already suffering the effects of PTSD. According to the Institute of Medicine (1999), “the question is not whether marijuana can be used as an herbal remedy but rather how well this remedy meets current standards of efficacy and safety” (p. 19). In the case of marijuana for the treatment of PTSD, it is not at all clear that these standards are met. Nearly 2 million individuals have been deployed in the US’s two recent wars, and 15–30% of returning military personnel may be affected by PTSD

(Tanielian and Jaycox, 2008). This defines an urgent national priority. Scientific analysis must continue, and its results disseminated in clear and comprehensible terms, in order to provide an empirical foundation for this national discussion.

POLICY AND LAW

Concurrent with increasing acceptance of medical marijuana, a seismic shift in the American public's view of recreational use of marijuana is ongoing (Steinhauser, 2014). This is resulting in rapid changes in policies and laws related to marijuana (Galston and Dionne, 2013), which in turn may further enhance cultural acceptance. In states that have implemented medical marijuana, prevalence of recreational use is greater, as are rates of abuse and dependence, and perception of riskiness is lower (Wall et al., 2011; Cerdá et al., 2012).

The broad availability of marijuana and the changing perceptions about risk will no doubt also promote its use for self-medication. Importantly, this will include veterans suffering the effects of posttraumatic stress, but will also likely include adolescents and other individuals at risk for suffering its negative consequences, by way of genetic predisposition, early life adversity, or other factors. The sea change in the laws pertaining to marijuana, particularly in the absence of the general public's understanding of its effects, substantially increases the urgency of the scientific mission to more fully characterize neurobehavioral mechanisms in PTSD and translate that information into informed preventive and treatment strategies.

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Cannabinoids and drug addiction

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DRUG ABUSE AND ADDICTION

Addiction is defined as obsessive thinking and compulsive need for something, like drugs, food or sex, despite the resulting negative consequences. In physiological conditions, the brain reward system reinforces behaviors required for species survival, including sexual activity, nursing, and eating. Drug abuse hijacks such a neural pathway and replaces normal reward-related behavior, leading over time to uncontrollable drug seeking and taking and, ultimately, to drug addiction. Yet, engaging in non-drug-related activities involving “natural” rewards such as food, sex, and playing activity could also activate the brain “pleasure circuit” and result in addiction. While it is quite intuitive to distinguish chemical from behavioral addictions, other important conceptual distinctions are necessary.

The terms “drug abuse,” “addiction,” and “dependence” are not interchangeable and cannot be used as synonyms, although they represent different ends of the same disease process.

While drug abuse refers to the taking of drugs for non-medical purposes, typically because of the drug’s positive subjective effects, “drug addiction” develops after repeated substance use and normally includes a pressing urge to take the drug and difficulties in controlling its consumption. Clinically, the occasional but limited use of a drug is different from escalated drug use and the emergence of chronic drug dependence. Yet, for some individuals drug addiction develops over time and usually begins with misuse, moving toward abuse and resulting in addiction (Figure 12.1). Once addicted, many drug users feel completely powerless and persevere in using the drug in the face of potentially dangerous health consequences.

Drug addiction is a chronically relapsing disorder characterized by (1) compulsion to seek and take the drug, (2) loss of control in limiting drug intake despite harmful consequences, (3) emergence of a withdrawal state and negative emotional

**FIGURE 12.1**

Different phases of the human addiction cycle.

state (e.g., dysphoria, irritability, anxiety) when access to the drug is prevented, (4) greater importance given to obtaining the drug than to other activities or goals, and (5) development of tolerance. Addiction involves a complex neuropharmacologic behavioral cycle in which positive reinforcement exerted by the drug and the negative state of withdrawal drive the user to extremes to obtain the drug. Conversely, *drug dependence* refers to the need to continue taking a drug to avoid withdrawal effects on drug discontinuation, and is not necessarily associated with the rewarding properties of the drug nor is related to its abuse liability. Several drugs, such as the selective serotonin reuptake inhibitors (SSRIs), can induce dependence but do not possess positive reinforcing properties.

Closely linked to the concept of drug dependence, drug *withdrawal* (e.g., abstinence) is caused by chronic drug use cessation, is characterized by signs and physical symptoms usually opposite to the acute effects of the drug, and is associated with the emergence of negative emotional feelings. Even if it is possible for drug addicts to desist from drug use, maintaining abstinence is extremely difficult. The inability to remain abstinent is often referred to as *relapse* and consists of a process by which an abstaining individual falls again into old behavioral patterns and substance use, i.e., the return to drug use after a period of withdrawal. Relapse is the most common outcome of recovery programs treating addictive behaviors, for which *craving* (i.e., the strong, often uncontrollable desire to use the drug) represents a major risk factor. At the clinical level, craving and relapse are now considered major challenges in drug addiction treatment, and preventing relapse when an abstinent patient is exposed to the drug or drug-related stimuli is still demanding.

Table 12.1 Factors Triggering Relapse in Both Humans and Animals

	Humans	Animals
Drug priming	A single exposure to the previously abused drug	Not contingent, not reinforced acute drug injection
Environmental cues	People, places or things associated with past drug use	Context, cue light or tone previously associated with drug delivery
Stress	Divorce Loss of employment or beloved person Violence Negative or challenging emotions	Intermittent footshock Acute 1 day food deprivation Yohimbine (chemical stress)

In human drug users, a variety of events or stimuli can precipitate drug craving and elicit the urge to use the drug, which ultimately results in relapse in abstinent individuals. The presentation of the drug itself, or stimuli previously associated with drug delivery (e.g., place, people, paraphernalia) or stressful events (e.g., loss of beloved person or employment, divorce), all increase the motivation to engage in drug taking and the likelihood of relapse (Bossert et al., 2013). Likewise, the same conditions that trigger relapse in humans are also able to reinstate drug-seeking behavior in laboratory animals, and include small doses of the drug itself, environmental stimuli (e.g., visual cues) previously associated with the drug delivery, and exposure to stressors such as electrical footshock or food deprivation (Shaham et al., 2003). Table 12.1 illustrates different factors triggering relapse in humans and drug-seeking reinstatement in laboratory animals.

THE MODULATING ROLE OF THE ENDOCANNABINOID SYSTEM IN DRUG-INDUCED REWARD

Cannabis is the most commonly abused illegal drug in the world and its main psychoactive ingredient, Δ^9 -tetrahydrocannabinol (THC), produces rewarding effects in humans (Hart et al., 2005), non-human primates (Tanda et al., 2000), and rodents (Braida et al., 2004). Over the last two decades, an endogenous system comprised of cannabinoid type 1 (CB₁) and type 2 (CB₂) receptors, several endogenous ligands (i.e., endocannabinoids) and enzymes responsible for their synthesis and degradation have been discovered and partially characterized (see elsewhere in this book).

Pleasurable behaviors such as sexual activity, eating, nursing, parenting, social interactions, and play activity are conserved strongly in evolution, are essential

for development and survival, and represent pleasant experiences with a high reward value. As such, they can act as robust rewarding stimuli in both humans and animals. Remarkably, rewarding behaviors activate the same brain circuits that mediate the positive reinforcing effects not only of drugs of abuse but also those of other forms of addiction, such as pathological gambling and food addiction. For decades, the dopaminergic and opioid endogenous systems have been considered the most important neurotransmission systems in mediating brain reward processes. Yet, given the involvement of the endocannabinoid system in a variety of physiological functions at both the central and peripheral level, it is not surprising that it takes part in the complex machinery that regulates gratification and perception of pleasure (Fattore et al., 2010a).

Experimental findings strongly suggest a major involvement of the endocannabinoid system in general brain reward functions including drug abuse, as natural and synthetic cannabinoids and endocannabinoids can produce rewarding effects in humans and laboratory animals (Fattore et al., 2001; Hart et al., 2005; Justinova et al., 2005; Seely et al., 2012). Accordingly, cannabinoid CB₁ receptors are present in brain areas involved in reward processes, and their activation by endogenous, plant-derived or synthetic agonists produces rewarding effects *per se* and also increases those of drugs of abuse (Vlachou and Panagis, 2014). Conversely, pharmacological or genetic blockade of cannabinoid CB₁ receptors reduces the rewarding effects of drugs of abuse and prevents their activation of dopaminergic neurotransmission (Cossu et al., 2001; Le Foll and Goldberg, 2005). Brain levels of the two best characterized endocannabinoids, anandamide and 2-arachidonoylglycerol (2-AG), are altered either by rewarding activity (Fattore et al., 2010a) or drugs of abuse (Basavarajappa et al., 2000; Viganò et al., 2003; Cippitelli et al., 2008). However, discrepant data concerning the facilitatory role played by the endocannabinoid system in brain stimulation reward have also been reported (Sañudo-Peña et al., 1997; Cheer et al., 2000). Actually, the influence of the endocannabinoid system on brain reward processes has been hypothesized to depend on the degree of activation of the different brain areas involved and to represent a mechanism for fine-tuning dopaminergic activity (Perra et al., 2005; Pillolla et al., 2007; Melis and Pistis, 2012). Notably, the endocannabinoid system appears to be also involved in the ability of drugs and drug-associated cues to reinstate drug-seeking behavior in animal models of relapse (Fattore et al., 2007a,b). Indeed, CB₁ receptor stimulation may elicit relapse not only to cannabinoid seeking (Spano et al., 2004; Fattore et al., 2010b) but also to heroin (De Vries et al., 2003; Fattore et al., 2003, 2005b) and nicotine seeking (Gamaledin et al., 2012), all effects that are significantly attenuated, when not fully prevented, by pretreatment with the CB₁ receptor antagonist rimonabant. However, corroborating data on the involvement of the cannabinoid system in stress-induced reinstatement are still rather scarce (Vaughn et al., 2012).

ASSOCIATED POLYMORPHISMS IN THE CANNABINOID CB₁ RECEPTOR (*CNR1*) AND FATTY-ACID AMIDE HYDROLASE (*FAAH*) GENES

Many works have repeatedly associated substance dependence and drug-related behaviors with polymorphisms in the *CNR1* and *FAAH* genes (López-Moreno et al., 2012). *CNR1* is located on chromosome 6 in the 6q15 cytogenetic band and encodes a seven-transmembrane domain protein of 472 amino acids, whereas *FAAH* is located on chromosome 1 in the 1p33 cytogenetic band and encodes one transmembrane domain protein of 579 amino acids. A number of mutations described in these genes lead to altered mRNA stability and transcription rate or to a reduction of the activity of the encoded protein. Increasing evidence shows that these functional mutations are related to marijuana, cocaine, alcohol, heroin, and nicotine dependence (Hoenicka et al., 2007; Proudnikov et al., 2010; Bidwell et al., 2013).

One of the most compelling associations is with the C385A single nucleotide polymorphism (SNP), which is found in the human *FAAH* gene (385C to A) and in homozygous form is over-represented in subjects with problem drug use. This SNP, which converts a conserved proline residue in *FAAH* to threonine (P129T), suggests a potential link between functional abnormalities in the endocannabinoid system and drug abuse (Tyndale et al., 2007).

Another SNP in *CNR1*, rs2023239, has been associated with cannabis dependence diagnosis and intermediate phenotypes, including abstinence-induced withdrawal, cue-elicited craving, and parahippocampal activation to cannabis cues (Schacht et al., 2012). The rs2023239 G allele was shown to predict lower volume of bilateral hippocampi among cannabis users relative to controls, suggesting that *CNR1* rs2023239 variation may predispose smaller hippocampal volume after heavy cannabis use (Schacht et al., 2012).

CB₁ RECEPTOR AND FAAH KNOCKOUT MICE IN CANNABINOID ADDICTION RESEARCH

The involvement of the endocannabinoid system in drug addiction was initially studied by a pharmacological approach, i.e., the use of compounds with different affinities for cannabinoid CB₁ or CB₂ receptors or for the enzymes involved in endocannabinoid inactivation. Genetically modified mice with selective mutations in specific components of the endocannabinoid system have been generated, which allowed essential progress in establishing the specific contribution of each component to drug addiction. In particular, mice lacking the CB₁ receptor or the enzyme responsible for catabolism of the endocannabinoid anandamide, i.e., fatty-acid amide hydrolase (*FAAH*), represent critical tools for the identification of the CB₁ receptor and anandamide as potential targets for drug addiction treatment.

As consequences of CB₁ receptor inactivation, CB₁ receptor knockout (CB1-KO) mice do not respond to cannabinoid drugs in the classical tetrad of behavioral test, demonstrating the exclusive role of the CB₁ receptor in mediating analgesia, hypothermia, hypolocomotion, and hypotension (Ledent et al., 1999). Also reinforcement resulted the number of affected, as CB1-KO mice do not self-administer CB₁ receptor agonists nor morphine (Ledent et al., 1999), while they readily self-administer cocaine, amphetamine, and nicotine to the same extent as in wild-type (WT) mice (Cossu et al., 2001). Yet, nicotine was reported to be unable to induce conditioned place preference (CPP) in CB1-KO mice (Castañé et al., 2002). CB₁ receptors were also shown to play an important role in alcohol preference, dependence, and stress-stimulated ethanol drinking, as CB1-KO mice fail to develop a clear alcohol CPP, display significantly lower baseline alcohol consumption compared to WT mice (Houchi et al., 2005; Thanos et al., 2005), and do not display ethanol withdrawal symptoms (Racz et al., 2003). Moreover, CB₁ receptors appear to be critical for the consolidation of cocaine reinforcement and, although to a lesser extent, for its acute rewarding effects (Soria et al., 2005). When compared to WT mice, CB1-KO animals display a significant reduction in the breakpoint level for cocaine self-administration under a progressive-ratio (PR) schedule of reinforcement (Xi et al., 2011). They also show reduced motor and striatal signaling responses to cocaine and amphetamines, and altered cocaine-induced sensitization (Corbillé et al., 2007).

These mutant mice were useful to also appreciate the involvement of the endocannabinoid system in the reinforcing effects of 3,4-methylenedioxymethamphetamine (MDMA). In fact, CB1-KO mice not only are less responsive than WT mice to the stimulating effect of MDMA on locomotor activity, body temperature, and anxiogenic-like responses, but they also fail to self-administer MDMA (Touriño et al., 2008), and do not develop sensitization to the locomotor stimulant effects of amphetamine (Thiemann et al., 2008).

The involvement of the CB₁ receptor in drug-induced reward may be ascribed, at least in part, to the general modulatory role of the endocannabinoid system in hedonic reward perception and processing (Friemel et al., 2014). In support of this, CB2-KO mice (1) do not develop nicotine CPP (Ignatowska-Jankowska et al., 2013), (2) do self-administer significantly less nicotine than WT mice, and (3) do not exhibit somatic signs of nicotine withdrawal (Navarrete et al., 2013). Moreover, deletion of CB₁ receptors decreases operant behavior and motivation to obtain highly palatable isocaloric food in mice, without affecting the operant responses to obtain standard pellets (Guegan et al., 2013). Notably, cannabinoid receptor-dependent changes in drug- and food-oriented appetitive behaviors may reflect more general changes in reward-learning processes, including those whereby the incentive value of the drug or food is assigned to instrumental outcomes or outcome-associated stimuli. Accordingly, CB1-KO mice show significant deficits in outcome-selective instrumental devaluation (Crombag et al., 2009), suggesting a crucial role for the CB₁ receptor in the capability to represent

and use sensory-specific outcome representations to alter appetitive behaviors. In line with this hypothesis, a reduced sensitivity to reward in CB1-KO mice has been described (Sanchis-Segura et al., 2004).

Genetic deletion of *FAAH* is associated with a pattern of intense CB₁ receptor-dependent behavioral responses, including hypomotility, analgesia, catalepsy, and hypothermia (Cravatt et al., 2001). Mice lacking this enzyme display (1) less severe THC precipitated withdrawal responses (Schlosburg et al., 2009), (2) an enhanced expression of nicotine CPP, (3) exacerbated physical somatic signs of spontaneous nicotine withdrawal, and (4) an increased conditioned place aversion (CPA) in a mecamylamine-precipitated model of nicotine withdrawal (Merritt et al., 2008). In line with the notion that the brain endocannabinoid system is critically involved in the neural circuitry regulating alcohol consumption and motivation to consume alcohol, *FAAH*-KO mice also show higher selective preference for, and higher intake of, alcohol than WT littermates (Blednov et al., 2007) as well as a reduction in the severity of handling-induced convulsions following withdrawal from chronic ethanol exposure (Vinod et al., 2008). Importantly, repeated anandamide administration in *FAAH*-KO mice causes smaller CB₁ receptor down-regulation and desensitization and shows lesser dependence liability than repeated THC (Falenski et al., 2010), which suggests that pharmacotherapies targeting endocannabinoid degrading enzymes are less likely to cause development of tolerance and dependence than direct CB₁ receptor stimulation.

When comparing findings from *FAAH*-KO mice with those from CB1-KO mice the increasing endogenous cannabinoid level amplifies, while lack of CB₁ receptor signaling lessens, nicotine reward and withdrawal. In support of this, a decreased cortical *FAAH* expression and elevated endocannabinoid transmission were observed in alcohol-preferring (AA) rats as compared with non-preferring (ANA) rats, which were accompanied by a compensatory down-regulation of CB₁ receptor signaling (Hansson et al., 2007).

THE ENDOCANNABINOID SYSTEM IN HUMAN DRUG ADDICTION

The endocannabinoid system is involved in reward mechanisms that facilitate the hedonic value of natural (Kirkham, 2003; De Luca et al., 2012) and drug (Fattore et al., 2008) rewards. This system participates in the primary rewarding effects of cannabinoids, nicotine, alcohol, and opioids (mostly through the release of endocannabinoids in the ventral tegmental area), and in the common mechanisms underlying drug addiction and relapse to drug-seeking behavior (by also mediating the motivational effects of drug-related environmental stimuli). In turn, many drugs of abuse, including cannabinoids, opioids, alcohol, and nicotine, can alter differently the levels of endocannabinoids in selected brain regions.

In rats chronically exposed to opiates, nicotine, ethanol, or cocaine, different changes in the brain contents of endocannabinoids and CB₁ receptor levels have been described. In particular, alcohol and nicotine increased anandamide levels in the limbic forebrain (González et al., 2004b; Buczynski et al., 2013), while chronic morphine modulated the contents of 2-AG in the rat brain (Viganò et al., 2003).

In humans, recent studies have revealed diverse response by the endocannabinoid system to long-term exposure to several drugs of abuse, and cannabis, ethanol, opioids, nicotine, and cocaine were found to alter the endocannabinoid system regardless of their diverse pharmacological mechanism of action. This section of the chapter will explore clinical evidence of the alterations of the endocannabinoid system induced by the consumption of drugs of abuse.

CANNABIS

According to the last World Drug Report, cannabis is still the most widely used illicit drug worldwide (UNODC, 2013). A new generation of potent synthetic cannabinoids, which can induce serious health risks, have recently been reported in virtually all European countries (EMCDDA, 2013), and are dominating the US market under the brand names of “K2” or “Spice” (Fattore and Fratta, 2011; Seely et al., 2012). Cannabis induces dependence less readily than the majority of other illicit drugs. Yet, about 9% of marijuana users become dependent, and this proportion increases up to 17% among individuals who initiate use at a young age, and up to 25–50% among daily users (SAMHSA, 2012). It is worth noting that 23% of all substance abuse treatment admissions in the United States are for cannabis-related disorders, second only to alcohol-related disorders (SAMHSA, 2012).

Epidemiological studies have demonstrated that chronic non-medical cannabis use can lead to severe adverse health effects such as dependence syndrome, impaired respiratory function, cardiovascular diseases, adverse effects on adolescent psychosocial development and mental health, and residual cognitive impairment (Hall and Degenhardt, 2009, 2014). For these reasons, alterations in endocannabinoid signaling have been extensively examined. It was found that after cannabis use, CB₁ or CB₂ receptor mRNA (Nong et al., 2002; Rotter et al., 2013) are increased in peripheral blood cells, and that 2-AG cerebrospinal serum levels are also increased while anandamide is absent (Morgan et al., 2013). Consistent with this study, among healthy people who had not used cannabis significantly in their lives (<five times) and those who were low-frequency users (<50 times in their lifetime) no differences in anandamide levels were observed (Leweke et al., 2007), suggesting that at least low-level cannabis use does not down-regulate endocannabinoid signaling. Another clinical study showed that in peripheral blood cells serum anandamide and CB₂ receptor mRNA, but not CB₁ receptor mRNA, are increased in high frequency users (who had smoked marijuana ≥20 times but had abstained from cannabis for ≥6 months) compared with no or infrequent (≤five times lifetime) cannabis use (Muhl et al., 2014). However, animals showed a depletion of anandamide following repeated THC administration (Di Marzo et al., 2000).

During frequent cannabis use a series of poorly understood neuroplastic changes occur, which lead to the development of dependence. Cannabis withdrawal in heavy users is commonly followed by increased anxiety, insomnia, loss of appetite, migraine, irritability, and restlessness (Haney, 2005). Tolerance to cannabis and cannabis withdrawal symptoms are believed to be the result of the desensitization of CB₁ receptors by THC. Abstinence in cannabinoid-dependent individuals elicits withdrawal symptoms that promote relapse into drug use, suggesting that pharmacological strategies aimed at alleviating cannabis withdrawal might prevent relapse and reduce dependence.

Cannabinoid replacement therapy and CB₁ receptor antagonism are two potential treatments for cannabis dependence that are under investigation (Huestis et al., 2001; Allsop et al., 2014). However, abuse liability and adverse side effects may limit the value of these approaches. A potential alternative stems from the recognition that (1) frequent cannabis use may cause an adaptive down-regulation of the brain endocannabinoid signaling, and (2) that genetic traits that favor hyperactivity of the endocannabinoid system in humans may decrease susceptibility to cannabis dependence. Altogether, these findings suggest that pharmacological agents that elevate the levels of the endocannabinoids in the brain might alleviate cannabis withdrawal and dependence. One such agent, the FAAH inhibitor URB597, selectively increases anandamide levels in the brain of rodents and primates. Preclinical studies showed that URB597 produces analgesic, anxiolytic-like, and antidepressant-like effects in rodents, which are not accompanied by evident signs of abuse liability, pointing to FAAH inhibitors as a possible therapeutic avenue for the treatment of cannabis withdrawal (Clapper et al., 2009).

ALCOHOL

Starting from the 1970s onward, a growing body of evidence has suggested a link between the neuropsychological effects of cannabis and ethanol consumption. Since then, the role of the brain endocannabinoid system in alcohol abuse and dependence as well as its comorbidity with mood disorders have been widely investigated (Schmidt et al., 2002; Wang et al., 2003; Vinod and Hungund, 2005; Pava and Woodward, 2012). A potential cross-tolerance to ethanol among cannabis users was corroborated by numerous studies investigating the cognitive and psychomotor effects of these two substances in humans (Jones and Stone, 1970; MacAvoy and Marks, 1975). In a clinical study conducted in adolescents with alcohol use disorders, over 70% reported use of cannabis within the past year, with a mean frequency of smoking marijuana ranging between 16 and 20 days per month (Martin et al., 1996). In turn, individuals in treatment for cannabis use disorders increased the frequency of alcohol drinking over a period of 1 year following treatment (Stephens et al., 1994). Accordingly, daily cannabis users significantly increased self-reported ethanol craving and consumption during a 2-week abstinence from marijuana (Peters and Hughes, 2010). Importantly, alcoholism often implies enhanced impulsivity and aggression that may induce suicide

(Koller et al., 2002; Conner et al., 2006; Modesto-Lowe et al., 2006). Levels of endocannabinoids and CB₁ receptors are altered in the prefrontal cortex of depressed and alcoholic suicide victims (Ashton and Moore, 2011; Erdozain et al., 2014), which further strengthens the role of the endocannabinoid signaling in alcoholism and suicide (Vinod and Hungund, 2006).

Besides the high comorbidity between alcohol use disorders and cannabis use disorders, much evidence has also been provided in support to the notion that some of the pharmacological and behavioral effects of alcohol may be mediated through the endocannabinoid signaling system. Recent studies have demonstrated a down-regulation of CB₁ receptor function and signal transduction by chronic alcohol intake, which probably results from the persistent stimulation of CB₁ receptors by the endogenous agonists anandamide and 2-AG, the synthesis of which is increased in the limbic forebrain by chronic alcohol treatment (González et al., 2002). This enhanced formation of endocannabinoids may subsequently influence the release of neurotransmitters.

DBA/2 mice, known to avoid alcohol intake, display reduced brain CB₁ receptor function, in line with other studies where the CB₁ receptor antagonist SR141716A was shown to prevent voluntary alcohol intake in rodents. Similarly, CB₁ receptor activation promoted alcohol craving, suggesting a role for the CB₁ receptor gene in excessive drinking and development of alcoholism (Basavarajappa and Hungund, 2002).

In humans, alcohol dependence has been associated with a down-regulation of CB₁ receptors, while suicide is thought to be related to the up-regulation of these receptors in the ventral striatum (Vinod et al., 2010). Besides chronic alcohol use, other studies indicate that acute consumption of a moderate amount of ethanol affects the levels of the endocannabinoids in some brain regions (Feuerecker et al., 2012). Indeed, both anandamide and 2-AG levels were found significantly reduced in healthy volunteers after consumption of a moderate amount (28 g of ethanol) of red wine (Feuerecker et al., 2012).

Accumulating evidence continues to link certain aspects of the endogenous cannabinoid system with alcohol dependence, negative-reinforcement learning, and the modulation of stress responses. Specific alterations in brain regions related to stress and negative-reinforcement learning have been reported to exist in type 1 alcoholics, which are anxiety prone and characterized by adult onset alcoholism, and in type 2 alcoholics, which are characterized by impulsive, antisocial behavior and teenage-onset alcoholism (Cloninger, 1995).

Endocannabinoids have both anxiogenic and anxiolytic properties (Bortolato et al., 2006; Moreira and Wotjak, 2010), and different brain regions seem to be responsible for the anxiogenic and anxiolytic properties of the endocannabinoids (Rubino et al., 2008). In a recent study, endocannabinoid levels were measured in the amygdala and hippocampus of type 1 and type 2 alcoholic postmortem brains, and compared with analogous samples from a group of non-alcoholic controls (Kärkkäinen et al., 2013). A statistically significant difference between the groups was found in the level of the putative endocannabinoid docosatetraenylethanolamine

(DHEA), an ethanolamide derivative of the omega-3 fatty acid docosahexaenoic acid (DHA), in the amygdala but not in the hippocampus. Another human postmortem brain study reported significant differences only in the levels of anandamide in the NAc and cortical regions (Lehtonen et al., 2010), confirming that in human alcoholism changes in 2-AG brain content may not be as important as suggested by animal studies (González et al., 2002, 2004b; Rubio et al., 2007, 2009; Malinen et al., 2009).

Notably, recent studies have suggested a possible protective effect of cannabidiol (CBD) in cannabis withdrawal syndrome and adverse psychological effects (Niesink and van Laar, 2013). A recent study described the case of a young woman with cannabis withdrawal syndrome treated with CBD for 10 days (Crippa et al., 2013). Daily symptom assessments demonstrated the absence of significant withdrawal, anxiety, and dissociative symptoms during the treatment.

NICOTINE

Nicotine is the primary psychoactive compound of tobacco smoke, and it determines and maintains tobacco dependence. Several lines of evidence suggest a functional interaction between central cholinergic and endocannabinoid systems (Castañé et al., 2005; Maldonado et al., 2006). Epidemiological, pharmacological, and behavioral studies in humans point out a clear link between cannabis and nicotine abuse (Viveros et al., 2006). Accordingly, many animal studies have shown that the pharmacological manipulation of the elements of the endocannabinoid system strongly influence important aspects of nicotine dependence (Scherma et al., 2008).

For example, CB₁ receptor activation and inhibition enhances and attenuates respectively the rewarding effects of nicotine, and nicotine addiction has been associated with altered endocannabinoid modulation of reward processing in the nucleus accumbens (Jansma et al., 2013). However, while FAAH inhibition results in increased brain anandamide levels and therefore increased endocannabinoid tone, distinct effects of FAAH inhibition on nicotine reward have been reported in mice and rats (Muldoon et al., 2013). A major PPAR- α receptor component appears to mediate the anti-nicotine reward effects of FAAH inhibitors in rats (Mascia et al., 2011).

In rats, nicotine and endocannabinoids seem to enhance the reinforcing effects of both systems (Viveros et al., 2006), and changes in endogenous cannabinoid levels have been observed in different brain regions chronically exposed to nicotine, such as the brainstem, hippocampus, cerebral cortex, and striatum (González et al., 2002). In mice, the administration of nicotine facilitates the pharmacological responses, tolerance, and physical dependence induced by THC (Valjent et al., 2002), while CB₁ receptor agonists decrease nicotine somatic withdrawal signs in mice (Balerio et al., 2004). Furthermore, no differences were reported on the effect of FAAH inhibition in nicotine somatic withdrawal signs in mice and rats. However, differences in affective signs of withdrawal, such as anxiety and aversion, after FAAH blockade seem to be emerging. More generally, the effects of FAAH inhibitors on nicotinic behavioral responses in animals may be influenced by procedural differences, species differences, level of nicotine exposure, and degree of FAAH inhibition. These factors

are likely to play an important role in understanding the physiological function of FAAH in nicotine reward and withdrawal. Pharmacological inhibition of FAAH blocks nicotine self-administration and prevent nicotine-induced reinstatement in rats, suggesting that FAAH is a promising molecular target for tobacco dependence (Scherma et al., 2008; Muldoon et al., 2013).

In humans, CB₁ receptor antagonists were evaluated as anti-smoking therapy, with some promising results (Cohen et al., 2005). For example, when used in combination with a nicotine patch, rimonabant was well tolerated and increased smoking cessation rates over rimonabant alone, with little weight gain after cessation in either group, even among weight-concerned smokers (Rigotti et al., 2009). However, evidence for using these antagonists in maintaining smoking abstinence remained inconclusive, mainly because of the adverse events observed, including nausea, upper respiratory tract infections and, more worryingly, depression and suicidal thoughts in people taking CB₁ receptor antagonists for weight control (Cahill and Ussher, 2007).

COCAINE

Cocaine use represents an important worldwide health problem due to the large number of physical, legal, social, cognitive, psychological, and psychiatric associated problems and comorbidity (O'Brien and Anthony, 2005; Karila et al., 2012). Cannabis is one of the most widely used illicit substances among users of psychostimulants such as cocaine and amphetamines. Interestingly, recent evidence points toward the involvement of the endocannabinoid system in the neurobiological processes related to stimulant addiction (Olière et al., 2013).

In cocaine addicts, a link between exposure to cocaine and dysregulated brain endocannabinoid signaling has been reported. Indeed, CB₁ but not CB₂ receptor protein and G-protein coupled receptor regulatory kinases (GRK) were reported to be significantly reduced in the prefrontal cortex with a simultaneous receptor redistribution and/or internalization, i.e., they were decreased in membranes and increased in cytosol (Álvarez-Bartolomé and García-Sevilla, 2013). Thus, in cocaine-addicted subjects the reductions of CB₁ receptors and GRK determines a receptor desensitization, a notion further strengthened by the finding that chronic cocaine reduces CB₁ receptor protein also in the cerebral cortex of mice and rats (Álvarez-Bartolomé and García-Sevilla, 2013).

The evaluation of plasma-free endocannabinoids and circulating endocannabinoid-related lipids in abstinent cocaine addicts can be useful for the identification of biomarkers for cocaine addiction. Pavón and colleagues (2013) found that plasma acyl derivatives are altered in abstinent cocaine-addicted subjects. In the same subjects, free N-acyl-ethanolamines were found to be increased while 2-acyl-glycerols were decreased. Intriguingly, the monounsaturated N-oleoyl-ethanolamine and N-palmitoleoyl-ethanolamine were significantly elevated in cocaine addicts diagnosed with mood and anxiety disorders when compared with non-comorbid cocaine-addicted subjects (Pavón et al., 2013).

Human studies also show that cannabinoid receptor *CNR1* gene polymorphisms might be related to cocaine addiction (López-Moreno et al., 2012). Two single nucleotide polymorphisms (SNPs) in *CNR1*, i.e., rs6454674 and rs806368, have been associated with cocaine dependence (Zuo et al., 2009; Clarke et al., 2013). Notably, also the (AAT)_n triplet repeat polymorphism in the close proximity of the *CNR1* gene has been associated with predisposition to cocaine dependence (Comings et al., 1997; Ballon et al., 2006), although non-significant association data have also been reported (Covault et al., 2001; Herman et al., 2006).

From a clinical perspective, much evidence suggests that cannabinoid ligands and endocannabinoid-level enhancers may be therapeutically useful against cocaine dependence (Tanda, 2007). For example, crack cocaine abusers reported smoking cannabis in order to get relief from cocaine-withdrawal symptoms (Labigalini et al., 1999). However, caution must be used before drawing any conclusions, especially in light of the potential negative clinical implications of cannabis use in cocaine-abstinent subjects (Aharonovich et al., 2005).

AMPHETAMINES

Amphetamine and its derivatives, i.e., methamphetamine (METH) and N-methyl-3,4-methylenedioxymethamphetamine (MDMA), are stimulant drugs that increase feelings of arousal and euphoria, decrease fatigue, and enhance attention and feelings of alertness. Co-abuse of cannabis and amphetamines is very common among polydrug users in Western societies. Yet, there are only a few and somewhat contradictory studies examining the effects of smoking marijuana and concomitant use of amphetamines on cognitive parameters (e.g., impulsivity, memory, executive functions). Similarly, animal studies examining long-term effects of amphetamine and cannabinoid co-administration and underlying neurobiology are quite limited.

Ecstasy users are typically polydrug abusers, and marijuana is commonly smoked among regular ecstasy users (Sindicich et al., 2009). In ecstasy users, regular cannabis and/or METH use confers additional risk of poor mental health and high levels of psychological distress, particularly with regard to paranoia, over regular ecstasy use alone (Scott et al., 2012). Regular cannabis and METH use was also associated with earlier initiation to use ecstasy (Scott et al., 2012). Marijuana is frequently smoked also by METH abusers (Simon et al., 2002; Gonzalez et al., 2004a); yet, whether it is smoked for the purpose of enhancing METH subjective effects or attenuating its adverse effects (self-medication purposes) is still unknown. Regular cannabis abuse in METH-dependent individuals has been associated with frontal, temporal, and striatal metabolic abnormalities compared to subjects that use METH only (Voytek et al., 2005). However, cannabis use was not found to exacerbate the neurotoxic effect of METH (Gonzalez et al., 2004a). Actually, a recent animal study indicates that METH-induced neurotoxicity in the caudate putamen and PFC can be attenuated by pre- and post-treatment with THC through inhibition of nNOS overexpression and astrocyte activation (Castelli et al., 2014).

Adolescent methamphetamine and cannabis abusers were found to have increased regional striatal volume and show stronger novelty-seeking behavior as compared to healthy controls (Churchwell et al., 2012). In the same study, degree of methamphetamine exposure was found to be positively correlated with regional striatal volume and novelty seeking in methamphetamine and cannabis users. Another clinical study evaluated whether the subjective responses of healthy volunteers to controlled administration of amphetamine are influenced by polymorphisms in the *FAAH* gene (Dlugos et al., 2010). Genotypes at rs3766246 and rs2295633 were found to be significantly associated with increased ratings of “arousal” in response to amphetamines, suggesting that the endocannabinoid system influences variation in subjective response to amphetamines. Yet, other studies found no significant association between methamphetamine dependence and the synonymous polymorphism of the *FAAH* gene, Pro129Thr (Morita et al., 2005). Regrettably, no studies have been conducted so far to quantify the brain level of endocannabinoids in amphetamine users, or to evaluate the density and functionality of CB₁ receptor in the brains of METH or MDMA users.

OPIOIDS

Cannabinoids and opioids share several pharmacological properties, including antinociception, hypothermia, sedation, and hypotension. Currently, a great body of evidence supports similarities of actions and interactions between central opioid and cannabinoid systems with reference to drug dependence and abuse (Fattore et al., 2004, 2005a; Spano et al., 2010; Scavone et al., 2013), including relapse phenomena (Fattore et al., 2003, 2005b, 2011; Spano et al., 2004). The first evidence for such an interaction in dependence-related phenomena, e.g., reward and withdrawal, dates to the middle 1970s, when it was reported that administration of THC attenuates naloxone-induced abstinence in morphine-dependent rats (Hine et al., 1975) and mice (Bhargava, 1976), whereas rats chronically treated with cannabinoids show opioid-like withdrawal signs following acute naloxone administration (Kaymakcalan et al., 1977).

Earlier clinical studies indicated that oral or smoked THC consistently induces changes in mood, usually euphoria, while higher doses are psychotomimetic producing, for example, marked distortion in visual and auditory perception (Isbell et al., 1967). When the effects of the opioid antagonist naltrexone on subjective responses to THC were examined in marijuana users, it was found that THC increases heart rate and self-reported drug effects (i.e., euphoria, marijuana-like effects) and decreases psychomotor performance, while naltrexone increases heart rate and decreases self-reported measures of vigor and hunger but does not alter any of the effects of THC (Wachtel and de Wit, 2000). Notably, more recent studies show that in morphine abusers cannabinoid receptors are up-regulated in the periphery blood mononuclear cells and that the expression of interleukin 4 (IL-4) mRNA in these cells is higher than that in healthy people (Zhang et al., 2012),

indicating that exposure to morphine can affect the expression of cannabinoid receptors and immune functions.

Cannabis is the most prevalent type of illicit drug used among heroin addicts. This implies at least two important issues to address, i.e., to determine the potential consequences associated with cannabis use in methadone-treated patients, and to evaluate the influence of concurrent marijuana use on treatment outcomes in opioid users on maintenance treatment. Concerning the first point, it was found that marijuana use does not impact on risk behavior for contracting acquired immunodeficiency syndrome (AIDS) (Saxon et al., 1990), but it can induce abnormalities in resting respiratory functions (Teichtahl et al., 2004). Moreover, when comparing outcomes of marijuana users and non-users enrolled in a methadone-maintenance program, no relation between cannabis consumption and the use of opioids was found (Saxon et al., 1990). Importantly, chronic marijuana smoking did not affect the normalization of the hypothalamic-pituitary-adrenal (HPA) axis induced by methadone in heroin addicts (Nava et al., 2007), which further supports the notion that marijuana use is not a risk factor for treatment outcome in methadone-maintenance treatment (Seivewright, 2003; Weizman et al., 2004).

Concerning the influence of opioid drugs on the brain endocannabinoid levels, it has been established that chronic morphine modulates the contents of 2-AG in the rat brain (Viganò et al., 2003), but no specific studies have been conducted so far on human opioid addicts. Similarly, it has been demonstrated that voluntary chronic intake of opioids or cannabinoids by rats induces reciprocal but differential regulation of mu-opioid and CB₁ receptor density and activity in brain structures underlying drug-taking and drug-seeking behavior (Fattore et al., 2007c), but whether or not similar effects also occur in the human brain remains to be investigated.

CONCLUSIONS

The motivational and addictive properties of drugs of abuse are mediated through drug-induced changes in neurotransmitter and neuromodulatory signaling within the brain. Endocannabinoids mediate retrograde signaling in neuronal tissues and are involved in the regulation of synaptic transmission to suppress neurotransmitter release by the presynaptic cannabinoid receptors. This powerful modulatory action on synaptic transmission has significant functional implications and interactions with the effects of abused substances. Although there are differences in the central effects caused by various classes of abused drugs, accumulating evidence indicates a central role for the ubiquitous endocannabinoid physiological control system in the regulation of the rewarding effects of these substances. Cannabinoids and endocannabinoids appear to boost the rewarding effects of addictive drugs, including alcohol, nicotine, cocaine, amphetamines, and opiates, suggesting that the endocannabinoid system may represent an important target for the treatment of addictive disorders.

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Cannabinoids and appetite (dys)regulation

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INTRODUCTION

Historically, marijuana intoxication in people has long been associated with reports of increased appetite and ravenous eating; an effect commonly referred to as “the munchies.” Anecdotal data supporting this phenomenon have been in existence for many years; indeed, reference to appetite stimulant properties can be found as far back as AD 300 (Chopra and Chopra, 1939), while in Western society, one of the first documented pieces of evidence for use of marijuana as an appetite stimulant was described by Dr. M’Meens in 1860. M’Meens reviewed the symptoms and conditions for which Indian hemp had been found useful to the Ohio State Medical Society, and, in relation to its effects on appetite, stated that “. . . digestion is not disturbed; the appetite rather increased. . .” (Grinspoon and Bakalar, 1997). In the following sections we will chart the history of the use of cannabis, cannabis extracts, and individual (endo)cannabinoids as modulators of feeding behavior, a phenomenon that is intimately associated with activation of CB₁ receptors within hypothalamic feeding circuits (Pagotto et al., 2006; Di Marzo et al., 2009). We will then reflect on the role of the endocannabinoid system mediating clinical eating disorders (EDs), obesity, and metabolic disorders in humans.

EARLY CLINICAL AND PRECLINICAL EVIDENCE FOR CANNABIS AS A MODULATOR OF FEEDING

By the 1950s, the potential therapeutic use of cannabis as an appetite stimulant was beginning to be explored. Parker and Wrigley (1950) administered pyrahexyl, a derivative of Δ^9 -THC (Δ^9 -tetrahydrocannabinol), to a group of psychotic patients. The drug did not produce any change in the psychiatric condition of their patients; however, following pyrahexyl treatment “some of the patients who had previously refused food began to demand it.” Similar improvements in appetite loss were also reported

by Thompson and Proctor (1953) in their treatment of alcoholics, and by Pond (1948) in his treatment of depressives. Hence, the general conclusion from early clinical literature was that cannabis and its homologs stimulated a desire for food in humans.

It was only in the 1980s that two fairly comprehensive, well-controlled, repeated-dose experiments were completed (Foltin et al., 1986, 1988). In their 1986 study, volunteer subjects were tested in a residential laboratory for periods of up to 25 days with each test day comprising three phases: a private work period, a performance task, and a period of social access. Marijuana cigarettes containing either 0 or 1.84% (w/w) Δ^9 -THC were supplied by NIDA and were smoked prior to private work periods and during the social access phases. Analysis of intake revealed little or no difference in food consumption during private work periods. However, marijuana markedly increased intake during social access with control subjects consuming ≈ 1000 kcal, while marijuana smokers significantly increased consumption to ≈ 2500 kcal. Detailed analysis of the food consumed showed that consumption of snack items was consistently and significantly increased by marijuana, increasing by 63% under drug-treatment compared to placebo.

In a follow-up to this study, Foltin et al. (1988) demonstrated that the increased snack food intake following marijuana treatment could be primarily ascribed to an increase in the intake of sweet solid items such as candy bars, biscuits, and chocolate, rather than sweet fluid or savoury solid items. Further corroboration of these effects was found by Mattes et al. (1994), who showed that increases in caloric intake following acute cannabinoid treatment were derived principally from an increase in snack consumption, rather than any self-selected meal. Mattes also examined the influence of satiety status on Δ^9 -THC effects (comparing subjects who were fasted overnight with those provided with a standard breakfast), but found no obvious interaction.

Despite the growing evidence that cannabis could stimulate appetite in humans, these data were not matched by studies from the literature on animals where the most common findings were either that of no effect on appetite (Sjoden et al., 1973; Graceffo and Robinson, 1998) or that of intake suppression (Jarbe and Henriksson, 1973; Sofia and Barry, 1974; Sofia and Knobloch, 1976). The failure to observe hyperphagia in these animal models can largely be attributed to the use of single doses that were either too low to exert any effect or too high resulting in a number of behavioral side effects (e.g., sedation, lack of motor coordination), which were incompatible with the normal expression of feeding. Only two investigations using animal models during this period demonstrated hyperphagic actions of Δ^9 -THC. In the first study, Glick and Milloy (1972) demonstrated that 1.0 mg/kg Δ^9 -THC administered i.p. to 24-hour food-deprived rats produced a modest (less than 2 g over 2 hours) increase in food intake. While Brown and colleagues, using more naturalistic methods such as nocturnal testing and non-deprived rats, showed significant, short-term increases in chow intake again the size of those increases were actually very small with animals consuming < 1 g over 1 hour (Brown et al., 1977).

The identification of the CB₁ receptor and consequential development of new pharmacological probes for the emerging endocannabinoid system in the late

1980s and early 1990s led to a resurgence of interest in the potential clinical application of cannabinoids, and a re-examination of the ability of cannabinoids to manipulate facets of feeding behavior.

ALTERATIONS TO FEEDING BEHAVIOR INDUCED BY Δ^9 -THC

The first comprehensive time—course and dose—response analysis of Δ^9 -THC effects on feeding in a rat model was performed in our laboratory in the late 1990s (Williams et al., 1998). Adapting the protocol of Brown et al. (1977), we used a pre-feed paradigm at lights off to ensure baseline intakes of chow would be low, thus maximizing our ability to detect any Δ^9 -THC-induced increases in food intake. Following 2-hour access to the pre-feed, a range of Δ^9 -THC doses (0.0–2.0 mg/kg) were given orally in a sesame oil vehicle, and after an hour to allow for drug assimilation, animals were given unrestricted access to their normal laboratory chow. We detected significant overeating at doses ≥ 0.5 mg/kg Δ^9 -THC with a greater than four-fold increase in consumption during the first hour produced by a 1.0 mg/kg Δ^9 -THC dose. Although we detected significant hyperphagia at the highest dose of 2.0 mg/kg Δ^9 -THC, this was accompanied by a number of non-specific behavioral effects such as impaired motor coordination.

Subsequent experiments from our laboratory further described these feeding-related actions by determining that the hyperphagia effect of Δ^9 -THC was specifically mediated by central CB₁ receptors (Williams and Kirkham, 2002a). Using an identical experimental protocol to our initial experiment, we co-administered Δ^9 -THC with either SR141716A, which at the time was described as a selective CB₁ receptor antagonist although is now recognized as a CB₁ receptor inverse agonist, or SR144528, a selective antagonist at the CB₂ receptor. Here, the feeding stimulation induced by Δ^9 -THC was significantly attenuated by SR141716A, but not SR144258 indicating that Δ^9 -THC's effects were mediated specifically by CB₁ receptors.

Having optimized a paradigm to reliably demonstrate hyperphagic effects of cannabinoids, our laboratory then performed a microstructural examination of the precise alterations to feeding that follow Δ^9 -THC administration (Williams and Kirkham, 2002b). This time using an open field arena rather than home cages, we administered Δ^9 -THC following 2-hour access to a pre-feed and observed the behavior of our rats. As expected, the vehicle-treated animals exhibited very little interest in food over the course of a 45-minute test with most animals failing to engage in any periods of feeding at all. In comparison, animals treated with 1.0 mg/kg Δ^9 -THC exhibited a marked reduction in latency to begin feeding and feeding became the predominant behavior during the test. These behavioral effects implied that the stimulation of feeding induced by Δ^9 -THC may be linked to the appetitive phase of feeding, perhaps orienting an animal toward food and increasing the salience or incentive (reward) value of food stimuli.

Further evidence for the role of Δ^9 -THC influencing reward was seen by Koch (2001) who investigated the effects of manipulating the palatability of the test food following Δ^9 -THC administration. Here, Koch (2001) showed that administration

of a range of doses of Δ^9 -THC (0.0, 0.5, 1.0, and 2.5 mg/kg; i.p.) to rats, followed by access to either standard laboratory chow, high fat (HF) or high fat plus sugar (HFS) diets, produced significant degrees of overeating comparable to the changes seen in our previous study (Williams et al., 1998). One hour post-drug, both 0.5 and 1.0 mg/kg Δ^9 -THC significantly increased food intake versus vehicle-treated animals within the HF diet group only, although non-significant increases in food intake were seen at these doses for both of the other food groups. During the second hour of testing, the 0.5 and 1.0 mg/kg Δ^9 -THC doses significantly increased intake versus vehicle treatment in all three food groups, with the 1.0 mg/kg dose caused the largest increases. Importantly, overall food intake across all doses was significantly greater in both the HF and HFS groups than the degree of overeating seen in the group fed the standard diet.

Similarly, work from our laboratory studied the effects of Δ^9 -THC on the microstructural characteristics of licking behavior in rats drinking a palatable 10% sucrose solution (Higgs et al., 2003). In this paradigm changes to the rate of licking or duration of bouts of licking provide information about orosensory influences on ingestion. Δ^9 -THC treatment significantly increased total number of licks, and inspection of cumulative lick curves revealed a pattern that is usually associated with an increase in palatability of the solution. Further evidence of a role for Δ^9 -THC in mediating palatability can also be seen due to the increase in the duration of licking bouts, while total number of bouts was unaffected.

The concept of cannabinoids influencing reward processes is not new, indeed earlier we described the data showing that marijuana administration promoted the consumption of palatable, sweet, snack-type foods (Foltin et al., 1988). An elegant experiment by Trojnar and Wise (1991) provided further evidence that cannabinoids could directly influence activity in brain reward systems. Here, they examined the actions of Δ^9 -THC on feeding evoked by electrical stimulation of the lateral hypothalamus showing that 0.4 mg/kg Δ^9 -THC treatment produced a facilitatory effect on feeding consistent with activation of brain reward pathways. Interestingly, 1 and 2 mg/kg naloxone (a general opioid receptor antagonist) reduced this facilitatory effect suggesting that the actions of Δ^9 -THC may be mediated by endogenous opioids.

Since the Trojnar and Wise (1991) study, evidence has accumulated to support overlapping opioid and cannabinoid mechanisms in relation to a range of physiological processes, including reward and feeding behavior. Using a “lick-based progressive ratio paradigm” in which an ever-increasing number of licks had to occur at a tube in order to receive a fixed unit of palatable beverage, Gallate and McGregor (1999) found that the facilitatory effects of 10, 30, and 50 mg/kg CP55,940, a cannabinoid CB₁ receptor-specific agonist, on the motivation to consume palatable solutions could be reversed by treatment with SR141716A and the opioid antagonist naloxone. Similarly, using our well-established pre-satiation paradigm, we have shown that Δ^9 -THC-induced hyperphagia can be attenuated by subnorectic doses of naloxone (0.1, 0.5, 1.0, and 5.0 mg/kg) providing further support for a functional relationship between cannabinoid and opioid systems in relation to appetite regulation.

The evidence we have reviewed so far has shown that animals work harder to obtain food after Δ^9 -THC treatment, and eat earlier and more frequently when food is freely available. Thus, Δ^9 -THC seems to actively provoke feeding, rather than merely prolong eating behavior that has been initiated through other mechanisms. Finally, we have also seen evidence that cannabinoids modulate the motivation to consume food via direct interaction with the endogenous cannabinoid system, but also modulate feeding indirectly by influencing activity within endogenous opioid circuits. It is these data that have led clinicians to assess Δ^9 -THC treatments for their potential efficacy in the treatment of clinical syndromes affecting food consumption. Indeed, commercial preparations of Δ^9 -THC, manufactured under the trade name Marinol (dronabinol; 2.5 or 5.0 mg THC in a sesame seed oil vehicle) have been licensed for clinical use.

ALTERATIONS TO FEEDING INDUCED BY CANNABIS EXTRACTS OR INDIVIDUAL PHYTOCANNABINOIDS

Despite the wealth of evidence showing a role for Δ^9 -THC in stimulating feeding behavior, few studies have investigated the contribution of non- Δ^9 -THC cannabinoids to this effect. Recently, we commenced a program of studies to investigate the appetite-stimulating actions of standardized cannabis extracts (botanical drug substances) and isolated phytocannabinoids (pCBs).

Using our standardized pre-feed paradigm, which we described in detail earlier, we initially compared the effects of purified Δ^9 -THC, synthetic Δ^9 -THC, and Δ^9 -THC standardized botanical drug substance (BDS), which contained a typical array of non- Δ^9 -THC pCBs found within the plant (Farrimond et al., 2010a). Importantly, all treatments were matched for Δ^9 -THC content across a range of doses known to induce hyperphagia (0.00, 0.34, 0.67, 1.34, and 2.68 mg/kg). As expected, both the synthetic and purified Δ^9 -THC doses induced significant hyperphagia that could be characterized by significant reductions in the latency to begin feeding and significantly increased duration of the first meal, mirroring the alterations to feeding microstructure that we described previously. Intriguingly, administration with Δ^9 -THC BDS produced a significantly reduced hyperphagia, which suggested that the combination of pCBs (and, potentially, non-cannabinoid components) in the Δ^9 -THC BDS attenuated the hyperphagic effects of Δ^9 -THC.

In a follow-up study we investigated the action of two different cannabis extracts, a standardized BDS containing 67% Δ^9 -THC and a BDS that contained 0% Δ^9 -THC (Farrimond et al., 2010b). Importantly, all remaining pCBs in the extracts were kept constant (cannabidiol [CBD]: 0.3%; cannabigerol [CBG]: 1.7%; cannabichromene [CBC]: 1.6%; tetrahydrocannabivarin [THCV]: 0.9%; tetrahydrocannabinolic acid [THCA]: 0.3%; cannabinol [CBN]: 1.5%). Both 0% Δ^9 -THC BDS and 67% Δ^9 -THC BDS induced dose-dependent increases in food intake in the hour after food was returned to the animals. Analysis of the feeding microstructure following 67% Δ^9 -THC BDS showed that this hyperphagia could be

accounted for by significant reductions in the latency to the onset of feeding and an increase in the duration of the first meal, effects that we would have predicted from previous studies (Williams et al., 1998; Farrimond et al., 2010a). Interestingly, while 0% Δ^9 -THC BDS did induce a highly significant reduction in the latency to onset of feeding, there was no increase in the duration of the first meal. These data suggest a role for non- Δ^9 -THC pCBs in the appetitive actions of feeding only.

Subsequent experiments from our laboratory then focused on investigating which of the pCBs found within these extracts could be capable of influencing appetite and feeding behavior, and, in particular, were responsible for the effects on the appetitive (but not the consummatory) aspects of feeding behavior. A review of the literature shows a relative paucity of data investigating non- Δ^9 -THC pCBs on feeding, with the majority of studies undertaken being either unreplicated or (where replications had been undertaken) contradictory. A summary of these studies and their effects is shown in Table 13.1.

In one of the earliest studies to investigate the actions of non- Δ^9 -THC pCBs, Sofia and Knobloch (1976) examined the effects of a single dose of CBN or CBD on the consumption of normal laboratory chow and sucrose solutions. Here, rather than utilize a naturalistic feeding paradigm, animals were given only 6 hours access per day to chow, water, and 5% or 20% sucrose solutions. Once animals were trained in this feeding regimen, they were administered 50 mg/kg CBN or CBD and intakes of food and sucrose were measured. Both CBN and CBD produced highly significant reductions in food intake, with intakes failing to return to baseline levels until 4–5 days post-drug administration. Similar reductions in sucrose intake were evident following drug administration, with intakes returning to baseline levels by days 3–4 post-administration. Sofia and Knobloch interpreted these findings as suggesting that CBN and CBD produced a preference for sweet calories because intake returned to baseline levels sooner than with standard chow. However, it should be noted that sucrose intake was not increased following treatment with CBN or CBD; rather, the naturally more palatable sucrose solution was more resistant to the appetite-suppressive actions of these drugs.

A number of other laboratories have examined the effects of CBD on feeding. Wiley et al. (2005) showed that a range of CBD doses between 3 and 100 mg/kg failed to significantly alter food intake in mice. Similarly, Scopinho et al. (2011) demonstrated that CBD (1–20 mg/kg) failed to alter feeding in both pre-satiated and fasted rats. Conversely, in our hands, using our standard pre-satiation regimen, CBD treatment (0.04–4.4 mg/kg) did not induce any significant change in food intake compared to our vehicle-treated animals in any single hour of our 4-hour test. However, a significant dose-dependent reduction in cumulative 4-hour food intake was evident (Farrimond et al., 2012). Detailed analysis of the meal patterns produced by CBD administration showed that although CBD administration significantly reduced the total amount of food consumed, it had no effect on latency to begin feeding, or the size or duration of the first meal. Finally, Ignatowska-Jankowska and colleagues investigated the effects of CBD administration on body weight gain in rats (Ignatowska-Jankowska et al., 2011).

Table 13.1 Summary of the Effects of Non- Δ^9 -THC pCBs on Feeding Behavior

pCB	Dose (mg/kg)	Intraperitoneal (i.p.) or per os (p.o.) Route of Drug Administration	Experimental Conditions	Food Offered	Effects on Feeding	Reference
CBN	50	i.p.	6 hr/day access to food, water, and sucrose	Laboratory chow, 5% and 20% sucrose	↓ chow intake ↓ sucrose intake	Sofia and Knobloch, 1976
	0.26, 2.6, and 26.0	p.o.	Pre-satiated	Laboratory chow	↑ intake	Farrimond et al., 2012
CBD	50	i.p.	6 hr/day access to food, water, and sucrose	Laboratory chow, 5% and 20% sucrose	↓ chow intake ↓ sucrose intake	Sofia and Knobloch, 1976
	3, 10, 30, and 100	i.p.	24-hour food deprived before drug	Laboratory chow	No effects	Wiley et al., 2005
	1, 10, and 20	i.p.	Free-feeding and 18-hr food deprived	Laboratory chow	No effects	Scopinho et al., 2011
	0.04, 0.44, and 4.40	p.o.	Pre-satiated	Laboratory chow	↓ intake	Farrimond et al., 2012
	3, 10, and 30	i.p.	Free-feeding	Laboratory chow	↓ intake	Riedel et al., 2009
Δ^9 -THCV						
Δ^9 -THCV BDS	0.48, 0.96, 1.44, 3, 10, and 30	i.p.	Free-feeding	Laboratory chow	No effects	Riedel et al., 2009
CBG	0.18, 1.76, and 17.6	p.o.	Pre-satiated	Laboratory chow	No effects	Farrimond et al., 2012

Although no measures of food intake were taken in this experiment, 14-day administration of 2.5 and 5 mg/kg/day CBD produced a dose-dependent decrease in body weight, which was blocked by co-administration with the selective cannabinoid CB₂ receptor agonist AM630 suggesting that CB₂ receptor mechanisms may be critical to the action of CBD on body weight.

Moving away from CBD, [Riedel et al. \(2009\)](#) investigated the feeding effects of the competitive CB₁ receptor antagonist Δ^9 -THCV (3, 10, and 30 mg/kg) and a Δ^9 -THCV BDS (containing between 0.1 and 0.3 mg/kg Δ^9 -THC). In the 12 hours following drug treatment, all doses of Δ^9 -THCV reduced food intake while in contrast Δ^9 -THCV BDS failed to significantly affect food consumption. No microstructural analysis of feeding was undertaken in this study; however, it is worth mentioning that this study was completed during the light phase of the rats' day during which time little feeding would be expected to occur.

In a final paper from our laboratory, utilizing our standard pre-feed paradigm, we assessed the effects of the individual phytocannabinoids CBG and CBN on feeding behavior ([Farrimond et al., 2012](#)). CBG (0.18–18 mg/kg) administration induced no significant alterations in feeding during any hour of the test. On the other hand, CBN (0.26–26 mg/kg) significantly increased food intake during hour 1 and total chow consumed during the test. Microstructural analysis of feeding showed that the observed increase in hour 1 food consumption was due to a significant dose-dependent increase in the size of the first meal and a reduction in the latency to the onset of the first meal, which shifted feeding into the first hour of the test. Together, these data indicate that CBN, like Δ^9 -THC, produces a significant effect on appetitive aspects of feeding behavior. In a final manipulation, we co-administered 26 mg/kg CBN with 1 mg/kg SR141716A. Co-administration of SR141716A with CBN blocked the previously observed CBN-mediated increases in hour 1 intake and total food intake during the 4 hours of the test. Similarly, SR141716A blocked the CBN-induced increases in meal 1 size, meal 1 duration and the latency to feed supporting a CB₁ receptor-mediated mechanism for CBN.

Thus, it is clear that Δ^9 -THC is not the only plant-derived cannabinoid that can significantly stimulate appetite. This is of particular interest because initial characterization of these other pCBs suggests that they exert their pharmacological effects through a range of mechanisms not limited to binding at cannabinoid receptors, e.g., via ion channels ([Lozovaya et al., 2009](#)). As these mechanisms and their behavioral effects have yet to be fully investigated, it is tempting to suggest that these compounds may provide at least the basis for novel therapies for the treatment of feeding disorders.

THE ROLE OF ENDOGENOUS CANNABINOIDS IN FEEDING BEHAVIOR

So far in this chapter, we have documented wide-ranging evidence showing that plant-derived cannabinoids can influence feeding behavior. However, endogenous

cannabinoids have also been investigated for their ability to modulate appetite and current results suggest significant involvement of the endocannabinoid system in the normal regulation of feeding behavior.

Using our optimized pre-satiation paradigm that reliably demonstrated the hyperphagic effects of some phytocannabinoids, we went on to examine the potential for endogenous cannabinoids to exert similar actions (Williams and Kirkham, 1999). Here, pre-satiated male rats received subcutaneous injections of anandamide (AEA; 0.5, 1.0, 5.0, 10.0 mg/kg) before 3-h nocturnal food intake tests. All doses of AEA induced significant overeating, with 1.0 mg/kg being most potent. However, it is noteworthy that the magnitude of AEA-induced hyperphagia was far less dramatic than the effects we had seen previously following Δ^9 -THC administration. In a second experiment, we co-administered our most significant hyperphagic dose of AEA (1 mg/kg) with a range of SR141716A doses (0.1, 0.5, and 1.0 mg/kg; Williams and Kirkham, 1999). Here, the AEA-induced hyperphagia was dose-dependently attenuated by SR141716A treatment indicating a CB₁ receptor-mediated effect.

Importantly, AEA's hyperphagic effect has been replicated by Hao et al. (2000), who showed that daily administration for 6 days of low doses of AEA (0.001 mg/kg, i.p.) in schedule-fed mice produced significant overeating on each successive treatment day. Moreover, Gomez et al. (2002) showed that AEA administration (0.1, 1, and 10 mg/kg; i.p.) produced significant hyperphagia in partially satiated rats, but not in rats that had been food deprived. Finally, a novel study by Jamshidi and Taylor (2001) used direct administration of AEA into the ventromedial hypothalamus, a brain area intimately linked with the control of daily food intake (Torelli et al., 2000). Under these circumstances, pre-satiated rats received an infusion of AEA (25, 50, and 150 ng $0.5 \mu\text{l}^{-1}$ infused over 10 s) before being given access to food for 3 hours. Administration of 50 ng AEA induced significant hyperphagia, an effect that could be blocked by pre-treatment with SR141716A (30 μg $0.5 \mu\text{l}^{-1}$).

Our next step was to ascertain the precise alterations in meal taking that resulted from AEA administration. Using pre-satiated rats, we assessed moment-to-moment changes in feeding behavior in the animal's home cage. Similarly to the changes in meal taking seen following Δ^9 -THC administration, the principal action of AEA was to markedly reduce the latency to the first meal consistent with an action to increase incentive motivation (Williams and Kirkham, 2002b). Here, very little eating occurred following vehicle treatment, with only two (out of six) animals engaging in any feeding behavior during the entire test. By contrast, AEA-treated animals engaged in eating within 15 minutes of being given access to food. In addition, we saw a significant increase in the number of eating bouts and an increase in total duration of time spent eating during the test.

AEA is not the only endocannabinoid that can modulate feeding. Kirkham et al. (2002) demonstrated that infusions of 2-arachidonoylglycerol (2-AG; 0.125, 0.5, 2.0 μg per rat) directly into the shell region of the nucleus accumbens, an area of limbic forebrain area strongly linked to eating motivation, can produce a

short-term stimulatory action on feeding. Here, 2-AG significantly increased the amount eaten during the first hour after the infusion, with particularly marked actions seen following 0.5 and 2.0 μg . Again this hyperphagic action could be reliably attenuated by pre-treatment with SR141716A (0.5 mg/kg, s.c.).

The demonstration that exogenously administered endocannabinoids (like AEA and 2-AG) have the potential to stimulate appetite has led to detailed investigations to identify the precise role of the endogenous cannabinoid system in the control of feeding behavior. In one of the first studies, we demonstrated that naturally occurring levels of AEA and 2-AG could be modulated dependent upon the feeding state of the animal (Kirkham et al., 2002). Here, animals were randomly allocated to one of four groups: (1) *ad libitum* access to food and killed during a period of low spontaneous feeding; (2) fed with a wet mash and then killed during feeding; (3) fed with a wet mash and killed once feeding had ceased; or (4) food deprived for 18 hours. We then measured levels of AEA and 2-AG in limbic forebrain, hypothalamus, and cerebellum. Food deprivation resulted in significant increases in both AEA and 2-AG levels in the limbic forebrain, and to a lesser extent 2-AG was also significantly increased in the hypothalamus. However, 2-AG levels were significantly reduced in the hypothalamus while animals were in the process of feeding. Thus, it appears that the increases in AEA and 2-AG levels in important reward-related brain areas during food deprivation play a role in motivating animals towards food. Similarly, the reduction of 2-AG levels in the hypothalamus during feeding suggests that 2-AG is actively suppressed in humans as they eat, to facilitate satiation.

The role for AEA and 2-AG in control of feeding behavior has led to the hypothesis that disturbances in the endocannabinoid system might underlie the manifestation of clinical eating disorders (EDs), obesity, and metabolic disorders in humans. In the next section, we review the evidence in support of cannabinoids as potential novel therapeutics for clinical EDs, obesity, and metabolic disorders.

OVERVIEW OF CLINICAL EATING DISORDERS

Anorexia nervosa (AN) is a severe psychiatric disorder characterized by an intense fear of gaining weight or becoming fat and refusal to maintain a minimum healthy body weight of the expected standard for age and height (American Psychiatric Association, 1994). Furthermore, individuals with AN are preoccupied with their own ability to control their weight and shape, as body image is extremely important to them (American Psychiatric Association, 1994). To date there is no clear evidence-based treatment for AN (Strober et al., 1997; Bulik et al., 2007) and the prognosis remains poor with 10–20% of cases following a chronic course with significant levels of impairment and elevated mortality (Kaye et al., 2009). Current treatments for AN involve psychological therapies such as cognitive behavioural therapy (CBT). The only pharmacological treatments available are those that treat the comorbid symptoms of anorexia such as depression,

anxiety, and obsessive compulsive disorder (OCD); they do not treat the anorexia itself (Melotto, 2014). Thus, discovering new pharmacological treatments for AN remains an ambition.

Bulimia nervosa (BN) is characterized by binge-eating episodes and loss of control over eating behavior. As seen in AN, self-esteem issues are present with patients reporting concerns related to their body shape and weight (American Psychiatric Association, 1994) and BN is currently treated with psychological therapies such as CBT although these are not always effective for everyone (Hollon and Wilson, 2014). As in AN, the current pharmacological treatments for BN only treat the comorbid features of increased anxiety, OCD, and depression in bulimia but unfortunately not the binge/purging that is the main characteristic of the disorder (Bergh et al., 2013). Drug treatments for these disorders are hindered by the lack of knowledge of the underlying neurobiology subserving the observable symptoms of restriction and overeating in eating disorders (see also Chapter 16).

PRECLINICAL RESEARCH ON CANNABINOIDS IN EDs

Animal models of AN and BN have mostly focused on the serotonergic and dopaminergic systems, yet the high rate of relapse despite treatment with these medications points to the need for another approach (Kaye et al., 2001; Walsh et al., 2006). Some evidence has been made available on the link between eating disorders and the cannabinoid system (for reviews see Horcujadas, 2007; Di Marzo, 2009). An example of this is work that examined feeding behaviors in CB₁ receptor knockout mice by Siegfried and colleagues. They found that although mice lacking the CB₁ receptor (CB₁^{-/-}) did not differ from their littermates in terms of weight or food consumption, they did eat less than wild-type mice after 18 h of fasting (Siegfried et al., 2003). This is an interesting result in that it might have some face validity in relation to AN where an interaction between neural cannabinoid dysfunction might only be triggered after significant dieting or weight loss.

Furthermore, others have found that when wild-type mice are treated with SR141716A, food intake is significantly reduced to the same level as in vehicle-treated CB₁^{-/-} mice, whereas in CB₁^{-/-} mice, SR141716A does not affect food intake (Di Marzo et al., 2001). The authors reported that this might indicate that, endogenous cannabinoids acting at the CB₁ receptor are involved in maintaining food intake in mice made hyperphagic by food deprivation.

More recently, a study examining reward processes and the exogenous administration of CB₁ receptor agonists set up to determine whether anorexia displayed in a rat activity-based anorexia model could be reversed (Verty et al., 2011). Animals housed with running wheels and subjected to daily food restriction show paradoxical reductions in food intake and increases in running wheel activity. This phenomenon, known as activity-based anorexia (ABA), leads to marked reductions in body weight that can ultimately lead to death. Thus, ABA has been proposed as a model of AN (Klenotich and Dulawa, 2012). Verty and colleagues examined the effect of subchronic (6 days) Δ^9 -THC treatment on chow and high

fat diet (HFD) intake, body weight, and running wheel activity (RWA) in rodents. They also measured thermogenesis in brown adipose tissue (BAT) and lipid metabolism in white adipose tissue (WAT). They induced anorexia by limiting time availability of food and allowing continuous access to running wheels, which resulted in significantly reduced body weight. The authors report that Δ^9 -THC treatment transiently stimulated chow intake with a moderate effect on RWA. Δ^9 -THC significantly reduced body weight loss and shifted markers of thermogenesis in BAT and lipid metabolism in WAT in directions consistent with reduced energy expenditure and lipolysis. Δ^9 -THC (2.0 mg/kg/day) combined with HFD produced a transient increase in food intake, reduction in RWA, attenuation of body weight loss, and changes in markers of thermogenesis in BAT and lipolysis in the WAT. Verty reported that these data showed for the first time the effectiveness of the endocannabinoid system in attenuating the weight loss associated with the development of ABA via a mechanism involving reduced energy expenditure (Verty et al., 2011).

Unfortunately the waters are muddied by opposing findings. Previous work from Lewis and Brett found, in the first study to examine the effects of Δ^9 -THC in a male mouse model of ABA, that there were increased mortality rates with high doses (0.5 mg/kg) but that feeding was increased in the surviving mice (Lewis and Brett, 2010). Furthermore, AM251, an inverse agonist at the CB₁ receptor, is found to be anxiogenic in mice and anxiolytic in rats whereas SR141716 is found to be anxiogenic in rats and anxiolytic in mice (Lafenêtre et al., 2007). Thus, it has been suggested that factors such as the drug used, the dose, and the task itself may all contribute to the differences observed across animal models (Lafenêtre et al., 2007; Parolaro et al., 2010). However, these findings remind us that caution is needed when interpreting the effects of cannabinoid manipulations on animal models of complex human disorders such as eating disorders that most likely have a combination of both psychological and biological origins.

Obesity and its possible relationship with a dysfunctional endocannabinoid system has been extensively investigated in animals models of feeding behavior as described earlier in this chapter. However, to briefly summarize again, we now know the important role of the cannabinoid system in feeding and how links have been made between genetic variants in the cannabinoid system that in turn influence the functioning of that system with increased susceptibility to a disorder like obesity (Gadzicki et al., 1999; De Luis et al., 2013). In animal models, drugs such as SR141716A (Rimonabant, trade name Acomplia) reduce food intake when access to food is unrestricted (Colombo et al., 1998). The same effects have also been found in starved animals and also following treatment with other CB₁ receptor antagonists (Gallate and McGregor, 1999; Freedland et al., 2001; Higgs et al., 2003). Studies have also examined whether the type of food fed to the animal could be related to the anorexic effects of these drugs, with studies showing a greater anorectic action of SR141716A for the intake of palatable food compared to less palatable foods or standard chow (Schwartz et al., 2000; Mechoulam and Fride, 2001; Cota et al., 2003). However, other authors have found that CB₁

receptor antagonists reduce intake of carbohydrate-rich, fat-rich, and standard diets, both in food-deprived and non-deprived animals (Berger et al., 2001; Fride et al., 2001; Werner and Koch, 2003). However, tolerance to these anorexigenic effects has also been seen after 4–5 days of administration, although weight loss persisted throughout the experiment (Colombo et al., 1998; Bensaid et al., 2003; Vickers et al., 2003). Interestingly it seems that tolerance to treatment developed more rapidly in lean than in obese animals (Vickers et al., 2003) but not when the animals received a palatable diet for 21 days (Gessa et al., 2006). Thus, it has been suggested that cannabinoid receptor antagonists are involved in weight loss outside of their anorexic effects as the animals continued to lose weight even after tolerance to the drug was evident. The authors concluded that perhaps their results were related to SR141716A having its effects through the hedonics of feeding. Perhaps also through metabolism regulation, SR141716A was found to stimulate energy expenditure and lipolysis and reduce adiposity and serum lipid levels in rats and mice (Bensaid et al., 2003; Jbilo et al., 2005; Poirier et al., 2005).

Binge-eating behavior can be modeled in animal protocols to investigate neurobiological substrates and pharmacological determinants of human bingeing disorders (Hancock and Olmstead, 2010; Berner et al., 2011). Animal models of binge-eating behavior have typically involved using stress to increase feeding of high-calorie foods (Gluck, 2006). A recent study by Scherma et al. (2013) examined the effects of CB₁ receptor inverse agonist/antagonist SR141716A on the aberrant eating behavior present in a validated rat model of binge eating (Corwin et al., 1998). They used a model that had female rats with limited access to an optional source of dietary fat (margarine). Rats were divided into three groups, all with *ad libitum* access to chow and water: control (C), with no access to margarine; low restriction (LR), with 2 h margarine access 7 days a week; high restriction (HR), with 2 h margarine access 3 days a week. They found that treatment with Δ^9 -THC proved to be effective in increasing margarine intake exclusively in the LR group, in line with the orexigenic effects of Δ^9 -THC in humans and rodents (Williams and Kirkham, 1999; Hart et al., 2002). They also reported that in LR rats, Δ^9 -THC increased the total food intake with a specific effect on palatable food, but not in the HR animals possibly due to a ceiling effect. Investigating the effects of URB597, which prevents intracellular inactivation of AEA by the AEA hydrolyzing enzyme fatty acid amide hydrolase (FAAH) and so prolongs its behavioral and neurochemical effects, the authors did not find an increase in the amount of margarine or chow consumed despite this having been shown previously in the literature (Soria-Gomez et al., 2007).

Scherma et al. suggested this might be due to the different route of administration used (i.p. rather than direct brain infusion), or to the fact that URB597 is unable to further enhance the pre-existing endocannabinergic tone in their bingeing rats. It is also possible, they note, that the result indicates the involvement of other neurotransmitter systems. However, they did find that SR141716A decreased margarine intake in HR rats when given acutely, yet it only reduced margarine intake in LR animals at high doses (3 mg/kg). In line with this, studies show that

administration of a different CB₁ receptor antagonist, SR147778, dose dependently attenuated binge-like intake of a sweet/fat diet in rats (Parylak et al., 2012). In addition, both doses of SR141716A reduced consumption of standard chow in all three diet groups. Notably, over time, SR141716A preserves its selective reducing effect on intake of fatty food as demonstrated by the finding that both HR and LR rats treated with SR141716A consumed less margarine but their intake of standard chow was unaltered. The authors conclude that their data suggest potential therapeutic utility of CB₁ receptor antagonists in the treatment of binge-like eating disorders (Scherma et al., 2013). Although the mechanism of action of drugs, such as SR141716A, has been thoroughly examined in animal models, much less has been done in human studies. This is an important next step in helping us understand why drugs like this have been unsuccessful as a human therapy to date.

CLINICAL RESEARCH ON CANNABINOIDS IN EDs

As described above, cannabinoid receptor agonists have been used successfully in treating anorexia associated with cancer chemotherapy and HIV (Beal et al., 1995, 1997; Jatoi et al., 2002). Recent studies have assessed the effects of CB₁ receptor agonist treatment in weight gain. For example, Nelson et al. showed a median weight gain of 1.3 kg over 28 days in patients with cancer-associated anorexia on relatively small daily doses of Δ^9 -THC (2.5 mg) in an interventional phase II study (Nelson et al., 1994). Further, in trials examining AIDS-induced anorexia, Δ^9 -THC daily treatment over 6 weeks was reported to produce minor weight gains.

Unfortunately, though, whether cannabinoids might be useful in AN has yet to be thoroughly examined. Despite studies on psychoactive drugs such as fluoxetine and olanzapine for AN, results have been scarce and hindered further by the lack of clinically controlled studies. As a result, to date there are no pharmacological treatments approved for anorexia nervosa. Δ^9 -THC, as described above, is documented as increasing intake, hunger ratings, and food appreciation. In an early study by Gross and colleagues, the authors compared the effects of diazepam (1.0–5.0 mg) and high doses of Δ^9 -THC in 11 patients with primary AN with high doses of synthetic Δ^9 -THC (from 7.5 mg/day to a maximum dose of 30 mg/day) tested against diazepam (Gross et al., 1983). They found that Δ^9 -THC-induced weight gain was slightly higher (1 kg) than that observed during diazepam treatment despite evidence that suggests that benzodiazepines may also increase food intake (Naruse, 1994; Patel and Ebenezer, 2008). However, the treatment was stopped over the weekends, occasional tube feeding was used, and three patients (27%) withdrew after experiencing severe dysphoric reactions during active treatment, leading the authors to conclude that Δ^9 -THC was an ineffective treatment for primary AN (Gross et al., 1983). However, it should be noted that Δ^9 -THC has been shown to cause anxiety in both animal and human studies (Marco et al., 2011), which therefore may hinder its utility in treating disorders like anorexia wherein comorbid anxiety disorders are prevalent.

More recently, studies have pointed to the disruption of neural substrates underlying feeding and the cannabinoid system in eating disorders (Stoving et al., 2009). Monteleone and colleagues have investigated the levels of AEA and 2-AG in BN and AN patients. They found that in AN and binge-eating disorder patients, but not in BN patients, there are increased blood levels of AEA (Monteleone et al., 2005). They also reported that there is an inverse correlation between AEA levels and plasma leptin concentrations in both healthy controls and anorexic women. Studies such as this and that of Holtkamp et al. (2006), who found reduced leptin in AN, point to the possibility of a relationship between leptin level and the cannabinoid system dysfunction in AN (for review see Bermudez-Silva et al., 2012).

Furthermore, studies examining levels of CB₁ receptor have found increases in the blood of females with AN and BN, further supporting the hypothesis of dysregulated endocannabinoid signaling in eating disorders (Frieling et al., 2009). Paradoxically, these authors found an association between lower CB₁ receptor expression and more severe forms of the disorders. Taken together it seems that there are biological differences in cannabinoid receptors and leptin levels that might be related to the development of eating disorders. If this is the case, then it further supports the investigation of the cannabinoid system as a possible target for treatment in eating disorders.

Moreover, Gerard and colleagues examined CB₁ receptor availability in AN and BN patients using PET imaging. They found that global CB₁ receptor availability was significantly increased in cortical and subcortical brain areas in AN patients compared with healthy control subjects (Gerard et al., 2011). Regionally, CB₁ receptor availability was increased in the insula in both AN and BN patients and the inferior frontal and temporal cortex in AN patients only. From this the authors concluded that global CB₁ receptor up-regulation in AN patients is a possible long-term compensatory mechanism resulting from an underactive endocannabinoid system in anorectic conditions. They also note that there is CB₁ receptor dysregulation across both AN and BN in areas such as the insular cortex, which is involved in the integration of interoceptive information, gustatory information, reward, and emotion processing (Gerard et al., 2011).

NEUROIMAGING OF REWARD AND CANNABINIDS

Disturbances in the regulation of reward and aversion in the human brain may underlie many psychiatric disorders from obesity to clinical eating disorders and addictions. SR141716A, as mentioned above, is an antagonist and possible inverse agonist (Pertwee, 2005) at the CB₁ receptor in the brain. In 2006, SR141716A was licensed (Rimonabant, trade name Acomplia) for the treatment of obesity in Europe but it was associated with an increased risk of depression, anxiety, and suicidal behavior (Le Foll et al., 2009; Moreira and Crippa, 2009). Thus, it was withdrawn from the market

in 2008 due to concerns over these side effects. Two meta-analyses found that patients given SR141716A were 2.5 times more likely to discontinue the treatment because of depressive mood disorders than were those given placebo when the data from four clinical trials were examined (Christensen et al., 2007a,b).

Furthermore, an FDA analysis also noted that 26% of SR141716A (20 mg) treated subjects vs. 14% of the placebo group reported psychiatric symptoms in the same trial data (US Food and Drug Administration Advisory Committee, 2007). The clinical trials revealed disturbing information such that those on the drug had increased rates of suicidal ideation and behavior and even more worryingly these trials excluded patients with a history of psychiatric illness, including depression. The importance of this oversight in clinical trials was borne out in the more recent STRADIVARIUS trial where patients with a history of psychiatric illness were not specifically excluded and thus the rate of reported psychiatric symptoms went up to 43% in the SR141716A 20 mg group vs. 28% in the placebo group (Nissen et al., 2008).

Thus, it is important when considering drugs for the human market to identify early on the presence of adverse side effects. SR141716A in humans induced depression, yet went through lengthy animal studies that yielded no depression-like profile and if anything showed an antidepressant effect in animal models (Griebel et al., 2005; Patel et al., 2005; Steiner et al., 2007; Takahashi et al., 2008). Therefore, it is important that we use human experimental models to examine the effects of these treatments, such as those that might induce eating but with anxiogenic side effects like Δ^9 -THC (D'Souza et al., 2004), or reduce eating with depressogenic side effects like SR141716A before they go through expensive and time-consuming human clinical trials.

We have developed a model that examines the human brain's responses to rewarding and aversive food stimuli. Using functional magnetic resonance imaging (fMRI), we presented the sight and taste of chocolate to produce a robust activation of the primary reward system in the brain in healthy volunteers (Rolls and McCabe, 2007). We showed previously that those vulnerable to depression have deficits in their neural reward responses to chocolate compared to healthy controls, despite no differences in their subjective experiences of reward (McCabe et al., 2009). We believe this might underlie the symptom of anhedonia (loss of pleasure) in depression, which is linked to abnormal reward processing and is a key symptom in all major diagnostic systems.

Using this methodology we examined the effects of SR141716A on reward processing in healthy volunteers. We hypothesized that our task would activate the key circuitry of ventral striatum, caudate, medial prefrontal, and orbitofrontal cortices, which we and others have shown to be sensitive to rewarding and aversive stimuli (McCabe and Rolls, 2007; Rolls and McCabe, 2007; McCabe et al., 2008). Notably, the CB₁ receptor is highly expressed in such reward areas including the basal ganglia (Herkenham et al., 1991). For this study we implemented 7 days of treatment with SR141716A, at a dose of 20 mg per day; this was the standard dose employed in the clinical treatment of obesity (Pi-Sunyer et al., 2006)

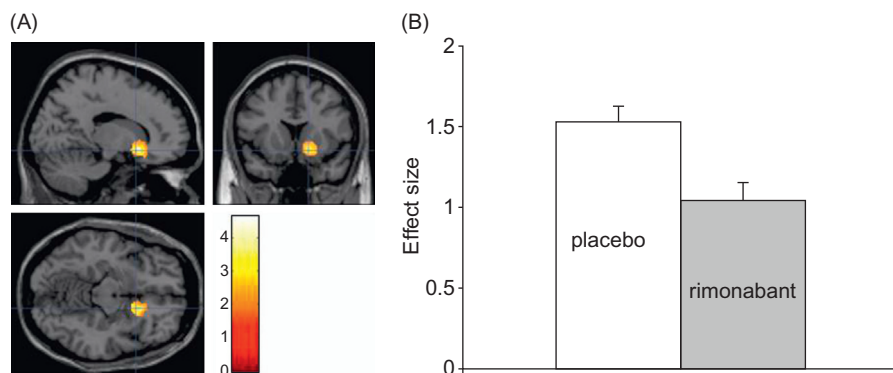


FIGURE 13.1

(A) Sight of chocolate condition (placebo > rimonabant). Axial, sagittal, and coronal image of decreased ventral striatal activation in the rimonabant group compared to the placebo group [(x = 16, y = 18, z = -10), Z = 3.76, $p < 0.05$ family-wise error corrected]. (B) Parameter estimates from 6 mm sphere centered at (x = 16, y = 18, z = -10) for rimonabant (gray) and placebo (white).

From [Horder et al. \(2010\)](#), with permission from the International Journal of Neuropsychopharmacology, Cambridge University Press.

and a dose at which psychiatric side effects have been observed ([Christensen et al., 2007a](#)).

We found that SR141716A reduced the neural response to chocolate stimuli, in key reward areas such as the ventral striatum and the orbitofrontal cortex ([Figure 13.1](#)). SR141716A also decreased neural responses to the aversive stimulus condition in the caudate nucleus and ventral striatum, but increased lateral orbitofrontal activations to the aversive sight and taste of strawberry condition ([Horder et al., 2010](#)). Our findings were the first to show that the anti-obesity drug SR141716A inhibited the neural processing of rewarding food stimuli in humans and we proposed that this may be a mechanism by which food intake was decreased but depressogenic symptoms increased. Hence, from our results we conclude that assessment of the effect of drug treatments on neural reward mechanisms could provide some early indication of their propensity to produce depression in clinical use. We also propose that fMRI in humans may be a useful method of screening novel agents for unwanted effects on reward and associated clinical adverse reactions.

Since our study, others have begun to examine other cannabinoids such as the CB agonist Δ^9 -THC on reward-related brain activity. [Van Hell et al. \(2012\)](#) used a monetary reward task (Knutson) and found that Δ^9 -THC induced a widespread attenuation of the brain response to feedback in reward trials but not in neutral trials. Anticipatory brain activity was not affected. They concluded that their results suggest a role for the endocannabinoid system in the appreciation of rewards. The involvement of endocannabinoids in feedback processing may be

relevant for disorders in which appreciation of natural rewards may be affected (van Hell et al., 2012). It will therefore be of interest to examine the effects of these treatments in human experimental models that might also be able to detect, early on, negative side effects such as depression and anxiety.

Furthermore, as reviewed more recently by Romero-Zerbo and Bermudez-Silva (2014), other constituents of cannabis could have anti-obesity properties. As described elsewhere in this chapter, pCBs such as Δ^9 -THCV and CBD also have potential anti-obesity qualities as they too counteract the orexigenic effects of cannabinoid agonists (Riedel et al., 2009; Scopinho et al., 2011; Farrimond et al., 2012). Also, evidence is emerging for CB₂ receptor involvement in mediating the effects of CBD, with increased expression of CB₂ receptors seen in the hypothalamus of lean phenotype animals. This might, therefore, be another avenue for research whereby human experimental models such as ours could be used to examine the effects in the human brain.

In fact we have just completed a new study examining the effects of Δ^9 -THCV, the component of *Cannabis sativa*, that, unlike SR141716A, acts as a neutral CB₁ receptor antagonist (Pertwee, 2008) and which is suggested as being free of depressogenic side effects (Le Foll et al., 2009). We used our model of reward and aversion and hypothesized that Δ^9 -THCV would, unlike SR141716A, leave intact the neural response to reward but augment the response to aversive stimuli.

Using a within-subject, double-blind design, 20 healthy volunteers were treated with a single dose of Δ^9 -THCV (10 mg) and placebo in a randomized order. We found that Δ^9 -THCV augmented activation to the chocolate stimuli, in key reward areas such as the anterior cingulate cortex and the thalamus. Δ^9 -THCV also increased neural responses to the aversive stimulus condition in the amygdala, insula, caudate, and hippocampus, and in the anterior cingulate, thalamus, medial frontal gyrus, and the putamen. Our findings are the first to show that treatment with the CB₁ receptor neutral antagonist Δ^9 -THCV potentiates neural responding to rewarding and aversive stimuli. The profile of effect of Δ^9 -THCV could suggest therapeutic activity in certain eating disorders, perhaps with a lowered risk of depressive side effects (Tudge et al., 2014, in preparation). Taken together the cannabinoid system seems a legitimate target for drug development for eating disorders. However, we suggest the need for validated human experimental models that can detect both beneficial weight loss effects and detrimental psychiatric effects of these drug developments before they go through clinical trials.

CONCLUSIONS

Recreational users of cannabis have long been aware of its effects upon appetite but it was not until the discovery of the endocannabinoid system in the late 1980s that both animal and human studies were able to begin revealing the underlying cellular, molecular, and neurophysiological processes that give rise to these

effects. While these studies have provided considerable advances in our understanding, and CB₁ receptor agonism as a treatment for drug-induced weight loss represents the longest standing licensed clinical use for cannabinoids, further drug development in this area, specifically in the area of eating disorders, has remained limited. While several early studies have suggested that certain non- Δ^9 -THC pCBs may hold some potential in this regard and remain under active investigation, the neuropsychiatric side effects associated with inverse agonism of CB₁ receptors and the anxiogenic effects of Δ^9 -THC, mainly at higher doses, present a significant challenge, particularly in clinical situations where a disorder has a significant psychiatric component.

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PART

Role of the
endocannabinoids
in impulsive
and compulsive
disorders

3

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The cannabinoid system and impulsive behavior

14

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BACKGROUND ON IMPULSIVITY

Daily life is full of impulsive actions and decisions ranging from speaking before your turn in a classroom or meeting, using money you had set aside for next month's rent to buy yourself a nice present, to postponing your regular visit to the dentist. A useful and commonly used definition of impulsivity has been provided by Barnes and Daruna stating that impulsivity comprises "actions which are poorly conceived, prematurely expressed, unduly risky or inappropriate to the situation and that often result in undesirable consequences" (Daruna and Barnes, 1993). Whereas this definition captures the key elements of impulsivity, one should keep in mind that being impulsive is not always maladaptive and to a certain extent can be advantageous and adaptive on an individual and group level (Williams and Taylor, 2006).

On the other hand, maladaptive levels of impulsivity are frequently associated with a variety of psychiatric and neurological disorders. In this respect, attention deficit/hyperactivity disorder (ADHD) is probably the best-known and most extensively studied disorder, whereas maladaptive impulsivity is also a prominent feature in bipolar disorder, Axis II personality disorders, and substance use disorders (American Psychiatric Association, 2000; Moeller et al., 2001). With regard to the latter, empirical evidence from longitudinal studies as well as preclinical studies indicate that trait impulsivity may be a vulnerability factor in the development and compulsive nature of substance use disorder and in turn drug abuse itself can lead to elevated impulsivity (Moeller et al., 2001; Verdejo-Garcia et al., 2008; De Wit, 2009; Winstanley et al., 2010; Dalley et al., 2011; Pattij and De Vries, 2013). In addition to the aforementioned psychiatric disorders, in a neurological disorder such as Parkinson's disease it has become increasingly well established that apart from the primary movement symptomatology, compromised impulse control is commonly observed as an adverse effect for dopamine replacement therapy (Voon et al., 2011; Vriend et al., 2014).

As becomes already apparent from the definition, impulsivity is multi-faceted and consists of various behavioral constructs. There is general consensus that impulsive behavior can be broadly divided into impulsive actions, that is, an inability to inhibit behavioral responses, and impulsive decision-making, that is, a distorted judgment with respect to choosing between different outcomes (see, for example, Evenden, 1999; Winstanley et al., 2006). Supported by recent empirical observations (Schachar et al., 2007; Sebastian et al., 2013), impulsive actions can be further conceptualized into two subcomponents, namely, action restraint (inhibiting a prepotent, inappropriate response) and action cancellation (response inhibition or volitional control over responding once the response has been initiated). Whereas impulsive decision-making is oftentimes operationalized by delay-based decisions and as such reflects delay aversion, one can argue that related decision-making processes including probability-based and effort-based decisions are also factors in impulsive decision-making. Nonetheless, even though there is some clinical evidence for cannabis effects on probability-based decision-making (for reviews see Crean et al., 2011; Lee et al., 2012), we will here only discuss the role of the endocannabinoid system in delay-based decision-making. Finally, next to impulsive actions and impulsive decision-making, a possible third modality of impulsivity comprises what is referred to as “reflection impulsivity.” This modality relates to the inability to make decisions before adequately sampling and evaluating available information and as such reflection impulsivity contains elements of both other modalities of impulsivity. Collectively, the scientific rationale for distinguishing these aspects of impulsive behavior may have clinical relevance, as it argues for individually-tailored (pharmacological) treatment of impulse control disorders. In addition to self-report measures of impulsivity, such as the Barratt Impulsivity Scale (Patton et al., 1995), various neuropsychological tasks have been developed to assess different modalities of impulsivity in humans. Importantly, over the last decades most of these tasks have been translated into rodent versions allowing simultaneous investigation of modalities of impulsivity in humans as well as experimental animals (Table 14.1; Broos et al., 2012).

Multidisciplinary and translational approaches incorporating neuroanatomical lesioning techniques and pharmacological interventions in rodents as well as neuroimaging and pharmacological interventions in humans have tremendously increased our understanding of the neural correlates of impulsivity. Briefly, cortico-striatal networks interacting with basal ganglia structures including the subthalamic nucleus and limbic regions such as the hippocampus and amygdala are crucially involved in impulsivity, and depending on the modality of impulsivity these networks are functionally segregated (Figure 14.1; see for recent reviews Pattij and Vanderschuren, 2008; Dalley et al., 2011). Furthermore, these networks are modulated via ascending monoaminergic projections from the locus coeruleus (norepinephrine), the raphe nuclei (serotonin), and the ventral tegmental area (dopamine) as evidenced by pharmacological approaches (Pattij and Vanderschuren, 2008; Winstanley, 2011; Dalley and Roiser, 2012). In this chapter, we will review and discuss the available evidence for cannabinoid involvement in impulsive behavior from a translational perspective.

Table 14.1 Overview of Different Modalities of Impulsive Behavior and Neuropsychological Tasks That Can Be Employed to Measure Those Behaviors in Humans and Rodents

Modality Impulsivity	Cognitive Domain	Description	Human Task	Rodent Task
Impulsive action	Action restraint; motor impulsivity	Ability to inhibit prepotent, inappropriate responses	CPT ^a ; IMT/DMT ^b	5-CSRTT ^c ; Go/No Go paradigm ^d
	Action cancellation; response inhibition	Ability to inhibit actions once initiated	SST ^e	SST ^e
Impulsive decision-making	Delay-based decision-making	Decision-making based on delay-aversion	Experiential or hypothetical DDT ^f	DDT ^f ; adjusting amount procedure ^g
Reflection impulsivity	Information sampling	Ability to evaluate available information prior to deciding	IST ^h	Uncertain visual discrimination test ⁱ

^aCPT, continuous performance task (Rosvold et al., 1956).

^bIMT/DMT, immediate and delayed memory task (Dougherty et al., 2002).

^c5-CSRTT, 5-choice serial reaction time task (Robbins, 2002).

^dsee (Terman and Terman, 1973).

^eSST, stop-signal task (Logan et al., 1984; Eagle and Robbins, 2003).

^fDDT, delay discounting task (Rachlin et al., 1991; Evenden and Ryan, 1996; Pietras et al., 2003).

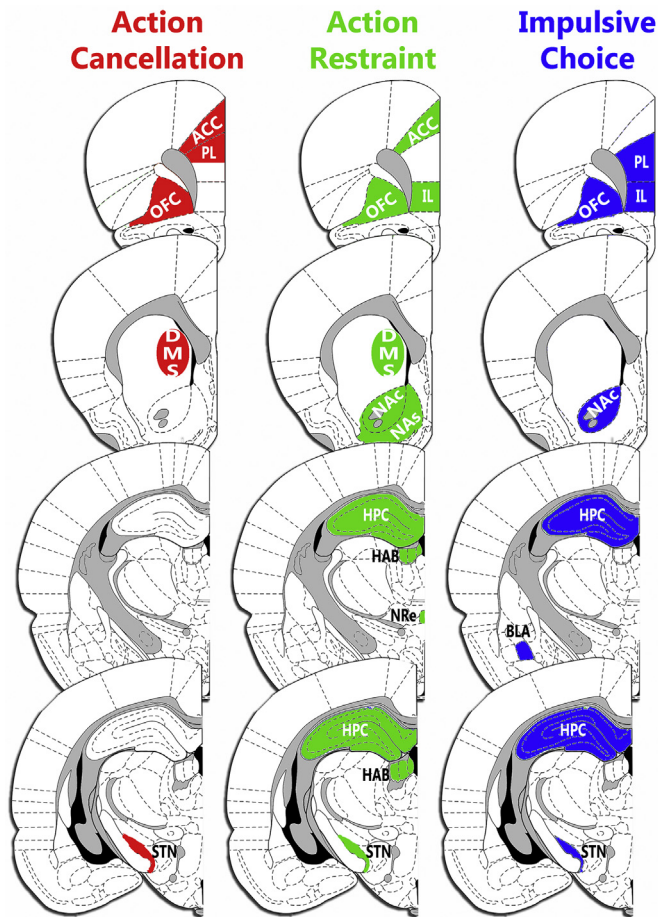
^gSee Richards et al. (1997).

^hIST, information sampling task (Clark et al., 2006).

ⁱSee Evenden (1999).

CANNABINOID MODULATION OF HUMAN IMPULSIVE BEHAVIOR

Given its widespread and abundant expression in the brain (Herkenham et al., 1990; Mailleux and Vanderhaeghen, 1992; Burns et al., 2007) and its known important role in other executive functions such as working memory, attention, time estimation, and behavioral flexibility (for reviews see Egerton et al., 2006; Pattij et al., 2008; Crean et al., 2011), it should come as no surprise that the endocannabinoid system mediates impulsive behavior. Evidence supporting cannabinoid involvement in human impulsive behavior ranges from acute effects of Δ^9 -THC (delta-9-tetrahydrocannabinol) on experimental measures of impulsivity to findings of impaired functioning of the endocannabinoid system in patients suffering from psychopathologies that are characterized by high levels of impulsive behavior. For example, ADHD patients were previously found to have reduced anandamide hydrolysis (Centonze et al., 2009). The first report on acute Δ^9 -THC-induced impulsivity stems from the late 1990s

**FIGURE 14.1**

Neuroanatomy of different modalities of impulsive behavior. Schematic overview of coronal sections (adapted from Paxinos and Watson, 1998) at various levels of one hemisphere of the rat brain (in anterior to posterior direction depicted from top to bottom) illustrating the overlap and distinctions in anatomical regions of the brain that have been shown to be involved in action cancellation (left, red), action restraint (middle, green), and delay-based impulsive decision-making/impulsive choice (right, blue). Dark gray/black areas indicate ventricles in the brain, whereas light gray areas indicate fiber tracts. Not shown are the midbrain nuclei containing the cell bodies of dopamine-, norepinephrine-, and 5-HT-neurons that are thought to be relevant for impulsivity, i.e., the ventral tegmental area, locus coeruleus, and raphe nuclei. Abbreviations: ACC, anterior cingulate cortex; BLA, basolateral amygdala; HAB, habenula; HPC, hippocampus; IL, infralimbic cortex; MS, medial striatum; NAC, nucleus accumbens core; NAS, nucleus accumbens shell; NRe, thalamic nucleus reuniens; OFC, orbitofrontal cortex; PL, prelimbic cortex; STN, subthalamic nucleus.

This figure was adapted, in part, from Pattij and Vanderschuren (2008), with permission from Elsevier.

(Hooker and Jones, 1987), when smoking low potency (1.2% Δ^9 -THC) marijuana was found to enhance the Stroop interference effect (Stroop, 1935). The Stroop effect, a slowing of reaction times when asked to name the color of a word that denotes a different color (e.g., the word “red” written in green letters) as compared to when the color and denotation of a word are the same (e.g., the word “red” written in red letters), reflects behavioral inhibition. Since the initial study by Hooker and Jones (1987), many studies have addressed the role of the endocannabinoid system in aspects of impulsive behavior.

The most consistently reported acute effect of Δ^9 -THC administration is reduced action cancellation as measured in the stop signal task (SST; McDonald et al., 2003; Ramaekers et al., 2006, 2009; Theunissen et al., 2012), although heavy cannabis users may become tolerant for this acute Δ^9 -THC effect (Ramaekers et al., 2009, 2011; Van Wel et al., 2013). It is conceivable that Δ^9 -THC-induced reductions in action cancellation abilities are related to findings of decreased activity in frontal cortical brain regions such as the anterior cingulate area and the inferior frontal gyrus following acute Δ^9 -THC administration in healthy volunteers (Borgwardt et al., 2008). Δ^9 -THC administration has also been shown to increase reflection impulsivity in a matching familiar figures task (Van Wel et al., 2013) as well as to enhance premature responding in cognitive tasks including the continuous performance task (CPT; Hart et al., 2001; Bossong et al., 2013). In contrast, performance in Go/No Go paradigms, measuring slightly different aspects of behavioral inhibition as compared to both the SST and CPT, seems unaffected by Δ^9 -THC (McDonald et al., 2003).

Interestingly, in the study by Bossong et al. (2013), Δ^9 -THC-induced increases in false alarm responses in the CPT (a measure of action restraint) correlated with a reduced deactivation of brain regions belonging to the default mode network. In contrast, no correlations were found with activity patterns in brain regions that showed task-related activation. The authors speculated that Δ^9 -THC-induced disruption of endocannabinoid-mediated GABA transmission may underlie these findings (Bossong et al., 2013), which fits with recent research interest in the importance of GABAergic signaling in impulsive behavior (Jupp et al., 2013; Silveri et al., 2013; Caprioli et al., 2014).

Finally, low doses of Δ^9 -THC do not seem to affect impulsive decision-making as measured in either a hypothetical or experiential delay discounting task (DDT; McDonald et al., 2003; Metrik et al., 2012). However, given the results from rodent studies that will be discussed in the next paragraph and the fact that the Δ^9 -THC content in marijuana nowadays tends to be high (e.g., Ramaekers et al., 2006), future studies should investigate the effects of higher doses of Δ^9 -THC on impulsive decision-making. Such studies should take into account that the expectancy of receiving Δ^9 -THC, irrespective of actually receiving the drug, can reduce impulsive decision-making (Metrik et al., 2012). Such an expectancy effect could either neutralize cannabinoid-induced increases in impulsive decision-making or occlude cannabinoid-induced decreases in this modality of impulsive behavior.

In addition to reports on acute effects of Δ^9 -THC on impulsivity, there is accumulating evidence suggesting that long-term cannabis users display impairments across various domains of impulsive behavior, including decreased action cancellation and action restraint (Moreno et al., 2012; Dougherty et al., 2013; Voon et al., 2014), increased reflection impulsivity (Clark et al., 2009; Solowij et al., 2012), and increased impulsive decision-making (Moreno et al., 2012; Dougherty et al., 2013). It should be noted though that the reported effects have generally been mild and that null effects on action cancellation, action restraint, and impulsive decision-making have also been reported (Johnson et al., 2010; Gonzalez et al., 2012; Dougherty et al., 2013). Such discrepancies could be arising from differences in subject populations or the experimental tasks that were used. For instance, the studies finding increased impulsive decision-making in cannabis users employed an experiential two-choice task in which subjects earn rewards per trial (Moreno et al., 2012; Dougherty et al., 2013), whereas null effects were found in cannabis users when hypothetical reward discounting paradigms were used (Johnson et al., 2010; Gonzalez et al., 2012). Similarly, inconsistent results have been obtained when cannabis users were asked to self-rate their impulsive behavior through various questionnaires (Johnson et al., 2010; Gonzalez et al., 2012; Moreno et al., 2012; Dougherty et al., 2013; Voon et al., 2014). Nonetheless, overall a picture is emerging of repeated cannabis users being slightly more impulsive than non-users.

It is to date largely unknown to what extent these impairments in impulse control persist beyond the period of cannabis use. Two recent studies seem to suggest that the impairments may be transient, since former cannabis users were found not to differ from control subjects on a delay discounting task (Johnson et al., 2010), and first episode psychosis patients that had given up cannabis use were reported to have lower levels of reflection impulsivity as compared to patients that currently still used cannabis (Huddy et al., 2013). Future studies on current and former cannabis users should combine behavioral readouts with imaging techniques such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET), since cannabis users may be able to mask the consequences of their cannabis use by allocating a larger portion of their brain capacity to executing impulsivity tasks (Batalla et al., 2013; Wrege et al., 2014). This would be reflected by increased activity in brain regions that traditionally mediate such behaviors, or even engagement of additional brain regions during the test session. For example, it was recently shown that former cannabis users (28 days of abstinence) showed increased task-related activity in areas such as the right dorsal lateral prefrontal cortex and parietal cortex as compared to non-users, while performing similarly on a Go/No Go paradigm (Tapert et al., 2007). Of note, an important caveat in studies on current and former cannabis users is the difficulty to infer whether enhanced levels of impulsive behavior were a consequence of cannabis use or a pre-existing trait that potentially made those individuals more vulnerable for long-term, heavy cannabis use, as has previously been postulated for other types of addictive drugs

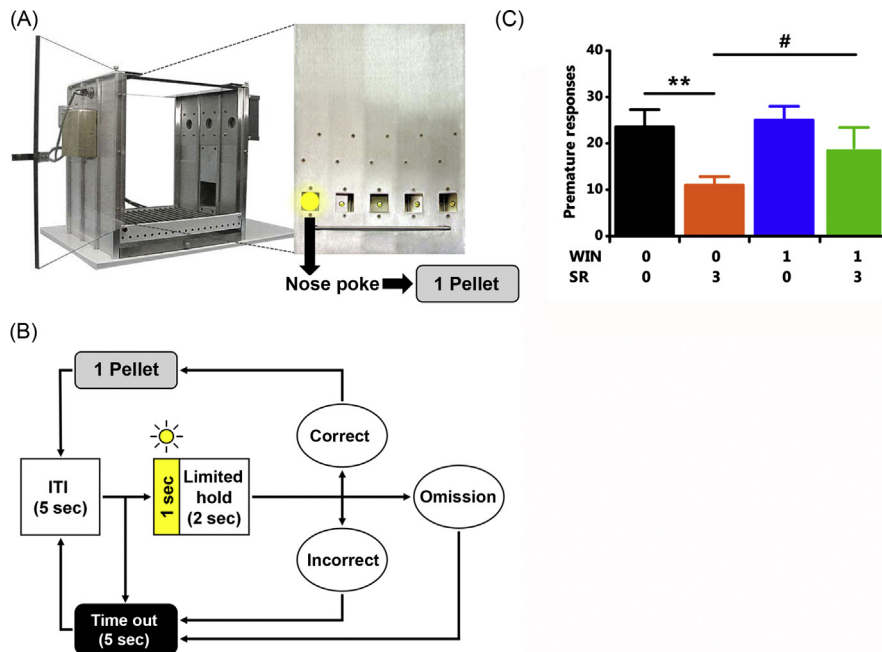
(for reviews see Jentsch and Taylor, 1999; Verdejo-Garcia et al., 2008; De Wit, 2009; Pattij and De Vries, 2013). Longitudinal studies in humans, following subjects from early childhood, and rodent studies in which animals are screened for individual differences in impulsive behavior prior to and following exposure to cannabinoid agonists, are warranted to this end.

The endocannabinoid system plays a critical role in the extensive neuroplasticity that occurs during adolescence (Spear, 2000; Harkany et al., 2008). Thus, it would be important for future studies on impulsivity in cannabis users to take age of onset of cannabis use into account, particularly since previous reports have underscored the importance of the age of onset of cannabis use on cannabis-induced cognitive deficits (for reviews see Pattij et al., 2008; Schneider, 2008; Trezza et al., 2012; Batalla et al., 2013). Indeed, it was recently found that early-onset (before age of 15 years) chronic cannabis users performed worse on experimental tasks measuring inhibitory control than later-onset users (Fontes et al., 2011). Moreover, positive correlations have been reported between the age of onset of cannabis usage and display of reflection impulsivity (Solowij et al., 2012). Another factor that is worth more attention in research on the role of the endocannabinoid system in impulsive behavior is individual variability. Several studies have linked polymorphisms in cannabinoid-related genes to impulsivity or impulse control disorders (Ponce et al., 2003; Ehlers et al., 2007; Lu et al., 2008; Hariri et al., 2009). Although it would require more subjects, bringing genetic variations in cannabinoid-related genes into the equation will likely help to clarify the exact role of the endocannabinoid system in impulsive behaviors.

TRANSLATIONAL EVIDENCE FOR CANNABINOID MODULATION OF IMPULSIVITY

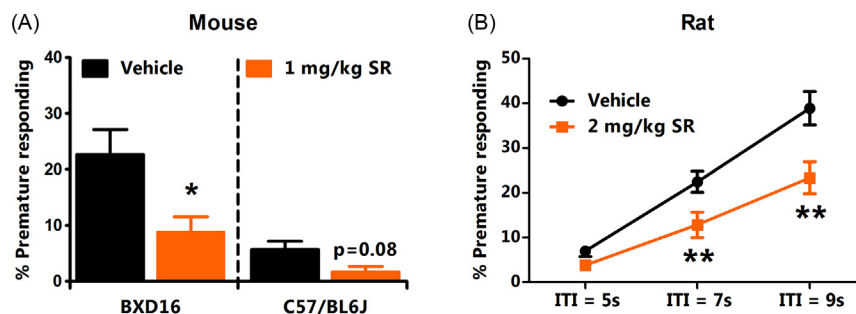
For most human neuropsychological tasks assessing different modalities of impulsivity, rodent analogues have been developed to measure similar behavioral parameters (Table 14.1; Winstanley et al., 2006). Recent studies employing pharmacological interventions in these animal models using cannabinoid ligands have generally corroborated human studies and have yielded further insight into the mechanisms by which the endocannabinoid system modulates impulsivity and how it interacts with other neurotransmitter systems.

The most widely used model to assess aspects of impulsive action, particularly action restraint, is an instrumental paradigm called the 5-choice serial reaction time task (5-CSRTT; Figure 14.2A,B). This task was developed in the early 1980s (Carli et al., 1983; Robbins, 2002) as a rodent version of the CPT (Rosvold et al., 1956), and as such has been designed to primarily be a test of sustained and visual spatial attention. In addition, action restraint can be assessed in both the CPT and 5-CSRTT by quantifying the number of responses that are expressed prematurely. In general, the 5-CSRTT is thought to have good validity (McKinney and Bunney, 1969)

**FIGURE 14.2**

Cannabinoid effects in the 5-choice serial reaction time task (5-CSRTT). (A, B) In the 5-CSRTT operant chamber, rats have to respond upon or shortly after (during limited hold) a brief visual stimulus that appears semi-randomly in one of five nose poke apertures (left curved wall) to earn food pellets (delivered in a receptacle in the right wall). In contrast, rats should refrain from responding during the inter-trial-interval (ITI) preceding each stimulus presentation to prevent a time-out penalty. Failure to do so, premature responding, reflects failed action restraint. Making a response in an incorrect hole, or making no response at all (omissions), will also result in a time-out penalty. (C) Data (mean $\pm$ SEM) adapted from [Pattij et al. \(2007a\)](#), demonstrating the effects of the CB₁ receptor antagonist SR141716A (SR) and the CB₁ receptor agonist WIN 55,212-2 (WIN), alone and together, on premature responding in the 5-CSRTT. Drug dosages are in mg/kg. ** $p < 0.005$ versus vehicle, # $p < 0.05$ versus SR alone.

as a translational task for action restraint, although it should be noted that certain differences have been found between the CPT and 5-CSRTT, particularly regarding the effects of dopaminergic compounds on impulsive behavior ([Winstanley, 2011](#)). Such discrepancies may be related to experimental differences between the two tasks. Alternatively, differences between findings in the 5-CSRTT and CPT may reflect species differences. The recently developed human analogue of the 5-CSRTT may help to shed some light on some of the discrepancies between CPT and 5-CSRTT results ([Voon et al., 2014](#)).

**FIGURE 14.3**

Rate-dependent effects of the CB₁ receptor antagonist SR141716A on action restraint in mice and rats. Unpublished data showing the effects of SR141716A on percentage premature responding ($\pm$ SEM) in the 5-choice serial reaction time task in (A) high impulsive BXD16 mice and low impulsive C57/BL6J mice (Loos et al., 2013) under conditions with a fixed (predictable) inter-trial-interval (ITI) of 7 s, and (B) Wistar rats tested in a 5-CSRTT with a variable (unpredictable) ITI (5–9 s). In both experiments, SR141716A-induced improvements in action restraint increased when the percentage premature responding under vehicle conditions was higher. Data were analyzed with paired Student t-tests (A) and repeated measures ANOVA followed by Newman–Keuls multiple comparisons *post-hoc* testing (B). * $p < 0.05$ and ** $p < 0.005$ versus vehicle.

In two initial preclinical studies on the role of the cannabinoid CB₁ receptor in impulsivity in rats (Figure 14.2C; Pattij et al., 2007a; Wiskerke et al., 2011), the non-selective CB₁ receptor agonists WIN 55,212-2 and Δ^9 -THC did not affect action restraint in the 5-CSRTT. These findings appear to contradict the above-discussed human findings on Δ^9 -THC-induced impairments in action restraint (Hart et al., 2001; Bossong et al., 2013). It would be interesting to test the effects of acute Δ^9 -THC administration in the novel human serial reaction time task (Voon et al., 2014). Despite the lack of effect of cannabinoid receptor agonists on premature responding in the 5-CSRTT, there is strong evidence for cannabinoid modulation of action restraint. Specifically, the cannabinoid CB₁ receptor antagonist SR141716A was shown to reduce premature responding in the 5-CSRTT, an effect that was prevented by increasing the tone at CB₁ receptors with the agonist WIN 55,212-2 (Pattij et al., 2007a). These effects of SR141716A were later confirmed using the CB₁ receptor antagonists SLV330 (De Bruin et al., 2011) and O-2050 (Wiskerke et al., 2011), of which the latter lacks inverse agonistic properties at the CB₁ receptor (Canals and Milligan, 2008; Wiley et al., 2010). Moreover, preliminary data from our laboratory indicate that SR141716A has similar alleviating effects on 5-CSRTT impulsive behavior in mice (Figure 14.3A). Thus, action restraint under baseline conditions seems to be modulated by an endogenous tone at the cannabinoid CB₁ receptor. Based on the lack of effect of CB₁ receptor agonists, this endogenous

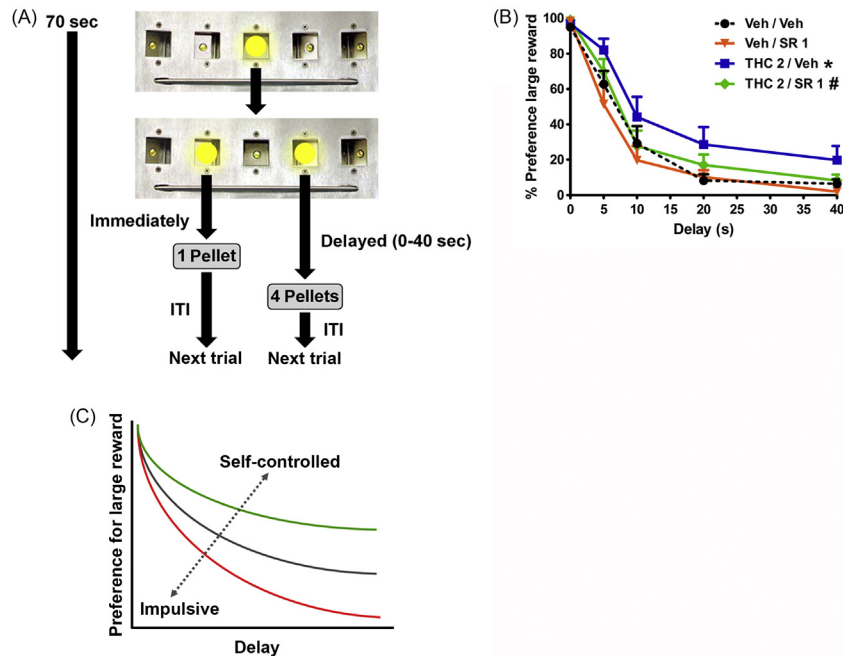
tone might be causing near maximal stimulation of the receptors involved in mediating this modality of impulsivity. Experiments involving measurement of impulsivity-related endocannabinoid release and administration of inhibitors of endocannabinoid hydrolysis, systemically but preferably intracranially, are warranted to further substantiate this conclusion.

There are several candidate brain regions where impulsivity-related endocannabinoid release may modulate action restraint, of which the ventral tegmental area seems particularly suited. Both in humans (Buckholtz et al., 2010) and in preclinical animal models (Cole and Robbins, 1987; Pattij et al., 2007b) increments in striatal dopamine release are associated with impairments in action restraint mechanisms. This is thought to be a pivotal mechanism underlying the impulsivity-enhancing effects psychostimulant drugs have in the 5-CSRTT (Cole and Robbins, 1989; Van Gaalen et al., 2006a; Pattij et al., 2007b). CB₁ receptor agonists including Δ^9 -THC and WIN 55,212-2 also increase dopamine release in the striatum of humans and rodents, most likely by disinhibition of midbrain dopamine neurons (Tanda et al., 1997; Gessa et al., 1998; Cheer et al., 2004; Bossong et al., 2009). Moreover, *in vivo* microdialysis studies have demonstrated that administration of the CB₁ receptor antagonist SR147176A, in a dose range that also reduces the impulsivity-related behavioral effects of these psychostimulants in the 5-CSRTT (Wiskerke et al., 2011, 2012), abolishes amphetamine- and nicotine-induced dopamine release in the nucleus accumbens shell (Cohen et al., 2002; Kleijn et al., 2012). Together, these findings may indicate that task-related endocannabinoid release in the ventral tegmental area maintains baseline levels of impulsive responding in the 5-CSRTT. This would explain why pharmacologically blocking CB₁ receptors improves action restraint in the 5-CSRTT. In further support of such an explanation is the finding that effects of CB₁ receptor antagonists in the 5-CSRTT appear rate dependent, that is, under behavioral conditions favoring elevated premature responding the beneficial effects of CB₁ receptor antagonists on action restraint become more pronounced (Wiskerke et al., 2011). For example, in 5-CSRTT experiments in which the length of the waiting period (inter-trial-interval, ITI) prior to the stimulus presentation is made variable, CB₁ receptor antagonist-induced improvement in action restraint has been demonstrated to be ITI length dependent (Figure 14.3B; De Bruin et al., 2011).

The prefrontal cortex comprises another possible brain region where endocannabinoids may influence action restraint, for instance, by modulating glutamate transmission in this brain region (Auclair et al., 2000). Recent evidence has pinpointed a role for glutamate transmission in the prefrontal cortex in action restraint. First, infusion of the NMDA receptor antagonist CPP into the medial prefrontal cortex has been demonstrated to increase premature responding in the 5-CSRTT (Murphy et al., 2005, 2012). Second, it was recently found that acute pharmacological activation of medial prefrontal cortex metabotropic glutamate 2/3 (mGluR2/3) receptors, which reduces local glutamatergic activity, induced impairments in action restraint as measured in the 5-CSRTT (Counotte et al., 2011). The hypothesis that endocannabinoid signaling may affect action restraint

through local modulation of prefrontal neurotransmission would be in line with the finding that the CB₁ receptor antagonist SR141716A ameliorated amphetamine- and nicotine-, but not GBR 12909-induced impulsivity in the 5-CSRTT (Wiskerke et al., 2011, 2012). In contrast to the psychostimulant drugs amphetamine and nicotine, the selective dopamine transporter inhibitor GBR 12909 does not directly change cortical neurotransmission due to very low expression of the dopamine transporter in the cortex (Freed et al., 1995; Sesack et al., 1998). However, it should be mentioned that these findings might also be related to the distinct pharmacodynamic profiles of amphetamine, nicotine, and GBR 12909, and particularly the way they activate the dopamine system (for discussion see Wiskerke et al., 2012). Taken together, the available data indicate that endocannabinoid signaling negatively and rate dependently regulates action restraint. Although more research is warranted, the underlying mechanisms might involve modulation of neurotransmission in brain regions such as the medial prefrontal cortex and the ventral tegmental area.

Delay-based decision-making in humans is best tested in a DDT (Rachlin et al., 1991; Pietras et al., 2003). In this task, subjects are asked to make choices between a small (monetary) reward that they can obtain immediately and a larger reward that will be obtained after some delay. Following a series of such choices, a delay discounting curve can be generated as a measure of impulsive decision-making (Mazur, 1987). In the rodent DDT (Figure 14.4A,B; Evenden and Ryan, 1996; Cardinal et al., 2000, 2006), rodents will similarly display a decrease in preference for the larger food reward over increasing delays. Data on cannabinoid modulation of impulsive decision-making to date remain rather inconclusive. CB₁ receptor antagonists (SR141716A and O-2050) have consistently been found not to affect baseline delay discounting in either a DDT or an adjusting-delay procedure (Pattij et al., 2007a; Wiskerke et al., 2011; Boomhower et al., 2013). Of note, however, (genotype-related) individual differences in the behavioral response to CB₁ receptor antagonist treatment might exist (Boomhower et al., 2013). Similarly to human studies (see above), the reported effects of CB₁ agonists on delay-based decision-making are rather inconsistent. In an initial study, WIN 55,212-2 was found not to affect impulsive decision-making (Pattij et al., 2007a). Subsequently, however, Δ^9 -THC was in one study found to reduce impulsive decision-making in a CB₁ receptor-dependent way (Figure 14.4C; Wiskerke et al., 2011), whereas in a different study this CB₁ receptor agonist seemed to increase impulsive decision-making (Tanno et al., 2014). Differences in the strain of rats studied (Chen et al., 1991), the experimental delay discounting task employed (Cardinal et al., 2000), and/or chemical structures and pharmacological profiles of the administered CB₁ receptor agonists administered (Pertwee, 2010; Pertwee et al., 2010) could explain these observed inconsistencies. It would therefore be interesting to investigate the effects of other CB₁ receptor agonists on DDT behavior, while taking into account the effects that varying different task parameters (levers vs. nose poke operanda, delay signaling, reward size) may have on (drug-induced) delay discounting. Perhaps first in line for such

**FIGURE 14.4**

Cannabinoid effects in the delay discounting task (DDT). The DDT can be performed in a 5-CSRTT operant chamber (see Figure 14.2). (A) Following an initial nose poke in the central aperture in the left wall, rats choose between a small immediate, or a larger delayed food reward by making a nose poke in one of two adjacent apertures. The delay for the larger reward increases over the course of a session. (B) As a result, rats gradually lose interest in the larger reward, similar to what is observed in humans performing a DDT. Importantly, by adjusting the duration of the inter-trial-interval (ITI), the total length of each trial is the same, irrespective of whether the small or large reward was chosen. (C) Data (mean $\pm$ SEM) adapted from Wiskerke et al. (2011), showing that acute administration of Δ^9 -THC reduces impulsive decision-making, an effect that can be blocked by administration of the CB1 receptor antagonist SR141716A. Of note, employing a similar DDT, the CB1 receptor agonist WIN 55,212-2 was previously found not to affect impulsive decision-making in rats (Pattij et al., 2007a), whereas Δ^9 -THC in a different DDT has been suggested to increase impulsive decision-making (Tanno et al., 2014). Drug dosages are in mg/kg. * $p < 0.05$ versus vehicle and # $p < 0.05$ versus THC alone.

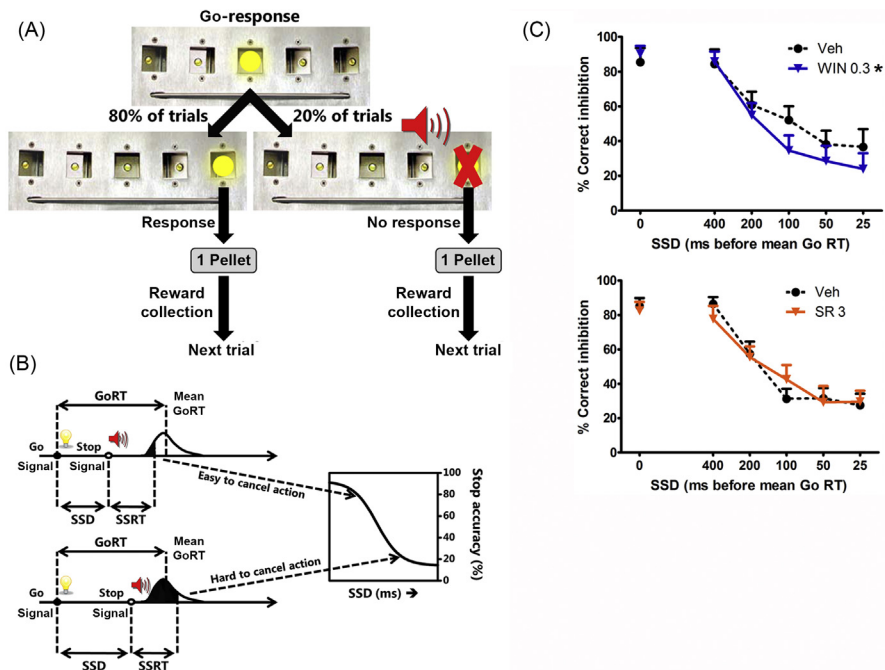
experiments should be exogenous administration of the endocannabinoid anandamide, since anandamide resembles Δ^9 -THC in being a partial CB₁ receptor agonist (Sugiura et al., 2002) and ADHD patients have been suggested to have impaired anandamide degradation as compared to healthy control subjects (Centonze et al., 2009).

In view of this latter finding, it is interesting that we showed that amphetamine-induced reductions in impulsive decision-making critically depend on CB₁ receptor activation (Wiskerke et al., 2011). Low-dose amphetamine (Adderall®) is one of the primary prescription drugs to treat maladaptive impulsivity, and at least its effects on action restraint and impulsive decision-making are known to critically depend on incremented dopamine release in the brain (Cole and Robbins, 1987; Winstanley et al., 2003; Van Gaalen et al., 2006a,b). Thus, our findings may have clinical implications in that targeted CB₁ receptor activation could be used to alleviate problems related to impulsive decision-making. Moreover, they also suggest that the endocannabinoid and dopamine neurotransmitter systems may interact to regulate impulsive decision-making. Accordingly, CB₁ receptor antagonist administration was recently demonstrated to prevent and reverse (1) impulsive decision-making induced by repeated cocaine injections, and (2) cocaine-induced perturbations in decision-making-related dopamine transients in the nucleus accumbens (Hernandez et al., 2013).

Finally, not much is known about endocannabinoid modulation of action cancellation and reflection impulsivity in rodents. The translational model for measuring action cancellation (Figure 14.5a,b), the SST (Eagle and Robbins, 2003), has perhaps the highest validity of all impulsivity models since it is almost identical to the human SST (Logan et al., 1984). Accordingly, akin to human studies with Δ^9 -THC, the CB₁ receptor agonist WIN 55,212-2 at low doses has been found to induce mild impairments in the rats' ability to cancel responses once initiated (Figure 14.5C; Pattij et al., 2007a). In the same study, acute administration of the CB₁ receptor antagonist SR141716A was found not to affect action cancellation. Although there is a rodent task to assay reflection impulsivity (uncertain visual discrimination test; Evenden, 1999), which is based on human paradigms such as the information sampling task (IST; Clark et al., 2006), the effects of cannabinoid ligands on reflection impulsivity have yet to be assessed.

CONCLUDING REMARKS

In this chapter we have reviewed the available evidence investigating (endo)cannabinoid involvement in impulsive behavior. Derived from human studies conducted in cannabis users or employing Δ^9 -THC challenges in healthy volunteers, the picture emerges that the (endo)cannabinoid system primarily modulates aspects of impulsive action. This notion is further strengthened and extended by preclinical work in rats and mice using pharmacological approaches with Δ^9 -THC and several other more selective cannabinoid ligands. Although it remains to be further substantiated, available evidence suggests that particularly CB₁ receptor activity in the prefrontal cortex and/or the ventral tegmental area may be relevant for the regulation of impulsive action. In these brain areas, cannabinoid signaling can modulate the activity of other neurotransmitter systems, including the dopamine, glutamate, and GABA neurotransmitter systems.

**FIGURE 14.5**

Cannabinoid effects in the Stop signal reaction time task (SST). The SST can be performed in a 5-CSRTT operant chamber (see Figure 14.2). (A) Each trial starts with a Go response (response in the middle of five apertures in the left wall). In 80% of the trials (Go trials) a light cue (Go stimulus) will be presented in the far right aperture and fast responding in that aperture will result in delivery of a food reward. In 20% of trials (Stop trials), however, a tone (Stop signal) is presented following the onset of the Go stimulus, with a variable delay (Stop signal delay, SSD) between the presentation of both cues. In these trials, successful inhibition of responding will be rewarded with a food reward. (B) Illustration of the theoretical race model thought to underlie SST performance (see, for example, Logan et al., 1984; Eagle and Robbins, 2003). The bell-shaped curves depict the distribution of the Go reaction times (GoRT) of a subject, with a vertical line to indicate the mean and the black filled area indicating the percentage of responses that will be executed. SSRT indicates the Stop signal reaction time of that subject. The longer the delay (SSD) between presentation of the Go stimulus and the Stop signal, the harder it is to inhibit a response. (C) Data (mean $\pm$ SEM) adapted from Pattij et al. (2007a), depicting impaired action cancellation (reduced % correct inhibition) following administration of the CB1 receptor agonist WIN 55,212-2 (top) but not the CB1 receptor antagonist SR141716A (bottom). Drug dosages are in mg/kg. * $p < 0.05$ compared to vehicle.

One important point to make here is that most of these studies have primarily focused on the role of cannabinoid CB₁ receptors in impulsivity. Recent work has demonstrated the presence of functional cannabinoid CB₂ receptors in the brain (Onaivi et al., 2008; Morgan et al., 2009), which were found to play a role in motivated behavior and dopamine transmission in the ventral striatum (Onaivi et al., 2008; Xi et al., 2011). Moreover, cannabinoid CB₂ receptors in the medial prefrontal cortex were found to control neuronal excitability in this brain region (den Boon et al., 2012). Given the importance of frontocortico-striatal activity in different modalities of impulsivity (Figure 14.1; Pattij and Vanderschuren, 2008; Dalley et al., 2011), it is possible that cannabinoid CB₂ receptors play a functional role in modulating impulsive behavior. Indeed, preliminary data obtained in mice have shown that the cannabinoid CB₂ receptor agonist JWH133 appeared to promote self-controlled choice in a between-session DDT (Navarrete et al., 2012). In view of these initial observations, it would be highly worthwhile to conduct pharmacological experiments with cannabinoid CB₂ receptor ligands in rats to substantiate these findings and to further unravel the involvement of cannabinoid CB₂ receptors in different modalities of impulsivity.

Finally, the development of adequate positron emission topography (PET) ligands for imaging of CB₁ receptors in humans and rodents, as well as the ongoing rapid improvements in pharmacological tools to more selectively manipulate endocannabinoid synthesis and hydrolysis, will certainly aid future research. Considering the evidence discussed in this chapter, the endocannabinoid system emerges as an interesting pharmacotherapeutic target to ameliorate impulsivity in psychopathology and neurological disorders, in particular when impulsive actions have gone awry.

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Cannabinoids and obsessive-compulsive disorder

15

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INTRODUCTION

Obsessive-compulsive disorder (OCD) is a neuropsychiatric condition that affects 2% to 3% of the population (Jenike, 2004). It has a chronic and fluctuating course and tends to be underdiagnosed (Jenike, 2004). OCD usually starts at the age of 22 to 36 years (OCD onset at ages above 35 years occurs only in 15% of patients) and is characterized by persistent, intrusive thoughts (obsessions) and repetitive ritualistic behaviors (compulsions) that cause marked distress and interfere with daily functioning (American Psychiatric Association (APA), 2000). Failure to perform a particular ritual can result in severe anxiety (Jenike, 2004; Wu et al., 2012).

The present therapeutic options can control but not completely eliminate OCD symptoms (Jenike, 2004; Simpson, 2010), with 25–40% of patients being refractory to treatment (Kessler et al., 2005; Abudy et al., 2011). The two main treatments are cognitive-behavioral therapy (Olatunji et al., 2013) and pharmacotherapy (Fineberg et al., 2013). The creation of neurosurgical lesions of structures in the basal ganglia-thalamo-cortical circuit can be attempted for severe OCD. Deep brain stimulation of the ventral striatum, subthalamic nucleus, and thalamo reticular and inferior thalamic peduncles has also been recently approved for refractory cases (Albelda and Joel, 2012; Heeramun-Aubeeluck and Lu, 2013).

Randomized controlled clinical trials have clearly established the serotonin uptake inhibitors as the main monotherapy for OCD (Simpson, 2010). They include the tricyclic antidepressant clomipramine and the serotonin selective reuptake inhibitors (SSRIs). Although the effectiveness of these drugs suggests that OCD involves abnormalities in the serotonin system (see below), the mechanisms of their therapeutic effects in this disorder remain poorly understood (Simpson, 2010).

NEUROBIOLOGY OF OCD

GENETIC AND OTHER PREDISPOSING FACTORS

Twin studies reveal a higher concordance of OCD in monozygotic (80–87%) than in dizygotic twins (47–50%) (Van Grootheest et al., 2005). Family studies have also indicated that the risk in first-degree relatives is 3–12 times greater than in the general population (Do Rosario-Campos et al., 2005; Hanna et al., 2005). Despite intensive efforts, no common alleles have been directly linked to the pathogenesis of OCD, which is likely to reflect a complex model of genetic susceptibility.

Autoimmune factors have also been postulated to play a role in the pathogenesis of OCD, particularly in a set of childhood neuropsychiatric disorders known as Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS) (Murphy et al., 2012). Swedo et al. (1998) first described a detailed case series of 50 children with OCD temporally related to streptococcal infections. The mechanisms of this association, however, are still unclear.

BRAIN STRUCTURES INVOLVED

The use of non-invasive neuroimaging methods to investigate abnormalities in brains of OCD patients have produced extensively replicated findings indicating the involvement of the cortico-striato-thalamo-cortical (CSTC) circuit in this disorder (Saxena and Rauch, 2000). Increased glucose metabolism and blood flow (detected at pretreatment baseline or during symptom provocation) have been consistently reported in one or more of the following regions: orbitofrontal and anterior cingulate cortices, caudate, and thalamus (Saxena and Rauch, 2000; Whiteside et al., 2004; Maia et al., 2008; Menzies et al., 2008). These regions are main components of the CSTC circuit (Saxena and Rauch, 2000). Effective pharmacotherapy or behavioral therapy normalizes activity in this circuit in OCD patients (Baxter et al., 1992; Saxena et al., 1999; Hansen et al., 2002).

Cortical neurons send dense projections to the striatum and establish glutamatergic synapses onto medium spiny neurons (MSNs). In turn, GABAergic MSNs connect to the major output structures of the basal ganglia, the globus pallidus pars internalis (GPi) and substantia nigra pars reticulata (SNr), by way of two anatomically distinct pathways thought to have opposing roles. Dopamine receptor type 1 expressing MSNs in the striatum compose the “direct pathway” (striato-nigral), whereas dopamine receptor type 2 expressing MSNs in the striatum compose the “indirect pathway” (striatopallidal) (Graybiel, 2000). The direct pathway axons terminate in the GPi/SNr, whereas the indirect pathway comprises a more complex relay, successively involving the globus pallidus pars externalis (GPe) and subthalamic nucleus (STN). In a simplified model, the output structures of the basal ganglia provide inhibitory tone to the thalamus, which in turn is modulated by the balance between activity of the direct and indirect pathways (Graybiel, 2000). A shift of this balance favoring activity in the direct pathway

will “disinhibit” the thalamus and thereby promote the selection of behavioral sequences. Conversely, a shift favoring activity in the indirect pathway has a net effect of reinforcing or strengthening the inhibitory tone to the thalamus and thereby inhibits the selection of behavioral sequences. The thalamus sends excitatory inputs back to the cortex, thereby completing the CSTC loop (Saxena and Rauch, 2000). The direct pathway is usually associated with facilitating motor behavior whereas activity of the indirect pathway more likely results in motor inhibition (Graybiel, 2000). It has been hypothesized that overactivity of the direct pathway or underactivity of the indirect pathway leads to OCD. Although highly simplified, this model provides a useful framework for understanding CSTC circuit dysfunction in OCD.

Other brain circuits are also likely to play a role in OCD. For example, there is evidence for involvement of the limbic system with respect to its anxiety component. Altered amygdala volume and dysfunctional amygdala activation have been reported in these patients (Szeszko et al., 1999, 2004). In addition, recent studies also suggest that the cerebellum is involved in the pathogenesis of OCD (Hou et al., 2012).

ANIMAL MODELS

Given that the cardinal symptoms of OCD involve intrusive and unwanted obsessional thoughts about uniquely human topics (e.g., being responsible for harm or mistakes, religion, morality, fear of contamination) and compulsive rituals are aimed at neutralizing these obsessions, it is difficult to conceive a true animal model of this condition (Abramowitz et al., 2011). Despite this limitation, several animal models of OCD have been developed based on ethological, pharmacological, and genetic approaches (for review see Pitman, 1989; Korff and Harvey, 2006; Joel, 2006a; Boulougouris et al., 2009; Albelda and Joel, 2012). Although a complete review of these models is out of the scope of the present chapter, two have been employed in the investigation of the effects of cannabinoids in OCD: the marble-burying test (MBT) and quinpirole-induced checking behavior.

The MBT was initially proposed as an animal model aimed at detecting possible anxiolytic drug effects in mice (Njung'e and Handley, 1991). However, subsequent studies showed that, contrary to most anxiety tests based on exploratory behavior, repeated exposure to the marbles does not cause behavioral habituation. This led to the proposal that this test, instead of measuring novelty-induced anxiety, is rather evaluating a natural, repetitive behavior that can become compulsive (Njung'e and Handley, 1991; Thomas et al., 2009). Recently, Thomas and co-workers (2009) performed a comprehensive analysis of the MBT under different conditions and found no correlation between the number of buried marbles and anxiety-related measures in the light-dark or open field tests. They also failed to find any association between marble-burying behavior and familiarity with the test or the burying material (Thomas et al., 2009). Moreover, because the marbles are non-reactive, they cannot provide the animal with the necessary stimuli to a natural ending of the investigation, and this “frustrated” investigation leads to

compulsive burying. This suggestion is in line with the view that compulsive behaviors result from an inability to achieve a sense of task completion (Szechtman and Woody, 2004). Based on these pieces of evidence, the MBT was proposed as a useful animal model to investigate repetitive responses involved in OCD (Korff and Harvey, 2006; Thomas et al., 2009; Greene-Schloesser et al., 2011). Another argument favoring the proposal that the MBT reflects compulsive-related behavior is the development of tolerance after repeated treatment with classical anxiolytic compounds such as diazepam (Ichimaru et al., 1995; Casarotto et al., 2010). The MBT is the most straightforward and cost-effective procedure of all current animal models of OCD.

Rats treated chronically with the dopamine D₂/D₃ receptor agonist quinpirole (0.5 mg/kg twice weekly for 5 weeks) develop locomotor sensitization (Einat and Szechtman, 1993; Culver et al., 2000) and exhibit compulsive checking of specific places in an open field arena, suggesting that this procedure could be an animal model of OCD (Szechtman et al., 1998). This proposal is based on three lines of evidence. First, the behavior of quinpirole-treated rats is similar to OCD-associated excessive checking. Second, this latter activity is an amplified form of normal checking in the rat, similar to what have been described in OCD patients where compulsive checking is an exaggerated form of normal behavior related to one's well-being and security (Szechtman et al., 2001). Finally, clomipramine can partially attenuate quinpirole-induced compulsive checking (Szechtman et al., 1998).

Other behavior/pharmacologically-based OCD models are as follows. (1) The signal attenuation model, where there is an excessive, "compulsive," lever-pressing behavior that is not accompanied by an attempt to collect a reward in subsequent extinction trials after attenuation of a signal indicating that a lever-press response is effective in producing a reward (Joel, 2006b; Friedlander and Desrocher, 2006; Joel and Klavir, 2006). (2) Schedule-induced polydipsia, observed in food-deprived rats exposed to a procedure in which food is delivered intermittently (Woods et al., 1993). (3) The 5-choice serial reaction time task (5-CSRTT) where the animal is tested in an apparatus containing five food magazines. In each trial a certain magazine is cued by a light stimulus in a random order and the animal learns to collect a food reward from the cued magazine. Perseveration in this task is defined as repeated responses to a specific magazine after it has been rewarded (Robbins, 2002). In male rats, lesions of the orbitofrontal cortex or medial striatum increase perseverative responses in the 5-CSRTT (Rogers et al., 2001; Chudasama et al., 2003). (4) Spontaneous alternation behavior, which refers to the natural tendency of rats to explore novel places sequentially and in succession (Montgomery, 1952; Yadin et al., 1991). An increased tendency to repeat a choice of the same goal arm appears analogous to the repetitive motor patterns seen in OCD patients (Whitaker-Azmitia et al., 1990; Yadin et al., 1991). (5) Acral lick dermatitis, a disorder seen in a variety of mammalian species, in particular in large breed canines. It responds to SSRIs and is characterized by excessive licking or biting of the extremities, which leads to localized alopecia and subsequent granulomatous lesions (Stein et al., 1992).

In addition to these behavioral tests, several OCD models involving genetically-modified mice have also been proposed. These models have not been planned on the basis of known mutations in humans but are largely based on face validity (behavioral similarity). They include the *Hoxb8* mutant and genetic manipulations of dopamine, serotonin, and glutamate function. *Hoxb8* is expressed in the orbital cortex, the anterior cingulate, the striatum, and the limbic system, all of which are implicated in the pathophysiology of OCD. Mice with mutations in this gene groomed excessively to the point of hair removal and skin lesions (Greer and Capecchi, 2002).

In terms of genetic manipulations of neurotransmitters, boosting D₁ receptor function in the pyriform cortex and amygdala produces perseveration and repetitive jumping behavior in D1CT-7 mice, probably mediated via striatal mechanisms (Campbell et al., 1999). Knockdown of the dopamine transporter (DAT) produces “sequential super-stereotypy” in mice with the perseverative performance of quite complex chains of grooming behavior (Berridge et al., 2005). A knockdown of the serotonin 5-HT_{2C} receptor leads similarly from perseverative “head-dipping” or the excessively orderly chewing of screen material (Chou-Green et al., 2003), to a compulsive behavior accompanied by other OCD-like responses such as stereotypic locomotion and excessive self-aggressive grooming.

Welch and colleagues (2007) found that at the age of 4–6 months *Sapap3* knockout mice show excessive self-grooming with no sign of peripheral cutaneous defects, as well as increased anxiety-like behaviors in several tests, with no change in general activity. Excessive grooming and increased anxiety-like behaviors were reduced following repeated injections (for 6 days) of the SSRI fluoxetine, supporting the relevance of these abnormal behaviors to OCD (Welch et al., 2007). SAP90/PSD95-associated protein 3 (SAPAP3) is expressed mainly in the striatum and is responsible for synaptic scaffolding and migration of glutamate nerve cells from the caudate to the orbitofrontal cortex. *Sapap3* knockout mice had specific defects in the structure of the postsynaptic complex of cortico-striatal synapses. Specifically, these mice exhibited reduced cortico-striatal synaptic transmission and defects in the functioning of NMDA and AMPA glutamate receptors (Welch et al., 2007). Another experiment found that *Sapap3* knockout mice that received intra-striatal injection of lentivirus expressing SAPAP3 showed less grooming and anxiety-like behavior compared with non-treated *Sapap3* knockout mice, demonstrating that loss of SAPAP3 in the striatum was critical for the development of these behaviors, and suggesting that the altered functioning of the glutamatergic striatal system is involved in producing excessive grooming and anxiety-like behaviors (Welch et al., 2007). Further experimentation showed that variation within the human *Sapap3* gene was associated with grooming disorders (pathologic nail biting, pathologic skin picking, and trichotillomania), suggesting that *Sapap3* is a promising functional candidate gene for human grooming disorders (Bienvenu et al., 2009). Additionally, a recent study in *Slitrk5* knockout mice (Shmelkov et al., 2010) demonstrated that loss of the neuron-specific transmembrane protein SLIT and NTRK-like

protein-5 (*Slitrk5*) leads to OCD-like behaviors, which manifests as excessive self-grooming, increased marble burying, and increased anxiety-like behaviors that are alleviated by the SSRI fluoxetine, supporting the relevance of these behaviors to OCD. *Slitrk5* knockout mice showed a selective overactivation of the orbitofrontal cortex, as well as abnormalities in striatal anatomy and cell morphology and alterations in glutamate receptor composition, which contribute to deficient cortico-striatal neurotransmission.

NEUROCHEMICAL CHANGES ASSOCIATED WITH OCD

Neurochemical changes of specific neurotransmitters, including serotonin, dopamine, GABA, and glutamate, have been associated with OCD by a large number of studies. The main findings are summarized in [Table 15.1](#).

SEROTONIN (5-HT)

Several studies support the hypothesis that the 5-HT system has a crucial role in OCD. First, long-term administration of 5-HT reuptake inhibitors (SRIs) is more effective than noradrenergic reuptake inhibitors in the treatment of this disorder. Early reports that clomipramine, a tricyclic antidepressant SRI, was effective in alleviating obsessions and compulsions in patients led to the hypothesis that dysregulation of the 5-HT system is involved in the pathophysiology of OCD ([Zohar and Insel, 1987](#); [Zohar et al., 1992](#)). Indeed, all SSRIs studied so far have been found to be effective for OCD treatment. Second, acute administration of 5-HT agonists, such as m-chlorophenylpiperazine (mCPP), exacerbates OCD symptoms ([Gross-Isseroff et al., 2004](#)). In addition, there is a positive correlation between clinical improvement and drug-induced decrease in 5-hydroxyindoleacetic acid (5-HIAA) levels, the major metabolite of 5-HT, in cerebrospinal fluid (CSF) ([Thoren et al., 1980](#); [Swedo et al., 1992](#)). Although there are contradictory results ([Leckman et al., 1995](#)), Insel and colleagues found higher CSF 5-HIAA levels in OCD patients than in controls ([Insel et al., 1985](#)), possibly reflecting increased brain 5-HT turnover. Furthermore, several studies have suggested an association between OCD and changes in the density of 5-HT transporter (5-HTT) as well as 5-HT receptors. For example, Pogarell and colleagues reported elevated 5-HTT density in the midbrain of OCD patients ([Pogarell et al., 2003](#)). In contrast to this finding, Hesse and colleagues reported a reduced 5-HTT density in the midbrain and thalamus of OCD patients with a negative correlation between 5-HTT density and symptom severity ([Hesse et al., 2005](#)). A reduced 5-HTT density in OCD patients has also been described by [Stengler-Wenzke et al. \(2004\)](#). Additionally, Adams and colleagues observed that 5-HT_{2A} binding in the caudate nucleus of untreated OCD patients was significantly higher compared to

Table 15.1 Neurochemical Changes in OCD Patients

Neurotransmitter	Changes in OCD	Method (Number of Patients)	Reference
Serotonin	↑ 5-HIAA levels (CSF)	HPLC (8 unmedicated adults with OCD vs. 23 controls)	Insel et al., 1985
	= 5-HIAA levels (CSF)	HPLC (33 unmedicated adults with OCD vs. 49 controls)	Leckman et al., 1995
Dopamine	= 5-HVA levels (plasma)	HPLC (13 unmedicated adults with OCD vs. 29 controls)	Benkelfat et al., 1991
	= 5-HVA levels (CSF)	HPLC (43 adolescents with OCD)	Swedo et al., 1992
Glutamate	↑ glutamate levels (CSF)	HPLC (21 unmedicated children with OCD vs. 18 controls)	Chakrabarty et al., 2005
	↑ glutamate levels (CSF)	HPLC (23 unmedicated adolescents with OCD vs. 23 controls)	Bhattacharyya et al., 2009
	↑ Glx levels (C)	¹ H MRS (11 unmedicated children with OCD vs. 11 controls)	Rosenberg et al., 2000
	↑ Glx levels (OFC)	¹ H MRS (18 unmedicated adults with OCD vs. 17 controls)	Whiteside et al., 2006
	↓ Glx levels (ACC)	¹ H MRS (14 unmedicated children with OCD vs. 14 controls)	Rosenberg et al., 2004
GABA	↓ GABA levels (mPFC) = GABA levels (dlPFC)	¹ H MRS (24 unmedicated adults with OCD vs. 22 controls)	Simpson et al., 2012

5-HIAA: 5-hydroxyindoleacetic acid, ACC: anterior cingulate cortex, C: caudate nucleus, CSF: cerebrospinal fluid, dlPFC: dorsolateral prefrontal cortex, Glx: a marker for combined glutamate and glutamine, ¹H MRS: proton magnetic resonance spectroscopy, HPLC: high-performance liquid chromatography, mPFC: medial prefrontal cortex, OCD: obsessive-compulsive disorder, OFC: orbitofrontal cortex.

controls (Adams et al., 2005). These pieces of evidence support the proposal that 5-HT plays a role in the pathogenesis of OCD. However, the current data do not converge into a coherent picture regarding the specific dysfunction of the 5-HT system in this disorder or the mechanisms by which SSRIs exert their therapeutic effect. Regarding the latter, it has been hypothesized that these drugs attenuate OCD symptoms by desensitization of presynaptic terminal 5-HT_{1A} and 5-HT_{1D} receptors (Bergqvist et al., 1999; El Mansari and Blier, 2006), resulting in an increase in serotonergic activity in subcortical regions of the brain, particularly the CSTC circuit (Korff and Harvey, 2006).

DOPAMINE

Dopamine may also be implicated in the pathophysiology of OCD. The combination of dopamine antagonists, such as haloperidol or risperidone, and SSRIs can be effective in refractory patients (McDougle et al., 1994, 2000), whereas dopamine agonists and dopamine reuptake inhibitors exacerbate symptoms in some OCD patients (Stein, 2002).

The neurochemical studies on the role of dopamine metabolites in OCD patients have yielded no evidence of abnormal dopamine function in this disorder (Benkelfat et al., 1991; Swedo et al., 1992). However, neuroimaging studies show higher density of the dopamine transporter (DAT) along with a down-regulation of the D₂ receptors in the basal ganglia of OCD patients (Denys et al., 2004, 2013; Perani et al., 2008). Higher DAT density, together with down-regulation of the D₂ receptor, suggests higher synaptic concentrations of dopamine in the basal ganglia. A possible increase in dopamine neurotransmission in the basal ganglia is in agreement with various working pathophysiology hypotheses of OCD such as the hyperactive CSTC circuitry. This model suggests an imbalance between the direct and indirect pathways that produces a hyperactive circuit responsible for the repetitive behaviors (Saxena and Rauch, 2000). Since D₁ preferentially activates the direct and D₂ the indirect pathway, and the density of D₁ in the basal ganglia is higher than that of D₂ receptors, increased concentrations of dopamine are most likely to result in a dominant D₁-regulated direct circuit, and consequently in a hyperactive cortico-striatal system.

GLUTAMATE

Frontocortical hyperglutamatergic dysfunction is suggested to underlie the CSTC circuitry abnormalities observed in imaging studies of OCD patients (Pittenger et al., 2011; Wu et al., 2012). Magnetic resonance spectroscopy studies of these patients show an association between symptom severity and the level of glutamate metabolites measured in various brain regions implicated in OCD, including the caudate nucleus, the orbitofrontal cortex, and the cingulate cortex (Rosenberg et al., 2000, 2004; Whiteside et al., 2006; Starck et al., 2008). Patients with OCD present increased glutamate levels in CSF compared to healthy subjects (Chakrabarty et al., 2005; Bhattacharyya et al., 2009). Moreover, both preclinical and clinical studies suggest that drugs that attenuate glutamate neurotransmission such as riluzole and memantine could be helpful in the treatment of OCD patients (Grant et al., 2007; Aboujaoude et al., 2009) and are effective in animal models of this disorder (Egashira et al., 2008; Iijima et al., 2010). Finally, several independent genetic studies have consistently implicated the *SLC1A1* gene, which encodes the neuronal glutamate transporter excitatory amino acid carrier 1 (EAAC1), in OCD (Arnold et al., 2006; Dickel et al., 2006; Stewart et al., 2007; Liang et al., 2008). No other OCD candidate gene has been associated with this disorder at this level of supportive evidence.

However, there are also contradictory results regarding the possible involvement of glutamate in this disorder. For example, a decrease in anterior cingulate cortex glutamate concentration was found in OCD patients (Arnold et al., 2009), and in transgenic models an anti-glutamatergic drug exacerbated OCD-associated behaviors (McGrath et al., 2000). Taken together, these data suggest that OCD could involve, rather than just generally increase, a dysregulation of glutamatergic neurotransmission in specific brain areas.

GABA

GABA modulates cortical glutamatergic neurons (Gonzalez-Burgos and Lewis, 2008) and there is evidence of abnormalities in cortical inhibitory processes in OCD patients (Greenberg et al., 2000; Richter et al., 2012). Simpson et al. (2012), using proton magnetic resonance spectroscopy, observed a decrease in GABA levels in the medial prefrontal cortex of OCD patients, suggesting a cortical inhibitory dysfunction (Greenberg et al., 2000; Maia et al., 2008; Pittenger et al., 2011; Richter et al., 2012; Wu et al., 2012). Reduced medial prefrontal cortex inhibitory tone could generate abnormal striatal activation either directly, due to anterior cingulate cortex projections to the striatum, or indirectly, due to anterior cingulate cortex projections to the orbitofrontal cortex (Price and Drevets, 2010).

OTHERS

Neuropeptides and monoaminergic systems interact in several brain regions. Elevated CSF levels of vasopressin, oxytocin, and somatostatin have been reported in patients with OCD (McDougle et al., 1999). In addition, these patients exhibit an increased growth hormone response after the administration of the acetylcholinesterase inhibitor pyridostigmine, providing evidence of cholinergic hypersensitivity and suggesting the involvement of the cholinergic system in OCD (Lucey et al., 1993). There are also data suggesting the involvement of nitric oxide (NO) in this disorder. Atmaca et al. (2005) found that OCD patients have higher plasma NO levels compared to healthy subjects and that these levels are positively correlated with the severity of OC symptoms (Atmaca et al., 2005). However, the functional significance of these findings is not yet known.

ENDOCANNABINOID SYSTEM IN OCD

LOCALIZATION OF CB₁ RECEPTOR IN THE CSTC CIRCUITRY

As discussed above, OCD has been associated with dysfunctions of the CSTC circuitry, a set of multiple reverberatory loops that embraces the key node structures regulating goal-directed and repetitive behaviors (Langen et al., 2011a,b). Cannabinoid CB₁ receptors are present in regions that participate in this circuitry,

such as the prefrontal and anterior cingulate cortices, striatum, and substantia nigra (Harkany et al., 2007; Diaz-Alonso et al., 2012). At least in the striatum these receptors are not homogeneously distributed, being more concentrated in the areas involved in sensory motor aspects (Van Waes et al., 2012). Cannabinoid CB₁ receptor expression is also high in other components of the basal ganglia, such as the substantia nigra and subthalamic nuclei (especially globus pallidus). It is, however, very low in the thalamus (Herkenham et al., 1991a,b; Matsuda et al., 1993).

Although present in the prefrontal cortex, CB₁ receptor expression is usually lower than in the basal ganglia (Herkenham et al., 1991b; Mailleux and Vanderhaeghen, 1992; Matsuda et al., 1993; Uchigashima et al., 2007; Heng et al., 2011). Interestingly, striatal regions with high CB₁ receptor expression tend to receive inputs from cortical areas with low expression of this receptor. Similarly, the associative/limbic areas of the striatum (with a relative lower expression of CB₁ receptor) receive inputs from the medial prefrontal cortex, which shows higher CB₁ receptor levels (Van Waes et al., 2012).

ROLE OF THE ENDOCANNABINOID SYSTEM IN THE CORTICO-STRIATAL-THALAMIC-CORTICAL CIRCUITRY

Endocannabinoids (eCBs) interact with glutamate, GABA, dopamine, and serotonin, the main neurotransmitters present in the CSTC circuitry. Even if their precise role in this circuitry is not fully understood, several pieces of evidence reviewed above (Table 15.1) suggest the presence of increased glutamatergic activity in cortical and striatal areas of OCD patients.

As discussed elsewhere in this book, CB₁ receptor activation decreases neurotransmitter release via G_{i/o} and modulation of Ca⁺⁺ or K⁺ channels and regulates short- and long-term synaptic plasticity (for review see Chevalleyre et al., 2006). Due to these effects, eCBs could normalize a possible excessive glutamatergic neurotransmission in the CSTC system of OCD patients. Corroborating this possibility, in the dorsal striatum, CB₁ receptors inhibit glutamate release and are necessary for the induction of long-term depression (LTD) of excitatory inputs to MSNs, an effect that also depends on concomitant D₂ receptor activation (Chevalleyre et al., 2006). LTD of GABAergic synapses in the striatum is equally mediated by eCBs (Adermark et al., 2009).

Serotonin neurotransmission is also regulated by eCBs. They reduce 5-HT release via CB₁ receptors and control the function of several 5-HT receptors (for review see Haj-Dahmane and Shen, 2011). In addition, eCBs modulate neuronal activity in the dorsal raphe nucleus, a major source of prosencephalic 5-HT innervation that projects to striatal, cortical, and limbic regions. CB₁ receptor agonists increase the firing rate of these neurons, probably via an indirect effect mediated by CB₁ receptor activation in the prefrontal cortex (Bambico et al., 2007).

The endocannabinoid and dopaminergic systems control each other by several mechanisms (van der Stelt and Di Marzo, 2003; El Khoury et al., 2012). CB₁ receptors can also form heterodimers with dopamine D₂ receptors in both the dorsal and

ventral (including the nucleus accumbens) parts of the striatum (Marcellino et al., 2008). This CB₁–D₂ coupling reduces the affinity of D₂ agonists to the binding sites and the locomotor effects of quinpirole (Marcellino et al., 2008), a drug that causes repetitive checking, a proposed model of OCD (see above).

In addition to regulating CSTC activity, eCBs could also be involved in the development of this circuitry. Activation of CB₁ receptors during brain development regulates neuronal maturation, neurite growth, and cell integration as fully functional neurons. CB₁ receptor labeling starts at embryonic day 14 (E14) to 18 (E18) and is very intense during the development of the central nervous system, especially in cortical areas of the embryonic brain (Diaz-Alonso et al., 2012). This labeling dramatically decreases after birth (Van Waes et al., 2012). Anandamide treatment of primary cell cultures derived from the embryonic cortex arrests cell differentiation and blocks the neurite outgrowth (Rueda et al., 2002). The mechanisms responsible for these effects are complex, involving not only CB₁ and CB₂ receptors but also the ERK and mTOR pathways (Palazuelos et al., 2012). Furthermore, CB₁ receptors interact with the BDNF/TRKB system to regulate neuronal migration and maturation in cortical cells. Activation of these receptors by anandamide or 2-arachidonoylglycerol (2-AG) leads to their coupling to TRK receptors, favoring their phosphorylation (Berghuis et al., 2005; Dalton and Howlett, 2012). This coupling prevents the neuronal differentiation induced by neurotrophins such as BDNF (Berghuis et al., 2005).

THE EFFECTS OF DRUGS THAT ACT ON THE ENDOCANNABINOID SYSTEM IN OCD

CLINICAL DATA

There are scarce data investigating a possible anti-OCD effect of cannabinoids in humans. Dronabinol, a pharmaceutical formulation of delta-9-tetrahydrocannabinol (Δ^9 -THC), had an “add-on” effect to clomipramine (Schindler et al., 2008) in two treatment-resistant OCD patients (Table 15.2). Also suggesting a possible role of cannabinoids in OCD treatment, drugs that modify glutamate- or dopamine-mediated neurotransmission could improve OCD symptoms. As discussed above, components of the endocannabinoid system are present in the CSTC circuitry and can interfere in these neurotransmitters systems. In a double-blind trial, ketamine, an NMDA non-competitive receptor antagonist, showed positive effects in OCD patients (Rodriguez et al., 2013). In addition, a limited number of studies indicate that other anti-glutamatergic drugs, such as N-acetylcysteine (Lafleur et al., 2006; Afshar et al., 2012) and riluzole (Pittenger et al., 2006; Grant et al., 2007) could alleviate OCD symptoms.

In relation to dopamine, even if there are contradictory reports regarding the effects of antipsychotics in OCD (Schonfelder et al., 2011; Maher and Theodore, 2012), atypical antipsychotics are proposed to reduce OCD symptoms (for reviews see Gao et al., 2006; Maher and Theodore, 2012). When

Table 15.2 Effects of Drugs that Act on the Endocannabinoid System in OCD

	Drug	Mechanism	Measurement
Clinical studies			
Schindler et al., 2008	Dronabinol	Synthetic THC (CB1 agonist)	YBOCS (↓)
Preclinical studies			
Casarotto et al., 2010	Cannabidiol	FAAH inhibition? (effect prevented by CB1 antagonist)	MBT (↓ buried marbles). Effect blocked by CB1 antagonist
Deiana et al., 2012	Cannabidiol		MBT (↓)
Nardo et al., 2014	Cannabidiol		MBT (↓ mCPP-induced increase in buried marbles)
Gomes et al., 2011	WIN 55,212-2	CB1, CB2, TRPV1 agonist	MBT (↓)
	AM404	FAAH and anandamide uptake inhibition	
	URB597	FAAH inhibition	
Umathe et al., 2011	Anandamide	CB1, CB2, TRPV1 agonist	MBT (↓)
	AM404	FAAH and anandamide uptake inhibition	
	URB597	FAAH inhibition	
Kinsey et al., 2011	PF3845	FAAH inhibition	MBT (↓)
	JZL184	MAGL inhibition	
Umathe et al., 2012	Anandamide		MBT (↓ or ↑). Effect blocked by CB1 or TRPV1 antagonist, respectively

FAAH: fatty acid amide hydrolase, MAGL: monoacylglycerol lipase, MBT: marble burying test, YBOCS: Yale-Brown Obsessive-Compulsive Scale.

added to an SSRI, aripiprazole reduced symptoms in refractory OCD patients (Matsunaga et al., 2011). Quetiapine was also effective as “add on” to SSRIs in an open-label trial (Zhornitsky et al., 2011). These positive effects could involve a CB₁–D₂ coupling-induced reduction in D₂ affinity as discussed above (Marcellino et al., 2008).

PRECLINICAL STUDIES

Most studies investigating the effects of cannabinoids in OCD have employed the MBT (Table 15.2). Direct CB₁ receptor agonists such as WIN 55,212-2 and

anandamide (Umathe et al., 2011, 2012) as well as inhibitors of the enzymes that metabolize endocannabinoids, fatty acid amide hydrolase (FAAH) (Gomes et al., 2011) and monoacylglycerol lipase (MAGL) (Kinsey et al., 2011), decrease the number of buried marbles in this test.

Another compound found in the *Cannabis sativa* plant, cannabidiol (CBD), is also effective in this model (Casarotto et al., 2010; Deiana et al., 2012). CBD has a complex pharmacology, being able to interact with several receptors in the central nervous system, including GPR55, TRPV1, and 5-HT_{1A}. It has a low affinity for CB₁ or CB₂ receptors but can inhibit the anandamide hydrolyzing enzyme FAAH, indirectly facilitating endocannabinoid-mediated neurotransmission (for review see Campos et al., 2012). Although the acute anxiolytic action of CBD has been attributed to a facilitation of 5-HT_{1A}-mediated neurotransmission (Campos and Guimaraes, 2008; Resstel et al., 2009), some of its behavioral effects could depend on CB₁ receptor activation (Campos et al., 2013). Accordingly, CBD-induced decrease in the number of buried marbles by mice was not blocked by a 5-HT_{1A}-receptor antagonist but only by the CB₁ receptor antagonist AM251 (Casarotto et al., 2010). This result, in addition to supporting a possible role of the endocannabinoid system in OCD, also reinforces the proposal that the MBT does not primarily evaluate anxiolytic-related but rather repetitive OCD-related behaviors (Thomas et al., 2009). However, an indirect modulation of the 5-HT system by CBD, maybe by facilitating eCB-mediated neurotransmission, cannot be ruled out. Favoring this possibility, we have recently found that CBD blocks the pro-compulsive effect of mCPP (a non-selective 5HT-receptor agonist that exacerbates OCD symptoms in patients) in the MBT (Nardo et al., 2014).

Although the brain sites involved in the anti-OCD-like effects of CBD have not yet been investigated, a functional neuroimaging study in healthy subjects suggested that this drug decreases activity of the anterior cingulate cortex (Kowal et al., 2013), a region that, as discussed above, shows increased glucose metabolism and blood flow in OCD patients. CBD can also decrease forward connectivity between the anterior cingulate and the amygdala, an effect that has been associated with its anxiolytic actions (Fusar-Poli et al., 2010).

Cannabinoids usually produce “bell-shaped” dose–response curves. Part of this effect could depend on activation of ion channels named TRPV1, which facilitate, rather than inhibit, glutamate release in several brain regions, including the striatum (Musella et al., 2009; Casarotto et al., 2012; Moreira et al., 2012). Corroborating this proposal, Umathe and colleagues have recently reported that higher doses of anandamide increase the number of buried marbles in the MBT, inducing a pro-compulsive effect (Umathe et al., 2011, 2012). Capsazepine, a TRPV1 receptor antagonist, was able to restore the effectiveness of anandamide. In these studies, the CB₁ receptor antagonist AM251 blocked the positive effects of the SSRI fluoxetine, suggesting that the serotonergic and endocannabinoid systems interact to control marble burying behavior (Umathe et al., 2011, 2012).

CONCLUSIONS

As discussed above, the number of preclinical and clinical studies investigating the effects of cannabinoids in OCD is still small. However, although its neurobiology is not completely understood, several studies point to dysfunctions of the CSTC circuit in OCD. Cannabinoid receptors are significantly expressed in most parts of this circuitry and can modulate the release of key neurotransmitters related to this disorder, including glutamate, dopamine, GABA, and serotonin. Together, these pieces of evidence indicate that the endocannabinoid system could be an important target for new therapeutic approaches in OCD. Additional studies are clearly needed to investigate the role of these neurotransmitters in this disorder.

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The endocannabinoid system: Anorexia nervosa and binge eating disorder

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INTRODUCTION

Eating disorders (EDs) include a range of chronic and disabling psychiatric pathologies characterized by aberrant eating patterns or weight-control behavior and distorted body image. The psychological and biological factors underlying EDs are complex and not yet completely understood. The endocannabinoid system plays a modulatory role in many physiological functions, such as control of motor behavior, nociception, memory, learning, and reward, and is an important constituent of neuronal substrates involved in regulation of feeding and energy balance through central and peripheral mechanisms. The appetite-stimulating effect of the cannabinoids is, in part, arbitrated by the activation of hypothalamic and mesocorticolimbic cannabinoid type-1 receptors (CB1Rs) (Matias and Di Marzo, 2007). Yet, the contribution of peripheral CB1Rs in cannabinoid modulation of feeding cannot be ruled out (Gómez et al., 2002). It is therefore not surprising that dysregulation of CB1Rs or other components of the endocannabinoid system in these central pathways may provoke clinical traits present in ED patients (Marco et al., 2012; Scherma et al., 2014).

EATING DISORDERS

According to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM)-V (APA, 2013), EDs are considered psychiatric pathologies characterized by aberrant eating patterns or weight-control behavior and an abnormal attitude or perception of body image. These disorders represent a significant public health problem, affect 2–5% of the general population, typically have their onset in

adolescence, and are more common in women (Hudson et al., 2007). Moreover, they are usually comorbid with other psychiatric conditions, including depression, anxiety, and substance abuse disorders and tend to become chronic and disabling, with considerable tolls on physical health and increased risk of mortality (Kaye et al., 2004; Blinder et al., 2006). EDs include anorexia nervosa (AN), bulimia nervosa (BN), and binge eating disorder (BED).

AN is characterized by a lower than minimal normal body weight (APA, 2013). Extreme weight loss in AN is a consequence of rigid dietary restriction, often coupled with excessive exercise (Shroff et al., 2006). Even when severely underweight, AN patients maintain an intense fear of weight gain due to their disturbed perceptions of body shape and size (APA, 2013). AN is often accompanied by psychological illnesses, such as depression, anxiety, or obsessive-compulsive disorder (Attia, 2010). The prevalence of AN is between 0.3 and 0.6% and is associated with high relapse and mortality rates (Birmingham et al., 2005; Hoek, 2006).

BN is characterized by recurrent and distressing binge eating episodes, defined by the uncontrolled consumption of a large amount of food (overeating) in a brief period of time. These episodes are followed by inappropriate compensatory weight loss behaviors, such as laxative use, induced vomiting, fasting, and excessive exercise (APA, 2013). Individuals with BN are disproportionately influenced by body shape and/or weight and exhibit a high comorbidity with psychiatric and substance abuse disorders (Brewerton et al., 1995). BN has a notably higher prevalence than AN, at $\approx 2\%$. Both AN and BN largely affect women, as men are affected in only 5–10% of cases (APA, 2013).

BED, like BN, is characterized by episodes of binge eating, but does not involve compensatory behaviors aimed at preventing weight gain; thus, individuals with BED have a heightened risk of excessive weight gain and obesity (Spitzer et al., 1993; Cooper and Fairburn, 2003; APA, 2013). BED is the most common ED, with a lifetime prevalence of $\approx 3.5\%$ (Hudson et al., 2007). In contrast to AN and BN, BED is more common in males; clinical studies have shown that males represent between 30 and 40% of cases. Nevertheless, like AN and BN, BED is associated with psychiatric comorbidities and other medical conditions (Grilo et al., 2009).

ETIOLOGY OF EDs

The etiologies of EDs are complex and not yet completely understood, although genetic, biological, and environmental factors are probably involved in their onset and progression. EDs involve a complex genetic component, with an estimated heritability of between 50 and 83% (Bulik and Tozzi, 2004; Bulik et al., 2007; Javaras et al., 2008). Previous ED genetic association studies have focused on genes regulating appetite, food intake, and body weight, as well as on serotonin (5-HT)- and dopamine-related genes (Trace et al., 2013). Alteration of neurotransmitters (dopamine, 5-HT, and norepinephrine), neuropeptides (opioids, neuropeptide-Y [NPY], and peptide YY), and peripheral peptides (vasopressin,

oxytocin, ghrelin, and leptin), which regulate eating behavior and energy homeostasis, have been found in acutely ill ED patients and in patients at follow-up (Monteleone, 2011; Avena and Bocarsly, 2012). Such alterations, whether a cause or consequence of malnutrition and/or aberrant eating behavior, may contribute to the persistence of ED symptoms, as well as relapse and chronicity.

In particular, dysfunction in the reward center of the brain, which plays a chief role in the hedonic aspect of eating, seems to be one of the main mechanisms accountable for ED development (Davis and Carter, 2009; Dichter et al., 2012). Brain imaging studies have shown structural and functional alterations in areas that contribute to reward processing that may predispose subjects to the onset and continuance of EDs, as well as relapse (Frank, 2013). For example, people with AN have decreased gray matter in a range of brain regions, including those involved in reward processing, whereas people with BN have increased gray matter volumes in frontal and ventral striatal areas (Van den Eynde et al., 2012; Titova et al., 2013). Both BN and BED patients were characterized by greater volumes of the medial orbitofrontal cortex (OFC) compared to healthy controls (Schäfer et al., 2010). Schienle and colleagues (2009) reported increased medial orbital frontal cortex activation in patients with BED and greater cingulate cortex and insula activation in BN patients in response to food images relative to controls. In response to food (versus non-food) images, women with BN showed greater neural activation in the visual cortex, right dorsolateral prefrontal cortex, right insular cortex, and precentral gyrus, while women with AN showed greater activation in the right dorsolateral prefrontal cortex, cerebellum, and right precuneus (Brooks et al., 2011). A recent positron emission tomographic (PET) study found dopamine dysfunction in the caudate of obese humans with BED compared to obese humans without BED (Wang et al., 2011). Moreover, PET showed an increase of D₂/D₃ receptor expression in the ventral striatum of people who recovered from AN (Frank et al., 2005).

Regrettably, the superfluous value placed on “thinness” in most societies at present simply encourages extreme dieting and weight loss. Other contributing environmental factors that can trigger ED behaviors include stressful events (Treasure et al., 2010; Keel and Forney, 2013). Unfortunately, drug-related therapies do not have an established place in ED management paradigms, with the exception of fluoxetine, the only US Food and Drug Administration (FDA)-approved drug for BN treatment (Romano et al., 2002).

IMPLICATION OF THE ENDOCANNABINOID SYSTEM IN FOOD INTAKE REGULATION

The endocannabinoid signaling system is composed of cannabinoid receptors, their endogenous ligands (e.g., arachidonylethanolamide or anandamide [AEA] and 2-arachidonoylglycerol [2-AG]), enzymes that produce and inactivate

endocannabinoids and transporters (Di Marzo, 2009). During the last decade, two subtypes of cannabinoid receptors, CB₁ and CB₂, have been characterized and cloned (Pertwee et al., 2010). The CB₁R is the most abundant G protein-coupled receptor in the brain and is expressed in multiple brain regions, including those involved in energy modulation (i.e., hypothalamus and mesocorticolimbic system). This receptor is also present in peripheral sites important for the control of metabolic function, such as adipose tissue, the gastrointestinal tract, skeletal muscle, and the endocrine pancreas and liver (Di Marzo and Matias, 2005; Pagotto and Pasquali, 2006). In contrast, the CB₂R has long been considered a peripheral immune receptor; but more recently it has been described in both the periphery and central nervous system (CNS) (Munro et al., 1993; Pertwee, 2005; Gong et al., 2006).

Endocannabinoids are lipophilic neuromodulators that are synthesized and released “on demand” by receptor-stimulated cleavage of lipid precursors within cell membranes in response to several physiological and pathological stimuli (Piomelli, 2003). After acting on presynaptic CB₁Rs, endocannabinoid signal molecules are imported into cells and terminated by metabolic degradation by specific enzymes; AEA is primarily metabolized by the intracellular hydrolyzing enzyme fatty-acid amide hydrolase (FAAH), while 2-AG is metabolized into arachidonic acid and glycerol via monoacylglycerol-lipase (MAGL). Both FAAH and MAGL have been cloned and characterized and are widely distributed within the CNS (Dinh et al., 2002; Kathuria et al., 2003).

Several lines of evidence have shown that endocannabinoid signaling plays a critical role in modulation of energy balance by controlling food intake through central and peripheral mechanisms (Di Marzo and Matias, 2005). For instance, pharmacological manipulation of this system through exogenous administration of cannabinoid agonists, such as Δ^9 -tetrahydrocannabinol (THC; the psychoactive ingredient in *Cannabis sativa*) or the synthetic compounds CP55940 and WIN 55212-2, revealed increased food intake in both humans and animals (Williams et al., 1998; Hart et al., 2002; Cota et al., 2003a; Dodd et al., 2009). In agreement with CB₁R distribution, cannabinoids promote food intake by acting at both hypothalamic and mesocorticolimbic levels (Matias and Di Marzo, 2007). Accordingly, it was demonstrated that THC and endocannabinoids, as well as the FAAH inhibitor N-arachidonoyl-5-HT (AA-5HT), increased food intake when directly infused into several areas of the forebrain, including multiple hypothalamic nuclei (Anderson-Baker et al., 1979; Jamshidi and Taylor, 2001; Verty et al., 2005) and the shell of the nucleus accumbens (NAc) (Kirkham et al., 2002; Soria-Gómez et al., 2007).

Multiple studies have confirmed that these hyperphagic actions are mediated by CB₁Rs since they are blocked by the CB₁R inverse agonist/antagonists rimonabant and AM251 (Williams and Kirkham, 1999, 2002; Soria-Gómez et al., 2007). Conversely, acute or chronic CB₁R inverse agonist/antagonist treatments have been shown to be hypophagic and reduce body weight (Carai et al., 2006). Likewise, the phytocannabinoid Δ^9 -tetrahydrocannabivarin (THCV), which

behaves like a CB1R antagonist, decreases food intake and body weight at low doses (Riedel et al., 2009). Food intake and weight gain seem to depend on the functionality of CB1Rs since CB1R knockout (CB1-KO) mice are lean and hypophagic (Wiley et al., 2005). Moreover, tonic endocannabinoid release may be crucial to the normal feeding since endocannabinoid levels change during fasting and feeding in rat hypothalamic and limbic areas. More specifically, starvation over 24 hours only led to a significant enhancement of AEA and 2-AG levels in the limbic forebrain and, to a lesser extent, of 2-AG in the hypothalamus. By contrast, hypothalamic 2-AG declined as animals ate, while no changes were detected in satiated rats (Kirkham et al., 2002).

THE ENDOCANNABINOID SYSTEM AND THE HOMEOSTATIC REGULATION OF FOOD INTAKE

Homeostatic regulation of food intake is a complex process that involves several brain regions, but most research has focused on the role of the hypothalamus. This brain structure is composed of several interconnected nuclei, namely the arcuate (ARC), paraventricular (PVN), dorsomedial hypothalamic (DMH), ventromedial hypothalamic (VMH), and lateral hypothalamic (LH) (Williams et al., 2001; Morton et al., 2006). Lesions or electrical stimulation of specific hypothalamic nuclei were shown to alter feeding behavior (Hetherington and Ranson, 1940; Anand and Brobeck, 1951; Stellar, 1954; He et al., 2006). Within this neural circuit, several molecules contribute to the regulation of food intake, including orexigenic (increased appetite and food intake) and anorexigenic factors (decreased appetite and food intake). For example, ARC neurons produce NPY and agouti gene-related protein (AGRP), both potent stimulators of food intake, as well as pro-opiomelanocortin (POMC; α -melanocyte-stimulating hormone [α -MSH] precursor) and cocaine- and amphetamine-regulated transcript (CART), which induce an anorexic response (Hahn et al., 1998; Kristensen et al., 1998).

The activity of ARC neurons is regulated by peripheral metabolic signals including hormones and gastrointestinal peptides. For example, leptin, which is produced in proportion to body fat content, activates leptin receptors present in ARC neurons to increase the expression and release of POMC and reduce the expression and release of NPY, resulting in decreased food intake (Schwartz et al., 2000). On the contrary, ghrelin-containing neurons project efferent fibers into neurons that contain NPY and AGRP, which can stimulate the release of these orexigenic peptides (Bagnasco et al., 2003). The PVN is an integrating center rich in terminals containing numerous appetite-modifying neurotransmitters, including NPY, α -MSH, corticotrophin-releasing factor, 5-HT, galanin, nor-adrenalin, and opioid peptides (Williams et al., 2001). The VMH has been long considered the satiety center and contains neurons that sense glucose and leptin (Williams et al., 2001). The DMH contains a high level of NPY and α -MSH terminals originating from the ARC, while the LH is comprised of neurons that

express orexins and melanin-concentrating hormone (MCH), both of which stimulate food intake (Williams et al., 2001).

As stated above, CB1Rs are expressed in the hypothalamus and regulate the expression and release of orexigenic and anorexic signals. Cota et al. (2003b) demonstrated that CB1R mRNA is co-expressed with mRNA encoding CRH, CART, MCH, and orexin/hypocretin. AEA and the CB1R agonist CP55940 significantly augment NPY release, which is blocked by the receptor antagonist AM251. Additionally, AM251 *per se* has been found to inhibit NPY release (Gamber et al., 2005). Accordingly, Poncelet et al. (2003) reported that rimonabant prevents NPY hyperphagia and that the ability of NPY to stimulate feeding is abolished in CB1R-KO mice. However, rimonabant is as effective in reducing food intake in NPY-KO as in wild-type (WT) mice (Di Marzo et al., 2001). Mice deficient in FAAH (responsible for *in vivo* AEA metabolism) have reduced levels of CART-immunoreactive nerve fibers and terminals in several brain regions implicated in appetite control, including the ARC, DMH, and PVN, which return to WT control levels following rimonabant treatment (Osei-Hyiaman et al., 2005).

In fasted rats, rimonabant significantly reduces ghrelin levels, while plasma ghrelin levels in rimonabant-treated rats were 35% lower than in vehicle-injected rats (Cani et al., 2004). Consistent with this study, Kola and colleagues (2008) used patch-clamp recording to show that ghrelin inhibits excitatory synaptic inputs in the PVN and that this effect can be abolished by AM251 or inhibition of cannabinoid synthesis. These authors also showed that both THC and ghrelin increase AMP-activated protein kinase (AMPK) activity, a key enzyme in appetite and metabolic regulation, in the hypothalamus of WT versus CB1R-KO mice. Rimonabant was also able to abolish the stimulatory effect of ghrelin on AMPK activity and reduce basal AMPK activity. Moreover, feeding stimulated by intra-hypothalamic injection of ghrelin is blocked by rimonabant pretreatment, suggesting the expression of ghrelin-induced hyperphagia is dependent on an intact endocannabinoid system (Tucci et al., 2004).

Considering leptin inhibits hypothalamic endocannabinoid production, hypothalamic endocannabinoids are increased in genetically obese rodents lacking leptin and/or its receptor (Di Marzo et al., 2001). In the PVN, glucocorticoid-induced suppression of glutamatergic inputs is mediated through retrograde release of endocannabinoids, an effect that is blocked by leptin (Di et al., 2003; Malcher-Lopes et al., 2006). Accordingly, leptin inhibition of glucocorticoid-induced, endocannabinoid-mediated suppression was absent in leptin receptor-deficient obese Zucker rats (Malcher-Lopes et al., 2006). AEA was found to suppress the inhibitory tone of MCH-releasing neurons of the LH by decreasing γ -aminobutyric acid (GABA) release to those neurons (Jo et al., 2005). Hentges and colleagues (2005) demonstrated that POMC neurons spontaneously released endocannabinoids that act as retrograde transmitters that selectively inhibit presynaptic GABA release from GABAergic terminals entering the ARC. In sub-anorectic rats, intra-cerebroventricular doses of melanocortin receptor 4 agonist or oxytocin and rimonabant work synergistically to suppress feeding (Verty et al., 2004a,b).

THE ENDOCANNABINOID SYSTEM AND THE HEDONIC REGULATION OF FOOD INTAKE

The mesocorticolimbic system of the CNS contains principal neural pathways that drive hedonic feeding (Meye and Adan, 2014) and consist of subpopulations of dopaminergic neurons, originating in the ventral tegmental area (VTA) and pars compacta of the substantia nigra, which project to the NAc, as well as to other limbic structures, such as the amygdala, hippocampus, and prefrontal cortex (PFC). These pathways have multifaceted functions that are among the hallmarks of reward-related behaviors (Wise and Rompre, 1989; Fields et al., 2007; Wise, 2009). It is well recognized that palatable food stimulates mesocorticolimbic dopaminergic signaling in a manner similar to that with drugs of abuse, by increasing dopamine release in the NAc shell (Martel and Fantino, 1996; Bassareo and Di Chiara, 1997). The mesocorticolimbic dopaminergic pathway is highly connected to hypothalamic areas and many molecules involved in homeostatic hypothalamic regulation of feeding (also present in NAc, VTA, and PFC) play an important role in the reward component of food intake (Kelley et al., 2005; Monteleone and Maj, 2013; Meye and Adan, 2014). For example, in the VTA, orexin neurons coming from the LH activate dopaminergic neurons, enhance dopamine signaling in the NAc, and increase intake of palatable food (Narita et al., 2006; Zheng et al., 2007). Leptin directly hyperpolarizes VTA dopaminergic neurons, plays an important role in regulating dopamine levels in the NAc, and suppresses the incentive value of food and other rewards (Figlewicz et al., 2006; Fulton et al., 2006; Hommel et al., 2006). Moreover, NPY can act on NPY receptors on VTA dopaminergic neurons to increase dopamine release in the NAc and regulate food-motivated behavior (Jewett et al., 1995; Korotkova et al., 2006; Tracy et al., 2008).

Several findings support the hypothesis that endocannabinoid signaling significantly contributes to the hedonic valuation of food. CB1Rs are present in the VTA, NAc, and several areas projecting into these two structures, including the PFC, central amygdala, and hippocampus, where they play an important role in brain reward processes (Gardner, 2005). These receptors are mainly located at the presynaptic level, and an important functional consequence of their activation is the inhibition of neurotransmitter release (Schlicker and Kathmann, 2001). Acting as retrograde messengers, endocannabinoids can modulate excitatory and inhibitory inputs that control dopaminergic neurons of the mesocorticolimbic system (Wilson and Nicoll, 2001). Thus, endocannabinoids can be released following NAc depolarization (Robbe et al., 2002) and also from dopaminergic neurons of the VTA (Melis et al., 2004). Activation of CB1Rs on axon terminals of GABAergic neurons in the VTA and glutamatergic neurons in both the VTA and NAc was shown to inhibit GABAergic and glutamatergic neurotransmission (Melis et al., 2004; Lupica and Riegel, 2005). The final effect on VTA dopaminergic activity depends on the relative level of input activation under distinct behavioral circumstances (Lupica and Riegel, 2005).

Various human and animal studies have shown that CB1R agonists increase total food intake, particularly consumption of palatable foods. For example, reports of human feeding behavior following marijuana intake include foods predominantly high in sugar and fat (Foltin et al., 1986, 1988). In rats, low doses of THC increase palatable food intake following both peripheral and central administration (Koch and Matthews, 2001). The CB1R agonist CP55940 was found to facilitate intake of palatable foods when injected into the hindbrain (Miller et al., 2004). Furthermore, parabrachial infusions of 2-AG and AA-5HT (FAAH inhibitor) selectively stimulate the intake of palatable foods, while both compounds failed to affect intake of standard rodent chow (Dipatrizio and Simansky, 2008).

On the other hand, CB1R antagonism decreases energy intake by preferentially reducing the consumption of palatable food in normal rats. Rimonabant, for example, suppresses intake of sucrose or sweets more efficiently than that of chow and water (Arnone et al., 1997; Simiand et al., 1998) and reduces the conditioned place preference induced by food (Chaperon et al., 1998). Previously, AM251 injections in mice given a choice between a low-fat or high-fat nutritionally complete diet were shown to selectively decrease high-fat food intake (South et al., 2007). Using a model of overconsumption with *ad libitum* access to standard chow and daily time-limited access to either a sugar gel or sugar-fat whip, Mathes and colleagues (2008) reported that both rimonabant (injected for 7 days) and AM251 (injected for 15 days) decreased 24-hour caloric intake, with specific reduction in sugar-fat whip consumption.

Further support for an endocannabinoid role in palatable food ingestion is provided by studies that demonstrate CB1R agonists and antagonists dose dependently increase and reduce, respectively, the motivation for food consumption in a progressive-ratio self-feeding schedule in rats (Gallate and McGregor, 1999; Gallate et al., 1999; Solinas and Goldberg, 2005). In addition, AEA microinjections into the medial part of the NAc shell were shown to specifically double the number of positive “liking” reactions elicited by intra-oral sucrose without altering negative “disliking” reactions to bitter quinine (Mahler et al., 2007). Similarly, THC ingestion specifically induces a rapid and robust potentiation of hedonic reactions to intra-oral sucrose, and CB1R-KO mice consume less sucrose than WT littermates (Poncelet et al., 2003; De Luca et al., 2012). All these data suggest that cannabinoid agonists act to facilitate intake of highly palatable foods. However, when given a choice between diets, cannabinoid effects are not limited to palatable food but also extend to normal chow (Arnone et al., 1997; Foltin and Haney, 2007).

IMPLICATION OF THE ENDOCANNABINOID SYSTEM IN AN AND BED

In recent years, both clinical and preclinical evidence have led to the hypothesis of a link between defects in the endocannabinoid system and AN and BED (Table 16.1).

Table 16.1 Link between Defects in the Endocannabinoid System and AN and BED

Anorexia Nervosa			Binge Eating Disorder		References
	Human	Animal	Human	Animal	
AEA	↑	nd	↑	nd	Monteleone et al., 2005 Monteleone et al., 2005 Frieling et al., 2009 Gérard et al., 2011 Bello et al., 2012 Casteels et al., 2013
2-AG	—	nd	—	nd	
CB1R	↑ mRNA ↑ Upregulation	↑ binding	nd	—	

↑: increase ; —: no changes; nd: not detected.

HUMAN STUDIES

A previous study demonstrated that elevated plasma AEA levels were found in women with AN and BED, but not BN, while circulating levels of 2-AG did not significantly differ between patients and healthy controls (Monteleone et al., 2005). Moreover, circulating leptin levels have been reported to be drastically reduced in AN patients and significantly increased in BED subjects, with no significant change in BN women. The increased levels of plasma AEA in AN patients were secondary to their leptin deficiency considering negative leptin modulation in endocannabinoid production has been demonstrated (Di Marzo et al., 2001). However, impaired leptin levels or signaling (not observed in BN) may explain why higher levels of AEA were found in subjects with BED. The authors speculated that elevated AEA levels may facilitate the rewarding properties of aberrant eating behaviors in AN and BED patients. However, the limitation of this work is that it is uncertain whether peripheral levels could reflect those at the level of the CNS.

Heightened levels of CB1R mRNA in the blood were found in AN and BN patients compared to healthy controls, further supporting the notion of impaired endocannabinoid signaling in EDs (Frieling et al., 2009). However, peripheral CB1R mRNA levels were reduced in a mixed sample of AN and BN patients with self-injurious behavior compared to those without this behavior and healthy controls (Schroeder et al., 2012). A CB1R PET imaging study conducted by Gérard and colleagues (2011) showed an increase in CB1Rs in cortical and sub-cortical brain areas in AN patients relative to age-matched healthy volunteers, which could be a consequence of a long-term compensatory mechanism up-regulated in response to underactive endocannabinoid signaling under anorectic conditions. The authors also found an increase of CB1Rs in the insular cortex of both AN and BN patients, a key area involved in the integration of interoceptive and gustatory information, reward, and emotion processing.

Genetic association studies between the endocannabinoid system and EDs have demonstrated significant associations between polymorphisms of the

gene encoding the CB1R and the gene encoding FAAH in AN and BN (Monteleone et al., 2009). Also, functional polymorphisms in the gene coding the orphan G protein-coupled receptor GPR55, recently reported to have an affinity for endocannabinoids, may be a risk factor for AN (Ishiguro et al., 2011). However, several other polymorphisms have shown no association with AN (Müller et al., 2008). Therefore, more in-depth genetic studies are needed to understand the genetic complexities of ED-related pathologies.

ANIMAL STUDIES

Animal models of AN and BED

Animal models are an important instrument for understanding the neurobiological, pathophysiological, pharmacological, environmental, and genetic determinants involved in the development of psychiatric pathologies. As mentioned above, multiple factors are involved in the etiology of EDs and only a few traits can be reproduced in animals. For example, psychological components, such as obsessing over body weight and shape, cannot be easily assessed in animals. Nonetheless, animal models are very useful for studying other, more measurable behavioral features (Casper et al., 2008). Table 16.2 shows an overview of the more common animal models of AN and BED. One of the most used animal models of AN is activity-based anorexia (ABA), generated by Routtenberg and Kuznesof in 1967, which reproduces key aspects of human AN, specifically hyperactivity and reduced food intake. In this experimental paradigm, animals (rats or mice) have free access to a running wheel situated in their home cage in combination with restricted feeding schedules (1–2 h/day). If applied simultaneously, these two factors cause a progressive increase in running wheel activity coupled with a massive decline in body weight. In fact, animals subjected only to restricted feeding schedules do not exhibit significant weight loss, while animals with free running wheel access and food *ad libitum* show stable levels of activity (Routtenberg and Kuznesof, 1967).

In addition to excessive activity, reduced food intake, and weight loss, the ABA animal model reproduces other clinical manifestations reported in AN patients, such as decreased leptin and increased ghrelin concentrations, as well as cessation of the estrous cycle in females (Adan et al., 2011). Weight loss and

Table 16.2 Overview of the More Common Animal Models of AN and BED

Anorexia Nervosa	Binge Eating Behavior
Self-starvation/activity-based anorexia (ABA) (Routtenberg and Kuznesof, 1967)	Restriction/refeeding/acute stress (Hagan et al., 2002)
Stress models (Shimizu et al., 1989; Smith, 1989)	Limited access (Corwin and Buda-Levin, 2004)
Diet restriction (Avraham et al., 2001)	Sugar addiction model (Avena et al., 2012)
Genetic models (Johansen et al., 2003)	

reduced food intake can also be obtained using physical stressors, such as tail pinching, cold swimming with or without food restriction, and direct brain stimulation (Shimizu et al., 1989; Smith, 1989). However, stress-based animal models of AN have several drawbacks; following administration of acute stress, animals often suffer physical injury. Excessive food restriction (less than half of daily *ad libitum* intake) can also be used as an AN model even if the restriction of food is not voluntary. Regardless, many changes in neuroendocrine function found in AN patients can be mimicked by this model, thus validating its use (Avraham et al., 2001; Kim, 2012). Lastly, the most commonly studied genetic model of AN is *anx/anx* mice. These mice are characterized by poor appetite, reduced stomach size, and inability to regulate food intake (Johansen et al., 2003). Other genetic models of AN include gene knockouts for evaluating the contribution of specific genes involved in feeding behavior, such as BDNF, tyrosine hydroxylase, δ -opioid receptor, 5-HT receptors, or M_3 muscarinic receptors (Kim, 2012).

Various animal models are currently being used to reproduce binge eating, a key behavior in both BN and BED. In the limited access model, binge eating behavior is induced in rats by alternating sporadic, time-limited access (e.g., 2 hours, 3 days/week) to palatable food with continuously available chow. Under these conditions, the intake of palatable food during periods of limited access escalates over several weeks, becoming significantly greater than in rats with daily access (Corwin and Buda-Levin, 2004; Corwin et al., 2011). Another ED model induces bingeing by predisposing rats to a history of dieting and external stress (Hagan et al., 2002). Dieting is simulated by administering cycles of food restriction (5 days with 66% of control animal chow) followed by refeeding (2 days of *ad libitum* palatable food and chow, followed by 4 days of only *ad libitum* chow). At the end of each cycle, rats received a foot shock stress just prior to the feeding test where they have *ad libitum* palatable food and chow in their home cages. During the feeding test, rats were found to eat more palatable food compared to rats that underwent only dieting or foot shock stress (Hagan et al., 2002). In another procedure, rats maintained on daily 12-hour food deprivation, followed by 12-hour access to a 10% sucrose solution and chow, increased their total daily sugar intake over time (Avena et al., 2012).

Preclinical studies

Using the ABA experimental model, Lewis and Brett (2010) found that THC (0.5 mg/kg) augments food intake, but not body weight, in AN mice compared to vehicle treatment. Moreover, excessive running wheel activity did not change with THC administration. Besides restoration of food intake, THC-treated AN mice also had high mortality rates, to which the hypothermic effect of THC may have contributed. The authors also demonstrated that the AEA uptake inhibitor (S)-N-oleoyl-(1'-hydroxybenzyl)-2'-ethanolamine (OMDM-2) was not able to reverse weight loss despite the increase in food intake at 1 and 3 mg/kg doses. Additionally, no significant changes in wheel running or survival were found in OMDM-2-treated compared to vehicle-treated mice.

In contrast, [Verty et al. \(2011\)](#) showed the effectiveness of THC in attenuating the weight loss associated with ABA development in female rats. More specifically, they found that subchronic THC treatment (2.0 mg/kg/day) transiently stimulated chow intake and significantly reduced weight loss with a moderate effect on running wheel activity. The ability of THC to retard AN progression was significantly amplified when high-fat palatable food was introduced in conjunction with THC treatment. Moreover, THC significantly shifted markers of thermogenesis in brown adipose tissue and lipid metabolism in white adipose tissue in directions consistent with reduced energy expenditure and lipolysis. The discrepancies between the above studies may reflect important differences related to the fact that administration of cannabinoids produces contrasting results in rats and mice that are also seen in other experimental models and procedures ([Berrendero and Maldonado, 2002](#); [Valjent et al., 2002](#); [Patel and Hillard, 2006](#)).

As mentioned above, limited access to highly palatable foods without food restriction or deprivation can successfully induce binge eating behavior in rodents ([Corwin and Buda-Levin, 2004](#)). Using intermittent, extended access to palatable food to induce compulsive eating in male rats, i.e., 2-day regular chow access, followed by 1-day access to highly palatable food (sugary diet), [Dore et al. \(2013\)](#) found that both 1 and 3 mg/kg injections of rimonabant reduced excessive intake of highly palatable food in diet-cycled rats more potently than it reduced chow intake in controls, with 3 mg/kg being most effective. Moreover, rimonabant reduced both risk-taking behavior and compulsive eating during withdrawal from intermittent, extended access to the sugary diet. Accordingly, our laboratory demonstrated that 0.3 and 3 mg/kg rimonabant dose dependently reduced binge eating behavior in female rats that was induced by providing limited access to a high-fat palatable food (margarine) ([Scherma et al., 2013](#)). Notably, chronic low-dose rimonabant (0.3 mg/kg) treatment selectively reduced margarine intake (compared to chow intake in the same animals), while significantly reducing body weight. Furthermore, using a different BED animal model where bingeing was induced by a combination of food restriction/refeeding (with both palatable food and normal chow) and acute stress cycles ([Hagan et al., 2002](#)), we confirmed that acute administration of 0.3 and 3 mg/kg rimonabant selectively reduced palatable food intake (Oreo cookies) in female bingeing rats relative to not-bingeing controls ([Scherma et al., 2014](#)). All of the above results are consistent with the notion that rimonabant can reduce the hedonic value of food and, therefore, preferentially decrease intake of highly palatable foods ([Arnone et al. 1997](#); [Simiand et al., 1998](#); [Mathes et al., 2008](#)).

As stated above, highly palatable food stimulates the mesocorticolimbic dopaminergic system in a manner similar to drugs of abuse, i.e., by increasing dopamine release in the NAc shell ([Martel and Fantino, 1996](#)). This release of dopamine disappears with repeated access to palatable food ([Bassareo and Di Chiara, 1999](#)), an effect not seen when rats were exposed to drugs of abuse ([Wise et al., 1995](#)). On the other hand, animals that binge on 10% sucrose solution ([Rada et al., 2005](#)) or a high-fat diet ([Liang et al., 2006](#)) repeatedly release dopamine in the NAc shell each time they binge. Importantly, [Melis et al. \(2007\)](#)

showed that the increase in dopamine in the NAc shell induced by palatable food is blocked by administration of rimonabant, suggesting that hedonic food responses depend on endocannabinoid signaling, probably through modulation of the mesocorticolimbic system. In view of these data, it would be important to assess the effect of rimonabant on release of dopamine during bingeing.

Several lines of evidence suggest that dietary conditions (specific dietary components, as well as compulsive or restrictive feeding) influence CB1R expression in multiple brain regions (Carr et al., 2008). For example, Harrold et al. (2002) found that in rats exposed to a sweet palatable food diet (sucrose and condensed milk) for 10 weeks, CB1R density significantly decreased by 30–50% in the hippocampus, cortex, NAc, and entopeduncular nucleus, inversely correlating with palatable food intake. However, CB1R density in the hypothalamus was low and unaltered in obese mice. Another study demonstrated that mice fed a high-fat diet for 20 weeks showed decreased CB1R density in the substantia nigra and VTA, as compared to mice fed a low-fat/high-carbohydrate diet (South and Huang, 2008). Consistently, long-term consumption of a palatable high-energy diet has been shown to significantly decrease CB1R mRNA expression levels in the cingulate cortex, VMH, and PVN (Timofeeva et al., 2009). While examining the consequences of dietary-induced binge eating in female rats on cerebral CB1R mRNA expression, Bello et al. (2012) found that CB1R mRNA expression and density were influenced by dietary conditions but without being specific to the dietary-induced binge eating paradigm used. In fact, an increase in nuclear CB1R mRNA levels of the solitary tract was found in animals receiving continuous access to a high palatable food (i.e., vegetable shortening with 10% sucrose); these animals also displayed a significant increase in body weight and adiposity. An $\approx 20\%$ reduction in CB1R mRNA was observed in the cingulate cortex of rats exposed to intermittent versus *ad libitum* feeding schedules. CB1R density was also reduced by $\approx 30\%$ in the NAc shell in groups receiving repeated access to high palatable food.

This decrease in the density of CB1Rs could be interpreted as a result of their increased activity by endogenous cannabinoids. Additionally, it has been demonstrated that both high-dose endocannabinoid ligand and chronic cannabinoid agonist treatment are associated with parallel compensatory decreases in CB1R density, mRNA expression, and G protein subunit expression (Rubino et al., 1994, 1997). Since decreased CB1R density is particularly evident in brain areas involved in hedonic aspects of eating, endocannabinoid system dysregulation in those areas may drive appetite for palatable food and thus determine total energy intake and the severity of diet-induced obesity, and may cause bingeing and reinforce the rewarding effects of palatable foods, further promoting the binge eating cycle. Casteels et al. (2013) investigated for the first time *in vivo* changes in cerebral CB1R binding in the ABA rat model using a small animal PET. Similarly to human studies, ABA rats showed increased CB1R availability in all cortical and subcortical areas that renormalized to baseline after weight gain. Increased CB1R availability might represent a possible attempt to compensate for an impaired endocannabinoid system (van der Stelt and Di Marzo, 2003; Di Marzo, 2008).

CONCLUSIONS

Despite the growing knowledge base of ED neurobiology, therapeutic interventions are still quite restricted and the incidence of relapse remains high. At present, no medications have been approved for the treatment of AN or BED, and the selective 5-HT reuptake inhibitor fluoxetine continues to be the only drug with FDA approval for the treatment of BN. Therefore, in addition to the standard psychotherapy, there is clearly a need for new, more effective ED treatments. The endocannabinoid system plays a key role in the homeostatic and hedonic regulation of eating behavior, and both clinical and preclinical studies suggest that dysregulation of its neuronal signaling may drive individuals with EDs to aberrant eating behaviors associated with these disorders (Monteleone et al., 2005, 2013). Thus, therapeutic strategies based on drugs that modulate endocannabinoid signaling may be useful in the treatment of EDs.

Different clinical trials investigated the effects of cannabinoid agonists such as THC and dronabinol in the management of cachexia in cancer and AIDS patients, reporting increased appetite and body weight. For example, Nelson et al. (1994) found that THC is an effective appetite stimulant in an interventional phase II study in patients with cancer-associated anorexia and was well tolerated at low doses (2.5 mg). Likewise, dronabinol (2.5 mg) was found to be safe and effective for anorexia associated with weight loss in patients with AIDS (Beal et al., 1995). With regard to AN, Gross et al. (1983) did not find any significant effect on weight gain in AN patients treated with THC compared to diazepam. Moreover, THC treatment resulted in increased sleep disturbances and interpersonal sensitivity. However, a very recent trial study of dronabinol (2.5 mg twice daily for 4 weeks) in women with severe, enduring AN, showed modest weight gain in the absence of severe adverse events (Andries et al., 2014).

Rimonabant was the first CB1R antagonist to be approved for the control of obesity, but it was removed from the market soon after its introduction in Europe due to severe psychiatric side effects associated with prolonged use (Di Marzo and Després, 2009). Clinical development of other brain-penetrating agents, such as taranabant and otenabant, were also terminated shortly thereafter. To date, there is only one randomized, placebo-controlled, double-blind trial that has assessed the effect of rimonabant in patients with BED (Pataky et al., 2013). In this study, rimonabant treatment significantly reduced body weight relative to placebo in obese subjects with BED. The rimonabant group also showed a greater reduction on the binge eating scale total score, and the incidence of treatment emergent adverse events was comparable in both the rimonabant and placebo groups.

The adverse psychiatric events related to rimonabant treatment have been mainly attributed to the CB1R antagonism in the CNS. Recent research is therefore oriented towards the development of new compounds based on the assumption that neutral and peripheral antagonists may lack central neuropsychiatric side effects but retain metabolic actions (Kirilly et al., 2012). Yet, not all researchers

are convinced of the feasibility of drugs acting exclusively as neutral antagonists (Kenakin, 2004; Giraldo et al., 2007). This skepticism is strengthened by the notion that some neutral CB1R antagonists induce an anxiety-like reaction, resembling rimonabant side effects (Järbe et al., 2008). However, definitive evidence that peripheral antagonists may also decrease food intake and body weight in animals is currently lacking. Thus, a better understanding of the mechanisms through which CB1Rs modulate depression and anxiety is urgently needed. Future research aimed at identifying drugs that act on endocannabinoid signaling and other associated neurotransmitter systems will likely lead to important pharmacotherapeutic breakthroughs in the treatment of AN and BED.

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The endocannabinoid system and trichotillomania: A promising target for treatment?

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INTRODUCTION TO TRICHOTILLOMANIA

Trichotillomania, also known as hair pulling disorder, is characterized by the repetitive pulling out of one's hair leading to hair loss and functional impairment (American Psychiatric Association, 2013). Trichotillomania is a relatively common disorder, with an estimated prevalence of between 1 and 3.4% in the population, and is associated with social disruption, co-occurring depression and anxiety, and reduced quality of life (Diefenbach et al., 2005; Odlaug et al., 2010, 2012). Onset of hair pulling is generally in late childhood/early adolescence, although it can initiate at any age (Flessner et al., 2010). In adults, trichotillomania has a large female preponderance; however, in childhood, the gender distribution has been found to be equal (Christenson et al., 1991; Lochner et al., 2010). In the *Diagnostic and Statistical Manual*, 5th edition (DSM-5), trichotillomania is listed in the category of "Obsessive-Compulsive and Related Disorders" (American Psychiatric Association, 2013), in recognition of parallels it shares with obsessive-compulsive disorder (OCD) in terms of phenomenology, co-morbidity, familiarity, and possibly neurobiology (Chamberlain et al., 2009). Similarly, overlap has been suggested between trichotillomania and Tourette's syndrome, with both being characterized by repetitive motor habits that are difficult to suppress (Ferrão et al., 2009).

Trichotillomania is often associated with reduced self-esteem and avoidance of social situations due to shame and embarrassment from the pulling and its

consequences (Diefenbach et al., 2005; Odlaug et al., 2010). The majority of individuals with trichotillomania have never sought treatment or discussed their pulling behaviors with healthcare professionals (Woods et al., 2006). Avoidance of activities, such as getting haircuts, swimming, being outside on a windy day, sporting activities, dating, or going out in public more than necessary, are commonplace (Christenson et al., 1991; Woods et al., 2006). Many individuals conceal areas in which they have pulled hair with hats, scarves, bandanas, or make-up, or by wearing concealing clothing (Christenson et al., 1991).

Although current diagnostic approaches regard trichotillomania as a unitary disorder, it is likely that the pathophysiological basis of symptoms is heterogeneous and that it will be possible in the future to identify distinct subtypes. For example, some individuals with trichotillomania report significant urges or a drive to pull their hair, and the triggers to pull vary from person to person (for example, the sight or feel of a hair may be “not right” or may feel coarse, fine, sharp, rough, oily, or dry, or appear too dark, curly, gray, or “out of place”). Cues prompting pulling episodes may include stress, boredom, “downtime,” fatigue, or the act of driving. Some individuals are aware of the act of pulling and seek out particular hair types and locations, a putative subtype referred to as “focused” pulling; while others report not being fully aware of their pulling behaviors (referred to as “automatic” pulling) and this may reflect a more “habitual” form of the disorder (Woods et al., 2006; Lochner et al., 2010). However, though focused and automatic pulling have been suggested to be potential subtypes of trichotillomania, this approach is problematic in that the majority of patients experience varying degrees of focused and automatic pulling (Flessner et al., 2008; Panza et al., 2013).

Trichotillomania can result in unwanted medical consequences. The most commonly discussed and the most serious medical consequence can result from ingestion of the hair. Approximately 20% of individuals who pull their hair eat the hair after pulling it (trichophagia) (Grant and Odlaug, 2008). This in turn may lead to gastrointestinal obstruction and the formation of intestinal hair-balls (trichobezoars) requiring surgical intervention (Gorter et al., 2010; Ramirez et al., 2011).

Various treatment approaches have shown promise (including cognitive and behavioral therapies, supportive counseling, support groups, hypnosis, and medications) in the treatment of trichotillomania, and psychotherapy in the form of habit reversal therapy is the treatment of choice, based on the available evidence (Franklin et al., 2011). Habit reversal therapy for trichotillomania generally includes awareness training (i.e., using self-monitoring to improve awareness of pulling and the awareness of the urge that precedes pulling), stimulus control (i.e., a variety of methods that serve to reduce the likelihood that pulling behavior begins), and competing response training (i.e., at the earliest sign of pulling or of the urge to pull, the individual should engage in a behavior that is physically incompatible with pulling for a brief period of time until the urge subsides) (Azrin and Nunn, 1973). Some habit reversal therapies have also included other techniques such as relaxation training and cognitive strategies to address

dysfunctional thoughts around pulling (Franklin et al., 2011). Although habit reversal therapy has provided benefit for many individuals with trichotillomania, long-term outcomes have seldom been studied and relapse following acute treatment is common (Bloch et al., 2007).

In terms of pharmacotherapy, there are currently no labeled medications for the treatment of trichotillomania in any country, despite almost two decades of research. Controlled clinical trials evaluating potential pharmacological interventions for trichotillomania have demonstrated that *N*-acetyl cysteine (Grant et al., 2009), olanzapine (Van Ameringen et al., 2010), and clomipramine (Swedo et al., 1989) all show some promise in reducing hair pulling behavior. Despite their promise, available studies are few in number, and these treatments do not appear effective for all individuals with trichotillomania, so additional options are needed (Rothbart et al., 2013).

THE CANNABINOID SYSTEM IN TOURETTE'S SYNDROME AND OCD

Understanding the pathophysiology of trichotillomania is one approach to the eventual development of more effective treatment options for this disorder. The current body of neuroscience research in trichotillomania is very limited; hence, clinicians and researchers can look to what is known of the neurobiology of related disorders such as Tourette's disorder and obsessive-compulsive disorder, in order to draw inferences regarding possible neurobiological and neurochemical involvement in hair pulling. The research on Tourette's disorder has led to an examination of the endocannabinoid system as a contributor to the motor symptoms of the disorder (see Chapter 10). Tourette's syndrome is associated with motor and vocal tics, as well as a spectrum of behavioral and cognitive features. Current evidence suggests that frontal–subcortical pathways and the dopaminergic system are all involved in the pathophysiology of Tourette's syndrome. The genetic research has been somewhat discouraging in terms of the endocannabinoid system (Gadzicki et al., 2004), but there has been a recognition that individuals with Tourette's syndrome often report a reduction of symptoms after smoking marijuana, the primary active ingredient of which is Δ^9 -tetrahydrocannabinol (THC) (Sandyk and Awerbuch, 1988; Müller-Vahl et al., 1998). One study examined 64 subjects with Tourette's syndrome and their use of marijuana and its influence on their symptoms (Müller-Vahl et al., 1998). Seventeen of the subjects (27%) reported prior use of marijuana, and 14 of the 17 (82%) experienced a reduction or complete remission of motor and vocal tics as well as a reduction in premonitory urges and obsessive-compulsive symptoms.

In addition to patient reports, randomized trials have shown that THC may effectively reduce tics and obsessive-compulsive symptoms in individuals with Tourette's syndrome (Müller-Vahl et al., 2002, 2003). The largest of these two

studies enrolled 24 subjects with Tourette's syndrome in a randomized, double-blind, placebo-controlled study for 6 weeks using doses up to 10 mg/day of THC. Tics were rated at six visits using several measures such as the Tourette Syndrome Clinical Global Impressions scale (TS-CGI) and the Yale Global Tic Severity Scale (YGTSS). Significant differences were noted on the main outcome measures comparing the THC group to the placebo group (Müller-Vahl et al., 2003).

What might explain this response to THC in people with Tourette's syndrome and what could these findings tell us about trichotillomania? Cannabinoid receptors are densely located in the basal ganglia (globus pallidus, substantia nigra pars reticulata), suggesting a role in regulating motor activity (Herkenham et al., 1990). In addition, cannabinoid transmission appears to exert a modulatory influence over other transmitter systems within the basal ganglia by increasing GABAergic transmission, inhibiting glutamate release, and affecting dopaminergic uptake. Endogenous cannabinoid tone therefore may play a role in the control of movements and possibly in the pathophysiology of a disorder such as Tourette's syndrome and, by extension, trichotillomania.

The data regarding the role, if any, of the cannabinoid system in obsessive-compulsive disorder is limited mostly to animal data (see Chapter 15). Marble-burying behavior (MBB) is a commonly used rodent model to reflect possible human symptoms of OCD (Broekkamp et al., 1986; Umathe et al., 2012). Recent studies demonstrate that THC exerts anti-compulsive effects in this animal model (Casarotto et al., 2010). Similarly, a synthetic cannabinoid CB₁ receptor agonist has been found to reduce MBB (Gomes et al., 2011). This has led investigators to suggest that CB₁ receptors may be a potential target in the treatment of OCD.

With regard to the scant human literature, a recent case report suggests a potential role for this system in OCD. The case involved a woman with severe OCD who received only partial response to first-line treatments (in her case cognitive behavioral therapy and clomipramine 300 mg). When her medications were augmented with dronabinol (10 mg tid), a cannabinoid agonist, her OCD symptoms decreased significantly within 10 days. The authors theorized that the cannabinoids reduced OCD symptoms via inhibition of glutamate release (Schindler et al., 2008).

NEUROIMAGING IN TRICHOTILLOMANIA

How does the limited understanding of the cannabinoid system in Tourette's syndrome and OCD apply to trichotillomania? To answer this, we must first briefly survey what is known about the neurobiology of trichotillomania and the relationship to motor behaviors. A structural magnetic resonance imaging (MRI) brain study found that individuals with trichotillomania compared to controls had

smaller left putamen volumes (O'Sullivan et al., 1997). The putamen appears to be involved in the generation of motor habits and responses (Singer et al., 1993). More recently, a study using voxel-based morphometry and MRI data in co-morbidity-free subjects with trichotillomania found that those with trichotillomania ($n = 18$) exhibited gray matter density increases, versus controls ($n = 19$) in several brain regions involved in affect regulation, motor habits, and top-down cognition (Chamberlain et al., 2008). These regions of abnormally increased gray matter density in trichotillomania included the putamen, amygdalo-hippocampal formation, anterior cingulate cortex, supplementary motor cortex, and bilateral frontal cortices.

Finally, a study using diffusion tensor imaging in subjects with trichotillomania compared to controls found that trichotillomania was associated with reduced integrity of white matter tracts connecting the bilateral orbitofrontal cortex (OFC) and anterior cingulate cortices, the left pre-supplementary motor area (pre-SMA), and the left temporal lobe (Chamberlain et al., 2010). These findings implicate dysconnectivity in white matter tracts connecting neural regions involved in motor generation and suppression as well as emotional processing in the pathophysiology. These white matter tracts found to be abnormal in trichotillomania are closely affiliated with gray regions mediating the generation and suppression of motor habits. The pre-SMA has classically been associated, across species, with the preparation and selection of movements and with high level functions relating to motor preparation (Picard and Strick, 1996). Multiple tiers of imaging evidence in humans suggest that the OFC and anterior cingulate cortices play important roles in response suppression, working alongside other neural regions such as the right inferior frontal gyrus and the pre-SMA to act as a break over ongoing behavior.

THE CANNABINOID SYSTEM AND TRICHOTILLOMANIA

As stated previously, effective treatments for trichotillomania are currently lacking. The neuroimaging data suggest abnormalities of both gray and white matter regions implicated in motor habit generation and suppression (Chamberlain et al., 2010). On a neurochemical level, these motor habits may rely at least partially on the endocannabinoid system. CB₁ receptors are plentiful in the basal ganglia nuclei, the hippocampus, cerebellum, and neocortex (Herkenham et al., 1990; Glass and Felder, 1997) and may attenuate glutamatergic excitotoxic damage by suppressing the neuronal release of glutamate via inhibition of calcium channels (Gerdeman et al., 2002; Marsicano et al., 2003). Activation of CB₁ receptors may therefore reduce glutamate release in the dorsal and ventral striatum and thereby modulate neurotransmission in the basal ganglia and mesolimbic reward system (van der Stelt and di Marzo, 2003).

This relationship of the cannabinoid system to the glutamate system is particularly relevant to trichotillomania given that glutamatergic dysfunction has been implicated in the pathophysiology of trichotillomania (Bienvenu et al., 2009; Grant et al., 2009). Pharmacotherapies, such as the cannabinoid agonist dronabinol, that target excessive glutamatergic drive may, therefore, be expected to ameliorate the presumptive underlying pathophysiology and symptoms of trichotillomania.

We conducted an open-label study to examine the tolerability of dronabinol, a cannabinoid agonist, in the treatment of trichotillomania. We enrolled 14 women (mean age = 33.3 ± 8.9) with trichotillomania. The participants reported a mean age of trichotillomania onset of 10.9 years. All subjects received 12 weeks of open-label dronabinol (dosing started at 2.5 mg/day for 3 weeks, increased to 5 mg/day for the next 3 weeks, at week 6 increased to 10 mg/day, and then to 15 mg/day at week 9). The study found that trichotillomania symptoms improved significantly in a majority (64.3%) of subjects with a mean effective dose of 11.6 ± 4.1 mg/day and no cognitive dysfunction (Grant et al., 2011). The study suggests that pharmacological modulation of the cannabinoid system was associated with reductions in the compulsive motoric aspect of trichotillomania and supports the idea of a functional role of cannabinoids in movement and behavior.

CONCLUSIONS

Despite trichotillomania being relatively common across the world, knowledge regarding its neurobiology is very limited and evidence-based treatments are few in number. Based on what is known of related disorders of OCD and Tourette's syndrome, there is reason to suppose that the endocannabinoid system may be implicated in trichotillomania, albeit that direct data testing this hypothesis are lacking. The involvement of the cannabinoid system in trichotillomania could be studied in future work by using complementary translational approaches, such as the use of recently developed radioligands relating to the cannabinoid system and positron emission tomography (PET) (Ceccarini et al., 2014), and via the elaboration of trichotillomania animal models, including whether cannabinoid abnormalities exist in such models (Camilla d'Angelo et al., 2014). Medications capable of targeting this system merit exploration as candidate treatments for trichotillomania. While the initial open-label study using dronabinol in trichotillomania was promising, this should now be followed up in controlled designs. It would also be valuable to examine possible relationships between cannabis consumption and trichotillomania symptoms using a cross-sectional design, to consider whether cannabis consumption is associated with frequency of hair pulling, or its nature (e.g., symptom severity).

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Future perspectives: Cannabinoid CB₂ receptor ligands and their therapeutic potential in mental diseases

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FRAMEWORK ON THE MOLECULAR BASIS FOR THE THERAPEUTIC POTENTIAL OF CANNABINOIDS

Part of the conclusions provided here on the therapeutic potential of cannabinoid CB₂ receptor (CB₂R) ligands is based on previous work and reviews by our group and others, on the emerging molecular features of the elements of the endocannabinoid system (ECS), and particularly on CB₁R and CB₂R and their therapeutic potential. The role of CB₁R and CB₂R and their interactions with one another cannot be ignored. The proposed therapeutic implications of targeting elements of the ECS for the treatment of neurological and mental illness may involve interaction between the CB₂R and the CB₁R, which remains one of the most abundant G-protein coupled receptors (GPCRs) in the brain. Therefore, significant advances with discoveries unraveling such a compelling puzzle and major breakthroughs in revealing the elements of the ECS can be described as paradigmatic (Onaivi, 2002). The discovery that specific genes code for cannabinoid receptors (CBRs) that are activated by marijuana use and endocannabinoids (eCBs) (Onaivi et al., 2006c), has provided surprising new knowledge about cannabinoid genomic and proteomic profiles as potential therapeutic targets. These remarkable progressions, new understanding, and advances indicate that the molecular, cellular, biochemical, and behavioral responses to marijuana, which remains one of the most widely used and abused drugs in the world, are coded in our genes and chromosomes. This increasing new knowledge from the decoding of the

human genome led to the acknowledgment that many aspects of genetic risk factors in the use of marijuana, whether as medicine or recreationally, including age of initiation, continuation and problem use, undoubtedly interact with environmental factors such as epigenetics and availability of marijuana along with the individual's genotype and phenotype. Therefore, the eCB signaling system has been described as a widespread, neuromodulatory system in the brain and is also widely utilized in the periphery to modulate metabolic functions and the immune system (Hillard et al., 2012).

These rapid advances in understanding the biological actions of marijuana, phytocannabinoids, cannabinoids, and eCBs are unraveling the genetic basis of marijuana use with implications not only for recreational use but also for therapeutic potentials for human health and disease. Thus, because of the ubiquitous distribution and role of the ECS in the regulation of human physiological processes, drugs that are targeted to different areas of this system are already benefiting cancer subjects and those with AIDS and metabolic syndromes (Jesudason and Wittert, 2008). So the cloning, identification, and characterization of some of elements of the ECS including the CB1Rs and CB2Rs, which are encoded by *CNR1* and *CNR2* genes, respectively, have involved mapping to human chromosomes 6 and 1, respectively. Intensive research, further progress, and milestones have continued after the respective cloning of *CB1R* and *CB2R* genes of rat (Matsuda et al., 1990; Griffin et al., 1999; Brown et al., 2002;), mouse (Chakrabarti et al., 1995; Shire et al., 1996), and human (Gerard et al., 1991; Munro et al., 1993). From the perspectives described above and from the other chapters in this book, new and interesting components of other elements of the ECS are emerging as potential therapeutic targets and are being uncovered using animal models of central nervous system (CNS) disorders. However, it is important to note that pharmacological actions of CB1Rs and CB2Rs in the CNS may be more diverse and complex than previously recognized (Onaivi et al., 2012) with their differential distribution patterns and species and subtype differences in mammalian cannabinoid receptors. Furthermore, the nature of the interaction between CB1Rs and CB2Rs has not been well studied and characterized (Onaivi, 2009; Onaivi et al., 2012), but emerging evidence suggests that CB₁ and CB₂ receptors may work independently and/or cooperatively in different neuronal populations to regulate diverse physiological and biological functions in mental and neurological disorders. For example, using the brain stimulation reward paradigm in the rat, opposing effects of CB1Rs and CB2Rs in modulating brain stimulation were demonstrated, with CB1Rs mediating brain stimulation and CB2Rs mediating brain inhibition (Onaivi et al., 2012).

The advances in biotechnology and molecular biology, and availability of precise tools and protocols using *in vitro* and various transgenic animals, are being used to explore and identify the involvement of elements of the ECS in models of CNS function and dysfunction. Specifically, a conditional mutagenesis approach in mice was used to investigate CB1R mutants revealing neuron subpopulation-specific effects on behavioral and neuroendocrine stress responses (Steiner et al., 2008). While this conditional mutagenesis approach has been utilized to generate conditional CB1R knockout mice using the Cre/loxP system, leading to a lack of CB1Rs in certain

neuronal subpopulations and thereby providing important clues as to potential therapeutic targets, the approach has yet to be used to generate mice lacking CB2Rs in glial and neuronal subpopulations. Such conditional as well as conventional gene knock strategies have been used to generate loss-of-function mutations to evaluate *in vivo* gene function, albeit with some limitations, and now methods of inducible and reversible regulation of endogenous genes (Sun et al., 2012) have been applied to CB1R mutant mice (Marongiu et al., 2012). It appears that using methods of inducible and reversible regulation of genes in the ECS may have advantages for studying the physiological and pathological roles of this system (Marongiu et al., 2012). In an emerging concept of eCB deficiency syndrome associated with the etiology in migraine, fibromyalgia, irritable bowel syndrome, and psychological disorders, potential clinical interventions that up-regulate the ECS have been proposed (McPartland et al., 2014).

In Table 18.1, we summarize some of the known polymorphisms associated or not associated with *CNR1* and *CNR2* genes involved in human neurological and mental disorders and other disease conditions as reviewed in this book. In the coming era of personalized medicine, genetic variants and haplotypes in *CNR1* and *CNR2* genes associated with obesity or addiction phenotypes may help to identify specific targets in conditions of eCB dysfunction (Onaivi, 2010). Our previous investigations had defined a number of features of the *CNR1* gene's structure, regulation, and variation (Zhang et al., 2004), but many features of *CNR2* gene structure, regulation, and variation still remain poorly defined. We and others have now demonstrated and reported that variants of the *CNR1* gene are associated with a number of disorders and substance abuse vulnerability in diverse ethnic groups including European-American, African-American, and Japanese subjects (Zhang et al., 2004). Most strikingly, variants of *CNR* genes co-occur with other genetic variations and share biological susceptibility that underlies comorbidity in most neuropsychiatric disturbances (Palomo et al., 2007). Thus, emerging evidence indicates that the ECS exerts a powerful modulatory action on retrograde signaling associated with inhibition of synaptic transmission (Lovinger, 2008). Interestingly, a role for variations in the *CNR1* gene has also been associated with striatal responses to happy but not disgusted faces (Chakrabarti et al., 2006) with the implication that functional variation of *CNR1* genotypes may be associated with disturbances of the brain involving emotional and social stimuli, such as autism (Chakrabarti et al., 2006) and depression (Domschke et al., 2008; Onaivi et al., 2013). Additional data from our group focus on these recent advances in cannabinoid genomics and the surprising new fundamental roles that the ECS plays in the genetic basis of marijuana use and cannabinoid pharmacotherapeutics. The powerful influence of cannabinoid-induced retrograde signaling on GABAergic and glutamatergic systems indicates that the main excitatory and inhibitory systems are in part under the influence of the ECS. Thus, the genetic basis of compulsive marijuana use may involve interaction of *CNR* genes with other genes and environmental factors. As with other dependencies with genetic risk factors, the risk for marijuana use is likely to be the result of *CNR* and other genes and environmental factors, each contributing a small fraction of the overall risk (Tyndale, 2003).

Table 18.1 Genetic Polymorphisms of Cannabinoid Receptor Genes (*CNR* Genes)^a

CNR Genes and Polymorphism(s)	Linkage or Association and References
CB2R	Associated with mouse model of impulsivity behavior (Navarrete et al., 2012)
CB ₂ , <i>CNR2</i> SNPs and haplotypes	Associated with human osteoporosis (Karsak et al., 2005)
<i>CNR2</i> SNPs	Not associated with cardiovascular risk factors (Reinhard et al., 2008)
<i>CNR2</i> SNPs	Associated with bone mass (Yamada et al., 2007)
<i>CNR2</i> (Q63R) SNP	Risk factor for autoimmune disorders (Sipe et al., 2005)
<i>CNR2</i> (Q63R) but not (H316Y)	Associated with alcoholism and depression (Ishiguro et al., 2007)
<i>CNR2</i> (rs41311993)	Associated with bipolar disorder (Minocci et al., 2011)
<i>CNR1</i> / <i>FAAH</i> gene	Associated impulsivity and the use of marijuana use (Bidwell et al., 2013; Buchmann et al., 2014)
CB ₁ , two allele DNA polymorphism	Associated with <i>CNR1</i> gene (Onaivi, 2006).
<i>CNR1</i> rs16880261	Associated with cannabis dependence (Agrawal et al., 2009)
<i>CNR1</i> rs4707436	Associated with endocannabinoid effects (Agrawal et al., 2012)
<i>CNR1</i> rs806377	Associated with endocannabinoid effects (Hopfer et al., 2007 ; Agrawal et al., 2009, 2012)
<i>CNR1</i> rs1049353	Associated with addictive disorders (Agrawal et al., 2009 ; Dinu et al., 2009 ; Benyamina et al., 2011)
<i>CNR1</i> rs2023239	Associated with endocannabinoid effects (Agrawal et al., 2009 ; Dinu et al., 2009 ; Filbey et al., 2010)
<i>CNR1</i> rs12720071	Associated with endocannabinoid effects (Agrawal et al., 2009 ; Dinu et al., 2009)
<i>CNR1</i> rs806375, rs806371, rs806368	Associated with drug addiction (Corley et al., 2008 ; Zuo et al., 2009)
1359 G/A <i>CNR1</i> variant	Associated with alcohol dependence (Gadzicki et al., 1999 ; Schmidt et al., 2002)
1359 G/A <i>CNR1</i> variant	Not associated with Tourette syndrome (Gadzicki et al., 2004)
1359 G/A <i>CNR1</i> variant	Not associated with alcohol withdrawal delirium tremens (Preuss et al., 2003)
1359 G/A <i>CNR1</i> variant	Associated with weight loss (Aberle et al., 2007, 2008)
3813 A/G and 4895 A/G variant	Associated with obesity in men (Russo et al., 2007)
<i>CNR1</i> SNPs	Not associated with obesity in German children (Müller et al., 2008)
<i>CNR1</i> SNPs	Associated with obesity and body mass index (BMI) (Gazzero et al., 2007 ; Peeters et al., 2007 ; Benzinou et al., 2008 ; Jaeger et al., 2008)

<i>CNR1</i> , <i>FAAH</i> , <i>DRD2</i> gene	Associated with comorbidity of alcoholism and antisocial behavior (Hoenicka et al., 2007)
(AAT)n repeat of <i>CNR1</i> gene	Conflicting associations with drug dependence (Jesudason and Wittert, 2008)
<i>CNR1</i> variants, SNPs, "TAG" haplotype	Associated with poly-substance abuse (Zhang et al., 2004)
<i>CNR1</i> SNPs	Not associated with poly-substance abuse (Herman et al., 2006)
<i>CNR1</i> SNPs	Associated with cannabis dependence (Agrawal et al., 2009, 2012)
CBR haplotype	Associated with fewer cannabis-dependent symptoms (Hopfer et al., 2007)
<i>CNR1</i> SNPs	Associated with alcohol and nicotine dependence (Hutchison et al., 2008)
<i>CNR1</i> SNPs	No association with anorexia nervosa (Müller et al., 2007, 2008)
<i>CNR1</i> (AAT)n repeats	Associated with restricting and binge/purging anorexia nervosa (Siegfried et al., 2004)
<i>CNR1</i> (AAT)n repeats	Associated with depression in Parkinson's disease (Barrero et al., 2005)
<i>CNR1</i> SNPs	Associated with striatal responses to facial expression (Chakrabarti, et al., 2006)
(AAT)n repeats	Association with attention-deficit hyperactivity disorder (ADHD) in alcoholics (Lu et al., 2008)
<i>CNR1</i> SNP haplotype	Risk factor for ADHD and post-traumatic stress disorder (PTSD) (Lu et al., 2008)
1359 G/A <i>CNR1</i> variant	Associated with schizophrenia (Leroy et al., 2001)
(AAT)n repeats	Not associated with schizophrenia and mood disorders (Li et al., 2000 ; Tsai et al., 2000)
(AAT)n repeats	Associated with schizophrenia (Martinez-Gras et al., 2006)
(AAT)n repeats	Associated with hebephrenic schizophrenia (Ujike et al., 2002 , Chavarria-Siles et al., 2008)
<i>CNR1</i> variants	Associated with depression and anxiety (Domschke et al., 2008)
<i>CNR1</i> variants and (AAT)n repeats	Associated with impulsivity (Ehlers et al., 2006 ; Schroeder et al., 2012)
1359 G/A <i>CNR1</i> tag SNP	Associated with antipsychotic response but not schizophrenia (Hamdani et al., 2008)
<i>CNR1</i> SNPs	No association with cognitive impairment in multiple sclerosis (Woolmore et al., 2008)

^aThere are inconsistencies in some of the association studies of variants in elements of the ECS in neurological and mental diseases, as some of the association studies are not replicable due to the heterogeneity of phenotype assessment ([Ehlers et al., 2007](#)), and the influence of genetic variation on impulsivity would be contingent on environmental factors ([Buchmann et al., 2014](#)).

Further evidence is provided by the complex *CNR1* and *CNR2* gene structures and their associated regulatory elements. In our current and ongoing studies many features of *CNR* gene structures, single nucleotide polymorphisms SNPs, copy number variants (CNVs), CPG islands (i.e., DNA sequences with a high frequency of —C—phosphate—G—, that is, cytosine and guanine separated by only one phosphate), microRNA regulation, and the impact of *CNR* gene variants in neuropsychiatry and, where possible, in rodent models, are being investigated. Although the *CNR1* gene has more CPG islands than the *CNR2* gene, both have CPG islands of less than 300 bases, but they may be regulated by DNA methylation. MicroRNA binding to the 3' untranslated region of the *CNR1* gene with two polyadenylation sites may also potentially regulate CB1R expression. The *CNR1* gene has four exons and there are 135 SNPs reported in more than 1% of the population, with no common SNP that changes amino acids of CB1R currently known or reported. A CNV, which is 19.5 kb and found in four out of 2026 people, covers exons 3 and 4 and codes amino acids that could alter the expression of CB1Rs. *CNR2* has four exons, with CB2A with three exons and CB2B with two exons; and there are about 100 SNPs found in more than 1% of the population, which include common cSNPs that change amino acids of CB2R, including R63Q, Q66R, and H316Y. CNVs in Asian and Yoruba populations have been reported. Therefore, the study of the CBR genomic structure, and its polymorphic nature, subtype specificity and their variants, and associated regulatory elements that confer vulnerabilities to a number of neuropsychiatric disturbances, may provide a deeper insight into the underlining mechanisms. Thus, understanding the ECS in the human body and brain will contribute to elucidating this natural regulatory mechanism and provide potential therapeutic targets in health and disease.

CB₂ CANNABINOID RECEPTORS AS A POTENTIAL THERAPEUTIC TARGET IN NEUROLOGICAL AND MENTAL DISEASES

For many years it was thought that marijuana use, phytocannabinoids, and eCBs act by activating brain type-1 cannabinoid receptors called CB1Rs. A second type of cannabinoid receptor was found in peripheral tissues and mainly in immune cells, and was referred to as the peripheral CB2R. This was because many investigators were not able to detect the presence of neuronal CB2Rs in healthy brains (Munro et al., 1993; Galiegue et al., 1995; Griffin et al., 1999), but CB2R expression was demonstrated in rat microglia cells and other brain-associated immune cells during inflammation (Ibrahim et al., 2003; Benito et al., 2003, 2005; Golech et al., 2004; Nunez et al., 2004; Sheng et al., 2005). Despite the evidence that CB2Rs might be present in the CNS, the expression of neuronal CB2Rs in the CNS has been much less well established and characterized in comparison with the expression of abundant brain CB1Rs.

We and others have reported the discovery and functional characterization of neuronal brain CB₂Rs. A number of studies including those ranging from mice to human subjects, using a variety of techniques including those used in pain models, and in histological, immunohistochemical, *in situ* hybridization, electron microscopy, molecular biological, behavioral and pharmacological, pharmacological MRI, cerebral occlusion and hemispherectomy, transgenic and cell culture approaches, show the functional presence of CB₂Rs in neural progenitor cells, neurons, glial and endothelial cells (Onaivi et al., 2006a,b,c; Palazuelos et al., 2006; Brusco et al., 2008a,b; Chin et al., 2008; Viscomi et al., 2009).

The functional neuronal CB₂Rs have ignited debate and controversy on their possible involvement in drug addiction and neuropsychiatric disorders. While the role of CB₂Rs in CNS disturbances involving neuroinflammation and neuropathic pain has been extensively reported, our studies provided the first evidence for a role of CB₂Rs in depression and substance abuse (Ishiguro et al., 2007; Onaivi et al., 2006a,b,c, 2008a,b,c). The controversy about the functional expression of brain neuronal CB₂Rs remains because the *CNR2* gene and CB₂Rs have received much less attention than *CNR1* and CB₁Rs. Although the expression of CB₁Rs in the brain and periphery has been well studied, many features of *CNR2* gene structure, regulation, and variation remain poorly characterized in comparison to the *CNR1* gene encoding the CB₁Rs. This poor characterization of *CNR2* gene structure and variants hampers progress in the determination of the functional role of CB₂Rs in a number of CNS disturbances. Additionally, the presence of CB₂Rs in the CNS may no longer be a subject of debate, but the neurobiological basis for CB₂R physiological activity and its potential interaction with CB₁Rs remains to be determined as discussed above.

An overwhelming number of studies now document CB₂R expression in neuronal, endothelial, and glial cells. Mounting evidence also shows that CB₂Rs and their gene variants may play possible roles in neuroinflammation occurring in multiple sclerosis, traumatic brain injury, HIV-induced encephalitis, and Alzheimer's, Parkinson's, and Huntington's diseases (Pazos et al., 2004; Benito et al., 2008). Central neuronal but glial-independent neuroprotection by CB₂R activation was reported to counteract apoptotic cell death that is induced by remote axonal damage achieved through PI3K/Akt signaling (Viscomi et al., 2009). Functional interactions between forebrain CB₂Rs and mu-opioid receptors (MORs) were demonstrated (Paldyova et al., 2008) and the CB₂R antagonist SR144528 was reported to decrease MOR expression and activation in mouse brainstem (Paldy et al., 2008). Following our discovery of the presence and functional expression of cannabinoid CB₂Rs in the brain (Onaivi et al., 2006a,b,c), most recent studies have confirmed that CB₂Rs are present in both cultured neural cells and the nervous system of several mammals such as rodents, monkeys, and humans under normal conditions (Fernandez-Ruiz et al., 2006). Thus, CB₂Rs have been implicated in the control of fundamental neural cell processes, such as cell proliferation and survival. It was therefore suggested that manipulating CB₂Rs might be useful for delaying the progression of neurodegenerative disorders and inhibiting the growth of glial tumors (Fernandez-Ruiz et al., 2006).

CB₂Rs have also been shown to subserve differential physiological roles in other neuroanatomical sites such as the brain stem, cortex, cerebellum, periaqueductal gray (PAG), substantia nigra, hippocampus, thalamus, pineal gland, and pinealocytes (Golech et al., 2004; Nunez et al., 2004; Van Sickle et al., 2005; Gong et al., 2006; Suarez et al., 2008, 2009). CB₂Rs in the pineal gland along with other components of the ECS may be involved in the control of pineal physiology (Koch et al., 2008). Gender-dependent changes in the expression of hippocampal CB₁Rs and CB₂Rs were demonstrated in the early maternal deprivation model in neonatal rats (Suarez et al., 2009). While the CB₁R remains one of the most ubiquitous G-protein coupled receptors in the mammalian brain, we have described the multifocal distribution of CB₂Rs, albeit at lower levels than the CB₁Rs, in neuronal and glial processes in a number of brain areas (Gong et al., 2006). This multifocal distribution and the presence of brain CB₂Rs suggest a need to re-evaluate the role of these receptors in neurotransmission. It is important to understand the role of the CB₂R and its gene variants in the CNS and its possible involvement in drug addiction and neuropsychiatric disorders. However, research on the involvement of CB₂Rs in neuroinflammatory conditions and in neuropathic pain has advanced in neuropsychiatry and drug addiction more than in other areas. Therefore, improved information about the *CNR2* gene and its human variants might add to our understanding of the role of brain CB₂Rs not only during neuroinflammatory conditions but also beyond neuroimmunocannabinoid activity.

Many previous studies were not able to detect the expression of CB₂Rs in the brain (Munro et al., 1993; Galiegue et al., 1995; Brown et al., 2002), because the polymerase chain reaction (PCR) primers may not have been specific to detect CB₂R isoforms. The specificity of the available antibodies for both CB₁Rs and CB₂Rs has also been controversial as some could not detect the native and in some cases the transfected cannabinoid CBR antigen, although they recognized proteins in Western blot and in immunohistochemical analysis (Grimsey et al., 2008). There are also problems with the antibodies because of the species differences between human and rodent CB₂ genes. We have resolved some of these issues by using CB₂R isoform-specific TaqMan probes that could differentiate the isoform-specific expression and are more sensitive and specific than the CB₂R antibodies that are currently available. The controversial CB₂R brain expression could also be due to the low expression levels of CB₂A isoform in brain regions and the less specific CB₂R commercial antibodies in immunohistochemical studies, especially in those studies using antibodies against human hCB₂ epitopes for rodent brain immunostaining. There are also problems with the use of the CB₂R-knockout (KO) mouse in Western blots and in behavioral analysis (Buckley et al., 2000). When we analyzed CB₂R-KO mice using the three TaqMan probes against two promoters of mouse the CB₂R gene and the deleted part of the CB₂R gene, we found that the promoters of the CB₂R-KO mouse were still active and that a CB₂R truncated version was expressed, indicating that the CB₂-KO mouse with ablation of the C-terminal peptides of 131 amino acids (Buckley et al., 2000) was an incomplete CB₂R knockout. Another CB₂R-KO mouse line that has now been generated with the ablation of N-terminal peptide

156 amino acid may clarify the specificity of the antibodies that were used against the N-terminal epitopes.

Comparison of these two CB₂R mutant mouse lines suggested that genetic background and/or unknown effects on other signaling pathways may contribute to the observed results obtained from the use of the currently available CB₂R mutant mice (Malfitano et al., 2014). Thus, contrary to prior reports that CB₂Rs were not functionally expressed in neurons, we and others have now reported the wide distribution of CB₂Rs in brain regions, suggesting a re-evaluation of the role of CB₂Rs in the CNS. Generating CB₂R flox mice will allow investigation of the involvement and specificity of the role of the CB₂R in various pathways including the immune system.

The complete gene structure, the 5'- and 3'-UTRs (-untranslated regions), and transcription initiation sites of human CB₂Rs, have not been fully characterized (Abood, 2005; Onaivi et al., 2006a), until now. We and others identified and reported on mouse CB₂R expression in brain regions (Van Sickle et al., 2005; Gong et al., 2006), the specific expression of human or mouse CB₂R isoforms in brain regions not being known. The published evidence had shown significant species differences of CB₂Rs in humans, mice, and rats in terms of peptides, mRNA sizes, gene structure, and pharmacology (Munro et al., 1993; Shire et al., 1996; Brown et al., 2002). Therefore, the discrepancies in the CB₂R mRNA sizes in the literature indicated incomplete gene structure of the CB₂ gene in different species or polymorphisms in the same species. We discovered a novel human *CNR2* gene promoter encoding testis isoform (CB₂A) starting at an exon located ca. 45 kb upstream from the previously identified promoter encoding the spleen isoform (CB₂B) (Liu et al., 2009). The size of the newly identified hCB₂A isoform is about twice that of the previously identified human hCB₂B gene. The 5' exons of both CB₂R isoforms are untranslated 5'-UTRs and alternatively spliced to the major protein coding exon of the *CNR2* gene. We found that CB₂A is expressed to a higher extent in testis and brain than CB₂B, which in turn is expressed in other peripheral tissues more intensively than CB₂A.

Using precise probes, species comparison found that the *CNR2* gene of human, rat, and mouse genomes deviated in terms of structure and isoform expression patterns and that the gene could be regulated by cannabinoid ligand treatment in the mouse model (Liu et al., 2009). The human *CB₂R* gene is almost four times larger than the CB₂ gene of mouse and rat respectively. If the transcription rates are similar between humans and rodents, the hCB₂A isoform would take much longer to be transcribed in the testis and brain. This would be unusual because other gene orthologues between humans and mice are usually within one-fold difference in genomic sizes. Our data show that there are two forms of the CB₂Rs in human, rat, and mouse with differential subtype distribution specificities in the brain and peripheral organ tissues. The promoter-specific CB₂R isoform distribution may in part explain why CB₂Rs were previously undetectable in both human and rodent brains (Munro et al., 1993; Galiegue et al., 1995; Brown et al., 2002). Our studies provided the first evidence for the CNS effects of the CB₂R and its possible involvement in drug addiction and neuropsychiatric disorders (Onaivi, 2006; Uhl et al., 2006; Onaivi et al., 2006a,b,c, 2008a,b,c; Ishiguro et al., 2007). We utilized behavioral and

molecular methods to study and determine whether there was a link between depression (which may be a risk factor in drug/alcohol addiction) and brain CB₂Rs. First, we established the use of the mouse model of depression, i.e., the chronic mild stress (CMS) model, which has been a validated and widely used model for the screening of antidepressants. Briefly, the CMS model measures one of the core symptoms of depression, which is anhedonia, the inability to experience pleasure. Mice were subjected to CMS daily for 4 weeks, and anhedonia was measured by the consumption of sucrose solution. Behavioral and rewarding effects of abused substances were determined in the CMS and control animals. The expression of CB₂Rs and their gene transcripts was compared in the brains of CMS and control animals by Western blotting and real-time PCR (RT-PCR). CMS induced gender-specific aversions in the test of anxiety, which were blocked by CB₁R and CB₂R agonists. In other studies, we demonstrated that direct CB₂R antisense oligonucleotide microinjection into the mouse brain induced anxiolysis, indicating that CB₂Rs are functionally present in the brain and may influence behavior (Onaivi, 2006; Uhl et al., 2006; Onaivi et al., 2006a, 2008a,b; Ishiguro et al., 2007).

The clinical and functional implications of neuronal CB₂Rs in the brain will gradually become clearer as more research unravels their contribution in drug addiction and neuropsychiatry. Knowledge from our investigations and other recent studies that neuronal CB₂Rs are present in the brain raises many questions about their possible roles in the nervous system. Our studies implicate the involvement of neuronal and glial CB₂Rs in the CMS model of depression and substance abuse. The immunohistochemical localization of brain CB₂Rs, when compared to that of CB₁Rs, may be an indication of other putative functional roles of CB₂Rs in the CNS. Therefore, both CB₁Rs and CB₂Rs seem likely to work independently and/or cooperatively in differing neuronal populations to regulate important physiological activities in the CNS.

Recent events in the clinic have linked the use of a CB₁R antagonist, Acomplia[®], as an anti-obesity drug and appetite suppressant with a higher risk of depression and suicide. Associations of the *CNR2* gene with depression, drug abuse, anorexia nervosa, and schizophrenia in a human population have also been reported (Onaivi et al., 2006a,b,c, 2008a,b), suggesting that CB₂Rs may be involved in eCB signaling mechanisms associated with the regulation of emotionality. More studies are therefore required to determine if CB₂R ligands carry the risk of depression or suicide, which has led to the withdrawal of rimonabant from use as an appetite suppressant in the control of obesity in Europe.

SUMMARY OF THE ROLE OF ENDOCANNABINOIDS IN NEUROINFLAMMATORY AND NEURODEGENERATIVE DISORDERS

The limited effectiveness of current therapies for most neurological and neurodegenerative disturbances including Alzheimer's, Parkinson's, and Huntington's diseases,

multiple sclerosis, epilepsy, and migraine underscores the need for intensifying research efforts aimed at developing new medications for preventing or retarding the disease process (Aso and Ferrer, 2014). There is evidence that endocannabinoid signaling modulates numerous concomitant pathological processes, including regulation of neuroinflammation, excitotoxicity, mitochondrial dysfunction, and oxidative stress (Aso and Ferrer, 2014; Kong et al., 2014), and both CB1Rs and CB2Rs are expressed in the immune system with higher CB2R expression in all immune subtypes (Basu and Dittel, 2011; Malfitano et al., 2014) along with higher CB1R expression in neurons. Extensive studies, reviewed also in previous chapters, have demonstrated *in vitro* and *in vivo* that CB2R is a potent regulator of immune function and therefore a prime target in neuroinflammatory and neurodegenerative disorders (Basu and Dittel, 2011; Malfitano et al., 2014). While targeting the CB2Rs in neuroinflammatory and neurodegenerative disorders may be clinically attractive, CB2R gene structures differ in mice, rats, and humans with different expression patterns in the brain and periphery. CB2Rs are the main mediator of the immunoregulatory effects of cannabinoids (Kong et al., 2014), and stroke or brain injury up-regulates the ECS, including CBRs, thereby contributing to immunosuppression that may limit neuroinflammation (Lehmann et al., 2014). Medical marijuana and formulation of mixtures of cannabinoids are touted as having positive effects in some neuroinflammatory and neurodegenerative disorders including multiple sclerosis, epilepsy, and migraine, which together will encourage progress towards clinical trials. Other lines of evidence have shown that elements of the endocannabinoid neurosignaling system have neuroprotective capabilities and therefore are potential targets for neurodegenerative disorders (Fagan and Campbell, 2014). However, more basic and clinical research is required for the development of therapeutically effective cannabinoid compounds, and the complexity of CB2R isoforms and their human and rodent variants should be carefully considered in the development of CB2R-based therapeutic agents (Zhang et al., 2004; Liu et al., 2009).

SUMMARY OF THE ROLE OF ENDOCANNABINOID IN PSYCHOLOGICAL AND PSYCHIATRIC DISORDERS

The ubiquitous CBRs and other elements of the ECS are probably the most abundant binding sites in the CNS and are known to be involved in most biological processes with impact on psychological and neuropsychiatric disorders. Therefore the ECS has been implicated in the regulation of a variety of physiological processes, including a crucial involvement in brain reward systems and the regulation of motivational processes (Vlachou and Panagis, 2014). Gene–environment interactions likely play a significant role in the pathogenesis of schizophrenia (Kannan et al., 2013) and underlie differences in pathological, behavioral, and clinical presentations (Kannan et al., 2013). Such gene–environment interactions can be extended to bipolar disorders, Tourette syndrome, drug reward and addiction, and appetite

(dys)regulation in obesity. Now many studies (summarized in [Table 18.1](#)) have shown that *CNR1* and *FAAH* SNPs may contribute to these disorders. In our ongoing studies, many features of CBR gene structures, SNPs, CNVs, CPG island, and microRNA regulation, and the impact in neuropsychiatry and, where possible, in rodent models, are evaluated. Accumulating evidence suggests the importance of CNVs in the etiology of neuropsychiatric disorders ([Horev et al., 2011](#)). The clinical consequences of CNV in the coding and non-coding *CNR* gene sequences associated with human phenotypes and disorders are mostly unknown and under investigation. Advances in genomic technologies and the analysis and identification of *CNR* gene CNVs may uncover the relationship (if any) between *CNR* gene CNVs to phenotype and disease. While *CNR1* and *CNR2* SNPs have been associated with a number of neuropsychiatric disorders (see [Table 18.1](#)), it is unclear to what extent *CNR* gene CNVs are involved in psychological and psychiatric disorders. Therefore, more studies are needed to determine the role and contribution of *CNR* gene CNVs to conditions of endocannabinoid dysregulation in psychological and psychiatric disorders.

SUMMARY OF THE ROLE OF ENDOCANNABINOIDS IN IMPULSIVE AND COMPULSIVE DISORDERS

Impulsivity and compulsive disorders are known to be present in many psychiatric disorders such as bipolar disorder, personality disorders, attention-deficit hyperactivity disorder (ADHD), eating disorders, and substance abuse ([Swann et al., 2004](#); [Winstanley et al., 2006](#); [Malloy-Diniz et al., 2007](#)). From mice to human subjects, altered endocannabinoid functioning has been associated with impulsive and compulsive behaviors ([Pattij and Vanderschuren, 2008](#); [Bidwell et al., 2013](#); [Buchmann et al., 2014](#)), and a number of studies using animal models have reported that the ECS is involved in obsessive-compulsive disorder (OCD) (e.g., [Gomes et al., 2010](#)). Enhanced eCB signaling has been implicated in a number of adolescent behavioral features, including increased risk taking, impulsivity, and novelty seeking ([Buchmann et al., 2014](#)). In human adults, the personality trait of novelty seeking was found to be inversely related to CB1R availability, which was assumed to be based on compensatory down-regulation of a high eCB tone ([Van Laere et al., 2009](#)). Impulsive self-injurious wrist cutting behavior has been associated with low levels of CB1R mRNA in female eating disorder patients ([Schroeder et al., 2012](#)). In their study, [Buchmann et al. \(2014\)](#) showed that the interaction of *CNR1* gene variants with experience of early life adversity may play a role in determining impulsive behavior in adolescents. Variation in the *CNR1* and *FAAH* genes along with marijuana-related problems have been associated with impulsivity ([Bidwell et al., 2013](#)). Interestingly, impulsivity-like traits and the involvement of the CB2R in the modulation of impulsivity have been evaluated in a mouse model ([Navarrete et al., 2012](#)). The investigators

concluded that CB2Rs might play an important role in regulating impulsive behaviors and should be considered a promising therapeutic target in the treatment of impulsivity-related disorders. There are, however, some inconsistencies in some of the association studies of variants in elements of the ECS in neurological and mental diseases, as some of these studies are not replicable due to the heterogeneity of phenotype assessment (Ehlers et al., 2007), and the influence of genetic variation on impulsivity would be contingent on environmental factors (Buchmann et al., 2014).

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