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Drug discovery strategies that focus on the endocannabinoid signaling system in psychiatric disease

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Abstract

Introduction—The endocannabinoid (eCB) system plays an important role in the control of mood, and its dysregulation has been implicated in several psychiatric disorders. Targeting the eCB system appears to represent an attractive and novel approach to the treatment of depression and other mood disorders. However, several failed clinical trials have diminished enthusiasm for the continued development of eCB-targeted therapeutics for psychiatric disorders, despite of the encouraging preclinical data and promising preliminary results obtained with the synthetic cannabinoid nabilone for treating post-traumatic stress disorder (PTSD).

Areas covered—This review describes the eCB system's role in modulating cell signaling within the brain. There is a specific focus on eCB's regulation of monoamine neurotransmission and the stress axis, as well as how dysfunction of this interaction can contribute to the development of psychiatric disorders. Additionally, the review provides discussion on compounds and drugs that target this system and might prove to be successful for the treatment of mood-related psychiatric disorders.

Expert opinion—The discovery of increasingly selective modulators of CB receptors should enable the identification of optimal therapeutic strategies. It should also maximize the likelihood of developing safe and effective treatments for debilitating psychiatric disorders.

1. Introduction

Cannabis sativa, or marijuana, is one of the most widely used illicit drugs, known to promote relaxation, euphoria, and a feeling of contentment [1–3]. Chronic use, however, can be accompanied by dysphoria, depressive mood, and increased anxiety [4] with the severity of symptoms being exacerbated by exposure to greater concentrations and increased frequency of usage [5]. Cessation of the clinical trial of the cannabinoid receptor antagonist, Rimonabant, due to serious side effects of depression and suicidal ideation brought increased attention to the role of the endocannabinoid (eCB) system on the regulation of mood [6]. CB receptors are widely distributed in the CNS and the eCB system modulates several key physiological systems involved in the control of mood. Recent studies have

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indicated that cannabis impacts stress-integrative neural circuits resulting in adaptive or maladaptive responses that may partially explain its biphasic effect on mood and emotional state. Targeting specific components of the eCB system may represent a novel and attractive approach to the treatment of multiple psychiatric disorders. The purpose of the present review is to (1) summarize current knowledge regarding cell signaling of the eCB system in brain, (2) present evidence for eCB regulation of monoamine transmitters and its implications for the regulation of mood, (3) discuss data showing the eCB system's role in the regulation of the stress response and how its dysfunction could lead to psychiatric disorders, and (4) highlight compounds, in various stages of development, that target this system and may provide therapeutic benefit for the treatment of mood-related psychiatric disorders.

2. Principles of Endocannabinoid Signaling

The first pharmacologically active compound identified in *Cannabis sativa*, Δ^9 -tetrahydrocannabinol (THC), was characterized in 1964 and has since been established as its primary psychoactive component [7, 8]. Since its discovery, over 100 additional active components of *Cannabis sativa* have been identified [8]. The first cannabinoid receptor, CB1r, was identified in 1988 and a second receptor, CB2r, was characterized in 1993 [3, 9]. Both are $G_{i/o}$ protein-coupled receptors with distinct distributions in the body [10]. CB1 receptors are one of the most abundant G protein coupled receptors (GPCR) in the brain and their activation most commonly results in the inhibition of neurotransmitter release [10, 11]. CB2 receptors are most prevalent in the immune system [10, 12]; however, recent studies suggest a presence in the central nervous system as well, showing CB2 receptor localization in the hippocampus, substantia nigra, periaqueductal gray matter, and parvocellular reticular nucleus [13, 14]. Following the identification of the CB receptors, endogenous cannabinoid ligands, or endocannabinoids (eCBs), were discovered. The first was N-arachidonyl ethanolamide (AEA), which was named "anandamide" after the Sanskrit word meaning "bliss" [3, 15]. Another well characterized eCB is 2-arachidonoylglycerol (2-AG), first isolated from canine intestines by Mechoulam et al. in 1995 [16]. It is now generally accepted that 2-AG is a full CB1r and CB2r agonist, whereas AEA, which is less potent, is a partial agonist [17]. Initially, the mechanism proposed for eCB release involved a depolarization-induced event followed by retrograde signaling and binding of the endogenous ligand to presynaptically distributed receptors [10, 18]. New evidence suggests that eCB can regulate synaptic transmission via non-retrograde and autocrine mechanisms, with CB1 receptors having been discovered postsynaptically [19]. Furthermore, eCBs can bind and activate transient receptor potential vanilloid receptor type 1 (TRPV1) receptors [10].

The rate of eCB synthesis and degradation determines their signaling profiles. Two primary mechanisms are known to be responsible for 2-AG synthesis: increases in intracellular Ca^{2+} via postsynaptic depolarization and activation of phospholipase C β (PLC β) via stimulation of group I metabotropic glutamate receptors. PLC β forms diacylglycerol from the hydrolysis of phosphatidylinositol, which diacylglycerol lipase α (DGL α) then converts to 2-AG [10]. Monoacylglycerol lipase (MGL) is the main enzyme responsible for breaking down 2-AG, rendering it inactive and thus controlling the strength and duration of its modulatory activity

[10, 20]. The synthesis and degradation of AEA is more complex. Though it is known that increases in intracellular Ca^{2+} and postsynaptic depolarization stimulate AEA formation, the mechanism underlying this process has yet to be elucidated [21]. N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase-D (NAPE-PLC) has been identified as a contributor to AEA synthesis, but other synthetic pathways have also been reported [22]. AEA is primarily degraded by fatty acid amide hydrolase (FAAH) and, similar to MGL for 2-AG, FAAH controls the spatiotemporal profile for AEA signaling [10]. In contrast to the activity-dependent classical eCB signaling, tonic eCB release has been observed in several brain regions [10].

3. Targeting the Endocannabinoid System

There are a wide variety of ways in which the eCB system can be targeted and modulated (Table 1). The most direct method is by utilizing CB1r or CB2r agonists and antagonists to increase or decrease eCB signaling, respectively. Numerous selective and nonselective agonists and antagonists have been synthesized and characterized (Table 1) and have been useful tools in elucidating the role of the eCB system. Several of these compounds have advanced to clinical trials over the past decade or two, though predominantly they have been tested for the treatment of pain, inflammation, neurodegenerative disorders, nausea, obesity, and nicotine and alcohol dependence (reviewed in [23]).

eCB signaling can also be modified by targeting the catabolic and metabolic enzymes of AEA and 2-AG (Table 1). By inhibiting FAAH and MGL, eCB levels can be increased, allowing for greater signaling to occur. Conversely, by inhibiting eCB-synthesis, eCB levels are decreased resulting in less signaling. Finally, the eCB degradative enzymes are located intracellularly, so by blocking eCB uptake into the pre- or post-synaptic cell, eCB levels will remain high and signaling will be increased [3]. These methods allow more fine-tuning of the eCB system as opposed to the CB1r agonists and antagonists, and many of these approaches have also been utilized to test the effects of altered eCB signaling in preclinical models of psychiatric disorders. More recently, eCB-based drugs have begun clinical testing for the treatment of various psychiatric disorders, including schizophrenia, posttraumatic stress disorder (PTSD), and depression (Table 2).

4. The Endocannabinoid System and Mood Regulation

Recognition of the involvement of the eCB system in the regulation of mood and specifically its role in depression and anxiety arose, in part, from observations obtained from symptomatic individuals [24, 25]. A significant increase in CB1r density and efficacy was reported in the dorsolateral prefrontal cortex (PFC) of depressed suicide victims, suggesting that altered functioning of the eCB system in the PFC could contribute to depression [24, 26]. Several other studies examined dysregulation of the eCB system in individuals suffering from post-traumatic stress disorder (PTSD) and discovered that PTSD patients had greater CB1r availability throughout the brain as well as a significant decrease in AEA plasma concentrations [25, 27].

Genetic manipulations of the eCB system in animal models, particularly CB1r knockout (KO) mice, have provided insight into how eCB signaling affects behavior. CB1 KO mice

exhibit an increase in passive behaviors compared to wild type (WT) mice in the forced swim test (FST), which is typically interpreted as a depressive phenotype [28]. They also show an increase in immobility time compared to WT mice in another animal model of depression, the tail suspension test (TST) [29]. Additionally, when exposed to chronic mild stress, KO mice develop anhedonia at a faster rate than WT mice, suggesting an increase in vulnerability to chronic stress [30]. In behavioral paradigms measuring anxiety-like behaviors, such as the elevated plus-maze, open-field test and light-dark box, CB1r KO mice exhibited increased anxiety-like behaviors [24]. CB1r KO mice display hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and higher levels of circulating corticosterone following stressor exposure, a response that is also commonly observed in depressed patients [31].

Pharmacological approaches also support a role for the eCB system in mediating depression and anxiety. Acute administration of CB1r agonists decreases the amount of behavioral despair observed in the FST, and similar anti-depressant-like effects are observed in the FST and TST following chronic administration [32, 33]. In support of this, injection of CB1r agonists directly into brain regions that are known to be involved with emotion reduce the depressive phenotype [24]. Consistent with these preclinical findings, Rimonabant, a CB1r antagonist originally intended as an anti-obesity drug was withdrawn from clinical trials due to significant undesirable side effects including depression and suicidal ideation [6]. Concerns about mood-altering side effects resulted in the withdrawal of several other CBR antagonists from clinical trials, including Taranabant and Otenabant from phase III trials and Ibipinabant and Surinabant from phase II trials [34]. These results provide evidence for a potential protective role of the eCB system in the development and treatment of depression and anxiety.

It has been postulated that cognitive impairment is associated with various psychiatric disorders [35]. Work by Robbe and Buzsaki has shown that cannabinoid administration causes memory impairment by altering the synchrony and temporal coordination in both the hippocampus and the thalamocortical region, and this impairment represents another potential mechanism for cannabinoid involvement in psychiatric disorders [36, 37]. Recent studies have also shown that the eCB system, particularly via CB2r actions on glial cells and microglia, interacts with the immune system whereby inflammation produces an increase in 2-AG production [38]. Additionally, various pro-inflammatory cytokines that are known to be upregulated in depression are reduced following cannabinoid treatment as well as antidepressants [38]. While this link between CB2r and the inflammatory response in depression and other psychiatric disorders should not be overlooked, for the scope of this review, the remainder of the paper will focus on how the eCB system modulates monoamine transmission and the stress response.

Putative mechanisms underlying a neuroprotective role of the eCB system arise from studies showing that increases in eCB signaling can stimulate neurogenesis [24, 39, 40] by increasing brain-derived neurotrophic factor (BDNF) levels [41]. As decreased expression of hippocampal BDNF has been linked to a depression, agents that increase eCB signaling may stimulate hippocampal neurogenesis thereby engaging neural circuitry mediating the regulation of mood [24, 39, 40]. Interestingly, THC administration has also been shown to

increase hippocampal BDNF levels [41]. Compounds that increase eCB neurotransmission have also been shown to attenuate the neuroendocrine response to stress and increase adaptive coping behaviors [42]. This effect is mediated, in part, by eCB modulation of noradrenergic circuitry [24]. Moreover, the ability of the eCB system to more broadly affect monoaminergic neurotransmission may underlie, in part, cannabinoids effects on mood. For example, FAAH inhibitors and CB1r antagonists enhance serotonergic neurotransmission [32, 33], CB1r activation can stimulate the release of norepinephrine (NE) and cannabinoid receptor agonists stimulate DA efflux in the cortex [33]. These findings indicate that eCBs may be neuroprotective, not only with respect to neurodegeneration but also against the development of psychiatric disorders. The next section of the review will focus on evidence supporting a role for the eCB system in the modulation of monoaminergic systems and the HPA axis.

5. The Endocannabinoid System and Monoamines

5.1 eCB and 5-HT

Serotonin (5-HT) is one of the major monoamines implicated in depressive disorders and selective-serotonin reuptake inhibitors (SSRIs) are a commonly prescribed antidepressant [8]. Recent research shows that the eCB system is positioned to modulate serotonergic transmission. Dorsal Raphe (DR)-5-HT neurons contain CB1r and the synthetic and catabolic eCB enzymes, suggesting that the eCB system is positioned to modulate DR-5-HT signaling [43]. Electrophysiological studies have shown that eCBs are synthesized and released in an activity-dependent fashion within the DR [44, 45]. *In vivo* extracellular recordings indicate that administration of the CB1r agonist, WIN 55,212-2, and FAAH inhibitors result in increased DR-5-HT firing [32, 33]. Studies have also shown that eCB-induced increases in serotonergic signaling involve CB1r activation in the PFC, as lesions of the PFC abrogate eCB-induced excitatory responses in DR-5HT neurons [32]. *In vitro* studies provide a more direct role for eCB regulation of the DR-5-HT system. Several studies have shown that CB1r stimulation and administration of WIN 55,212-2 decrease glutamatergic signaling in DR-5-HT neurons [44, 45]; however, other findings indicate that eCB tone regulates DR-5-HT signaling by modulating GABAergic inputs to serotonergic neurons [46]. Neuroanatomical studies examining the synaptic relationship of CB1r with GABAergic and glutamatergic neurons are needed to better define sites of action of eCBs in the modulation of DR-5-HT signaling [46].

The 5-HT system plays a role in the neuroendocrine response to stress and eCB modulation of 5-HT circuits impact the regulation of mood [43]. 5-HT1A receptor activation decreases stress-induced ACTH and corticosterone production, and pharmacological antagonism of these receptors enhances the stress response and contributes to an anxiety phenotype [47, 48]. For example, stress-induced increases in anxiety-like behaviors are attenuated by cannabidiol (CBD) administration and this involves 5-HT neurotransmission as WAY100635, a 5-HT1A receptor antagonist, prevents the anxiolytic effect [49]. Additionally, in an adolescent mouse model of social withdrawal, hippocampal CB1 and 5-HT1A receptor densities were increased and HPA stress responses were exaggerated, further illustrating how the eCB and 5-HT systems are both involved in anxiety-like behaviors [50].

Further, one study using micro-positron emission tomography observed that both non-stressed CB1 KO mice and chronically stressed wild type mice exhibited similar decreases in 5-HT transporter levels, showing an interplay between the stress, 5-HT, and eCB systems in the development of mood disorders [51]. In contrast, chronic exposure to cannabinoids may elevate stress responses via an increase in 5-HT_{2A} receptor expression in the paraventricular nuclei (PVN) resulting in increased production of prolactin and corticosterone [52]. Taken together, these data illustrate that the eCB system modulates serotonergic neurotransmission to impact behavior but the extent to which the exposure produces an anxiogenic or depressive response depends on duration of exposure and the involvement of stress-integrative circuitry.

5.2 eCB and NE

High levels of NE have been correlated with an increased duration of remission in previously depressed patients, implicating a potentially protective role of NE [53, 54]. It is well accepted that NE signaling is important in the pathophysiology of depression [54] and compounds that increase NE levels, such as serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants and MAO inhibitors are effective antidepressants. The locus coeruleus (LC) provides NE to the PFC, and high resolution neuroanatomical studies have demonstrated co-existence of CB1r with noradrenergic axon terminals in this brain region [55]. CB1 receptors are localized both pre- and post-synaptically in the LC [56]. Most of the pre-synaptic CB1r were localized to symmetric synapses, indicating that they are most likely regulating GABAergic transmission [54]. The presence of CB1r in noradrenergic neurons [56] further suggests that the eCB system may modulate noradrenergic activity directly without pre-synaptic modulation of amino acid signaling [54], potentially acting as a subsequent brake on LC activation. The opposing effects of CB1 receptors on noradrenergic terminals decreasing NE release versus CB1 receptors on GABAergic and serotonergic terminals increasing NE release also demonstrates the importance of local eCB levels in alteration of monoamine neurotransmission [57].

Additional studies support eCB regulation of NE signaling. CB receptor agonists CP55940 and WIN 55,212-2 increase spontaneous firing and stimulate immediate early gene c-Fos expression in LC-NE neurons [58, 59]. Additionally, an increase in Fos expression was observed in dopaminergic neurons following treatment with CB agonists; however, this increase was blocked by co-treatment with either an α 1-AR antagonist or an α 2-AR agonist, indicating that the CB agonist-induced increase in dopaminergic activation is likely due to LC-NE activation [54, 59]. The dose-dependent increase in LC-NE firing observed after both systemic and central CB agonist administration is blocked by co-administration with SR141716A, a CB1r antagonist [58, 60]. Interestingly, SR141716A administration by itself results in a decrease in LC activity, suggesting that tonic eCB production controls the LC under basal conditions [54]. Also, administration of a FAAH inhibitor increases the spontaneous firing rate of LC-NE neurons, supporting the notion of tonic eCB regulation of LC neurons [33]. Finally, CBs also alter NE neurotransmission in other noradrenergic circuits, such as the nucleus of the solitary tract (NTS), that influence limbic circuitry including the amygdala and the HPA axis via the hypothalamus [61]. As the NTS is involved in interoceptive responses to stress, a better understanding of how the eCB system

is positioned to influence neuronal activity in this region would help elucidate how potential cannabinoid-based therapeutics might alter stress responses in humans.

Following CB exposure, increases in NE levels have been observed and may involve mechanisms other than disinhibition of LC noradrenergic neurons [62]. For example, *in vitro* studies have shown that CBs can inhibit monoamine oxidase (MAO) [63]. MAO metabolizes monoamine neurotransmitters, so inhibition would produce increased NE levels. CB-induced decreases in $\alpha 2$ -AR expression in the LC have been observed which would result in an increase in NE release at postsynaptic targets [54]. Increases in NE efflux in the PFC have been observed following acute and chronic CB administration and pretreatment with SR141716A blocks CB-induced increases in NE levels [64, 65]. Taken together, these data illustrate alterations in NE signaling following CB administration in regions where dysregulation is associated with stress and depressive- and anxiety-like effects.

5.3 eCB and DA

There is evidence that the eCB system is also positioned to modulate and alter dopamine (DA) neurotransmission. Several eCBs, including 2-AG and AEA have been found in dopaminergic brain regions and CB1r expression has been observed in the substantia nigra (SN) and ventral tegmental area (VTA), as well as the dorsal and ventral striatum, and various other targets of dopamine innervation [3, 66–68]. Unlike serotonergic neurons in the DR and noradrenergic neurons in the LC, CB1r expression is not very prominent in tyrosine hydroxylase positive, dopamine-producing neurons [69]. eCB modulation of dopaminergic signaling most likely occurs indirectly via eCB modulation of glutamatergic or GABAergic neurons [69]. Exogenous cannabinoids also appear to affect dopaminergic transmission differently than eCBs. It has shown that cannabinoid agonists increase the spontaneous firing of dopaminergic cells within the VTA through CB1r signaling [69, 70]. In olfactory-bulbectomized rats, which is an animal model of depression, self-administration of WIN 55,212-2 was increased, suggesting that the increase in the CB1r agonist administration could be an attempt to compensate for the decreased DA neurotransmission and rewarding effects observed in some animal models of depression [71]. Additionally, several other studies have similarly reported that cannabinoid agonists cause an increase in DA efflux within the cortex [72, 73]. As mentioned previously, increases in monoamine levels in the PFC represent one putative mechanism underlying the effectiveness of antidepressant therapeutics [74].

Dysregulation of dopaminergic neurotransmission has been implicated in major psychiatric disorders such as schizophrenia. Low levels of dopamine in the mesocortical pathway and increased levels of dopamine in the mesolimbic pathway are thought to underlie the pathophysiology of schizophrenia [75, 76]. Based on evidence showing that cannabinoids can increase dopaminergic signaling in the mesolimbic pathway, contributing to the positive symptoms of schizophrenia, a cannabinoid hypothesis of schizophrenia has been proposed [77]. This theory suggests that overactivity of the eCB system could play a role in the development of schizophrenia [77]. In support of this, several studies have shown that paranoid schizophrenic patients, without a drug history, have heightened AEA levels in their cerebrospinal fluid (CSF) [78–80]. Also, postmortem examination of schizophrenic patients

revealed that an increase in CB1r binding in several brain regions, including the dorsolateral PFC and cingulate cortex [77]. Additionally, many studies have indicated that adolescent cannabis use is correlated with an earlier onset of schizophrenic symptoms and more severe psychosis [81]. More recently, however, there has been an abundance of compelling data gathered that disproves this hypothesis. First, CB1r antagonism using Rimonabant proved unsuccessful in clinical trials at alleviating psychotic symptoms [77]. Second, AEA levels in the CRF of schizophrenic patients have been negatively correlated with psychosis [79, 80]. Electrophysiological and behavioral studies have shown that increasing AEA levels further increases the search for reward in mice, counteracting the effects of dopaminergic dysfunction [68]. Therefore, this increase in AEA levels might indicate a compensatory mechanism in schizophrenic patients, since it is thought that alterations in reward-related processes could contribute to the emotional disturbances seen in schizophrenic individuals [68]. Finally, direct measurement of CB1r expression in the dorsolateral PFC showed a decrease in receptor density [82], suggesting that the eCB system could be exploited to protect against schizophrenic symptoms. Current CB1r agonists are being tested in clinical trials and could hopefully become efficacious drugs for alleviating the psychosis associated with schizophrenia [83, 84].

5.4 eCB and MAO Inhibition

Monoamine oxidase (MAO) is the main enzyme responsible for metabolizing catecholamines and other neurotransmitters, resulting in their inactivation and termination of their signaling [74]. It exists as two different isoforms. MAO-A is responsible for metabolizing NE and 5-HT while MAO-B is responsible for breaking down benzylamine [74]. Both MAO-A and -B metabolize DA and tyramine; however, since MAO-B is the predominant isoform in the basal ganglia, it plays a much greater role in the metabolism of DA compared to MAO-A [74, 85]. In the 1950s and 60s, it was discovered serendipitously that treatment of tuberculosis with isoniazid resulted in patients experiencing euphoria and antidepressant phenotypes [74]. Subsequent studies then showed that isoniazid also functions to inhibit MAO, which led to the development of the first class of antidepressant medications and the monoamine hypothesis of depression, suggesting that an increase in monoamine levels can alleviate symptoms [74]. While these drugs are very successful at treating depression, they also can result in extreme side effects, such as liver toxicity and hypertension due to dietary tyramine intake (the cheese reaction) [86]. Reversible MAO inhibitors have since been developed, which help alleviate the drug-food interactions by allowing tyramine to compete for the binding site [74].

In 2010, Fisar reported that several cannabinoids alter MAO functioning in vitro. By performing radiochemical assays on the mitochondria from pig brains, the effects of THC, AEA and WIN 55,212-2 on MAO-A and -B activities were determined. All three were nonselective and weak MAO inhibitors, with THC having the greatest potency for both enzymes. AEA has the lowest potency of the tested cannabinoids, and competitively binds to MAO-A while noncompetitively binding to MAO-B. Finally, WIN 55,212-2 had equal potency to THC for MAO-A; however, it is uncompetitive at MAO-B, not fully inhibiting its functioning, even when at high concentrations [63]. It is worth noting that the IC₅₀ values of the cannabinoids were much larger than that of iproniazid, indicating that only at very

high concentrations, typically larger than physiologically active levels, do these compounds function to inhibit MAO. Since cannabinoids are very lipophilic and MAO is localized to the outer membrane of the mitochondria, it is possible that they can affect MAO activity by accumulating in the mitochondrial membrane following chronic administration [63, 74]. Considering that strong MAO inhibition results in many negative side effects, potentially weaker inhibition by physiological eCB levels could result in a slight increase in monoamine levels without causing toxicity.

6. Effects of Cannabinoids on the Stress Response

HPA hyperactivity is very common in individuals suffering from depression and anxiety [31, 42]. While glucocorticoid release initially is beneficial, priming the body physiologically and metabolically to deal with threats, long-term secretion can result in maladaptive cardiovascular, metabolic, and even neurological conditions [87]. There are negative feedback mechanisms in place, allowing glucocorticoids to attenuate HPA axis activity, and studies have shown the PFC to be the critical site for this termination [88]. Immunohistochemical data and electron microscopy provide evidence that CB1r in layer V of the prelimbic PFC region are found on GABAergic terminals [88]. Genetic deletion of CB1r and injection of CB1r antagonists directly into the PFC produce an increase in the stress response and corticosterone levels, further implicating the importance of the eCB system in the negative feedback mechanisms on the HPA axis [88]. Activation of the GABAergic circuits located in either the prelimbic PFC or the bed nucleus of the stria terminalis (BNST) result in a decrease in corticotropin-releasing factor (CRF) release from the paraventricular nucleus (PVN) [89].

In vivo rat studies have demonstrated that peripheral injection of corticosterone causes a swift escalation of eCB levels in the PVN, indicating that stress upregulates hypothalamic eCB levels via a glucocorticoid-mediated mechanism [90]. This has led Hill et al. to propose a model for the influence of the eCB system on the temporal phases observed in glucocorticoid feedback. It is known that stress causes the production and release of glucocorticoids into the circulation. According to the model, rapid attenuation of the HPA axis occurs via an increase in eCB synthesis and release in the PVN, resulting in the suppression of glutamatergic signaling on CRF-releasing neurons [88]. A longer, time-delayed feedback loop centers on eCB production in the mPFC. Circulating glucocorticoids stimulate 2-AG synthesis and release in the prelimbic mPFC, which then binds to CB1r on GABAergic neurons. This results in the disinhibition of projection neurons that synapse with GABAergic neurons in the BNST, ultimately causing a decrease in signals projecting to the PVN and a subsequent decrease in CRF release [88]. While it appears that eCBs are produced on demand in the above pathways, it has been proposed that tonic AEA signaling in the basolateral amygdala (BLA) occurs [91]. This is the basis for a gatekeeper function, in which tonic eCB tone results in basal inhibition of the HPA axis [90]. During acute stress, there is an increase in FAAH activity, resulting in a decrease in AEA levels [91]. This causes a disinhibition of the principal neurons located in the BLA, subsequently leading to an increased amygdalar output to various regions including the PVN, stimulating the HPA axis [91].

The amygdala is a key structure involved in the regulation of fear and emotional memory, and the eCB system plays a role in regulating the amygdala's response to stress. As previously mentioned, stress causes a rapid decrease in AEA levels within the amygdala however, when stress is absent and corticosterone is administered, an increase in AEA levels within the amygdala is observed [90, 91]. While this might seem contradictory, such a mechanism may be adaptive. Activation of the HPA axis is important for escaping and managing an acute stress; however, problems arise from over-activation of the HPA axis. Therefore, though stress initially causes a decrease in amygdalar AEA levels via a glucocorticoid-independent pathway, the subsequent glucocorticoid release caused by HPA axis stimulation feeds back to increase amygdalar AEA levels, attenuating the HPA axis activity [90]. While acute stressors only affect the production of AEA in the amygdala, repeated chronic stressors can increase amygdalar 2-AG signaling temporarily, with levels beginning to return to normal one hour after the stressful event [92]. Therefore, elevated levels of 2-AG following repeated stressors represents another mechanism by which eCBs protect from HPA axis overactivation. Consistent with this hypothesis, injection of a CB1r antagonist locally into the BLA reverses this stress habituation [93].

7. Effects of Cannabinoids on Fear and PTSD

A direct role for eCB modulation of the emotional components of amygdalar function has been observed in animal studies. Classical associative fear conditioning and extinction behavioral models show that eCB levels are increased in the amygdala during the extinction session; however, these increases were not observed during the initial fear condition, and fear behaviors persisted longer in the extinction sessions in CB1r KO mice compared to WT mice [94]. Rats exposed to a footshock followed by situational reminders, which is a potential model for PTSD, exhibit impaired extinction of the traumatic memory and increased CB1r levels in the hippocampus (CA1) and PFC, and these alterations were prevented by WIN 55,212-2 administration following exposure to the traumatic event [95]. Combined with results from other studies, it has been concluded that amygdalar eCB signaling is critical for both within- and between-session habituation and adaption of fear-related behaviors [94, 96]. These data suggest that the eCB system is essential for regulating amygdalar function and that the amygdala is a nucleus where eCB signaling can affect both neuroendocrine and stress adaptation behaviors [90].

In addition to affecting fear related behaviors, the eCB system is also involved in the consolidation, retrieval, and extinction of emotionally charged and distressing memories [97]. As previously mentioned, glucocorticoids that are released following a stressor can stimulate eCB synthesis, which in turn inhibits GABAergic neurotransmission. This disinhibition of GABAergic projections from the BLA to the LC results in increased NE release and its subsequent binding to β -ARs, causing the consolidation of stressful and potentially traumatic memories [91]. Administration of WIN 55,212 or glucocorticoid receptor antagonist RU-486 into the BLA before exposure to a stressful stimuli prevented the enhancement in memory consolidation that is normally observed [98]. Since stress also leads to rapid increases in FAAH levels, FAAH inhibitors prevent the degradation of AEA, which in turn promotes long-term fear extinction in animal models via CB1r binding in the BLA and provides protection against stress-induced alterations to eCB signaling [99]. It has

been suggested that since eCBs are released within the BLA during fear extinction, the resulting eCB-dependent negative feedback on the HPA axis is critical for the extinction of traumatic memories [94, 100]. Therefore, compounds that enhance the eCB system could serve as therapeutics for PTSD.

8. Therapeutic Potential for the Treatment of Psychiatric Disorders

The ability of the eCB system to modulate monoaminergic neurotransmission and the HPA axis make it a potentially attractive therapeutic target for the treatment of numerous psychiatric disorders, which involve abnormal function of these systems. As described earlier in this review, there is an extensive body of preclinical data demonstrating that various compounds and manipulations that increase CB signaling produce effects in behavioral assays that are predictive of therapeutic efficacy. Although several CB compounds have been evaluated in clinical trials for non-psychiatric disorders such as obesity and pain, it is only recently that some of these compounds have begun to be tested for psychiatric disorders including schizophrenia, PTSD and depression (Table 2) [101, 102]. While only a limited number of studies have released information on the results of their trials, some of them seem particularly promising. For example, cannabidiol resulted in relief from psychotic symptoms in acute schizophrenic patients that was comparable to a potent antipsychotic while resulting in fewer side effects. In a study of PTSD, Nabilone, a synthetic cannabinoid, greatly improved the quality of sleep and decreased the number of daytime flashbacks in treatment resistant patients [101]. Nabilone also significantly improved PTSD-associated insomnia, chronic pain, and nightmares in a retrospective study of 104 mentally ill men [103]. A second study found that THC treatment, twice a day over the course of three weeks, decreased the number of nightmares and increased sleep quality in ten patients suffering from chronic PTSD [104]. PTSD patients are plagued with debilitating flashbacks of a horrific event, potentially due to dysfunctional retrieval and extinction of emotional memories [105, 106]. These results are consistent with clinical studies showing that many individuals afflicted with PTSD self-medicate with cannabis to help alleviate their symptoms [107]. *Cannabis* use is correlated with both the onset and severity of PTSD symptoms [108, 109]. Since it is known that the eCB system is involved in these processes and that people suffering from PTSD often self-medicate with cannabis, other compounds that increase eCB signaling could prove to be therapeutic as well.

9. Novel Modulators of Cannabinoid Receptors

Recently, allosteric CB1r agonists have been identified, which has important implications for drug discovery, as allosteric compounds allow for the modulation of signaling without completely inducing or blocking receptor responses as traditional agonists and antagonists would do [110]. Prince et al identified the first allosteric CB1r modulators in 2005. They discovered three Organon compounds that all enhanced agonist affinity for the CB1r; however, they function as insurmountable antagonists, decreasing the Emax value for CB1r agonists and increasing the length of time it takes for the agonists to dissociate from the receptor [111]. Subsequent research has shown that Org-27569 might in fact function as a biased ligand, decreasing coupling to cAMP and β -arrestin signaling while increasing G α -independent ERK phosphorylation and stimulating receptor internalization [112]. This

compound was tested in several *in vivo* rodent studies but failed to alter CB1-mediated effects of AEA, CP 55,940 (a CB1r agonist), and THC in anti-nociception, catalepsy, and hypothermia [113]; however, other eCBs were not tested in conjunction with Org-27569, nor were psychiatric effects evaluated. PSNCBAM-1 appears to have a similar profile to Org-27596, functioning as a negative allosteric CB1r modulator. *In vivo* studies, though, have shown it to be effective in an acute food-intake model [114]. Additionally, several positive allosteric modulators of CB1r activity have been identified and in particular, carbozamides have been found to selectively enhance CB1r activity [115]. Lipoxin A₄ enhances AEA induced nociception and catalepsy in various mouse models [116, 117]. Finally, RTI-371, a selective DAT inhibitor, has also been found to increase CP 55940 signaling *in vitro* in a concentration-dependent fashion [118].

10. Conclusion

There is a growing body of evidence that the eCB system plays an important role in the control of mood. Anatomical, electrophysiological and *in vivo* behavioral studies have revealed that the eCB system can modulate both the monoamine systems and the HPA axis. Since current antidepressant therapy is designed to modulate monoamine transmission and dysfunction of the HPA axis is a hallmark of depression, targeting the eCB system appears to represent an attractive and novel approach to the treatment of depression and other mood disorders. In support of this notion, studies in animal models of depression and anxiety predict that activation of CB receptors is likely to be efficacious in the treatment of these disorders. However, there are reports that chronic use of cannabis can actually result in dysphoria and panic. This suggests that over-activation of the eCB system may have undesirable consequences and it will be critical to take this into consideration when targeting the eCB system. One approach with the potential to produce therapeutic levels of CB activation without risking the undesirable side effects associated with over-activation is inhibition of the degradation of the endogenous eCBs, AEA and 2-AG. In theory, this should result in indirect, activity-dependent increases in CB receptor stimulation due to elevation of endogenous eCB levels while minimizing the likelihood of over-activation. More specifically, inhibition of FAAH, the enzyme that degrades AEA, would appear to be particularly promising, because AEA is a partial agonist that would be even less likely to produce over-activation of CB receptors. It is worth noting that virtually all current antidepressants rely on activity-dependent mechanisms to increase monoamine transmission. Interestingly, cannabidiol, which is an inhibitor of AEA degradation, has been reported to have therapeutic effects in a clinical trial for schizophrenia. One possible concern with this strategy is that AEA and 2-AG are not completely selective for CB receptors and have been shown to bind to and activate TRPV1 receptors. The recent development of allosteric modulators of CBR1 may provide an attractive alternative to modulation of eCB levels. Both positive and negative allosteric modulators of CB1r have been identified and compounds that appear to be biased agonists of CB1r have also been reported. These classes of compounds have the potential to specifically activate CB1r in an activity-dependent manner and minimize the risk of over-activation or undesirable side effects.

11. Expert Opinion

The eCB and noradrenergic systems are significantly and dynamically impacted by stress [119–123] and noradrenergic transmission is responsible for cannabinoid-induced activation of the HPA axis [124]. Under conditions of acute stress, NE is increased centrally and peripherally [120, 125–130] while the eCB system tonically constrains activation of neural circuits, including the hypothalamic-pituitary-adrenal (HPA) axis [123, 131]. However, disrupted noradrenergic and eCB signaling is associated with an inability to adapt to chronic stress [121, 123, 127, 132–134]. Our work indicates a different consequence to the regulation of NE by cannabinoids under stress conditions. Specifically, stress-induced increases in cortical NE levels are significantly attenuated by prior treatment with a cannabinoid receptor agonist suggesting complex actions of cannabinoids on noradrenergic circuitry that vary under basal vs stress conditions. The working model posits that, under basal conditions, decreased signaling of pre-synaptically distributed CB1r localized to noradrenergic afferents contribute to local increases in cortical NE and adrenergic receptor (AR) desensitization. Under conditions of stress where NE levels are elevated, increased release of eCB from cortical neurons attenuates pre-synaptic release of NE potentially leading to AR sensitization.

There is significant potential for establishing cannabinoid-adrenergic interactions as a novel target in the development of improved treatment strategies for stress-induced anxiety. The pathophysiology underlying anxiety disorders, and specifically PTSD, may be related to an inability to *extinguish* aversive memories [135]. Increased salience of aversive memories due to activation of limbic circuits and poor cognitive inhibition/flexibility due to decreased cortical activity may contribute to the behavioral expression of anxiety. Understanding the cellular mechanism responsible for *extinction* of fear memories may provide the basis for more effective forms of clinical treatment of anxiety. Patients with PTSD suffer from recurrent retrieval of traumatic memories in the form of context-induced flashbacks and repeated nightmares. Repeated *re-consolidation* of fear memories in limbic circuits and inability to *extinguish* fear memories [136] are thought to underlie the pathophysiology of PTSD. *Consolidation* of emotionally arousing memories involves, in part, noradrenergic circuits targeting the amygdala [126, 137], while *extinction* of memory is dependent on the mPFC [138, 139]. Pharmacological manipulation of AR systems has provided symptomatic relief in PTSD patients [140, 141] suggesting that therapeutic improvement may result, in part, from attenuation of signaling of sensitized ARs. Moreover, the cannabinoid receptor agonist, nabilone, has recently been reported to be effective in management of symptoms of PTSD [101]. Taken with recent evidence that the EC and noradrenergic systems interact in stress-related memory consolidation [122, 142], targeting interactions between these two systems may represent a novel approach for the treatment of stress-induced anxiety disorders. Elucidating reciprocal interactions between the cannabinoid-adrenergic systems in stress-integrative circuits is vital for demonstrating that interaction of the two is important in modulating stress-induced anxiety and extinction of conditioned fear. Given that the PFC represents a critical region in mediating the *extinction* of traumatic/aversive memories, treatments that target this region may help alleviate symptoms of anxiety disorders by increasing *extinction* of such memories. Achieving the proper balance in frontal cortical

activity by targeting cannabinoid-adrenergic interactions may result in enhancing *extinction* of aversive memories and diminish anxiety-like behaviors that are precipitated by stress.

The increasing availability of different classes of compounds that target discrete aspects of the eCB system provide a unique opportunity to more thoroughly evaluate the importance of cannabinoid-adrenergic interactions on anxious behaviors in both preclinical and clinical studies. As mentioned earlier, the preliminary results obtained with the synthetic cannabinoid Nabilone as well as the natural cannabinoid THC and cannabis itself for the treatment of PTSD have been very promising. Additional insight provided by detailed preclinical studies and the discovery of increasingly selective modulators of CB receptors should enable the identification of optimal therapeutic strategies and maximize the likelihood of developing safe and effective treatments for debilitating disorders such as PTSD.

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Highlights Box

- Dysregulation of the eCB system has been implicated in several psychiatric disorders.
- Much evidence has been gathered in support of eCB regulation of monoamine neurotransmission and its ability to alter mood.
- Stress response is regulated by the eCB system, and its dysfunction can lead to HPA axis dysregulation and the development of psychiatric disorders.
- Many compounds have been developed that target the eCB system and could prove to be successful therapeutics for psychiatric disorders, especially the more selective modulators of CB receptors.
- The cannabinoid-adrenergic interaction represents a promising novel target for the symptomatic treatment of stress-induced anxiety and PTSD, since these systems are heavily involved in memory consolidation and extinction.

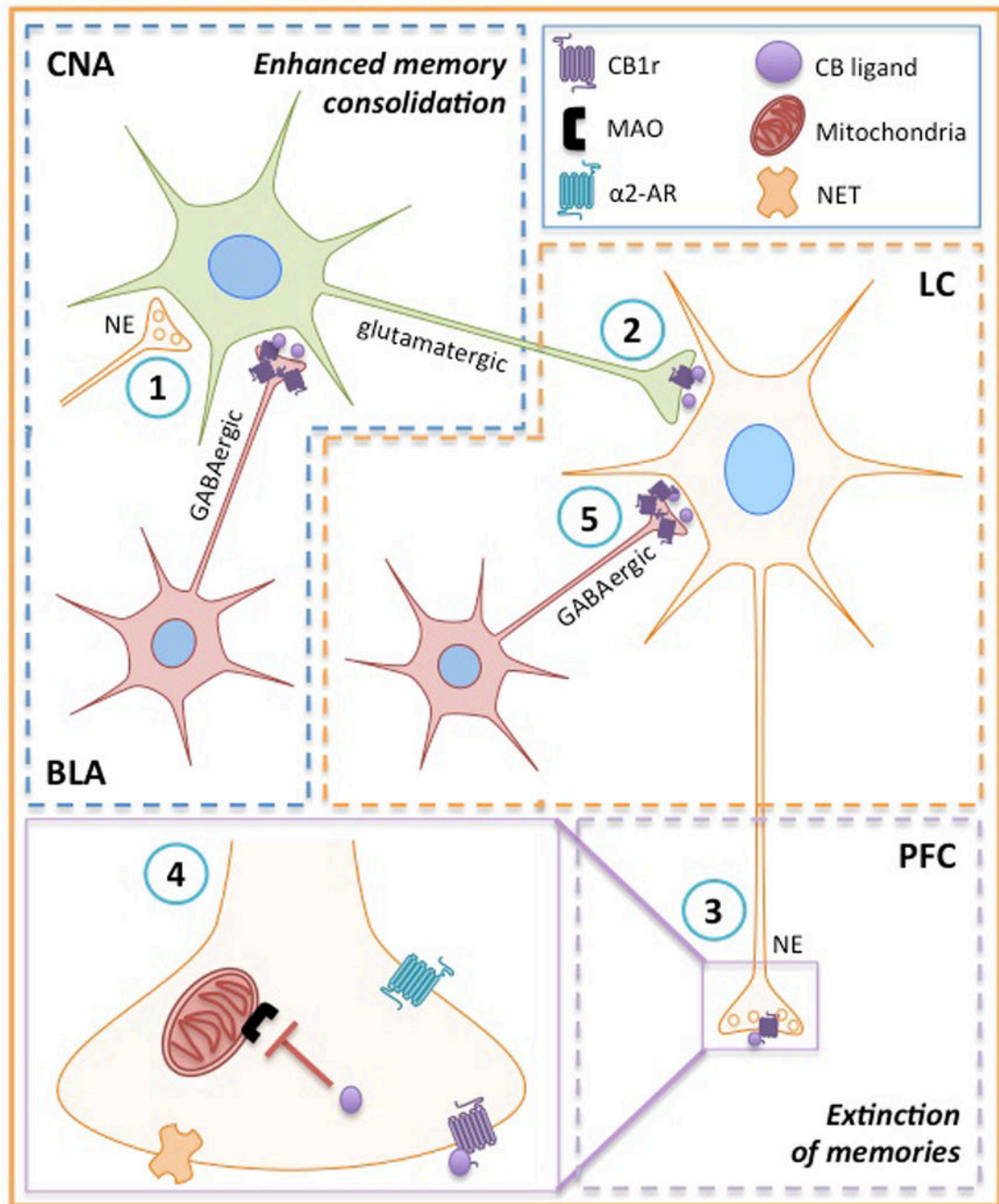


Figure 1.

Schematic diagram depicting cannabinoid-adrenergic interactions in stress-integrative circuitry. The basolateral complex of the amygdala (BLA) has been implicated in the consolidation of emotionally arousing experiences and involves glucocorticoid-mediated increases in eCB release and interactions with norepinephrine [146]. (1) eCBs are posited to increase BLA activity by decreasing GABAergic neurotransmission [147]. (2) Disinhibition of GABAergic interneurons results in an increase of glutamatergic signaling in the central nucleus of the amygdala (CNA), a source of excitatory afferents to the LC [148]. (3)

Activation of the LC causes an increase in noradrenergic signaling and norepinephrine (NE) release in postsynaptic targets, such as the prefrontal cortex (PFC). Given that the PFC represents a critical region in mediating the *extinction* of traumatic/aversive memories, treatments involving the eCB system that target this region may help alleviate symptoms of anxiety disorders by increasing *extinction* of such memories. For example, (4) CBs have been shown to inhibit monoamine oxidase (MAO), representing another mechanism in which CB signaling can regulate NE levels. (5) Targeting GABAergic projections to the LC with CB ligands can potentially modulate LC afferent activity to the PFC. Achieving the proper balance in frontal cortical activity by targeting cannabinoid-adrenergic interactions may result in enhancing *extinction* of aversive memories and diminish anxiety-like behaviors that are precipitated by stress.

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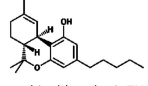
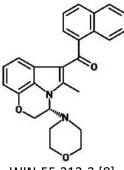
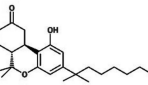
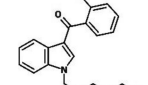
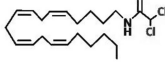
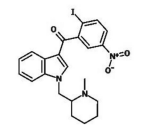
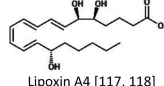
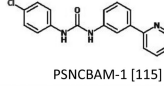
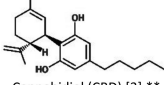
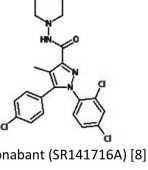
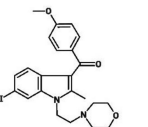
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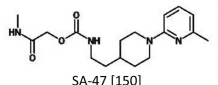
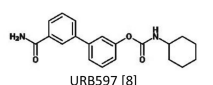
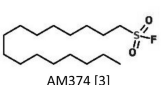
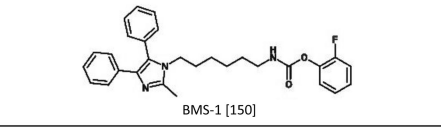
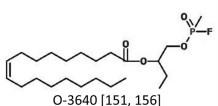
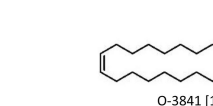
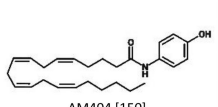
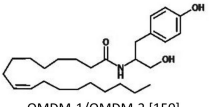
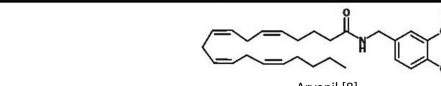
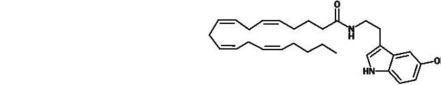
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Table 1

Summary of drugs and compounds that are known to target the eCB system. Each class of drug is described with respect to its putative target and effect on eCB signaling.

	Target	Drug		Effect on eCB Signaling		
CB1r/ CB2r agonists	Dronabinol (synthetic THC) [150,151]		CP-55,940 [8]	 WIN-55,212-2 [8]	INCREASE	
			Nabilone [101]			Bay 38-7271 [3]
	Arachidonyl-2'-chloroethylamide (ACEA) [8]					
	CB1r agonists		M122, WO0128557 [3]			 M123, WO0128498 [3]
			HU-210 [151]			
	CB2r agonists		AM-1241 [151]	JWH-015 [8, 152]	HU-308 [3]	INCREASE
				JWH-133 [8]		
				GW405833 [84]		
	CB1r positive allosteric agonists		Lipoxin A4 [117, 118]	Indole-2-carboxamides [116]	POSITIVE MODULATION	
				RTI-371		
CB1r negative allosteric agonists		PSNCBAM-1 [115]	Org-27596 [112-114]	NEGATIVE MODULATION		
			Org-29647 [112-114]			
CB1r/ CB2r antagonists		Cannabidiol (CBD) [3] **	Otenabant (CP-945,598) [150]	INCREASE/ DECREASE		
			WO0170700 [3]			
			TM38837 [8]			
			AM-251 [8]			
			AM-4113 [8]			
			AM-281 [3]			
			NESS0327 [8]			
CB1r antagonists/ inverse agonists		Rimobant (SR141716A) [8]	Drinabant (AVE-1625) [150]	DECREASE		
		Surinabant (SR147778) [150]	Taranabant (MK-0364) [150]			
CB2r antagonists/ inverse agonists		AM-630 (6-Iodopravadoline) [8]	JTE-907 [150]	SR144528 [150]		
			Sch.336 [150]	SR144228 [151]		
			Sch.336 [150]	SR1414528 [150]		

eCB METABOLISM	FAAH inhibitors	 SA-47 [150]	 URB597 [8]	 AM374 [3]	INCREASE	
		 BMS-1 [150]		AM3506 [8]		JNJ5003 [8]
		Methyl arachidonoyl fluorophosphonate (MAFP) [151]		OL-135 [150]		AAWCF3 [8]
		PF-3845 [8]	SSR-411298 [154]	PF-750 [150]		
				Compound 7 [3]		
eCB CATABOLISM	DAGL inhibitors	 O-3640 [151, 156]	 O-3841 [151, 156]		DECREASE	
eCB REUPTAKE INHIBITORS	eCBRIIs	 AM404 [150]	 OMDM-1/OMDM-2 [150]	AM1172 [150]	INCREASE	
		LY-2183240 [150]	VDM-11 [150]	O-3246/O-3262 [150]		
				UCM707 [150]		
TARGETING TRPV1	TRPV1 agonists	Capsaicin [151]	Olvanil [151]	Resiniferatoxin (RTX) [151]	INCREASE	
	Dual TRPV1/CB1r agonists	 Arvanil [8]			INCREASE	
	TRPV1 antagonists	Capsazepine [151]	Iodo-resiniferatoxin [151]	SB-366791 [151]	DECREASE	
	Dual FAAH/TRPV1 blockers	 N-arachidonoyl-serotonin (AA-5-HT) [151]			INCREASE/ DECREASE	

** While CBD acts as a CB1r antagonist and CB2r inverse agonist, it can increase the eCB response by upregulating CB1r levels and by inhibiting FAAH [143–145].

Table 2

Summary of the clinical progression of eCB-based drugs tested for the treatment of psychiatric disorders.

TARGET	DRUG	FOR THE TREATMENT OF	CLINICAL PROGRESSION
CB1r/CB2r AGONISTS	Dronabinol	Schizophrenia [83]	Phase IIa
		Fear extinction [156]	Phase II (completed)
	THC	PTSD [157]	Phase IV
	Nabilone	PTSD (nightmares) [101]	Phase II
	Sativex (THC & CBD)	Cannabis dependence w/ anxiety [158]	Phase I
Multiple Sclerosis (cognitive function & mood) [159]		Phase IV	
CB1r/CB2r ANTAGONISTS	Cannabidiol (CBD)	Schizophrenia (psychotic symptoms) [160]	Phase II (completed)
	GWP42003 (CBD)	Schizophrenia [84]	Phase II
CB1r ANTAGONIST	AVE-1625	Schizophrenia (cotreatment w/ anti-psychotics) [161]	Phase II (terminated)
FAAH INHIBITORS	URB597	Schizophrenia [162]	Phase I
	SSR411298	Major Depressive Disorder [153]	Phase II