Don't Worry, Be Happy: Endocannabinoids and Cannabis at the Intersection of Stress and Reward

Nora D. Volkow, Aidan J. Hampson, and Ruben D. Baler

National Institute on Drug Abuse, National Institutes of Health, Bethesda, Maryland 20892; email: nvolkow@nida.nih.gov

Annu. Rev. Pharmacol. Toxicol. 2017. 57:285–308

First published online as a Review in Advance on September 2, 2016

The *Annual Review of Pharmacology and Toxicology* is online at pharmtox.annualreviews.org

This article's doi: 10.1146/annurev-pharmtox-010716-104615

Keywords

cannabis, marijuana, endocannabinoids, THC, stress, reward

Abstract

Cannabis enables and enhances the subjective sense of well-being by stimulating the endocannabinoid system (ECS), which plays a key role in modulating the response to stress, reward, and their interactions. However, over time, repeated activation of the ECS by cannabis can trigger neuroadaptations that may impair the sensitivity to stress and reward. This effect, in vulnerable individuals, can lead to addiction and other adverse consequences. The recent shift toward legalization of medical or recreational cannabis has renewed interest in investigating the physiological role of the ECS as well as the potential health effects, both adverse and beneficial, of cannabis. Here we review our current understanding of the ECS and its complex physiological roles. We discuss the implications of this understanding vis-á-vis the ECS's modulation of stress and reward and its relevance to mental disorders in which these processes are disrupted (i.e., addiction, depression, posttraumatic stress disorder, schizophrenia), along with the therapeutic potential of strategies to manipulate the ECS for these conditions.

INTRODUCTION

The search for a state of mental relaxation and well-being is one of the factors driving the widespread consumption of cannabis. The most frequently abused illicit substance worldwide, cannabis is consumed regularly by about 2.4% of the world population (approximately 181 million people in 2013) (1). The principal psychoactive component of cannabis is Δ^9-tetrahydrocannabinol (THC), which acts as an orthosteric agonist for cannabinoid receptors and mediates both the positive and negative effects of cannabis. The cannabinoid receptors are part of the brain's endocannabinoid system (ECS), which modulates multiple neurobiological processes including reward and stress, a fact that is relevant for understanding not just the recreational use of cannabis but also its therapeutic potential. This review focuses on the role of the ECS in the modulation of stress responses, its interaction with the reward system in the brain, and the implications of this emerging understanding for cannabis abuse, mental illnesses, and therapeutics.

COMPONENTS OF THE ENDOCANNABINOID SYSTEM

The ECS is a receptor-based signaling system composed of three core elements: enzymes that synthesize endocannabinoid agonists (ECBs), receptors that respond to agonists by triggering intracellular signaling cascades, and transporters and enzymes that remove ECBs from the receptor vicinity. Two ECBs—*N*-arachidonoylethanolamide (AEA, also known as anandamide) and 2-arachidonyl glycerol (2-AG)—are structurally similar but synthesized and degraded by separate pathways. Both agonists can act on the two known cannabinoid receptors described in the next section, although AEA is also an agonist for transient receptor potential vanilloid 1 (TRPV1) receptors. Additionally, there is debate about whether ECBs interact with lysophosphatidylinositol-sensitive receptors and peroxisome proliferator-activated receptors under physiological conditions.

Receptors

The two known cannabinoid receptors, designated type 1 (CB1R) and type 2 (CB2R), are Gi/o protein–coupled, seven-transmembrane domain receptors (GPCRs) with different tissue distributions and functions. The CB1R is the most abundantly expressed GPCR in the central nervous system (CNS) (2, 3), and in the human brain the highest levels of *CNR1* (the gene encoding for CB1R) transcription occur in hippocampus, striatum, cortex, and amygdala, with intermediate levels in cerebellum (4, 5). The level of CB1R expression in human cerebellum (3) is lower than in primates and rodents, possibly explaining why catalepsy is one of the tetrad of definitive behaviors seen in rodents exposed to cannabinoids (6) but cannabis-induced catatonia is rarely observed in humans (7) (**Supplemental Figure 1*a***; follow the **Supplemental Materials link** from the Annual Reviews home page at **http://www.annualreviews.org**). In contrast, CB2R expression is generally low in the CNS, where it is restricted mostly to microglia, but is high in peripheral immune cells and tissues (8). Microglial CB2R expression increases dramatically following neuropathic injury or inflammatory encephalomyelitis (9), which is why CB2R is an attractive pain management and immune modulatory target, particularly because CB2R agonists do not induce THC-like psychoactivity (2). Even so, there is some evidence of CB2R expression in the rodent brain (10), although a consensus on whether CB2R is expressed at a significant functional level has not yet been achieved. However, several groups have produced data demonstrating CB2R in different neural tissues and have shown CB2R knockout animals demonstrate not only altered electrophysiological responses (10a) but also behavioral memory deficits (10b). Human positron emission tomography (PET) brain imaging studies show negligible, if any,

Supplemental Material

CB2R ligand-specific binding in the brain of healthy individuals, which might reflect the poor sensitivity of these ligands for detecting low levels of CB2R (**Supplemental Figure 1*b***).

Supplemental Material

The CB1Rs are located primarily on GABAergic and glutamatergic neuronal presynaptic terminals, where they serve to restrict neurotransmitter release and modulate neuronal firing. This is accomplished by inhibiting the voltage-gated calcium channels that control neurotransmitter release and by activating inwardly rectifying potassium channels that reduce the probability of neuronal firing (2). Upon CB1R activation, the receptors' associated G protein subunits uncouple; these subunits interact with ion channels, thereby modulating protein kinase A (PKA) (by inhibiting cyclic adenosine monophosphate synthesis) and upregulating extracellular signal–regulated kinase (ERK) pathways (11). Whereas ion channel modulation allows ECBs to very rapidly downregulate neuronal circuits, it is the kinases that mediate the long-lasting effects of cannabinoids. One example is the tolerance that develops with chronic cannabis exposure, which is a function of CB1R expression downregulation and is modulated by ERK–β-arrestin interactions (12). CB1R expression is a dynamic event: Soon after the receptors are activated, they are internalized from the membrane into endosomes. Some of these CB1Rs will return to the membrane, whereas others become destined for proteolysis. The balance between the rate of membrane expression and internalization regulates the number of active receptors on both pre- and postsynaptic membranes (13) (see the sidebar, Synaptic Distribution of Endocannabinoid Agonists). The ERK stimulation that follows CB1R activation plays a role in tuning this overall system, which controls CB1R levels during periods of both high CB1R activation and high synaptic activity (12). Receptor internalization is driven by the binding of β-arrestin to CB1R as soon as the G proteins disassociate, but the affinity of the interaction between the receptor and β-arrestin determines whether it will be returned to the membrane or broken down (14). Different CB1R agonists induce varying levels of engagement between the receptor and β-arrestin and therefore are more or less powerful inducers of CB1R downregulation and tolerance (14). However, the degree to which an agonist induces a tight bond with β-arrestin may not be related to its relative efficacy at inducing other CB1R-related downstream signals. For example, THC is not as efficacious as AEA or 2-AG at activating PKA or ERK pathways (15), but it is significantly more effective at promoting β-arrestin association, receptor internalization, and ultimately tolerance to its effects (16).

SYNAPTIC DISTRIBUTION OF ENDOCANNABINOID AGONISTS

The synaptic distribution of CB1R is consistent with its role as a retrograde regulator of synaptic signaling. CB1R expression is high in presynaptic axon membranes and low on postsynaptic dendrites and soma. This polarization is driven by the push-pull combination of high monoacylglycerol lipase (MGL) levels on axons and high diacylglycerol lipase (DGL) expression on dendrites and soma (25). The DGL on dendrites maintains a tonic state of CB1R activation, and because CB1Rs are internalized rapidly once activated (14, 16), DGL is responsible for maintaining low levels of externalized CB1Rs in the dendrites. In contrast, the high level of MGL expression on presynaptic axons ensures 2-AG is present only transiently, and thus most of the CB1R population remains externalized on the plasma membrane (26). Puzzlingly, the enzyme that metabolizes AEA [fatty acid amide hydrolase (FAAH)] is expressed predominately on the postsynapse (27, 28), whereas an AEA synthetic enzyme [*N*-acylphosphatidylethanolamine precursor–D-type phospholipase (NAPE-PLD)] is associated primarily with axons. It is unclear whether this indicates that AEA exerts an anterograde mode of ECS action or whether the expression pattern suggests that AEA commonly acts as an endovannilloid (agonist of TRPV1) (29).

Endocannabinoid Agonists

The two endogenous agonists for cannabinoid receptors, AEA and 2-AG, appear to have both distinct and overlapping roles. In the striatum, both AEA and 2-AG can induce long-term depression (LTD) in response to various forms of associative neural activity (17), but in other regions, some important functions appear to be mediated by a single ECB (see the next section).

State Dependence of the Synthesis and Degradation of Endocannabinoids

Unlike many other transmitters, ECBs are synthesized and secreted on demand (as opposed to synthesis and storage prior to demand), which indicates that network activity tightly controls (and in turn is modulated by) the synthesis and elimination of ECBs (18). In other words, ECS signaling is highly dependent on the state of synaptic activity. The degradation pathways of both AEA and 2-AG are relatively straightforward, but ECB synthesis is more complicated. In the brain, 2-AG signaling is terminated following cleavage of the glycerol group by monoacylglycerol lipase (MGL), which is responsible for approximately 80% of 2-AG cleavage (19), and by α/β hydrolase domain 6, which plays a lesser role (20). Cleavage of AEA is analogous, with the ethanolamide moiety cleaved by fatty acid amide hydrolase (FAAH). The synthesis of 2-AG also appears to be straightforward: Diacylglycerol (DAG) lipase (DGL) cleaves an acyl group from a 1-acyl 2-arachidonoyl-*sn*-glycerol precursor in a single step (21). In contrast, two distinct AEA synthetic pathways have been elucidated, but more remain to be characterized. In one pathway, AEA is liberated in a single step from an *N*-acylphosphatidyl-ethanolamine precursor (NAPE) by a D-type phospholipase (NAPE-PLD). The second pathway is a multistep process in which an A/B-type phospholipase conducts two rounds of acyl cleavage on a diacyl-NAPE to yield an AEA precursor (glycerophospho-AEA), from which glycerophosphodiesterase 1 (GDE1) then ultimately liberates AEA (22). Surprisingly, however, mice that lack both NAPE-PLD and GDE1 retain most of their capacity to generate AEA, indicating that there are still other pathways that generate this ECB in the brain (23).

Endocannabinoid Signaling Mechanisms

The best-characterized mode of ECB-mediated signaling is the retrograde release of 2-AG from the postsynapse, which acts on presynaptic CB1R to suppress neurotransmitter release (see the sidebar, Synaptic Distribution of Endocannabinoid Agonists) (24). This retrograde 2-AG modulation was discovered as the mechanism underlying an electrophysiological property of GABAergic synapses known as depolarization-induced suppression of inhibition (DSI) and its glutamatergic equivalent, depolarization-induced suppression of excitation (DSE) (21).

Control of both DSE and DSI by 2-AG depends more on the rate of depolarization than on substrate concentration. Especially during strong chains of depolarizing stimuli, calcium accumulates in the postsynapse, and 2-AG is synthesized in response to this accumulation (30). However, 2-AG can also be produced by a second, calcium-independent synthetic mechanism. Metabotropic glutamate receptors (mGLuRs) and muscarinic acetylcholine receptors can both stimulate 2-AG synthesis by activating phospholipase Cβ (PLCB), which causes DAG (the DGL substrate) release from phospholipids (31). This metabotropic release of 2-AG is controlled primarily by availability of DAG (32), although PLCB is somewhat calcium sensitive and can act as a coincidence detector for the two pathways (33). Metabotropic receptors are located on the synaptic periphery, and when extreme synaptic activity causes neurotransmitter spillover from the synapse (34), this pathway cooperativity, centered on PLCB, allows the ECS to enhance synaptic suppression greatly (31, 35, 36).

The duration of DSI/DSE is controlled largely by MGL, which tends to be located on presynaptic neurons (19, 26), although in the hippocampal dentate gyrus, MGL is located exclusively in perisynaptic astrocytes (37, 38), demonstrating that MGL can be located on astrocytes or neurons. One study deleted MGL selectively from astrocytes and neurons to examine the relative roles of these cell types in controlling DSI/DSE (38). In the different regions examined, deletion of MGL from either astrocytes or neurons did not have a profound effect, but if MGL was deleted from both cell types, DSI/DSE was increased significantly. This physiological redundancy of MGL expression illustrates the importance of tight regulation of ECB-mediated neuromodulation.

In most brain regions, both DSI/DSE and mGluR-facilitated suppression of neurotransmission is mediated almost exclusively by 2-AG (39, 40). Anandamide appears to mediate DSI only in specialized situations (41), which leaves its role in the ECS open to question. Anandamide is a partial agonist at CB1R and has been hypothesized to functionally reduce (antagonize) the effect of 2-AG (42). However, the concentration of 2-AG in the brain is several orders of magnitude greater than AEA (on the order of nanomoles per gram compared with 10–40 pmol/g, respectively) (43), whereas their affinity for CB1R is comparable, which suggests the main function of AEA is not 2-AG antagonism. However, in addition to being a CB1R agonist, AEA is also a TRPV1 agonist (29), and evidence suggests that AEA can suppress 2-AG release via this mechanism (26). However, the specifics of the inhibitory relationship between AEA and 2-AG synthesis are unclear in that TRPV1 increases intracellular calcium, and calcium enhances DGL synthesis of 2-AG. Glutathione has been proposed as a link between AEA and TRPV1 and between 2-AG and DGL, but glutathione enhances both processes similarly (44). Although the exact mechanism is unclear, serendipitous data confirm the presence of coordination between 2-AG and AEA (45). In different studies, researchers knocked out the α and β DGL isoforms (46, 47), which led to an 80% reduction in 2-AG but also to a 40% reduction in AEA levels. Moreover, in one of the studies, AEA levels were restored by MGL inhibition (46).

Although the predominant picture of ECB function centers on the retrograde DSI/DSE effects of 2-AG, evidence suggests AEA can also mediate communication between axons (48). In the rat nucleus tractus solitarius, activation of presynaptic (but not postsynaptic) NMDA receptors induces LTD. This presynaptic LTD is CB1R-dependent and is probably due to AEA, in that it is potentiated by FAAH inhibitors, not perturbed by DGL inhibition and, unlike DSI/DSE, does not require postsynaptic calcium influx. It is unclear how universally expressed this mechanism is, but its ultimate effect is similar to DSI, namely a reduction in neurotransmitter release and synaptic activity. This finding would explain why the AEA synthetic enzyme NAPE-PLD is principally located presynaptically and not on postsynapse, as one might expect for a retrograde messenger (see the sidebar, Synaptic Distribution of Endocannabinoid Agonists). In addition, AEA-mediated axon-axon communication represents a potentially three-dimensional effect (volumetric effect on a region of synapses), unlike the retrograde DSI, which is contained within individual synapses.

The differences between 2-AG and AEA signaling may be primarily temporal; that is, 2-AG is involved in rapid (phasic) effects on neuroplasticity, whereas AEA imparts longer-term tonic information—such as setting basal levels of responsivity (24). The exclusivity of 2-AG in controlling DSI/DSE supports its role in rapid responses (39, 49), and the relative roles of 2-AG and AEA in stress-induced analgesia may be an example of their temporal differences. In the footshock model of stress-induced analgesia (see the sidebar, Endocannabinoid System Analgesia and Stress-Pain Interactions), 2-AG levels rise quickly but remain high only momentarily, whereas AEA levels rise in a slower and somewhat delayed fashion (50) but have a more sustained elevation. However, other studies have found 2-AG to be involved in tonic as well as phasic neuroplasticity (26), and 2-AG appears as likely to mediate the behavioral effects described in the next section as AEA.

FUNCTIONS OF THE ENDOCANNABINOID SYSTEM IN STRESS AND REWARD

Among the most studied functions of the ECS in the brain are its effects on reward [via modulation of dopamine (DA)] (51), cognition and memory [fine-tuning neuronal oscillations and synaptic plasticity in the prefrontal cortex (PFC) and hippocampal networks] (52), stress regulation (mostly anxiolytic effects) (53), and pain perception (mostly analgesic effects) (50, 54). In this review, we focus on the role of the ECS in the regulation of stress and reward and their interactions. The stress and reward domains are highly interactive and relevant to both the recreational use of cannabis and its putative therapeutic effects.

Stress Regulation

The brain processes stress to determine the behavioral and physiological responses necessary to cope with it (55). A major brain component of this system is the hypothalamo-pituitary-adrenocortical (HPA) axis that mediates autonomic and neuroendocrine responses to stress. Additionally, the amygdala, extended amygdala [including the bed nucleus of the stria terminalis (BNST)], hippocampus, and PFC also respond to stress and influence behavior and HPA responses (55).

Several studies have demonstrated the role of ECBs as stress modulators. Knocking out DGLα in mice resulted in an 80–90% reduction in brain 2-AG levels and led to an anxious phenotype with reduced fear extinction (46). Similarly, in a rat model of panic, a FAAH inhibitor alleviated panic responses and a CB1R antagonist exacerbated them, suggesting a role of AEA in panic control (56). A FAAH inhibitor also increased the rate of fear extinction in a rodent model of persistent stress disorders (57). It has also been reported that, in humans, individuals with a low-expressing *FAAH* allele (385A; rs324420) habituate to threats more rapidly than average and have low stress reactivity (57). Numerous studies have documented the analgesic role of the ECS and cannabinoids, and ECBs also mediate stress-induced analgesia (see the sidebar, Endocannabinoid System Analgesia and Stress-Pain Interactions).

The ECS regulates the various brain regions involved with stress and relevant stress-modulating molecules [i.e., corticotropin-releasing factor (CRF), glucocorticoids, norepinephrine, and serotonin] (53, 68). All major components of the ECS are expressed prominently in the amygdala, hippocampus, and PFC, where they modulate stress responses (53). Stress, in turn, modulates the ECS. Preclinical studies indicate that acute and chronic stress alter CB1R expression level and increase 2-AG production, although AEA levels are decreased (53). The decreases in AEA levels upon stress exposure occur rapidly; are mediated in part by CRF stimulation; and contribute to the expression of stress responses, including anxiety behaviors, HPA activation, and corticosterone release (see the sidebar, Endocannabinoid System Analgesia and Stress-Pain Interactions). However, stress-induced increases in 2-AG in the PFC and paraventricular nucleus of the hypothalamus are slower and are mediated in part by corticosterone (69) and serve to inhibit the HPA axis and terminate the stress responses (53). It is postulated that the distinct effects of AEA and 2-AG ultimately have an inhibitory role on stress: The fast AEA decreases are relevant for the initiation and manifestation of the effects of stress, whereas the slower increase in 2-AG ultimately tempers and terminates the stress responses (53). This is an example wherein the purported predominant involvement of 2-AG in phasic and AEA in tonic responses does not apply.

A human brain imaging study found significant increases in CB1R levels in the brain of patients with posttraumatic stress disorder (PTSD) (greater in females than males) and reduced plasma AEA levels (70). Consistent with these findings, a study in adults with diverse trauma-related psychopathologies showed that increased CB1R in amygdala was associated with increased attentional

ENDOCANNABINOID SYSTEM ANALGESIA AND STRESS-PAIN INTERACTIONS

The ECS modulates analgesia through both central and peripheral mechanisms (58, 59). Reduced heat sensitivity is an example of 2-AG–mediated central analgesia (signaling through the mGluR pathway) (54), as is AEA-mediated analgesia in inflammatory allodynia (60). However, the deletion of CB1R from peripheral neurons has shown that peripheral ECS sets basal sensitivity to a wide range of painful stimuli (59), and locally applied AEA and *N*-palmitoyl-ethanolamine—CB1R and CB2R agonists, respectively—can affect locally induced pain (61).

The ECS also plays a role in stress-induced analgesia. Mild electric foot shock induces analgesia and increases 2-AG and AEA in the periaqueductal gray, a key pain modulatory center. Furthermore, this analgesia is increased by MGL and FAAH inhibitors and blocked by a CB1R antagonist (50). As with heat sensitivity, foot shock–induced 2-AG synthesis appears to occur via postsynaptic mGluR activation (54) rather than through DSI/DSE phenomena.

The ECS also appears to modulate the risk of developing a neuropsychiatric disorder such as anxiety or depression as a result of extreme or unabated stress (62). For example, in a sciatic nerve ligation model, both $Cb1^{-/-}$ and wild-type mice develop allodynia, but $Cb1^{-/-}$ mice also develop long-lasting anxiety and depression-related behaviors (63), a result consistent with the roles of the ECS in moderating the intertwined pain and anxiety/mood responses.

Finally, the ECS also reduces anxiety and pain caused by everyday stress, such as that seen during and following prolonged exercise. Conditional $Cb1^{-/-}$ mice do not develop exercise-induced anxiolysis and pain insensitivity, unlike wild-type mice given extensive access to a running wheel (64). Similarly, humans exposed to moderately strenuous exercise demonstrate an opioid-independent antinociceptive effect and increased plasma levels of 2-AG and AEA (65). Another study also showed increased plasma AEA levels after 60 min of moderate exercise and the following 15 min recovery period, whereas neither 2-AG nor endorphin levels were affected (66). Notably, however, the correlation between plasma ECB levels and brain concentrations is uncertain (67).

bias to threats, whereas greater AEA plasma levels were associated with decreased bias (71). These studies postulated that the increases in CB1R probably reflect reduced receptor internalization because of the low tone of AEA stimulation, an example of the tight coordination of CB1R expression as a function of stimulation level (see the section titled Receptors). Similarly, a preclinical stress study found that THC treatment was acutely effective as an anxiolytic, but the therapeutic effect did not continue with chronic administration because of CB1R downregulation (72).

Regulation of Reward and Interaction with Stress

The involvement of the ECS in reward processing has been studied extensively (see References 51 and 73 for reviews). ECBs regulate reward seeking by modulating DA signaling in the ventral tegmental area (VTA) (74), a central component of the brain reward system. CB1R blockade in the VTA reduces reward seeking dramatically, whereas augmenting 2-AG (but not AEA) increases it (75). The 2-AG synthetic enzyme DGLα is expressed in VTA dopaminergic and nondopaminergic neurons in opposition to CB1R-expressing glutamate and GABA terminals (76), which is consistent with 2-AG's involvement in regulating VTA function through modulation of glutamate and GABA release (77). Burst firing of DA neurons, driven by glutamate excitation of DA cells, results in larger levels of DA being released in the nucleus accumbens (NAc) (78), which is essential to the reinforcing and conditioning effects of rewarding stimuli, including those induced by drugs. The burst firing of DA neurons enhances retrograde ECS modulation of its synaptic inputs (79), thus modulating the levels of DA released into the NAc (75). In addition, the ECS also modulates glutamate projections into the NAc, possibly those arising from PFC, an additional mechanism by which the ECS regulates DA release in the NAc (78). In the NAc, DA influences the motivation for reward seeking, integrating convergent cortical, hippocampal,

ENDOCANNABINOID SYSTEM, SOCIAL REWARD, AND OXYTOCIN

The ECS is implicated in the modulation of social rewards, including those needed for bonding. When mice are isolated and then resocialized, AEA increases in brain regions involved with reward and emotion or mood (the NAc and ventral hippocampus, respectively). *FAAH*$^{-/-}$ mice show increased sociability, which is inhibited by treatment with CB1R antagonists (83). The increases in AEA induced by socialization are stimulated by activation of oxytocin receptors (OTRs), and an OTR antagonist blocks both socialization and the AEA increase. Furthermore, the OTR antagonist does not reduce sociability in the presence of a FAAH inhibitor or in *FAAH*$^{-/-}$ mice. The exact coupling between OTRs and AEA synthesis is not yet known, but the data suggest OTR activation is upstream of increased AEA availability, which is upstream of social reward. This convergence of the ECB and oxytocin systems in social reward also extends to maternal care, in that hippocampal CB1R and OTR expression is higher in mouse dams than in unmated females, but not in *Cb1*$^{-/-}$ females that are less attentive to their pups (84). In addition to social bonding and rewarding effects, oxytocin also exhibits antihyperalgesic effects that are partially mediated via CB1R activation (85).

and amygdalar inputs (80). The ECS is involved in the motivational aspects of reward-directed behavior for palatable foods (81), cannabis and other drugs of abuse (including alcohol, opiates, cocaine, and nicotine) (51, 73), social rewards (see the sidebar, Endocannabinoid System, Social Reward, and Oxytocin), sexual behaviors (82), and cue-motivated behaviors (75). Recent work also suggests it plays a role in reward associated with physical activity (64, 66).

The reward system is also involved in motivating behaviors to avoid aversive states. DA levels in the NAc are decreased by stress, aversive stimuli, or withdrawal from drugs or palatable foods (reviewed in Reference 51). Repeated drug exposures result in blunted signaling in DA reward circuits, along with an enhanced sensitivity of stress circuits that involve enhanced signaling through CRF and dynorphin and contribute to the negative mood states in addiction (86). Recent studies have shown neuroadaptations in the ECS with chronic exposure to drugs, including cannabis (reviewed in Reference 51), that suggest reductions in ECS signaling might also contribute to the enhanced sensitivity to stress and negative mood states in addiction (87).

The stress and reward networks are highly interactive, and ECBs modulate these interactions. Specifically, the ECS influences the responses of the reward circuitry to stress by modulating the stress-induced changes in sensitivity to natural rewards that can result in anhedonia when stress becomes chronic. The ECS also enhances the incentive salience of strong rewards that, with chronic stress, can increase the vulnerability to drug use. Pharmacological or genetic manipulations of ECB signaling, through CB1R, can modify these stress-induced changes in the reward circuitry (53).

One mechanism by which ECS affects stress-reward interactions involves the prefrontal BNST circuitry, which is crucial to stress responses. The PFC projects to the VTA, exciting DA cell firing, and CB1Rs in the BNST control this PFC-mediated excitation. Infusion of a CB1R agonist into the BNST inhibits excitation of VTA DA cells evoked by PFC stimulation (88), and neuroadaptations in this circuit with chronic nicotine exposures have been proposed to contribute to drug taking and relapse (89).

Considering the prominent links between the stress and reward systems and the behavioral actions of the ECS (**Figure 1**), it is tempting to hypothesize that the ECS and DA systems play similar but distinct roles along a related continuum between distress and well-being (**Figure 2**). In this model, we postulate that the ECS plays a prominent role in modulating the behavioral stress/anxiety to calmness/contentedness axis, whereas DA is prominent in the related

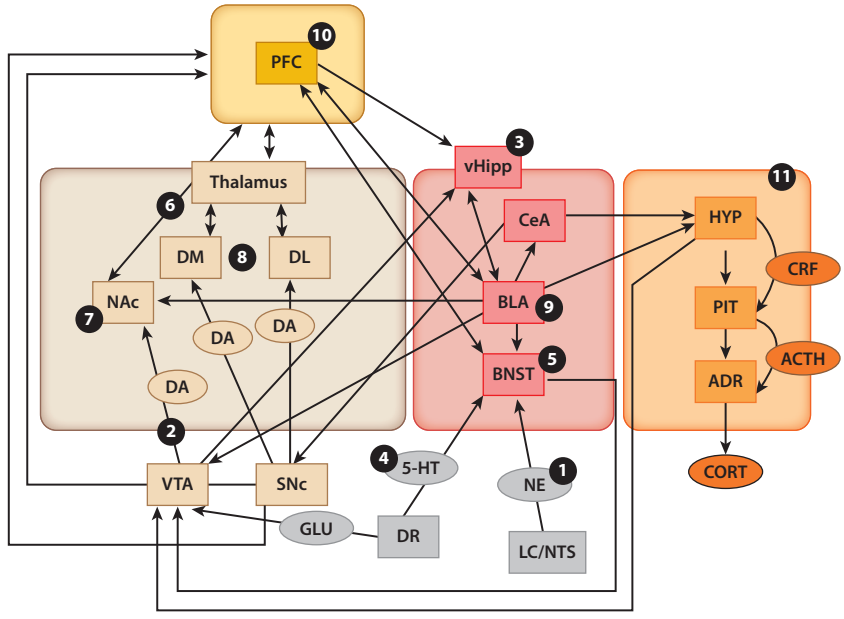

Figure 1

Diagram of the interactions between stress and reward circuits. Several brain regions are implicated in the interacting networks that respond to stress and reward. They include corticolimbic regions involved in stress responses (*red*), the hypothalamo-pituitary-adrenocortical (HPA) axis (*orange*), and brain regions involved with reward (*brown*). Key brain regions mediating stress responses include the basolateral amygdala (BLA); the central nucleus of the amygdala (CeA); the bed nucleus of the stria terminalis (BNST); the ventral hippocampus (vHipp); the prefrontal cortex (PFC) (*yellow*); and the hypothalamus (HYP), which activates the HPA axis via release of corticotropin-releasing factor (CRF) that stimulates adrenocorticotropic hormone (ACTH) by the pituitary (PIT) that then stimulates corticosterone (CORT) release by the adrenals (ADR). Key reward-processing regions include the ventral tegmental area (VTA), where the dopamine (DA) neurons are located; the nucleus accumbens (NAc) in the ventral striatum; and the dorsomedial (DM) and dorsolateral (DL) aspects of the dorsal striatum, which integrate subcortical inputs from the VTA and substantia nigra compacta (SNc) with cortical inputs (as well as limbic and thalamic inputs), which then send projections back into the cortex via the thalamus. In addition, the locus coeruleus (LC) sends noradrenergic projections (*gray*) to the amygdala, modulating stress responses, and the dorsal raphe (DR) (*gray*) sends glutamatergic projections to the VTA and serotonergic (5-HT) projections to the BNST, modulating stress and reward responses. The BLA, which plays a central role in stress responses, is reciprocally connected with PFC and vHipp and is greatly affected by excitatory projections from these regions while it sends predominantly unidirectional outputs to the NAc, BNST, and CeA. The BNST and the hypothalamus send projections to the VTA that influence DA neuronal firing. This is a selective diagram of projections that are relevant for the stress-reward interaction and is not meant to offer a comprehensive description of all anatomical connections. The numbered circles illustrate some of the key points along this complex regulatory network that have been shown to be modulated by the ECS. See **Supplemental References** for specific citations. Although not directly implicated in fear conditioning, projections from BLA to VTA and from CeA to SN have been described (164, 165). Other abbreviations: 5-HT, 5-hydroxytryptamine; GLU, glutamate; NE, norepinephrine; NTS, nucleus tractus solitarius.

Supplemental Material

motivational axis that runs from avoidance/anhedonia to motivation/reward. The two systems do communicate with each other, such as via CB1R expression on DA D1 receptor–expressing neurons, wherein *Cb1* deletion results in a fearful phenotype with attenuated fear extinction (90), or on DA D2 receptor–expressing neurons, wherein cannabinoid and dopamine receptors regulate each other's expression (91). Given the greater ability of THC to downregulate CB1R expression

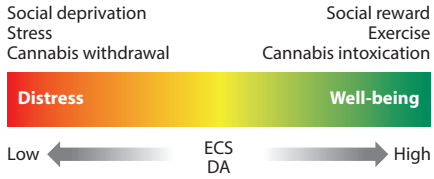

Figure 2

The stress and reward networks are highly interactive, and endocannabinoids modulate these interactions. The endocannabinoid system (ECS), dopamine (DA) system, and hypothalamo-pituitary-adrenocortical axis help establish the balance between distress (*red*) and well-being (*green*) gradients. Interaction with friends and family, exercise, and cannabis intoxication can result in a sought-after state of calmness or contentedness. This state is mediated by the interactive anxiolytic effects of increased cannabinoid and oxytocin receptor activation, together with the rewarding effects of elevated dopamine. Conversely, the persistent distress associated with stress exposure, social deprivation, and cannabis withdrawal results from the anxiogenic effects of lower ECS tone, partially mediated by stress hormone release (corticotropin-releasing factor and dynorphin) and reduced dopamine levels.

in striatal neurons relative to that of ECBs (16), one could speculate that the extent and nature of ECS-DA interactions would differ in individuals with different cannabis use trajectories, with different risks for mental illnesses, or even at different stages of development.

ENDOCANNABINOID SYSTEM–CANNABIS INTERACTIONS

The main psychoactive constituent of cannabis, THC, has generated significant interest as a potential therapeutic, as has the minor constituent cannabidiol (CBD). However, CBD has a much lower affinity for CB1R than THC [1.5–3 μM versus 20–50 nM, respectively (2)] and is present at a much lower concentration in recreational cannabis (<0.5% versus >10%) (**Figure 3**).

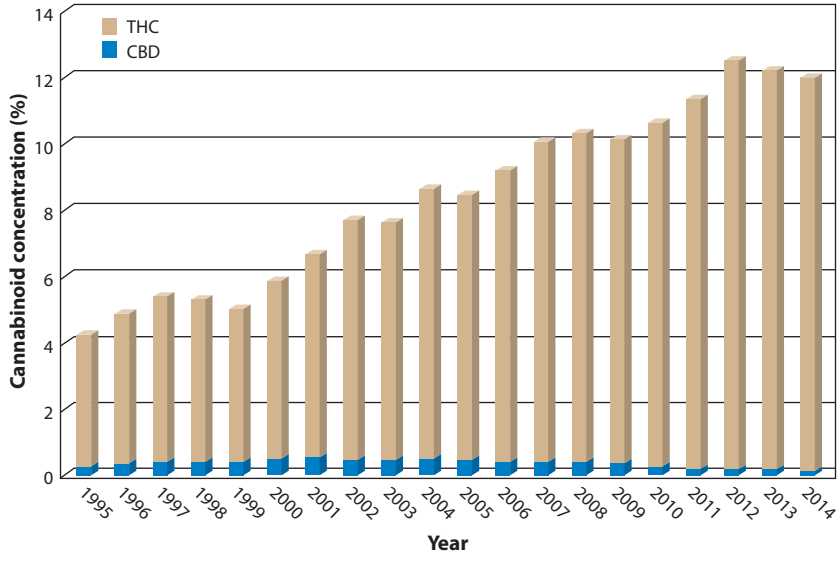

Figure 3

Average cannabinoid concentration of cannabis samples confiscated by the US Drug Enforcement Administration from 1995 to 2014 (163). Abbreviations: CBD, cannabidiol; THC, Δ^9-tetrahydrocannabinol.

ROLE OF THE ENDOCANNABINOID SYSTEM IN BRAIN DEVELOPMENT

The various components of the ECS (CB1R, AEA, 2-AG, and their synthetic and hydrolytic enzymes) appear in the very early stages of development (96). The regional brain distribution of CB1R during early development is similar to that in adults (97), with high levels in basal ganglia; limbic, cortical, and cerebellar regions; and in the white matter of the developing brain (98).

The ECS regulates interneuron migration, morphogenesis, and cell fate regulation (99, 100). In developing neurons, the ECS modulates axonal elongation (101) and guidance (102). Signaling at both CB1R and CB2R influences neural progenitor expansion (100, 103) and neuronal migration (104). The ECS is also involved in synaptogenesis (105) and microtubule turnover during directional axonal growth (106).

Few studies have evaluated the consequences of fetal THC exposure on the human brain. Consistent with preclinical data (107), studies in human fetuses suggest that cannabis exposure in utero can interfere with the proper development and regulation of the mesolimbic DA system in children (108) and with axonal connections between neurons in utero (106).

Other recent studies have demonstrated that adolescents are particularly sensitive to chronic THC exposure, with females responding differently than males, often exhibiting more profound and longer-lasting effects. Chronic THC exposure downregulates CB1R and induces changes in the volume of adolescent hippocampi in both sexes, but these effects are greater in young females (108a, 108b). This is consistent with the broader emerging literature demonstrating sex differences in the effects of THC exposure (108c, 108d).

Finally, the ECS may be generally implicated in adolescent behavior (109). Specifically, a gain-of-function mutation in CB1R leads to an adolescent-like phenotype in adult rats that includes increased peer interaction, enhanced impulsivity, high-risk and novelty-seeking behaviors, and increased sensitivity for drug and nondrug rewards.

Limited clinical studies demonstrating pharmacological effects of CBD against social anxiety (92) and intractable epilepsy (93) have reported effects at CBD doses exceeding 200 mg/day (usually 300–600 mg/day), which means that, with the exception of some CBD oil extracts, the amount of CBD in most cannabis products is pharmacologically negligible. The rewarding and negative effects of cannabis are primarily due to THC, and, not surprisingly, the negative effects are more likely to be observed with regular use or in developing brains (e.g., from fetal to young adult) (see the sidebar, Role of the Endocannabinoid System in Brain Development) (94). Consumption of high-potency cannabis has been associated with a greater risk of negative effects (e.g., psychosis, addiction, car accidents), although the increased toxicity may be somewhat offset by experienced users inhaling lower smoke volumes when using high potency cannabis (self-titration) (95).

Investigations have produced a picture of the various ways in which THC affects synaptic function and plasticity (110). A chief mechanism whereby THC affects network activity stems from its ability to inhibit release of both GABA and glutamate throughout the brain (76, 77). It is also likely that some of the adaptive phenomena triggered by chronic THC stem partly from downregulation of CB1R in brain regions involved in reward, memory, cognition, and emotions (72). For example, a recent study found that chronic administration of THC led to a dose-dependent reduction in hippocampal CB1R expression and a concomitantly lower efficacy of cannabinoids modulating GABA release (13). It took several weeks of THC-free recovery for CB1R to return to basal levels. It follows that chronic THC use would homeostatically increase FAAH activity, but a recent PET study revealed the reverse to be true. The reasons are unclear (110a).

Cannabis Effects in the Human Brain: Consequences to Behavior and Psychopathology

Acute cannabis intoxication produces changes in a wide variety of behavioral, cognitive, and motor functions (111), but there are also indirect consequences of cannabis use. Particularly significant are the effects of THC on motor coordination (probably due to effects on cerebellar fine motor coordination) (112), which at least partially explain cannabis-associated risk of traffic accidents and fatalities (113). The motor-coordinating impairments from cannabis are temporary and do not persist after intoxication subsides, even though brain imaging studies show the cerebellum to be structurally and functionally disrupted in cannabis abusers (114–118). Acute cannabis exposure also interferes with cognitive operations such as memory, disrupting both information encoding and short-term recall (119, 120). The extent to which the cognitive deficits persist after cannabis detoxification continues to be debated (121), but even temporary disruption of memory and learning in cannabis-using adolescents could interfere with their educational performance (122).

The most frequent negative effect of chronic cannabis exposure is addiction. Approximately 9% of those exposed to cannabis will become addicted (cannabis use disorder as per DSM-5), and this estimate rises to 16% when cannabis use is initiated during adolescence and 50% when cannabis is used daily (123). Based on epidemiological data, researchers have proposed that cannabis might be a gateway drug that facilitates the rewarding effects of other drugs (124). However, although cannabis use frequently precedes the abuse of other drugs, this could reflect common vulnerability factors for substance use disorders in general and the easier access to cannabis relative to other illicit substances. Because the ECS modulates brain reward systems, neuroadaptations triggered by early exposure to cannabis could alter the ECS and prime the brain to the rewarding effects of other drugs (51). Indeed, the ECS participates in the orchestration of such effects for many different drugs, including nicotine, alcohol, cocaine, and opioids such as heroin (51), and in the neuroadaptations associated with their chronic use (87). In humans, brain CB1Rs are downregulated not just in cannabis abusers (5, 125) but also in alcoholics (126), although one study reported CB1R upregulation in alcoholics (127).

The potential for chronic cannabis exposure to produce psychopathology other than addiction, particularly its potential role in anxiety disorders and schizophrenia, is an area of debate. Cannabis use represents a risk factor for precipitating certain types of psychopathology (128), possibly because of its modulatory role in neurotransmission. Additionally, the ECS also plays a prominent role in managing neuronal synchrony (129), which might be relevant to its association with psychoses. Endocannabinoids optimize inhibitory and excitatory balance in a state-dependent manner (130), and THC perturbs this balance. For example, cannabis use induces disruptions in γ and θ bands of brain-wave activity that resemble those found in psychotic patients (131, 132). In addition, magnetic resonance imaging studies have found several brain regions with high CB1R whose volumes are reduced in cannabis users. Not surprisingly, they include regions involved in emotions (amygdala and hippocampus) and executive function (PFC) that are known to play prominent roles in the neuropathology of PTSD and schizophrenia. Thus, one could speculate that cannabis-induced atrophy in these brain regions could exacerbate psychopathology in PTSD and schizophrenia. Although some researchers have documented that these volume reductions persist after long periods of abstinence (116), others have failed to find such changes (133). Changes in brain connectivity (structural and functional) that are relevant to psychopathology have also been reported in cannabis abusers. Specifically, the reduced neuronal connectivity in the PFC and in subcortical networks reported in adults who smoked cannabis during adolescence (134) could exacerbate the impairments in executive function in schizophrenia and the impaired emotional regulation in PTSD. In juxtaposition to these findings, functional deficits have been

reported by imaging studies that measured brain activation in response to specific emotional tasks that are opposite to those seen in patients with PTSD. Specifically, the decreased activation of the anterior cingulate and amygdala to masked affective stimuli (happy and fearful faces) in cannabis abusers, which is consistent with an attenuated reactivity to affective stimuli (135), is opposite to the enhanced reactivity reported in PTSD (136). The findings in schizophrenia showed mixed results, documenting both reduced as well as enhanced activation to emotional stimuli (137).

Studies using PET have also allowed us to investigate changes in neurotransmitters that are relevant to mental illnesses. For example, PET studies have shown that cannabis abusers have a reduced capacity to synthesize DA in their brain, which was associated with reduced motivation (138) and apathy (139) and with decreased DA release that was associated with negative symptoms (140). These findings suggest that downregulation of DA signaling could underlie the amotivation syndrome and negative emotionality associated with chronic cannabis use (121) and thus exacerbate mood and negative symptoms in patients with schizophrenia. A study of individuals at high risk for schizophrenia reported that those who were cannabis abusers showed stress-induced DA reductions in striatum, whereas noncannabis users showed DA increases (125, 141). Although one could interpret these findings as an indication that cannabis might normalize the enhanced brain DA release reported in schizophrenia (142), one has to consider that enhanced striatal DA signaling is associated with the early stages of schizophrenia, along with the occurrence of positive symptoms (hallucinations and delusions), whereas reduced DA signaling in the PFC is associated with the later stages of schizophrenia, along with negative symptoms (poverty of thought, anhedonia, and concreteness) (143). Thus, in this context, the effects of cannabis are expected to differ when consumed in the early and later stages (144) of the condition and might accelerate the progression of the disorder (145). PET studies of CB1R levels are also relevant, and imaging and postmortem studies have shown, more or less consistently, that these levels are increased in PTSD and schizophrenia. Because CB1Rs are downregulated by ECBs as well as by cannabinoid agonists, the findings of CB1R increases in PTSD could reflect reduced ECB signaling resulting in reduced CB1R internalization (70, 146), which could help explain the anecdotal reports of amelioration of PTSD symptoms with cannabis intoxication. However, chronic cannabis consumption is associated with low CB1R levels (5, 125), presumably from increased internalization, which could explain the recovery of CB1R after one month of cannabis detoxification (125). Thus, although acute cannabis use could help compensate temporarily for the ECB deficits and ameliorate symptoms in PTSD, the eventual downregulation of CB1R with chronic use could result in symptom exacerbation when users are not under the influence of THC. Imaging studies in schizophrenia patients have reported an upregulation of CB1R. In schizophrenia, the high CB1R levels do not seem to reflect reduced internalization, as studies have reported high concentrations of AEA and 2-AG in the cerebrospinal fluid of patients with this disease (147). Increased ECB signaling, alongside high CB1R levels, could explain the exacerbation of psychosis associated with cannabis consumption (121).

ENDOCANNABINOID SYSTEM AND MENTAL ILLNESS: THERAPEUTIC POTENTIAL

Given the complex and ubiquitous role of the ECS in brain function, it is not surprising that an increasing number of psychiatric disorders, including substance use disorders (148), are becoming linked to various aspects of ECS dysregulation.

The role of the ECS in downregulating hypersensitized stress systems, including those that interact with the reward neurocircuitry, may be the key to understanding its involvement in a host of psychiatric disorders. An impaired capacity to modulate stress reactivity is relevant for

schizophrenia, depression, bipolar disorder (149), PTSD, and other anxiety disorders and addiction. ECS regulation of the reward system is directly relevant not only to substance use disorders and drug relapse behavior (87) but also to eating disorders [anorexia nervosa (150) and binge eating disorders] and certain instances of obesity (51). Specifically, an impaired modulation by ECBs of the sensitized (heightened) responses to conditioned stimuli (or to stress) could contribute to relapse and compulsive drug taking. The reported ECS system abnormalities that occur in these disorders may represent compensatory changes as the brain attempts to buffer hyperstimulated networks. If so, then the ECS would represent a new point of vulnerability for these disorders, a notion that would be consistent with the genetic associations that have been reported between genetic polymorphisms in the ECS and stress and reward response disorders (**Supplemental Table 1**). This reconceptualization also marks the ECS as a viable target for the development of therapeutics for these disorders.

Supplemental Material

Cannabis and the ECS have long been seen as targets for the development of medications for the treatment of many psychiatric disorders (e.g., PTSD, anxiety, depression, addiction, schizophrenia, attention deficit hyperactivity disorder) (151). Of particular interest has been the potential for the treatment of anxiety disorders such as PTSD. This hypothesis is further strengthened by a vast amount of anecdotal stories and case reports, often presented as so-called evidence that smoking cannabis can dramatically reduce PTSD-related flashbacks, panic attacks, and self-mutilation (152). There are also reports that smoking cannabis can relieve stress or insomnia in a population consisting mostly of chronic pain sufferers (153). However, when considering cannabis (or other orthosteric CB1R agonists) for the treatment of PTSD, other anxiety disorders, or depression-related stress (154), it should be remembered that repeated use of such medications is bound to downregulate CB1R expression (125). Such reduction in CB1R expression would result in tolerance to the medication effects (72, 155), thus increasing the risk for depression (156) and perpetuating cannabis dependence (154, 157). As of now, no reported randomized controlled trials have shown benefits of cannabis in anxiety disorders, including PTSD, depression, or addiction. Another significant problem with cannabis-based medicinal preparations is the difficulty associated with the production of standardized medications from plant extracts that are acceptable to the US Food and Drug Administration (FDA). One company has generated such a purified THC/CBD product (nabiximols, approved in Canada and Europe and on fast track by FDA), and two synthetic cannabinoids (dronabinol and nabilone) have made it into the US pharmacopeia. However, whether herbal cannabis, a purified extract, or a synthetic cannabinoid, the same issue faces all cannabinoid drugs: Namely, orthosteric agonists are not state dependent. Orthosteric agonists activate CB1R without regard to the location of the synapse in which it resides or the timing of the signaling inhibition. The same problem exists with CB1R antagonists such as rimonabant, which showed efficacy in diminishing obesity and could have been a viable strategy to treat drug addictions. Unfortunately, rimonabant is also state insensitive, resulting in a lack of subtlety and excessive hedonic pathway inhibition, which resulted in some weight loss but also in negative affect and even suicidal ideation.

A different, more rational strategy would entail the modulation of ECB concentrations by reducing their rate of metabolism or synthesis, thereby affecting neurotransmission in a more subtle manner than exogenous orthosteric ligands. In animal models, manipulation of ECB metabolism has provided good examples of the potential for beneficial effects that are intrinsic to the ECS for modulating anxiety, pain, stress responses, and stress-induced analgesia (46, 50, 54, 56, 60, 158). Clinical studies of FAAH inhibitors, which increase AEA availability, are more advanced than for MGL inhibitors that are only just starting to undergo clinical testing. The Pfizer drug PF-04457845 and Janssen compound JNJ-42165279 are FAAH inhibitors that have been demonstrated to be safe in humans (**http://clinicaltrials.gov** identifiers NCT00836082

and NCT01964651) and have been examined for pain (NCT00981357), anxiety and depression (NCT02498392), and cannabis withdrawal (NCT01618656) in clinical efficacy trials. Unfortunately, in January 2016, the future of FAAH inhibitors was put into question owing to the death of a participant and brain damage to four of six subjects in a cohort exposed to the FAAH inhibitor BIA 10-2474 in a first-in-human research study. The report from the French National Agency for Medicines and Health Products Safety issued on March 7, 2016, noted that the symptoms were all similar, rapid onset, and appeared to affect only the CNS, particularly the hippocampus and pons, in a manner distinct from that seen previously in any known disease or toxicity. The report noted that BIA 10-2474 has a relatively low affinity for FAAH compared to previously tested compounds and therefore may be more likely to exhibit off-target effects. BIA 10-2474 was dosed at levels considerably higher than needed to completely (and irreversibly) block FAAH, in a study design that was surprising given that significant preclinical toxicity had been observed at high doses. In August 2016, an FDA safety review concluded that "BIA 10-2474 exhibits a unique toxicity that does not extend to other drugs in the class" (158a). It is hoped, therefore, that the pharmaceutical industry will continue to view enzymatic components of the ECB system as viable therapeutic targets.

Allosteric modulation of CB1R is another pharmacological approach that maintains the state dependencies of the ECS. An allosteric ligand binds to the receptor at a site distinct from that of orthosteric agonists but exerts no effect unless an orthosteric agonist is present (159). Allosteric modulators simply enhance or inhibit receptor responses to ECBs and so maintain the state dependence of the ECS. Currently, there is considerable interest in developing GPCR allosteric modulator medications, but in the case of CB1R, the field is in its infancy (15). However, the positive allosteric CB1R modulator ZCZ011 has been shown to be antinociceptive without being reinforcing (160), whereas the negative allosteric modulator pregnenolone reduces some of the behavioral effects of THC (161). The pharmaceutical company Aelis Farma is currently using the pregnenolone pharmacophore to develop medications to treat cannabis abuse disorder.

Finally, functionally selective (biased) agonists represent another attractive strategy to pharmacologically target the ECS. Agonists such as THC and AEA are only partial agonists, i.e., the maximal effect that THC or AEA can exert on G protein activation is less than the maximal effect exerted by other cannabinoids (2). Recently, it has become clear that the ratio of two drugs' efficacy could differ depending on the endpoint being measured and the cell type being evaluated (162). This has raised the possibility of developing medications that are more or less efficacious at activating different pathways, such as a drug with low activity on β-arrestin recruitment (16) that may result in a slower rate of tolerance development (12). Simple biased agonists still suppress the subtle state dependence of ECB release in one synapse versus another; some such biased allosteric modulators have already been identified. Although work in this field is still in its infancy, it offers the exciting possibility of developing compounds that have both state dependence and pathway selectivity (15).

CONCLUSIONS

Cannabis has been used for centuries across the globe. However, the recent changes in laws regarding legalization of recreational or medicinal cannabis, along with the availability of cannabis with increasingly higher THC levels (94, 163) (**Figure 3**), are generating a sense of urgency for understanding the potential adverse effects of cannabis exposure as well as its purportedly medicinal actions. Although many studies have been published on deleterious effects of chronic cannabis vis-á-vis cognition, emotion, and psychiatric symptoms, the findings are inconsistent, which has made it easier for proponents of cannabis legalization to dismiss them and wrongly claim that

cannabis use has no harmful effects. At the same time, major advances in our understanding of ECS neurobiology have opened exciting new opportunities for the development of novel, smarter medications for psychiatric and neurological disorders.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. WHO (World Health Organ.). 2016. *Management of Substance Abuse*. Geneva: World Health Organ. **http://www.who.int/substance_abuse/en/**
2. Pertwee RG, Howlett AC, Abood ME, Alexander SPH, Di Marzo V, et al. 2010. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB_1 and CB_2. *Pharmacol. Rev.* 62:588–631
3. Burns HD, Van Laere K, Sanabria-Bohórquez S, Hamill TG, Bormans G, et al. 2007. [^{18}F]MK-9470, a positron emission tomography (PET) tracer for in vivo human PET brain imaging of the cannabinoid-1 receptor. *PNAS* 104:9800–5
4. Allen Inst. Brain Sci. 2016. *Allen Brain Atlas.* Seattle, WA: Allen Inst. **http://www.brain-map.org/**
5. Ceccarini J, Kuepper R, Kemels D, van Os J, Henquet C, Van Laere K. 2015. [^{18}F]MK-9470 PET measurement of cannabinoid CB_1 receptor availability in chronic cannabis users. *Addict. Biol.* 20:357–67
6. Sañudo-Peña MC, Romero J, Seale GE, Fernandez-Ruiz JJ, Walker JM. 2000. Activational role of cannabinoids on movement. *Eur. J. Pharmacol.* 391:269–74
7. Bajaj V, Pathak P, Mehrotra S, Singh V, Govil S, Khanna A. 2011. Cannabis induced periodic catatonia: a case report. *Int. J. Mental Health Addict.* 9:162–64
8. Galiègue S, Mary S, Marchand J, Dussossoy D, Carrière D, et al. 1995. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur. J. Biochem.* 232:54–61
9. Rom S, Persidsky Y. 2013. Cannabinoid receptor 2: potential role in immunomodulation and neuroinflammation. *J. NeuroImmune Pharmacol.* 8:608–20
10. Li Y, Kim J. 2015. Deletion of CB2 cannabinoid receptors reduces synaptic transmission and long-term potentiation in the mouse hippocampus. *Hippocampus* 26:275–81

10a. García-Gutiérrez MS, Ortega-Álvaro A, Busquets-García A, Pérez-Ortiz JM, Caltana L, et al. 2013. Synaptic plasticity alterations associated with memory impairment induced by deletion of CB_2 cannabinoid receptors. *Neuropharmacology* 73:388–96

10b. Li Y, Kim J. 2016. CB_2 cannabinoid receptor knockout in mice impairs contextual long-term memory and enhances spatial working memory. *Neural Plast.* 2016:9817089

11. Turu G, Hunyady L. 2010. Signal transduction of the CB_1 cannabinoid receptor. *J. Mol. Endocrinol.* 44:75–85
12. Rubino T, Viganò D, Premoli F, Castiglioni C, Bianchessi S, et al. 2006. Changes in the expression of G protein-coupled receptor kinases and β-arrestins in mouse brain during cannabinoid tolerance: a role for RAS–ERK cascade. *Mol. Neurobiol.* 33:199–213
13. Dudok B, Barna L, Ledri M, Szabo SI, Szabadits E, et al. 2015. Cell-specific STORM super-resolution imaging reveals nanoscale organization of cannabinoid signaling. *Nat. Neurosci.* 18:75–86
14. Gyombolai P, Boros E, Hunyady L, Turu G. 2013. Differential β-arrestin2 requirements for constitutive and agonist-induced internalization of the CB_1 cannabinoid receptor. *Mol. Cell. Endocrinol.* 372:116–27
15. Khajehali E, Malone DT, Glass M, Sexton PM, Christopoulos A, Leach K. 2015. Biased agonism and biased allosteric modulation at the CB_1 cannabinoid receptor. *Mol. Pharmacol.* 88:368–79
16. Laprairie RB, Bagher AM, Kelly ME, Dupré DJ, Denovan-Wright EM. 2014. Type 1 cannabinoid receptor ligands display functional selectivity in a cell culture model of striatal medium spiny projection neurons. *J. Biol. Chem.* 289:24845–62

17. Lerner TN, Kreitzer AC. 2012. RGS4 is required for dopaminergic control of striatal LTD and susceptibility to parkinsonian motor deficits. *Neuron* 73:347–59
18. Alger BE, Kim J. 2011. Supply and demand for endocannabinoids. *Trends Neurosci.* 34:304–15
19. Gulyas AI, Cravatt BF, Bracey MH, Dinh TP, Piomelli D, et al. 2004. Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. *Eur. J. Neurosci.* 20:441–58
20. Marrs WR, Blankman JL, Horne EA, Thomazeau A, Lin YH, et al. 2010. The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. *Nat. Neurosci.* 13:951–57
21. Ohno-Shosaku T, Tanimura A, Hashimotodani Y, Kano M. 2012. Endocannabinoids and retrograde modulation of synaptic transmission. *Neuroscientist* 18:119–32
22. Okamoto Y, Tsuboi K, Ueda N. 2009. Enzymatic formation of anandamide. *Vitam. Horm.* 81:1–24
23. Simon GM, Cravatt BF. 2008. Anandamide biosynthesis catalyzed by the phosphodiesterase GDE1 and detection of glycerophospho-*N*-acyl ethanolamine precursors in mouse brain. *J. Biol. Chem.* 283:9341–49
24. Ohno-Shosaku T, Kano M. 2014. Endocannabinoid-mediated retrograde modulation of synaptic transmission. *Curr. Opin. Neurobiol.* 29:1–8
25. Ladarre D, Roland AB, Biedzinski S, Ricobaraza A, Lenkei Z. 2014. Polarized cellular patterns of endocannabinoid production and detection shape cannabinoid signaling in neurons. *Front. Cell. Neurosci.* 8:426
26. Lee SH, Ledri M, Toth B, Marchionni I, Henstridge CM, et al. 2015. Multiple forms of endocannabinoid and endovanilloid signaling regulate the tonic control of GABA release. *J. Neurosci.* 35:10039–57
27. Egertová M, Cravatt BF, Elphick MR. 2003. Comparative analysis of fatty acid amide hydrolase and CB_1 cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. *Neuroscience* 119:481–96
28. Cristino L, Starowicz K, De Petrocellis L, Morishita J, Ueda N, et al. 2008. Immunohistochemical localization of anabolic and catabolic enzymes for anandamide and other putative endovanilloids in the hippocampus and cerebellar cortex of the mouse brain. *Neuroscience* 151:955–68
29. Ross RA. 2003. Anandamide and vanilloid TRPV1 receptors. *Br. J. Pharmacol.* 140:790–801
30. Shonesy BC, Winder DG, Patel S, Colbran RJ. 2015. The initiation of synaptic 2-AG mobilization requires both an increased supply of diacylglycerol precursor and increased postsynaptic calcium. *Neuropharmacology* 91:57–62
31. Maejima T, Oka S, Hashimotodani Y, Ohno-Shosaku T, Aiba A, et al. 2005. Synaptically driven endocannabinoid release requires Ca^{2+}-assisted metabotropic glutamate receptor subtype 1 to phospholipase Cβ4 signaling cascade in the cerebellum. *J. Neurosci.* 25:6826–35
32. Ramikie TS, Nyilas R, Bluett RJ, Gamble-George JC, Hartley ND, et al. 2014. Multiple mechanistically distinct modes of endocannabinoid mobilization at central amygdala glutamatergic synapses. *Neuron* 81:1111–25
33. Hashimotodani Y, Ohno-Shosaku T, Tsubokawa H, Ogata H, Emoto K, et al. 2005. Phospholipase Cβ serves as a coincidence detector through its Ca^{2+} dependency for triggering retrograde endocannabinoid signal. *Neuron* 45:257–68
34. Yamasaki M, Matsui M, Watanabe M. 2010. Preferential localization of muscarinic M1 receptor on dendritic shaft and spine of cortical pyramidal cells and its anatomical evidence for volume transmission. *J. Neurosci.* 30:4408–18
35. Ohno-Shosaku T, Shosaku J, Tsubokawa H, Kano M. 2002. Cooperative endocannabinoid production by neuronal depolarization and group I metabotropic glutamate receptor activation. *Eur. J. Neurosci.* 15:953–61
36. Kim J, Isokawa M, Ledent C, Alger BE. 2002. Activation of muscarinic acetylcholine receptors enhances the release of endogenous cannabinoids in the hippocampus. *J. Neurosci.* 22:10182–91
37. Uchigashima M, Yamazaki M, Yamasaki M, Tanimura A, Sakimura K, et al. 2011. Molecular and morphological configuration for 2-arachidonoylglycerol-mediated retrograde signaling at mossy cell-granule cell synapses in the dentate gyrus. *J. Neurosci.* 31:7700–14
38. Viader A, Blankman JL, Zhong P, Liu X, Schlosburg JE, et al. 2015. Metabolic interplay between astrocytes and neurons regulates endocannabinoid action. *Cell Rep.* 12:798–808

39. Pan B, Wang W, Long JZ, Sun D, Hillard CJ, et al. 2009. Blockade of 2-arachidonoylglycerol hydrolysis by selective monoacylglycerol lipase inhibitor 4-nitrophenyl 4-(dibenzo[*d*][1,3]dioxol-5-yl(hydroxy)methyl)piperidine-1-carboxylate (JZL184) enhances retrograde endocannabinoid signaling. *J. Pharmacol. Exp. Ther.* 331:591–97
40. Jung KM, Mangieri R, Stapleton C, Kim J, Fegley D, et al. 2005. Stimulation of endocannabinoid formation in brain slice cultures through activation of group I metabotropic glutamate receptors. *Mol. Pharmacol.* 68:1196–202
41. Huang GZ, Woolley CS. 2012. Estradiol acutely suppresses inhibition in the hippocampus through a sex-specific endocannabinoid and mGluR-dependent mechanism. *Neuron* 74:801–8
42. Gonsiorek W, Lunn C, Fan X, Narula S, Lundell D, Hipkin RW. 2000. Endocannabinoid 2-arachidonyl glycerol is a full agonist through human type 2 cannabinoid receptor: antagonism by anandamide. *Mol. Pharmacol.* 57:1045–50
43. Smaga I, Bystrowska B, Gawliński D, Pomierny B, Stankowicz P, Filip M. 2014. Antidepressants and changes in concentration of endocannabinoids and *N*-acylethanolamines in rat brain structures. *Neurotoxicity Res.* 26:190–206
44. Maccarrone M, Rossi S, Bari M, De Chiara V, Fezza F, et al. 2008. Anandamide inhibits metabolism and physiological actions of 2-arachidonoylglycerol in the striatum. *Nat. Neurosci.* 11:152–59
45. Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, et al. 2003. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J. Cell Biol.* 163:463–68
46. Jenniches I, Ternes S, Albayram O, Otte DM, Bach K, et al. 2015. Anxiety, stress, and fear response in mice with reduced endocannabinoid levels. *Biol. Psychiatry* 79:858–68
47. Gao Y, Vasilyev DV, Goncalves MB, Howell FV, Hobbs C, et al. 2010. Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. *J. Neurosci.* 30:2017–24
48. Khlaifia A, Farah H, Gackiere F, Tell F. 2013. Anandamide, cannabinoid type 1 receptor, and NMDA receptor activation mediate non-Hebbian presynaptically expressed long-term depression at the first central synapse for visceral afferent fibers. *J. Neurosci.* 33:12627–37
49. Straiker A, Hu SS, Long JZ, Arnold A, Wager-Miller J, et al. 2009. Monoacylglycerol lipase limits the duration of endocannabinoid-mediated depolarization-induced suppression of excitation in autaptic hippocampal neurons. *Mol. Pharmacol.* 76:1220–27
50. Hohmann AG, Suplita RL II, Bolton NM, Neely MH, Fegley D, et al. 2005. An endocannabinoid mechanism for stress-induced analgesia. *Nature* 435:1108–12
51. Parsons LH, Hurd YL. 2015. Endocannabinoid signalling in reward and addiction. *Nat. Rev. Neurosci.* 16:579–94
52. Busquets-Garcia A, Desprez T, Metna-Laurent M, Bellocchio L, Marsicano G, Soria-Gomez E. 2015. Dissecting the cannabinergic control of behavior: The *where* matters. *BioEssays* 37:1215–25
53. Morena M, Patel S, Bains JS, Hill MN. 2016. Neurobiological interactions between stress and the endocannabinoid system. *Neuropsychopharmacology* 41:80–102
54. Gregg LC, Jung KM, Spradley JM, Nyilas R, Suplita RL II, et al. 2012. Activation of type 5 metabotropic glutamate receptors and diacylglycerol lipase-alpha initiates 2-arachidonoylglycerol formation and endocannabinoid-mediated analgesia. *J. Neurosci.* 32:9457–68
55. McEwen BS. 2007. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol. Rev.* 87:873–904
56. Viana TG, Hott SC, Resstel LB, Aguiar DC, Moreira FA. 2015. Anti-aversive role of the endocannabinoid system in the periaqueductal gray stimulation model of panic attacks in rats. *Psychopharmacology* 232:1545–53
57. Gunduz-Cinar O, MacPherson KP, Cinar R, Gamble-George J, Sugden K, et al. 2013. Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. *Mol. Psychiatry* 18:813–23
58. Woodhams SG, Sagar DR, Burston JJ, Chapman V. 2015. The role of the endocannabinoid system in pain. *Handb. Exp. Pharmacol.* 227:119–43

59. Agarwal N, Pacher P, Tegeder I, Amaya F, Constantin CE, et al. 2007. Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. *Nat. Neurosci.* 10:870–79
60. Booker L, Kinsey SG, Abdullah RA, Blankman JL, Long JZ, et al. 2012. The fatty acid amide hydrolase (FAAH) inhibitor PF-3845 acts in the nervous system to reverse LPS-induced tactile allodynia in mice. *Br. J. Pharmacol.* 165:2485–96
61. Romero TR, Resende LC, Guzzo LS, Duarte ID. 2013. CB1 and CB2 cannabinoid receptor agonists induce peripheral antinociception by activation of the endogenous noradrenergic system. *Anesth. Analg.* 116:463–72
62. Lim J, Igarashi M, Jung KM, Butini S, Campiani G, Piomelli D. 2015. Endocannabinoid modulation of predator stress-induced long-term anxiety in rats. *Neuropsychopharmacology* 41:1329–39
63. Rácz I, Nent E, Erxlebe E, Zimmer A. 2015. CB1 receptors modulate affective behaviour induced by neuropathic pain. *Brain Res. Bull.* 114:42–48
64. Fuss J, Steinle J, Bindila L, Auer MK, Kirchherr H, et al. 2015. A runner's high depends on cannabinoid receptors in mice. *PNAS* 112:13105–8
65. Koltyn KF, Brellenthin AG, Cook DB, Sehgal N, Hillard C. 2014. Mechanisms of exercise-induced hypoalgesia. *J. Pain* 15:1294–304
66. Heyman E, Gamelin FX, Goekint M, Piscitelli F, Roelands B, et al. 2012. Intense exercise increases circulating endocannabinoid and BDNF levels in humans—possible implications for reward and depression. *Psychoneuroendocrinology* 37:844–51
67. Morgan CJA, Page E, Schaefer C, Chatten K, Manocha A, et al. 2013. Cerebrospinal fluid anandamide levels, cannabis use and psychotic-like symptoms. *Br. J. Psychiatry* 202:381–82
68. Häring M, Enk V, Aparisi Rey A, Loch S, Ruiz de Azua I, et al. 2015. Cannabinoid type-1 receptor signaling in central serotonergic neurons regulates anxiety-like behavior and sociability. *Front. Behav. Neurosci.* 9:235
69. Hill MN, McLaughlin RJ, Pan B, Fitzgerald ML, Roberts CJ, et al. 2011. Recruitment of prefrontal cortical endocannabinoid signaling by glucocorticoids contributes to termination of the stress response. *J. Neurosci.* 31:10506–15
70. Neumeister A, Normandin MD, Pietrzak RH, Piomelli D, Zheng MQ, et al. 2013. Elevated brain cannabinoid CB_1 receptor availability in post-traumatic stress disorder: a positron emission tomography study. *Mol. Psychiatry* 18:1034–40
71. Pietrzak RH, Huang Y, Corsi-Travali S, Zheng MQ, Lin SF, et al. 2014. Cannabinoid type 1 receptor availability in the amygdala mediates threat processing in trauma survivors. *Neuropsychopharmacology* 39:2519–28
72. Mayer TA, Matar MA, Kaplan Z, Zohar J, Cohen H. 2014. Blunting of the HPA-axis underlies the lack of preventive efficacy of early post-stressor single-dose Delta-9-tetrahydrocannabinol (THC). *Pharmacol. Biochem. Behav.* 122:307–18
73. Solinas M, Goldberg SR, Piomelli D. 2008. The endocannabinoid system in brain reward processes. *Br. J. Pharmacol.* 154:369–83
74. Wang H, Lupica CR. 2014. Release of endogenous cannabinoids from ventral tegmental area dopamine neurons and the modulation of synaptic processes. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 52:24–27
75. Oleson EB, Beckert MV, Morra JT, Lansink CS, Cachope R, et al. 2012. Endocannabinoids shape accumbal encoding of cue-motivated behavior via CB1 receptor activation in the ventral tegmentum. *Neuron* 73:360–73
76. Matyas F, Urban GM, Watanabe M, Mackie K, Zimmer A, et al. 2008. Identification of the sites of 2-arachidonoylglycerol synthesis and action imply retrograde endocannabinoid signaling at both GABAergic and glutamatergic synapses in the ventral tegmental area. *Neuropharmacology* 54:95–107
77. Melis M, Pistis M, Perra S, Muntoni AL, Pillolla G, Gessa GL. 2004. Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors. *J. Neurosci.* 24:53–62
78. Alvarez-Jaimes L, Polis I, Parsons LH. 2008. Attenuation of cue-induced heroin-seeking behavior by cannabinoid CB_1 antagonist infusions into the nucleus accumbens core and prefrontal cortex, but not basolateral amygdala. *Neuropsychopharmacology* 33:2483–93

79. Riegel AC, Lupica CR. 2004. Independent presynaptic and postsynaptic mechanisms regulate endocannabinoid signaling at multiple synapses in the ventral tegmental area. *J. Neurosci.* 24:11070–78
80. Floresco SB, Blaha CD, Yang CR, Phillips AG. 2001. Modulation of hippocampal and amygdalar-evoked activity of nucleus accumbens neurons by dopamine: cellular mechanisms of input selection. *J. Neurosci.* 21:2851–60
81. Ward SJ, Dykstra LA. 2005. The role of CB1 receptors in sweet versus fat reinforcement: effect of CB1 receptor deletion, CB1 receptor antagonism (SR141716A) and CB1 receptor agonism (CP-55940). *Behav. Pharmacol.* 16:381–88
82. Fattore L, Melis M, Fadda P, Pistis M, Fratta W. 2010. The endocannabinoid system and nondrug rewarding behaviours. *Exp. Neurol.* 224:23–36
83. Wei D, Lee D, Cox CD, Karsten CA, Peñagarikano O, et al. 2015. Endocannabinoid signaling mediates oxytocin-driven social reward. *PNAS* 112:14084–89
84. Schechter M, Weller A, Pittel Z, Gross M, Zimmer A, Pinhasov A. 2013. Endocannabinoid receptor deficiency affects maternal care and alters the dam's hippocampal oxytocin receptor and brain-derived neurotrophic factor expression. *J. Neuroendocrinol.* 25:898–909
85. Russo R, D'Agostino G, Mattace Raso G, Avagliano C, Cristiano C, et al. 2012. Central administration of oxytocin reduces hyperalgesia in mice: implication for cannabinoid and opioid systems. *Peptides* 38:81–88
86. Koob GF, Volkow ND. 2010. Neurocircuitry of addiction. *Neuropsychopharmacology* 35:217–38
87. Sidhpura N, Parsons LH. 2011. Endocannabinoid-mediated synaptic plasticity and addiction-related behavior. *Neuropharmacology* 61:1070–87
88. Massi L, Elezgarai I, Puente N, Reguero L, Grandes P, et al. 2008. Cannabinoid receptors in the bed nucleus of the stria terminalis control cortical excitation of midbrain dopamine cells in vivo. *J. Neurosci.* 28:10496–508
89. Reisiger AR, Kaufling J, Manzoni O, Cador M, Georges F, Caillé S. 2014. Nicotine self-administration induces CB1-dependent LTP in the bed nucleus of the stria terminalis. *J. Neurosci.* 34:4285–92
90. Terzian AL, Drago F, Wotjak CT, Micale V. 2011. The dopamine and cannabinoid interaction in the modulation of emotions and cognition: assessing the role of cannabinoid CB1 receptor in neurons expressing dopamine D1 receptors. *Front. Behav. Neurosci.* 5:49
91. Blume LC, Bass CE, Childers SR, Dalton GD, Roberts DCS, et al. 2013. Striatal CB_1 and D_2 receptors regulate expression of each other, CRIP1A and delta opioid systems. *J. Neurochem.* 124(6):808–20
92. Bergamaschi MM, Queiroz RH, Chagas MH, de Oliveira DC, De Martinis BS, et al. 2011. Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naïve social phobia patients. *Neuropsychopharmacology* 36(6):1219–26
93. Devinsky O, Marsh E, Friedman D, Thiele E, Laux L, et al. 2016. Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. *Lancet Neurol.* 15(3):270–78
94. Volkow ND, Baler RD, Compton WM, Weiss SR. 2014. Adverse health effects of marijuana use. *N. Engl. J. Med.* 370:2219–27
95. van der Pol P, Liebregts N, Brunt T, van Amsterdam J, de Graaf R, et al. 2014. Cross-sectional and prospective relation of cannabis potency, dosing and smoking behaviour with cannabis dependence: an ecological study. *Addiction* 109:1101–9
96. Maccarrone M, Guzman M, Mackie K, Doherty P, Harkany T. 2014. Programming of neural cells by (endo)cannabinoids: from physiological rules to emerging therapies. *Nat. Rev. Neurosci.* 15:786–801
97. Glass M, Dragunow M, Faull RL. 1997. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* 77:299–318
98. Romero J, Garcia-Palomero E, Berrendero F, Garcia-Gil L, Hernandez ML, et al. 1997. Atypical location of cannabinoid receptors in white matter areas during rat brain development. *Synapse* 26:317–23
99. Kowalczyk T, Pontious A, Englund C, Daza RA, Bedogni F, et al. 2009. Intermediate neuronal progenitors (basal progenitors) produce pyramidal-projection neurons for all layers of cerebral cortex. *Cereb. Cortex* 19:2439–50

100. Mulder J, Aguado T, Keimpema E, Barabás K, Ballester Rosado CJ, et al. 2008. Endocannabinoid signaling controls pyramidal cell specification and long-range axon patterning. *PNAS* 105:8760–65
101. Keimpema E, Barabás K, Morozov YM, Tortoriello G, Torii M, et al. 2010. Differential subcellular recruitment of monoacylglycerol lipase generates spatial specificity of 2-arachidonoyl glycerol signaling during axonal pathfinding. *J. Neurosci.* 30:13992–4007
102. Berghuis P, Rajnicek AM, Morozov YM, Ross RA, Mulder J, et al. 2007. Hardwiring the brain: Endocannabinoids shape neuronal connectivity. *Science* 316:1212–16
103. Aguado T, Monory K, Palazuelos J, Stella N, Cravatt B, et al. 2005. The endocannabinoid system drives neural progenitor proliferation. *FASEB J.* 19:1704–6
104. Saez TMM, Aronne MP, Caltana L, Brusco AH. 2014. Prenatal exposure to the CB_1 and CB_2 cannabinoid receptor agonist WIN 55,212-2 alters migration of early-born glutamatergic neurons and GABAergic interneurons in the rat cerebral cortex. *J. Neurochem.* 129:637–48
105. Kim D, Thayer SA. 2001. Cannabinoids inhibit the formation of new synapses between hippocampal neurons in culture. *J. Neurosci.* 21:RC146
106. Tortoriello G, Morris CV, Alpar A, Fuzik J, Shirran SL, et al. 2014. Miswiring the brain: Δ^9-tetrahydrocannabinol disrupts cortical development by inducing an SCG10/stathmin-2 degradation pathway. *EMBO J.* 33:668–85
107. DiNieri JA, Hurd YL. 2012. Rat models of prenatal and adolescent cannabis exposure. *Methods Mol. Biol.* 829:231–42
108. DiNieri JA, Wang X, Szutorisz H, Spano SM, Kaur J, et al. 2011. Maternal cannabis use alters ventral striatal dopamine D2 gene regulation in the offspring. *Biol. Psychiatry* 70:763–69
108a. Silva L, Harte-Hargrove L, Izenwasser S, Frank A, Wade D, Dow-Edwards D. 2015. Sex-specific alterations in hippocampal cannabinoid 1 receptor expression following adolescent delta-9-tetrahydrocannabinol treatment in the rat. *Neurosci. Lett.* 602:89–94
108b. Keeley RJ, Trow J, McDonald RJ. 2015. Strain and sex differences in puberty onset and the effects of THC administration on weight gain and brain volumes. *Neuroscience* 305:328–42
108c. Cooper ZD, Haney M. 2016. Sex-dependent effects of cannabis-induced analgesia. *Drug Alcohol Depend.* 167:112–20
108d. Fogel JS, Kelly TH, Westgate PM, Lile JA. 2016. Sex differences in the subjective effects of oral Δ^9-THC in cannabis users. *Pharmacol. Biochem. Behav.* In press. **https://doi.org/10.1016/j.pbb.2016.01.007**
109. Schneider M, Kasanetz F, Lynch DL, Friemel CM, Lassalle O, et al. 2015. Enhanced functional activity of the cannabinoid type-1 receptor mediates adolescent behavior. *J. Neurosci.* 35:13975–88
110. Hoffman AF, Lupica CR. 2013. Synaptic targets of Δ^9-tetrahydrocannabinol in the central nervous system. *Cold Spring Harb. Perspect. Med.* 3:a012203
110a. Boileau I, Mansouri E, Williams B, Le Foll B, Rusjan P, et al. 2016. Fatty acid amide hydrolase binding in brain of cannabis users: imaging with the novel radiotracer [^{11}C]CURB. *Biol. Psychiatry*. 80:691–701
111. Iversen L. 2003. Cannabis and the brain. *Brain* 126:1252–70
112. Volkow ND, Gillespie H, Mullani N, Tancredi L, Grant C, et al. 1991. Cerebellar metabolic activation by delta-9-tetrahydro-cannabinol in human brain: a study with positron emission tomography and ^{18}F-2-fluoro-2-deoxyglucose. *Psychiatry Res.* 40:69–78
113. Ramaekers JG, Berghaus G, van Laar M, Drummer OH. 2004. Dose related risk of motor vehicle crashes after cannabis use. *Drug Alcohol Depend.* 73:109–19
114. Batalla A, Bhattacharyya S, Yucel M, Fusar-Poli P, Crippa JA, et al. 2013. Structural and functional imaging studies in chronic cannabis users: a systematic review of adolescent and adult findings. *PLOS ONE* 8:e55821
115. Yucel M, Solowij N, Respondek C, Whittle S, Fornito A, et al. 2008. Regional brain abnormalities associated with long-term heavy cannabis use. *Arch. Gen. Psychiatry* 65:694–701
116. Ashtari M, Avants B, Cyckowski L, Cervellione KL, Roofeh D, et al. 2011. Medial temporal structures and memory functions in adolescents with heavy cannabis use. *J. Psychiatric Res.* 45:1055–66
117. Cousijn J, Wiers RW, Ridderinkhof KR, van den Brink W, Veltman DJ, Goudriaan AE. 2012. Grey matter alterations associated with cannabis use: results of a VBM study in heavy cannabis users and healthy controls. *NeuroImage* 59:3845–51

118. Matochik JA, Eldreth DA, Cadet JL, Bolla KI. 2005. Altered brain tissue composition in heavy marijuana users. *Drug Alcohol Depend.* 77:23–30
119. Abel EL. 1971. Marihuana and memory: acquisition or retrieval? *Science* 173:1038–40
120. Miller LL, Cornett TL, Brightwell DR, McFarland DJ, Drew WG, Wikler A. 1977. Marijuana: effects on storage and retrieval of prose material. *Psychopharmacology* 51:311–16
121. Volkow ND, Swanson JM, Evins AE, DeLisi LE, Meier MH, et al. 2016. Effects of cannabis use on human behavior, including cognition, motivation, and psychosis: a review. *JAMA Psychiatry* 73:292–97
122. Ranganathan M, D'Souza DC. 2006. The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology* 188:425–44
123. Anthony JC. 2006. The epidemiology of cannabis dependence. In *Cannabis Dependence: Its Nature, Consequences and Treatment*, ed. R Roffman, RS Stephens, pp. 58–105. Cambridge, UK: Cambridge Univ. Press
124. Anthony JC. 2012. Steppingstone and gateway ideas: a discussion of origins, research challenges, and promising lines of research for the future. *Drug Alcohol Depend.* 123(Suppl. 1):S99–104
125. Hirvonen J, Goodwin RS, Li CT, Terry GE, Zoghbi SS, et al. 2012. Reversible and regionally selective downregulation of brain cannabinoid CB_1 receptors in chronic daily cannabis smokers. *Mol. Psychiatry* 17:642–49
126. Ceccarini J, Hompes T, Verhaeghen A, Casteels C, Peuskens H, et al. 2014. Changes in cerebral CB_1 receptor availability after acute and chronic alcohol abuse and monitored abstinence. *J. Neurosci.* 34:2822–31
127. Neumeister A, Normandin MD, Murrough JW, Henry S, Bailey CR, et al. 2012. Positron emission tomography shows elevated cannabinoid CB_1 receptor binding in men with alcohol dependence. *Alcohol. Clin. Exp. Res.* 36:2104–9
128. Gage SH, Hickman M, Zammit S. 2015. Association between cannabis and psychosis: epidemiologic evidence. *Biol. Psychiatry* 79:549–56
129. Sales-Carbonell C, Rueda-Orozco PE, Soria-Gómez E, Buzsáki G, Marsicano G, Robbe D. 2013. Striatal GABAergic and cortical glutamatergic neurons mediate contrasting effects of cannabinoids on cortical network synchrony. *PNAS* 110:719–24
130. den Boon FS, Werkman TR, Schaafsma-Zhao Q, Houthuijs K, Vitalis T, et al. 2015. Activation of type-1 cannabinoid receptor shifts the balance between excitation and inhibition towards excitation in layer II/III pyramidal neurons of the rat prelimbic cortex. *Pflügers Archiv.: Eur. J. Physiol.* 467:1551–64
131. Cortes-Briones J, Skosnik PD, Mathalon D, Cahill J, Pittman B, et al. 2015. Δ^9-THC disrupts gamma (γ)-band neural oscillations in humans. *Neuropsychopharmacology* 40:2124–34
132. Morrison PD, Nottage J, Stone JM, Bhattacharyya S, Tunstall N, et al. 2011. Disruption of frontal theta coherence by Δ^9-tetrahydrocannabinol is associated with positive psychotic symptoms. *Neuropsychopharmacology* 36:827–36
133. Tzilos GK, Cintron CB, Wood JBR, Simpson NS, Young AD, et al. 2005. Lack of hippocampal volume change in long-term heavy cannabis users. *Am. J. Addict.* 14:64–72
134. Filbey F, Yezhuvath U. 2013. Functional connectivity in inhibitory control networks and severity of cannabis use disorder. *Am. J. Drug Alcohol Abuse* 39:382–91
135. Gruber SA, Rogowska J, Yurgelun-Todd DA. 2009. Altered affective response in marijuana smokers: an FMRI study. *Drug Alcohol Depend.* 105:139–53
136. Liberzon I, Phan KL. 2003. Brain-imaging studies of posttraumatic stress disorder. *CNS Spectrums* 8:641–50
137. Taylor SF, Kang J, Brege IS, Tso IF, Hosanagar A, Johnson TD. 2012. Meta-analysis of functional neuroimaging studies of emotion perception and experience in schizophrenia. *Biol. Psychiatry* 71:136–45
138. Bloomfield MAP, Morgan CJA, Egerton A, Kapur S, Curran HV, Howes OD. 2014. Dopaminergic function in cannabis users and its relationship to cannabis-induced psychotic symptoms. *Biol. Psychiatry* 75:470–78
139. Bloomfield MAP, Morgan CJA, Kapur S, Curran HV, Howes OD. 2014. The link between dopamine function and apathy in cannabis users: an [^{18}F]-DOPA PET imaging study. *Psychopharmacology* 231:2251–59

140. van de Giessen E, Weinstein JJ, Cassidy CM, Haney M, Dong Z, et al. 2016. Deficits in striatal dopamine release in cannabis dependence. *Mol. Psychiatry.* In press. **https://doi.org/10.1038/mp.2016.21**
141. Mizrahi R, Kenk M, Suridjan I, Boileau I, George TP, et al. 2014. Stress-induced dopamine response in subjects at clinical high risk for schizophrenia with and without concurrent cannabis use. *Neuropsychopharmacology* 39:1479–89
142. Kegeles LS, Abi-Dargham A, Frankle WG, Gil R, Cooper TB, et al. 2010. Increased synaptic dopamine function in associative regions of the striatum in schizophrenia. *Arch. Gen. Psychiatry* 67:231–39
143. Durstewitz D, Seamans JK. 2008. The dual-state theory of prefrontal cortex dopamine function with relevance to catechol-*O*-methyltransferase genotypes and schizophrenia. *Biol. Psychiatry* 64:739–49
144. Kelley ME, Wan CR, Broussard B, Crisafio A, Cristofaro S, et al. 2016. Marijuana use in the immediate 5-year premorbid period is associated with increased risk of onset of schizophrenia and related psychotic disorders. *Schizophr. Res.* 171(1–3):62–67
145. van der Meer FJ, Velthorst E, Genet. Risk Outcome Psychos. (GROUP) Investig. 2015. Course of cannabis use and clinical outcome in patients with non-affective psychosis: a 3-year follow-up study. *Psychol. Med.* 45(9):1977–88
146. Volk DW, Eggan SM, Horti AG, Wong DF, Lewis DA. 2014. Reciprocal alterations in cortical cannabinoid receptor 1 binding relative to protein immunoreactivity and transcript levels in schizophrenia. *Schizophr. Res.* 159:124–29
147. Ujike H, Morita Y. 2004. New perspectives in the studies on endocannabinoid and cannabis: cannabinoid receptors and schizophrenia. *J. Pharmacol. Sci.* 96:376–81
148. Ghosh A, Basu D. 2015. Cannabis and psychopathology: the meandering journey of the last decade. *Indian J. Psychiatry* 57:140–49
149. Monteleone P, Bifulco M, Maina G, Tortorella A, Gazzerro P, et al. 2010. Investigation of *CNR1* and *FAAH* endocannabinoid gene polymorphisms in bipolar disorder and major depression. *Pharmacol. Res.* 61:400–4
150. Andries A, Frystyk J, Flyvbjerg A, Stoving RK. 2014. Dronabinol in severe, enduring anorexia nervosa: a randomized controlled trial. *Int. J. Eating Disord.* 47:18–23
151. Pacher P, Batkai S, Kunos G. 2006. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol. Rev.* 58:389–462
152. Passie T, Emrich HM, Karst M, Brandt SD, Halpern JH. 2012. Mitigation of post-traumatic stress symptoms by *Cannabis* resin: a review of the clinical and neurobiological evidence. *Drug Testing Anal.* 4:649–59
153. Webb CW, Webb SM. 2014. Therapeutic benefits of cannabis: a patient survey. *Hawai'i J. Med. Public Health* 73:109–11
154. Smaga I, Bystrowska B, Gawliński D, Przegaliński E, Filip M. 2014. The endocannabinoid/endovanilloid system and depression. *Curr. Neuropharmacol.* 12:462–74
155. Suplita RL II, Eisenstein SA, Neely MH, Moise AM, Hohmann AG. 2008. Cross-sensitization and cross-tolerance between exogenous cannabinoid antinociception and endocannabinoid-mediated stress-induced analgesia. *Neuropharmacology* 54:161–71
156. Beyer CE, Dwyer JM, Piesla MJ, Platt BJ, Shen R, et al. 2010. Depression-like phenotype following chronic CB_1 receptor antagonism. *Neurobiol. Disease* 39:148–55
157. Kedzior KK, Laeber LT. 2014. A positive association between anxiety disorders and cannabis use or cannabis use disorders in the general population—a meta-analysis of 31 studies. *BMC Psychiatry* 14:136
158. Ghosh S, Kinsey SG, Liu QS, Hruba L, McMahon LR, et al. 2015. Full fatty acid amide hydrolase inhibition combined with partial monoacylglycerol lipase inhibition: augmented and sustained antinociceptive effects with reduced cannabimimetic side effects in mice. *J. Pharmacol. Exp. Ther.* 354:111–20
158a. US Food Drug Admin. 2016. *FDA finds drugs under investigation in the U.S. related to French BIA 10-2474 drug do not pose similar safety risks*. News Release, Aug. 12. **http://www.fda.gov/Drugs/DrugSafety/ucm482740.htm**
159. Dohlman HG. 2015. Thematic Minireview Series: new directions in G protein-coupled receptor pharmacology. *J. Biol. Chem.* 290:19469–70

160. Ignatowska-Jankowska BM, Baillie GL, Kinsey S, Crowe M, Ghosh S, et al. 2015. A cannabinoid CB_1 receptor-positive allosteric modulator reduces neuropathic pain in the mouse with no psychoactive effects. *Neuropsychopharmacology* 40:2948–59
161. Vallée M, Vitiello S, Bellocchio L, Hébert-Chatelain E, Monlezun S, et al. 2014. Pregnenolone can protect the brain from cannabis intoxication. *Science* 343:94–98
162. Kenakin T, Christopoulos A. 2013. Signalling bias in new drug discovery: detection, quantification and therapeutic impact. *Nat. Rev. Drug Discov.* 12:205–16
163. ElSohly MA, Mehmedic Z, Foster S, Gon C, Chandra S, Church JC. 2016. Changes in cannabis potency over the last 2 decades (1995–2014): analysis of current data in the United States. *Biol. Psychiatry* 79:613–19
164. Lee HJ, Groshek F, Petrovich GD, Cantalini JP, Gallagher M, Holland PC. 2005. Role of amygdalonigral circuitry in conditioning of a visual stimulus paired with food. *J. Neurosci.* 25:3881–88
165. Okate K, Nakamura Y. 2000. Possible pathways through which neurons of the shell of the nucleus accumbens influence the outflow of the core of the nucleus accumbens. *Brain Dev.* 22(Suppl. 1):S17–26