REVIEW ARTICLE
Brain activity of anandamide: a rewarding bliss?

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Anandamide is a lipid mediator that acts as an endogenous ligand of CB1 receptors. These receptors are also the primary molecular target responsible for the pharmacological effects of Δ9-tetrahydrocannabinol, the psychoactive ingredient in *Cannabis sativa*. Several studies demonstrate that anandamide exerts an overall modulatory effect on the brain reward circuitry. Several reports suggest its involvement in the addiction-producing actions of other abused drugs, and it can also act as a behavioral reinforcer in animal models of drug abuse. Importantly, all these effects of anandamide appear to be potentiated by pharmacological inhibition of its metabolic degradation. Enhanced brain levels of anandamide after treatment with inhibitors of fatty acid amide hydrolase, the main enzyme responsible for its degradation, seem to affect the rewarding and reinforcing actions of many drugs of abuse. In this review, we will provide an overview from a preclinical perspective of the current state of knowledge regarding the behavioral pharmacology of anandamide, with a particular emphasis on its motivational/reinforcing properties. We will also discuss how modulation of anandamide levels through inhibition of enzymatic metabolic pathways could provide a basis for developing new pharmaco-therapeutic tools for the treatment of substance use disorders.

Keywords: endocannabinoids; 2-AG; anandamide; cannabinoid receptor; brain reward; drug use disorder; drug addiction; FAAH; cocaine; morphine; nicotine; alcohol

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INTRODUCTION
Cannabis sativa and its derivatives, i.e., marijuana, are among the best-known mind-altering substances used by man in ancient times and are still among the most abused substances worldwide. The major psychoactive component of marijuana, Δ9-tetrahydrocannabinol (THC), was isolated and its chemical structure clarified by Gaoni and Mechoulam in 1964 [1]. Since then, THC has been synthesized, and many studies have been conducted on its activity [2]. Nevertheless, until the discovery of CB1 receptors in 1988 [3], the mechanisms involved in the action of THC on the brain were elusive and were suggested to consist of non-specific activity on the neuronal cell membrane [4, 5]. The discovery of CB1 receptors [6], the primary pharmacological target of THC, prompted research to find the endogenous ligands for these receptors. The first endocannabinoid to be discovered was anandamide (AEA), which was isolated from the pig brain by William Devane and co-workers in 1992 [7]. Its name comes from the Sanskrit word "ananda" (*internal bliss*), which emphasizes the interesting role of AEA as an endogenous marijuana-like substance self-delivered by the brain. 2-Arachidonoylglycerol (2-AG) was the second endocannabinoid to be identified; it was isolated from the rat brain and the canine gut [8, 9]. Since the discovery of these first two endogenous cannabinoids, numerous studies have been carried out demonstrating that their behavioral and molecular effects only partially overlap between compounds, as well as between each compound and the most studied CB1 agonist, THC [10]. The discovery of CBRs and endocannabinoids led to the identification of other components of what was then called the "endocannabinoid system", which also includes the enzymes that are responsible for both the biosynthesis and the metabolism of endocannabinoids [11]. The endocannabinoid system is an important lipid signaling system that emerged before the evolution of vertebrates and it is well preserved across species [12]. In mammals, it has been recently recognized as a modulator of a large variety of physiological processes, including inflammation and pain [13, 14], appetite [15], mood [16], and pre-and postnatal development [17]. Furthermore, the endocannabinoid system is an important constituent of the neuronal substrates involved in the reinforcement and reward processes of the brain [18, 19]. It modulates the rewarding and pharmacological responses induced by cannabinoids [20], as well as those induced by other drugs of abuse [19, 21, 22].

With regard to its involvement in the mechanisms of reward, AEA has been the most studied among the endocannabinoids. Indeed, several studies indicate that AEA exerts an overall modulatory effect on the reward circuitry. It may possess reinforcing effects of its own, and it is implicated in the reinforcing and addictive effects of various drugs of abuse, including non-cannabinoids [19, 21, 23]. For instance, AEA may play important roles in the sequence of events from experiencing the euphoric effects of a drug to abuse and drug use disorders. The aim of the present manuscript is to explore and discuss preclinical behavioral studies about the best-known brain endocannabinoid, AEA, and how its direct effects or changes in its abundance in the brain might (or might not) suggest a role in drug use

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disorders. We have reviewed results obtained through specific behavioral pharmacology methodologies such as brain stimulation reward place conditioning, self-administration, reinstatement, and relapse in order to provide discussion material about the potential of AEA as a reinforcer and/or as a treatment for drug addiction. Then, we briefly introduce some notions about the endocannabinoid system. The last part of the review focuses on specific preclinical brain/behavioral activities of AEA as a reinforcer or as a modulator of rewarding/reinforcing effects of non-cannabinoid drugs of abuse.

ENDOCANNABINOIDS

As mentioned in the introduction, discoveries regarding candidate endogenous ligands of the CBRs started in the early 1990s, soon after the identification of CBRs as specific receptors for THC. Both AEA and 2-AG are bioactive lipids, belonging to the subclasses known as N-acylethanolamines (NAEs) and mono-acylglycerols (MAGs), respectively [24]. Numerous studies have shown that these compounds play key roles in several biological activities within the central nervous system (CNS) and the periphery [25]. Although similar in structure (AEA and 2-AG are arachidonic acid derivatives conjugated with ethanolamine or glycerol, respectively) (Fig. 1), these compounds show some crucial distinctions that are responsible for their different physiological and pathophysiological roles: (i) they are regulated by different biosynthetic and degradative pathways [26]; (ii) brain tissue levels of AEA are 10–100 times lower than those of 2-AG [27]; (iii) AEA (as well as THC) activates CBRs with low intrinsic efficacy (partial agonist), whereas 2-AG is an agonist with high intrinsic efficacy (full agonist) [28]. In addition to AEA and 2-AG, several other lipids with endocannabinoid-like activity have been isolated [29], including 2-arachidonylglyceryl ether (2-AGE, noladin), O-arachidonylethanolamine (virodhamine), and N-arachidonyldopamine (NADA) [30–32]. However, their physiological role is not clear, and thus it remains to be ascertained whether they are true endocannabinoids.

Endocannabinoid biosynthesis and metabolism
Given their hydrophobic nature, endocannabinoids are precluded from stable uptake and storage into synaptic vesicles, and there are no known cannabinoid neurons or cannabinoid neuronal pathways. However, virtually all cells possess AEA/2-AG precursors, which are usually found as components of the cell membrane, and the end products themselves can be synthesized on demand in a Ca^{2+}-dependent manner after cellular depolarization or

receptor stimulation [24, 33]. Several pathways exist for the biosynthesis of AEA and 2-AG; these pathways, together with the catabolic pathways, represent the key points in the regulation of their tissue levels (Fig. 2). AEA is derived mainly from the cleavage of a phospholipid precursor, N-arachidonoyl phosphatidylethanolamine (NAPE); NAPE, in turn, is derived from the enzymatic transfer, catalyzed by N-acyltransferase (NAT), of an acyl group from the sn-1 position of arachidonic acid to the amino group of a phosphatidylethanolamine (PE). NAPE is hydrolyzed to AEA and phosphatidic acid by a phosphodiesterase, a substrate-specific phospholipase D (NAPE-PLD). In addition to NAPE-PLD, NAPE can also be hydrolyzed by other enzymes such as phospholipase A2 (PA2), phospholipase C (PLC) and α/β-hydrolase 4 (Abh4) [34]. The formation of 2-AG proceeds mainly from inositol phospholipids (PI) via diacylglycerol (DAG), by the phospholipase C (PLC)/DAG lipase pathway [35–37]. In addition, 2-AG may be synthesized from PI by sequential hydrolysis catalyzed by PI-specific phospholipase A1 (PLA1) and lyso-PI-specific PLC [38]. As already mentioned above, endocannabinoids are not stored in vesicles, and their half-life is very short, as they undergo rapid metabolic deactivation by specific enzymes after being taken up into the cell. Degradation of AEA occurs mainly through the action of the intracellular enzyme fatty acid amide hydrolase (FAAH), which breaks it down into free arachidonic acid and ethanolamine [26, 39]. In addition to FAAH, AEA is also degraded by N-acylethanolamine acid amidase (NAAA) [40]. Both FAAH (which most prefers AEA as a substrate) and NAAA also hydrolyze oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), two endogenous fatty acid amides that bind primarily to the α-type of peroxisome proliferator-activated receptors (PPARα) but that seem to enhance AEA activity through an entourage effect [41] . In addition to hydrolytic pathways, AEA can be oxygenated by cyclooxygenase-2 (COX-2), lipoxygenase (LOX) isoenzymes, and by cytochrome P-450 [25].

FAAH may represent an attractive therapeutic target for the treatment of pain, depression and other CNS conditions [42]. For example, knocking out the FAAH enzyme in mice leads to more than 10-fold elevation of AEA levels within many brain regions [43] and gives rise to a CBR-mediated analgesic phenotype [44]. The discovery of this FAAH knockout phenotype led researchers to focus on FAAH inhibitors, such as URB597 ([3-(3-carbamoylphenyl) phenyl] N-cyclohexylcarbamate). Administration of an FAAH inhibitor prolongs the half-life and increases the activity of AEA [45]. Furthermore, URB597 has been demonstrated to be active in neuropathic [46], inflammatory [47], and acute pain [48]; depression [49]; and anxiety [48]. Several α-keto-heterocycles, such as OL-135, have also been found to increase AEA levels and display in vivo activity, producing analgesic effects in acute thermal and noxious chemical pain assays in mice [50]. While degradative enzymatic pathways for AEA have been evidenced during the last 20 years, AEA transport through the neuronal cell membrane is still a debated issue in the scientific community [25, 51–53]. To date, molecular identification and cloning of the AEA transporter has not been achieved. Nevertheless, a model has been proposed claiming that AEA uptake is also related to FAAH [54]. For this reason, recently identified AEA transport inhibitors, such as N-arachidonoyl-aminophenol (AM404) [55], N-arachidonyl-2-methyl,4-hydroxyphenylamine (VDM11) [56] and LY218240 [51], which prevent the recycling of AEA back into the cells from the synaptic cleft, also lead to FAAH inhibition. In contrast, other transport inhibitors, such as N-(5Z, 8Z, 11Z, 14Z eicosatetraenyl)-4-hydroxybenzamide (AM1172), N-arachidonyl-3-furylmethylamine (UCM707), and (R)-N-(1-(4-hydroxyphenyl)-2-hydroxyethyl)oleamide (OMDM2), inhibit FAAH weakly or not at all in vitro [57].

Similar to AEA, 2-AG is rapidly degraded by different enzymes to yield arachidonic acid and glycerol. The most ubiquitous
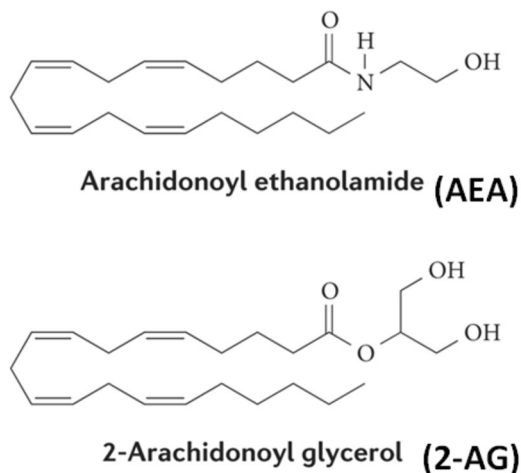

Arachidonoyl ethanolamide (AEA)

2-Arachidonoyl glycerol (2-AG)

Fig. 1 Structures of AEA and 2-AG

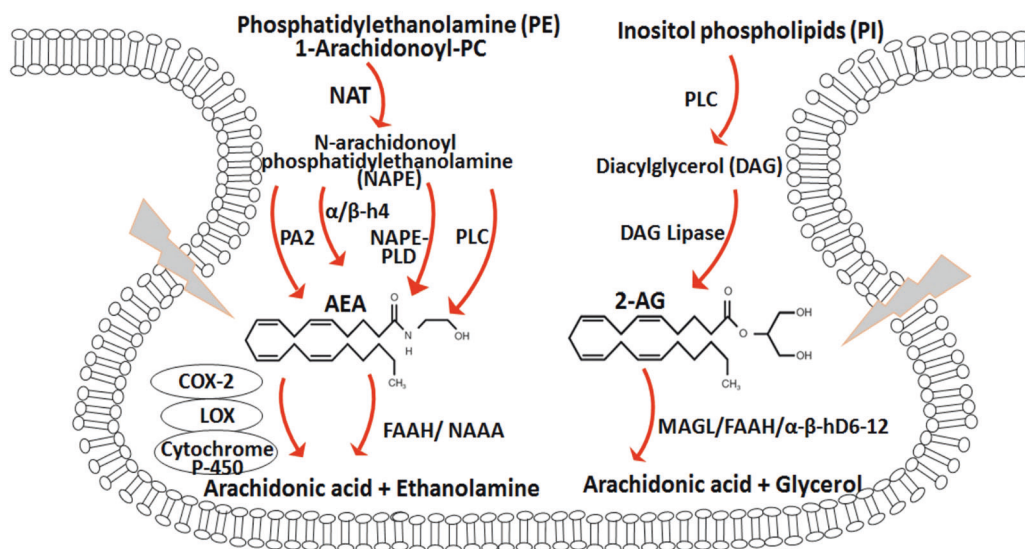Brain activity of anandamide: a rewarding bliss?
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Fig. 2 Schematic representation of AEA and 2-AG biosynthesis and degradation

mechanism of degradation of 2-AG is by monoacylglycerol lipase [34]. However, some studies have provided evidence that 2-AG is also degraded by FAAH [58, 59]. Finally, 2-AG can be degraded by a series of serine hydrolase α-β-hydrolase domain 6 or 12 that accounts for approximately 4%–9% of total brain 2-AG hydrolysis [60, 61]. Similar to FAAH inhibitors, the irreversible MAGL inhibitor N-arachidonyl maleimide (NAM) decreases 2-AG hydrolysis by ~85% [62]. Despite conflicting results [63], other MAGL inhibitors such as URB602 and URB754 have been reported to increase 2-AG brain levels and produce anti-hyperalgesia in rodents [64, 65].

Endocannabinoid signaling

It is well established that endocannabinoids retrogradely regulate synaptic neurotransmission, controlling the activity of a range of different neurotransmitters such as glutamate and gamma-aminobutyric acid (GABA) and thus controlling both excitatory and inhibitory inputs [66, 67]. Once synthesized from cell membrane phospholipids, endocannabinoids are immediately released by the postsynaptic terminal, and after traveling through the synaptic cleft, they bind to and activate CBRs in the presynaptic membrane, inhibiting neurotransmission release by activating presynaptic K^+ channels and inhibiting N- and P/Q-type Ca^{2+} channels [66, 68, 69] (Fig. 3). Presynaptic inhibition of neurotransmitter release by endocannabinoids can lead to two different forms of synaptic plasticity depending on the involvement of GABA or glutamate transmission: activation of CBRs on axon terminals of GABAergic neurons mediates depolarization-induced suppression of inhibition (DSI), whereas activation of CBRs on axon terminals of glutamatergic neurons mediates depolarization-induced suppression of excitation (DSE) [70]. Moreover, the suppression of neurotransmitter release can be either transient (endocannabinoid-mediated short-term depression) or persistent (endocannabinoid-mediated long-term depression) [66]. This retrograde signaling function of endocannabinoids appears to be widely distributed throughout the CNS [71, 72].

Cannabinoid receptors

The pharmacological actions of marijuana and of its principal psychoactive ingredient THC were attributed in early studies to the ability of THC to penetrate cell membranes and to alter specific membrane properties due to its very high lipophilicity [4, 5]. This was the best scientific explanation possible before the discovery of specific receptors for this compound. The existence of
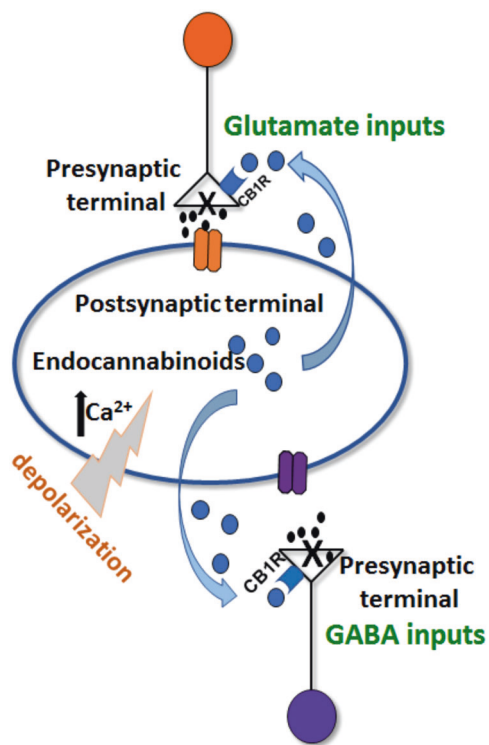

Fig. 3 Schematic representation of the "retrograde" mechanism of action of endocannabinoids: after depolarization, endocannabinoids are released by the postsynaptic terminal and activate CBRs in the presynaptic terminal, inhibiting neurotransmission release

CBRs was initially suggested in the late 1980s by ligand-binding studies and then confirmed by cloning the receptors, demonstrating unequivocally that CBRs were the receptors mediating the behavioral-pharmacological effects of marijuana [3, 6]. Thus, CBRs, revealed years after the discovery of most of the other known neurotransmitter receptors, were shown to be the most expressed receptors in the brain. However, there are no cannabinoid neurons that release endogenous cannabinoids to activate these receptors. Indeed, endogenous ligands are released on demand by cleavage
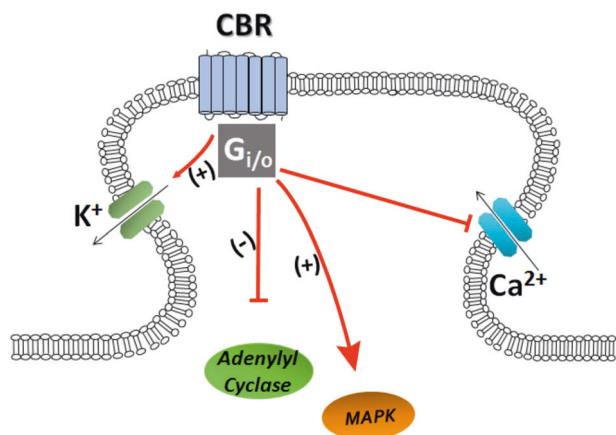
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Fig. 4 Schematic representation of CBR stimulation: activation of CBR leads to inhibition of adenylyl cyclase, inhibition of Ca^{2+} channels, an increase in K^+ channel conductance and activation of the mitogen-activated protein kinases (MAPKs)

of lipid precursors. It is interesting to note that while most of the classical neurotransmitter-neuromodulator receptor systems have only one endogenous ligand, CBRs have at least 2, likely more, endogenous ligands [29]. It is also interesting to note that another receptor system showing more than one endogenous ligand is the opioid system, and members of these two families of endogenous ligands show reinforcing effects in self-administration studies in animals.

To date, two CBRs have been characterized: the CB1 receptor (CB1R) identified in 1988 [3], and cloned in the early 1990s from rat cerebral cortex [6], and later from human [73] and mouse brain [74]; the second CBR subtype, CB2 receptor (CB2R), was derived from human promyelocytic-leukemia cells (HL-60 cells) [75]. Both CB1R and CB2R are members of the G-protein-coupled receptor (GPCRs) that belong to the rhodopsin GPCR family (Class A). AEA, 2-AG, and THC, as well as synthetic cannabinoids of other structural classes, such as CP55940,5 and WIN 55,212-2 are able to bind with high affinity to CB1R. CB1R represents the most abundant G-protein-coupled receptor in the brain: it has been identified at extremely high density in the cingulate gyrus, frontal cortex, hippocampus, cerebellum and basal ganglia [76–78]; at moderate density in the basal forebrain, amygdala, nucleus accumbens, periaqueductal gray and hypothalamus; and at low density in the midbrain, pons, medulla, primary motor cortex and thalamus [79]. CB1R is expressed mostly on axons and axon terminals of neurons but also on interneurons and astrocytes [80, 81]. Once CB1R is activated, it couples predominantly with G proteins of the αi and αo subtypes (Fig. 4). Its general mechanism comprises a signaling cascade that leads to inhibition of adenylyl cyclase [82], inhibition of the opening of voltage gated calcium channels [83], an increase in potassium channel conductance [83, 84], activation of the mitogen-activated protein kinases (MAPKs) [85], and overall suppression of neurotransmitter release. Nevertheless, the endocannabinoid system is characterized by a great complexity such that the signaling pathways and interacting proteins used by CBRs are much more various than the originally established ones [86, 87]. Furthermore, CB1R have also been described in peripheral sympathetic axon terminals, where they inhibit norepinephrine release [88, 89], and throughout the enteric nervous system, where they act inhibiting both intestinal motility and secretion [90]. In contrast to the view that CB2R is restrictively expressed in the immune system, such as in the marginal zone of the spleen, the thymus, the tonsils, and the surface of immune cells [75], various evidence has more

recently shown that CB2Rs are also present within the brain, especially in microglial cells [91]. Despite controversy surrounding the data, they may also be involved in functions regulating substance abuse [92, 93]. Recent reports also demonstrated that CB2Rs are expressed in midbrain DA neurons [94, 95], where they modulate alcohol preference and the reinforcing and neurochemical effects of cocaine [92, 96, 97]. However, it appears that species differences related to CB2R genes splicing and their functional expression might produce different behavioral outcomes related to cocaine self-administration. Indeed, brain gene-expression studies on mice and rats have shown rat-specific CB2R isoforms, together with different regional levels of CB2R mRNA and cell-type localization [98, 99]. In humans, CB2R, encoded by the CNR2 gene, shares 68% identity with human CB1R within the transmembrane regions and only 44% homology throughout the total protein [75]. Despite belonging to the same family of G proteins and sharing some ligands, CB1R and CB2R differ significantly from one another in their signaling: CB2R poorly modulates calcium channels and inwardly rectifying potassium channels [100]. Moreover, CB2Rs from different species have often shown distinct pharmacological results in response to activation by identical drugs [101, 102].

Although AEA and 2-AG are widely accepted as endocannabinoids due to their interactions with CB1R and CB2R, they also stimulate other receptor types [31, 103]. A large body of evidence demonstrated that lipid-based molecules, in particular AEA (but not 2-AG), can bind TRPV1 vanilloid receptors, the molecular target of capsaicin [104]. Indeed, Zygmunt et al. [105] reported that AEA induces vasodilation by activating TRPV1 receptors on perivascular sensory nerves. The interaction of AEA with TRPV1 receptors is specific and depends on the ability of AEA to reach the intracellular binding site [34]. While AEA and capsaicin have similar affinity for TRPV1, the former has significantly lower potency, and higher concentrations of AEA are necessary to induce the typical TRPV1 responses than are required for CB1 activation [106, 107]. Furthermore, the activity of AEA at TRPV1 receptor may be modulated by molecular mechanisms that regulate its internalization and degradation [108]. AEA seems to act as a partial agonist when the TRPV1 receptor expression is low, while it is a full agonist when receptor expression is high [104, 106]. Additionally, TRPV1 sensitivity to AEA is enhanced by entourage effects of other endogenous lipids and by phosphorylation of TRPV1 itself. The activation of TRPV1 receptors by AEA has potential implications in the treatment of inflammatory, respiratory, and cardiovascular disorders [106].

Different studies have established the orphan G-protein-coupled receptor GPR55 as a cannabinoid receptor with signaling distinct from that of the CB1 and CB2 receptors [109–111]. Specifically, recent studies revealed that GPR55 could be a pharmacological target for AEA [112]. GPR55 is highly expressed in large dorsal root ganglion neurons and, upon activation, increases intracellular calcium in these neurons. GPR55 possesses numerous signaling pathways through which it exerts its physiological effects. Examination of its signaling pathway in HEK293 cells transiently expressing GPR55 indicated that the calcium increase involves G(q), G(12), RhoA, actin, phospholipase C, and calcium release from IP(3)R-gated stores [112, 113]. GPR55 activation also inhibits M currents [114].

A growing body of evidence identifies PPARs as cannabinoid targets and suggests that this interaction could be responsible for some effects of endogenous cannabinoid-like molecules [115, 116]. PPARs are a family of nuclear receptors that regulate the transcription and expression of different genes [117] and control numerous physiological functions such as inflammation, cell differentiation and homeostasis [115, 118, 119]. Recently, several studies have produced evidence supporting the structural and functional analogy between endogenous PPAR ligands and

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AEA, demonstrating the capacity of the latter to activate some members of this family directly [116, 120]. Specifically, it was found that AEA is a weak PPARα ligand and is also able to activate PPARγ [115, 120]. Regarding PPARγ, AEA has anti-inflammatory effects inhibiting the release of the pro-inflammatory cytokine IL-2 in a CB1R/CB2R-independent manner [121]. Furthermore, the possibility that AEA causes PPARγ and CB1 receptor upregulation has been suggested [122].

THE ENDOCANNABINOID SYSTEM IN BRAIN REWARD PROCESSES

Neurochemistry

The mesocorticolimbic system of the CNS consists of subpopulations of dopaminergic neurons, originating in the ventral tegmental area (VTA) and pars compacta of the substantia nigra, which project to the nucleus accumbens, as well as to other limbic structures, such as the prefrontal cortex (PFC), amygdala and hippocampus. The mesocorticolimbic system has multifaceted functions that are among the hallmarks of reward processing and motivated behavior [123–125].

It is well established that dopaminergic neurons of the mesocorticolimbic pathway are under the control of excitatory (primarily glutamatergic) and inhibitory (primarily GABAergic) inputs that regulate their neuronal activity [126–129]. Several findings support the hypothesis that the endocannabinoid system also contributes to the brain reward processes modulating the activity of dopaminergic neurons, even if indirectly [130–132]. CB1Rs are present in the VTA and in the accumbens, as well as in the PFC, central amygdala, and hippocampus, where they are mainly located at the presynaptic terminal [133]. An important functional consequence of their activation is that they inhibit neurotransmitter release by acting as retrograde messengers [134, 135]. Thus, endocannabinoids can be released in both the accumbens and the VTA following depolarization [136, 137]. Activation of CB1Rs on axon terminals of GABAergic neurons in the VTA and glutamatergic neurons in both the VTA and accumbens was shown to inhibit GABAergic and glutamatergic neurotransmission [136, 138]. The final effect on VTA dopaminergic activity depends on the relative level of input activation under distinct behavioral circumstances [138].

A shared feature of most drugs abused by humans is their ability to activate dopaminergic neurotransmission, resulting in increased extracellular levels of dopamine (DA) in the accumbens and particularly in its ventromedial portion, the shell [139–141]. Moreover, more natural rewards such as palatable food increase DA release in the accumbens shell [142–144]. It is well established that THC, as well as AEA and 2-AG, also produce increases in the extracellular levels of DA in the shell of the nucleus accumbens, suggesting that they themselves could have either rewarding or reinforcing effects [131, 145, 146]. Moreover, this effect is blocked by the CB1 antagonist rimonabant, indicating that the dopaminergic effects of endocannabinoids involve CB1Rs [131, 146]. It should be emphasized that pharmacological blockade of CB1Rs also prevents or reduces the transient increases in DA in the shell of the accumbens produced by the administration of several drugs of abuse such as nicotine, ethanol and cocaine [147]. Moreover, CB1R blockade reduces the increase in DA release in the rat accumbens induced by a novel highly palatable food [148].

Finally, it is interesting to note that brain levels of AEA and 2-AG have been found to be altered by activation of reward processes after exposure to different drugs of abuse. For example, Gonzàlez et al. [149] found that chronic exposure to nicotine or ethanol did produce a significant increase in AEA content in the limbic forebrain, a brain structure that, among other nuclei, includes the accumbens. In contrast, the same authors found that chronic alcohol exposure caused a decrease in the contents of both AEA and 2-AG in the midbrain, as well as within the hippocampus, the striatum and the cerebral cortex. A decrease in AEA and/or 2-AG after chronic nicotine exposure has also been observed [149]. Changes in AEA and 2-AG content were also found in different brain areas, including the hippocampus and PFC, of morphine-sensitized rats [150]. Furthermore, ethanol self-administration causes an increase in dialysate 2-AG levels from the accumbens of rats with no concomitant changes in dialysate AEA concentrations. On the other hand, heroin self-administration was found to increase dialysate AEA levels and to induce a subtle but significant decrease in dialysate 2-AG levels [151]. Finally, various studies have shown that psychostimulants, such as cocaine or amphetamine, also alter AEA and 2-AG contents in the striatum [152, 153].

Behavioral studies in animals

Several animal models have been used to study and characterize the effects of AEA and its potential role in the reinforcing/rewarding actions of drugs. Here, we review the discoveries yielded by the most commonly used behavioral tests over the past few decades.

These behavioral tests include (1) the brain stimulation reward procedure, which evaluates the effects of the drug in the brain reward circuits; (2) conditioned place preference, which assesses conditioned responses related to the rewarding effects of drugs; (3) self-administration procedures, which directly measures the reinforcing properties of drugs; and (4) the reinstatement model of relapse, used to investigate mechanisms underlying drug-seeking behavior.

Brain stimulation reward. This procedure [154], called also intracranial self-stimulation (ICSS), allows an indirect preclinical assessment of the potential reinforcing effects of drugs in vivo (see for review: [155]). Indeed, when lever pressing causes the delivery of electrical stimulation to selected brain regions, the stimulation may produce reward-like feelings, which, in turn, maintain the lever-pressing behavior. In general, systemic administration of drugs of abuse reduces the electrical threshold at which the behavior is maintained, thus facilitating the reward-like experience. However, the same method also allows the testing substances that might interfere negatively with the reward-like feelings, thus increasing the threshold for reward. As discussed by Tanda [156], cannabinoids tested with ICSS procedures have provided inconsistent, sometimes opposite, outcomes. Indeed, administration of cannabinoid CB1 receptor agonists such as THC, WIN-55212,2 or CP55940 have produced mixed results [156–158]. For example, it has been shown that THC, like other drugs of abuse, might facilitate reward mechanism(s) under the ICSS procedure [159–161]. In contrast, a lack of effect or even an increase in brain stimulation thresholds by THC and other cannabinoid agonists has also been reported [160, 162, 163]. Moreover, these effects were all reversed by pretreatments with CB1 antagonists, indicating the involvement of CB1Rs in these actions [160, 162, 163]. Thus, as with many behavioral assessments, THC shows an inverted U-shaped dose-response function for behaviors maintained by electrical brain self-stimulation, with positive and negative modulation of the stimulation threshold depending on dose and experimental conditions. To the best of our knowledge, AEA and its more metabolically stable analog meth-anandamide (methAEA) have never been tested in the ICSS procedure. However, the effects of administration of drugs able to increase the circulating brain levels of AEA have been assessed with this procedure. For example, ICSS testing of cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB597), N-3-pyridinyl-4-[[3-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenyl]methyl]-1-piperidinecarboxamide(PF-3845) and phenylmethylsulfonyl-fluoride (PMSF), which block the FAAH enzyme, thus inhibiting the degradation of AEA, and OMDM2, an inhibitor of the cellular reuptake of AEA, have been reported [164–166]. URB-597 significantly increased the ICSS electrical threshold in rats at doses

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of 1, 3, and 10 mg/kg, but not at the 0.3 mg/kg dose that has already been shown to be already very effective in increasing the levels of AEA in rats and efficacious in several behavioral tests [48, 49, 167, 168]. However, the selectivity of URB-597 effects on CB1Rs in this paradigm was confirmed by blunting the effects with administration of a low dose of rimonabant, an antagonist of CB1 receptors [165], which by itself does not affect brain stimulation thresholds [163]. PMFS increased the threshold for ICSS at all doses tested, but its effects were not blunted by antagonism at CB1 receptors, suggesting that PMSF would increase levels of endogenous compounds, other than endocannabinoids, that activate non-cannabinoid receptors to produce behavioral effects in the ICSS procedure. Only the highest doses of OMDM2 and PF-3845 (30 mg/kg) enhanced the threshold for ICSS in rats. The effects of OMDM2 were blocked by a low dose of rimonabant. Blockade of FAAH in rats would increase the endogenous levels of AEA but also the levels of other endogenous substances, such as OEA and PEA, which, as mentioned before, are PPARα agonists. This effect would raise a question about the effects obtained in the ICSS after FAAH blockade: are those effects the result of a cooperative/synergistic action of AEA/OEA/PEA on CB1 and/or PPARα and/or TRPV1? Thus, the increased ICSS threshold observed in these published reports indicates a possible anhedonic effect of AEA due to its increased levels after FAAH blockade. This would be in agreement with other reports showing, for example, that AEA elicits place aversion in rats [169]. However, blockade of FAAH by URB597 has been shown to elicit anti-anxiety effects in rats [170–175]. Furthermore, to complicate this picture, AEA has been shown to be intravenously self-administered in squirrel monkeys, suggesting that under some circumstances it works as a behavioral reinforcer. Clearly, species differences might be a factor in the effects of cannabinoids, although more studies would be required to better understand the neurobiology underlying the effects of AEA in brain stimulation reward procedures in rodents.

Place conditioning. Conditioned place preference (CPP) is an experimental protocol largely used for studying drug reward in animal models [176, 177]. In the context of reward and addiction, CPP measures the motivational effects of drug and non-drug stimuli in animals, typically mice or rats, as subjects [178]. In such experiments, conditioning sessions start with administering a drug or its vehicle to animals in one of two distinct, contiguous environments at separate times. Then, after one or more conditioning sessions, animals are allowed to freely explore the two environments. Depending on the amount of time the animal spends in the drug-conditioned versus vehicle-conditioned environment, it is possible to infer the animals' preference for the drug or vehicle stimulus [179]. This paradigm is valid for aversive stimuli as well, in which use it is known as conditioned place aversion (CPA). It is well established that virtually all drugs abused by humans increase, at specific doses, the time spent by the animals in the drug-conditioned environment, thus producing robust CPP. In contrast, as in the test reported for the ICSS procedures, administration of cannabinoids agonists such as THC or WIN55,212-2 did not produce consistent results in place-conditioning procedures: both CPP and CPA have been reported in different studies (see, for review: [156, 157]). For example, dose- and injection-time-dependent effects of THC on place-conditioning procedures have been reported in rats and mice [180–183], providing evidence for both CPP and CPA, thus also suggesting the biphasic effects of this drug. However, other research groups have reported only CPA induced by administration of CB1R agonists [184–186]. Although AEA has been scarcely investigated in the CPP paradigm, interesting insights have been provided in the literature. Mallet and Beringer found neither CPP nor CPA for AEA in rats when the endogenous agonist was delivered intraperitoneally (i.p.) across a large dose range

(0.031–16 mg/kg) [187]. It should be noted that the latter authors administered AEA in combination with the protease inhibitor PMSF, capable of blocking AEA metabolism and thus prolonging its half-life. In the same report, different results were obtained with a non-endogenous cannabinoid, THC, which produced significant CPA at doses of 1.0 and 1.5 mg/kg. In our recent study, we reported that intravenously administered AEA (0.03–3 mg/kg), by itself, produced neither CPP nor CPA [169]. However, in contrast to Mallet and Beringer, we found that AEA produced dose-related CPA when rats were pretreated with URB597 [169], which, similar to PMSF in the experiments by Mallet and Beringer, prolonged the half-life of AEA by blocking its FAAH-driven enzymatic metabolism. The aversive effects of AEA during FAAH inhibition appeared to be mediated mainly by CB1Rs, since pretreatment with the CB1R antagonist AM251 blocked the development of CPA. Consistent with previous findings by Gobbi et al.[49], we also found that URB597 by itself, at a dose (0.3 mg/kg) that almost completely inhibits FAAH activity, produced neither CPP nor CPA. Finally, similarly to what reported by Mallet and Beringer, we observed that WIN 55,212-2 (50–300 μg/kg, intravenous) produced dose-dependent CPA [169].

Unlike URB597, the AEA transport inhibitor AM404, at the dose of 2.5 mg/kg given i.p., has been reported to produce CPP in rats, but only when they are housed under enriched conditions [188], suggesting that environmental factors might play a pivotal role. In contrast, we found that when administered at a higher dose of 10 mg/kg, AM404 induced significant CPP in rats housed under standard conditions [189].

Drug self-administration. As mentioned in the previous sections, AEA acts as a reinforcer in squirrel monkeys, and it is one of the few endogenous substances that maintain self-administration behavior in animal models. While a few CB1 receptor agonists are self-administered in rats and mice, for example, WIN55-212,2, JWH-018 and 2-AG [156], other CBR agonists, such as THC, do not act as behavioral reinforcers in rats when administered systemically. However, it should be mentioned that rats learned to lever-press to receive local THC injections into brain areas known to play important roles in drug abuse and addiction, such as the VTA and the shell of the nucleus accumbens [182, 190]. Intra-VTA injection of THC was effective only in the posterior region and not in the anterior region of the VTA. Intra-accumbens delivery of THC maintained self-administration behavior only when injections were directed to the shell rather than the core subdivision of this nucleus [190]. Additionally, THC-maintained behavior was dependent on CB1 receptor activation, as demonstrated by CB1 antagonist pretreatments [182, 190]. However, even recent attempts to obtain intravenous AEA or methAEA self-administration behavior in rats in our laboratories have been unsuccessful, as with intravenous self-administration of THC [156]. It seems clear that species differences play an important role in self-administration behavior, which is maintained by THC and AEA in squirrel monkeys but not in other monkeys or in rodents (see for review: [156, 158]). Thus, specific differences in reward-related circuitry in the brains of different species might play a larger role in the rewarding effects of cannabinoids than of other abused drugs. Indeed, cannabinoid receptors are highly expressed throughout the brain in all species, but differences in their specific expression in selected areas/pathways could be among the reasons for such differences in response. AEA has been successfully demonstrated to act as a behavioral reinforcer in drug-naïve squirrel monkeys [191]. Rates of responding for AEA and its analog methAEA were comparable to those obtained under similar conditions for THC or cocaine. However, when compared to THC (highest rate of responding obtained at 4 μg/kg), it appeared that significantly increased doses of intravenous AEA or methAEA (for both of these drugs, the highest rate of responding was obtained at 40 μg/kg) were necessary to maintain

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a similar pattern of behavior. While the higher dose of AEA might reflect its short half-life because of its metabolic instability, methAEA is a metabolically stable analog of anandamide; thus, its circulating levels will not be reduced quickly by metabolism. As discussed by Justinova and colleagues [191], it is likely that potential pharmacokinetic differences (for example, hepatic first-pass metabolism) are reduced by administering drugs intravenously. It is also important to note that AEA and methAEA self-administration behavior was blunted by rimonabant administered before the session, which also blocked THC-maintained self-administration [192]. Thus, the efficacy of plant-derived (THC), endogenous (AEA) and synthetic (methAEA) cannabinoid drugs as behavioral reinforcers in the squirrel monkey is mediated by activation of CB1 receptors. In a different series of experiments, reinstatement of extinguished anandamide self-administration behavior was not obtained by pretreatment doses of URB597 fully capable of blocking the enzyme FAAH. Indeed, after URB597 administration, it was found that anandamide levels were higher and 2-AG levels were lower than their basal levels. Additionally, URB597 alone did not maintain self-administration behavior in squirrel monkeys, but it potentiated the effects of anandamide when given in combination. On the other hand, in contrast to URB597, the anandamide transporter inhibitor AM404 was shown to work as a behavioral reinforcer in squirrel monkeys [193]. In the same series of experiments, AM404 pretreatment potentiated anandamide- but not cocaine-maintained self-administration behavior, suggesting that activation of cannabinoid signaling is not involved in the self-administration of cocaine. In the same report, anandamide and AM404 pretreatments could reinstate extinguished self-administration behavior previously maintained by intravenous injections of anandamide, THC, or cocaine. This might not be surprising, since the CB1R antagonist/inverse agonist rimonabant blocked reinstatement of cocaine-seeking behavior in rats induced by cocaine itself or by cocaine-associated cues. Rimonabant also blocked the effects of AM404 on relapse to both anandamide- and cocaine-seeking behavior. Thus, while cannabinoid receptor activation might not be a factor influencing cocaine-reinforced versus AEA-reinforced self-administration behavior, CB1R seems to be involved in the reinstatement of seeking behavior for both drugs. A strong suggestion about the potential involvement of increased anandamide levels in these behaviors elicited by AM404 came from the potentiation of its effects by pretreatment with URB597 before self-administration sessions maintained by AM404, similar to what occurs when self-administration behavior is maintained by AEA.

Reinstatement of drug-seeking behavior. Relapse is defined as a setback that occurs during the process of behavioral change, such that progress toward the initiation or maintenance of a behavioral change goal (e.g., abstinence from drug use) is interrupted by a reversion to the target behavior [194]. The reinstatement model is currently the foremost method used in research to investigate the mechanisms underlying relapse to drug seeking. Reinstatement is typically studied using animals with a history of self-administration. Subsequent to the self-administration procedure and different periods of drug withdrawal, extinction training and tests for reinstatement are conducted [195]. The test for reinstatement for drug-seeking behavior can be achieved with a priming injection of a compound (drug-induced reinstatement) or with exposure to other experimental manipulations, such as a cue or context previously associated with the self-administration of the drug (cue- or context-induced reinstatement), or a stressor (stress-induced reinstatement) [196]. Using the discontinued delivery of THC and/or THC-associated cues as reinstatement model of relapse, interesting insights have been recently provided about the effects of AEA on cannabinoid reinstatement. For example, Justinova and colleagues [197] found that priming administrations of AEA

or methAEA reinstate THC seeking [197].

Cannabinoids can produce reinstatement of drug-seeking behaviors for agonists of CB1Rs [198] and for other drugs of abuse [22, 198–201]. For instance, in the study by Spano and colleagues [198], rats previously trained to intravenously self-administer the CB1R agonist WIN 55,212–2 were found to be susceptible to intraperitoneal priming injections of the same drug, which reinstated cannabinoid-seeking behavior following extinction [198]. Furthermore, interesting insights have been provided recently about the effects of AEA in cannabinoid-reinstatement models of relapse using the discontinued delivery of THC and/or THC-associated cues. On this subject, Justinova and co-workers [197] found that priming administration of AEA (30–560 µg/kg) or its longer-acting congener methAEA (3–100 µg/kg) in monkeys produced dose-dependent reinstatement of THC seeking [197].

MODULATION OF ANANDAMIDE BRAIN LEVELS AND ITS POTENTIAL THERAPEUTIC EFFICACY ON SUBSTANCE USE DISORDERS
FAAH inhibition as a new therapeutic tool for drug use disorders
Although the use of cannabis for medicinal purposes remains controversial [202], the discovery of the endocannabinoids and the characterization of their degradative enzymes, FAAH and MAGL, has opened new horizons for cannabinoid-based pharmacological strategies for treating pain and several brain disorders. Cannabis has been used for centuries as a therapeutic drug, and some of its main components, THC and cannabidiol, are widely demonstrated to exert strong analgesic actions [203, 204]. In human subjects, repeated "global" activation of brain CB1Rs by cannabis derivatives containing THC may result in cannabis use disorders. AEA, similar to THC, is a partial agonist of CB1Rs. As such, its systemic administration could mimic several behavioral actions of typical agonists of CB1Rs. Among these actions, AEA may elicit potential reinforcing effects, likely due to its "global" activation of brain systems, as demonstrated by Justinova and colleagues [191]. One of the more convenient ways to elicit the activation of brain CB1Rs in specific areas without the "global" activation could be obtained by administration of AEA level modulators, such as FAAH inhibitors or putative blockers of AEA neuronal membrane uptake.

Taking into account the aforementioned considerations about the crucial importance of FAAH inhibition in developing novel pharmacological solutions, we will now review the role of AEA in the context of reward and drug use disorders and the potential use of AEA level enhancers as potential therapeutic strategies against substance use disorders.

Nicotine use disorders
CB1R activation increases the rewarding effects of nicotine [199], while decreasing CB1R function has the opposite effect [205]. Accordingly, Merritt and her group found that FAAH knockout or inhibition enhanced nicotine CPP [206]. However, several works contradict the results obtained by Merritt and colleagues. For example, the FAAH inhibitor URB597 has been found to alter the abuse-related effects of nicotine in rats. When administered at a certain dose (0.3 mg/kg, ip), which does not give rise to behavioral side effects, it is able to prevent the development of nicotine-induced CPP and the acquisition of nicotine self-administration [207]. URB597 is also able to reduce nicotine-induced reinstatement in both CPP and self-administration models of drug abuse, as well as nicotine-induced elevation of DA outflow in the accumbens shell and nicotine-induced excitation of mesolimbic dopaminergic neurons in rodents [207, 208]. Similarly, URB597 decreased the reinstatement of nicotine seeking induced either by presentation of nicotine-

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associated stimuli or by nicotine priming, although it failed to reduce the breakpoint of nicotine self-administration on a progressive-ratio schedule in rats [209]. Not only URB597 but also VDM11 was found to attenuate nicotine reinstatement induced by nicotine-associated cues and nicotine priming [210], and finally, AM404 was reported to prevent nicotine CPP as well as the reinstatement of nicotine CPP [189]. It is well established that chronic administration of nicotine in rodents can result in a state of "physical dependence" that can be unmasked and characterized by abruptly interrupting the administration of nicotine or by administering a nicotine receptor antagonist such as mecamylamine, resulting in the occurrence of a nicotine withdrawal syndrome consisting of somatic signs as well as aversive motivational and affective states [167]. AEA has also been implicated in alleviating the somatic and motivational effects of nicotine withdrawal.

Indeed, pharmacological inhibition of FAAH mediated by URB597 (0.1 and 0.3 mg/kg, i.p.) has been found to reduce the aversive effects of nicotine-withdrawal-induced anxiety as demonstrated in the elevated plus maze test and in the shock-probe defensive burying paradigm, suggesting that an increase in the endocannabinoid signal could be useful to treat the negative affective state produced by nicotine withdrawal [167]. In a recent report, blockade of the enzyme FAAH by administration of URB597 or URB694 reduced the reinforcing actions of nicotine in squirrel monkeys [211]. The FAAH blockers produced a rightward shift in the dose-response curve of nicotine self-administration under fixed-ratio schedules, suggesting an attenuation of nicotine-induced reward. Additionally, FAAH blockade counter-acted the reinstatement of nicotine-seeking behaviors elicited by priming administration of nicotine or by cues previously associated with nicotine self-administration. These effects of FAAH on nicotine were attenuated but not completely blocked by an antagonist of PPARα, suggesting the involvement of this receptor in the actions of FAAH [211]. Indeed, blockade of FAAH increases the endogenous levels of AEA, OEA, and PEA, which are endogenous ligands for PPARα. In a recent study, blockade of the enzyme MAGL by JZL184 treatment, which increases brain levels of 2-AG, potentiated the reinstatement of nicotine-seeking behavior induced in mice by extinguished nicotine-associated cues, without alteration of behaviors maintained by fixed-ratio or progressive-ratio schedules of nicotine self-administration [212]. These effects are likely due to activation of CB1Rs, since 2-AG is a full agonist of those receptors. AEA is a partial agonist of CB1Rs. Thus, differences in the activity of increased levels of AEA and 2-AG could result from their differential activation of CB1Rs, where changes in tonic levels of AEA and/or 2AG might shift the activation and thus the functionality of CB1R. Taken together, these studies of FAAH blockade under the reported experimental conditions in rodents and squirrel monkeys provide a significant contribution to the search for potential pharmacological therapies for nicotine use disorders.

Alcohol use disorders

The relation between FAAH inhibition and drug dependence is not exclusive to nicotine abuse but is also a matter of discussion relevant to a wider variety of abuse behaviors. For example, Cippitelli and co-workers found that AM404 reduced alcohol self-administration in rats [213]. In contrast, AM404 did not potentiate alcohol-induced hypothermia or hypolocomotion and did not affect cue-induced reinstatement of alcohol seeking [214]. However, the same authors reported that administration of URB597 failed to decrease the risk of alcohol abuse in voluntary home-cage drinking and self-administration models [214]. In contrast, Blednov et al. [215] reported that FAAH knockout mice consumed more alcohol than wild-type (WT) littermates in self-administration studies. Additionally, treatment with URB597 increased alcohol intake in WT mice [215]. Similarly, Hansson

and colleagues showed that intra-PFC administration of URB597 increased operant alcohol self-administration in non-selected Wistar rats [216]. More recently, Stopponi et al. [175] investigated the blockade of FAAH by URB597 in the central (CeA) and basolateral (BLA) amygdala in Marchigian Sardinian alcohol-preferring (msP) and control rats. They reported, partially in contrast to Hansson and co-workers, that intra-CeA URB597 as well as intra-BLA URB597 (in a less pronounced manner) leads to a reduction of the anxiogenic effects of restraint stress and alcohol self-administration in msP but not in control rats [175]. Recently, URB597 effects were tested in a voluntary alcohol drinking model in mice.[217] Dependence-induced anxiety is a significant risk factor for relapse to alcohol use during withdrawal. As already shown for nicotine, URB597 prevents the anxiogenic response measured during withdrawal after acute administration of alcohol [217]. Moreover, pretreatment with URB597 reduced alcohol intake and preference after acute withdrawal, and such reduction was reversed by the CB1R antagonist AM251. URB597 prevented relapse to alcohol drinking after 1-week withdrawal when administered in single or multiple doses. Furthermore, AEA levels increased in several brain regions, including the accumbens and basolateral amygdala, as a result of acute (1-day) alcohol withdrawal. Based on the authors' interpretation, the increased levels of AEA may provide a kind of protection from stress during alcohol withdrawal [217]. Together, these results demonstrate that activation of AEA signaling by selective inhibition of FAAH reduces anxiety associated with alcohol withdrawal. On this issue, a study on a human single-nucleotide polymorphism of the FAAH gene that would decrease the enzymatic rate of FAAH [218] has been recently reported. This polymorphism, when transferred into genetically modified knockin mice, increased the endocannabinoid tone, leading to increased alcohol intake and preference compared to control WT mice. These experiments suggest that this FAAH gene polymorphism might be involved in alcohol binge drinking. Additionally, an altered endocannabinoid tone at CB1Rs, caused by increased levels of AEA, could be mediating those effects, which were indeed blocked by the CB1R antagonist AM251 [218].

From the aforementioned considerations, it is clear (in rodents, at least) that the upregulation of CB1 transmission gives rise to an increase in alcohol drinking, whereas downregulation of CB1 transmission causes a decrease in this behavior [219]. Nevertheless, this assessment is more complicated in humans and has yet to be ascertained. For example, while rimonabant and other CB1 antagonists suppress rodent alcohol consumption, no significant effect of rimonabant was reported in clinical trials [220], and there were no effects of rimonabant on alcohol self-administration [221]. Recently, a clinical study of the association between FAAH functional genetic polymorphisms (FAAH Pro129Thr, rs324420) and alcohol dependence and severity was conducted in European-American and African-American subjects [222]. Results show that, in European-American but not in African-American subjects, there was a higher frequency of the Thr129 allele in the alcohol-dependent group than in the non-dependent controls. Thus, although the clinical trials cited above did not report significant interactions between CB1 antagonism and alcohol consumption, there is a possibility that alterations in endocannabinoid levels might play a role in alcohol use disorders and in their pharmacological treatment.

Opioid use disorders

In a series of experiments by Solinas et al. [223], the CB1R agonists THC and WIN55,212-2 did not modify the fixed-ratio 1 self-administration of heroin but significantly increased the breakpoint for heroin injections at all doses tested. In contrast, blockade of FAAH or AEA transport by URB597 and AM404, respectively, or their combination did not alter the efficacy of heroin as a reinforcer. Thus, it seems that an interaction between CB1Rs and

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opioid receptors facilitates the reinforcing action of heroin, while the lack of effects of AM404 and URB597 might be explained by lack of effect of heroin on the release of AEA [223]. On the other hand, endocannabinoid catabolic enzyme inhibitors have been shown to reduce abrupt withdrawal in morphine-dependent mice, as reported by Ramesh et al. [224]. They used the FAAH inhibitor PF-3845, the MAGL inhibitor JZL184 (at a low dose and in combination with PF-3845), and the dual FAAH-MAGL inhibitor SA-57. The combination of JZL184 and PF-3845, as well as SA-57 by itself, increased brain AEA levels (SA-57 more than the others) compared to those of controls and reduced all of the examined opioid withdrawal symptoms (i.e., platform jumping, paw flutters, head shakes, diarrhea, and total body weight loss) [224]. These results suggest a very interesting interaction between the cannabinoid and opioid systems, which might be important at the translational level. Thus, endocannabinoid level enhancers could open a new way to potential pharmacotherapies for alleviating withdrawal symptoms. These symptoms related to pain and stress are likely among the main reasons that prevent individuals from starting on the road to recovery from addiction. Attenuation of spontaneous opiate withdrawal has also been reported after administration of AM404 [225], and the direct action of AEA administration has been shown to attenuate naloxone-precipitated withdrawal in mice chronically treated with morphine [226]. However, URB597 was reported ineffective in modifying morphine-induced reinstatement of conditioned floor preference and naloxone-precipitated withdrawal-induced conditioned floor avoidance [227]. In a more recent study, SA57 dose-dependently reduced heroin drug-seeking behavior in mice.[228], Additionally, when mice were assessed in the progressive-ratio self-administration procedure, SA57-treated mice showed significantly lower heroin breakpoints than control mice. Since the breakpoint in a progressive-ratio schedule of self-administration could be a measure of the willingness of the animals to self-administer a certain dose of a drug, this experiment suggests that mice treated with SA57 had a decreased level of motivation to self-inject heroin. However, SA57 failed to significantly modify heroin-induced reinstatement of drug-seeking in mice under behavioral extinction [228].

Psychostimulant use disorders
In contrast to other drugs of abuse, psychostimulants produce direct effects on the mesolimbic dopaminergic neurons by acting on the reuptake of monoamines, thus enhancing the overall activity of DA neurons [229, 230]. The endocannabinoid system is implicated in different mechanisms and relative behavioral outcomes, such as rewarding, motivational and seeking-related effects of diverse psychostimulants. In fact, it has been shown by various authors that some of the effects of both acute and chronic administration of psychostimulants are absent in CB1 knockout mice [21].

Other interesting insights come from studies of FAAH inhibition and behavioral sensitization to cocaine, one of the most abused psychostimulants worldwide. Cocaine sensitization has been described as one of the steps occurring during the transition from simple taking of the drug to abuse and dependence [231–233]. Several different methodologies have been tested to induce cocaine behavioral sensitization [234–239]. A role of endocannabinoids in the neuroplasticity induced by repeated injections of cocaine has been shown [23, 234, 235]. However, the role of cannabinoids in cocaine sensitization is still debated [240], as is the evidence that cocaine administration induces the release of endocannabinoids [149, 151–153, 241, 242]. In a recent study, a single injection of cocaine was shown to induce behavioral sensitization that was related to increased stimulation of extracellular DA levels in the core, but not in the shell, of the accumbens in mice [23]. These effects were significantly reversed by pretreatment with the CB1R antagonist rimonabant, suggesting

the possibility that an increase in endocannabinoid tone could be involved in the neuroadaptation induced by cocaine. Indeed, a dose of cocaine lower than the dose able to induce behavioral sensitization had no effect unless the animals were pretreated with the FAAH inhibitor URB597. Thus, blockade of anandamide metabolism was likely magnifying the cocaine-induced release of AEA, suggesting its involvement in the neuroadaptation that occurs during the early stages of cocaine use [23]. The involvement of the endocannabinoid system in cocaine use disorders has also been shown in studies where URB597 reduced cocaine-induced seizures and cocaine-induced activation and death of hippocampal neurons, both in primary cell culture and in mice [243]. Furthermore, Luchicchi and colleagues found that URB597 blocked the inhibitory effects of nicotine and cocaine in medium spiny neurons of the accumbens shell in rats by activating both CB1Rs and PPARα or only PPARα, respectively. Additionally, URB597 did not alter the effects of either cocaine or morphine on VTA DA neurons [244]. Finally, in the monkey brain, it has been found that pretreatment with URB597 did not modify self-administration of cocaine but significantly potentiated the behavior maintained by AEA [245]. In the same experiments, it was also shown that URB597 enhanced AEA levels while causing a compensatory downregulation in 2-AG levels [245]. The effects of FAAH inhibitors on cocaine self-administration and reinstatement have also been tested in rodents [246]. Intravenous cocaine self-administration, 0.5 mg/kg/inj, fixed ratio 5 in rats was not altered by pretreatments with the AEA metabolism blockers PMSF and URB597 at any dose tested. Both drugs attenuated cocaine- and cue-induced reinstatement of cocaine-seeking behavior, but URB597 did so without behavioral disruptions. Based on the authors' interpretation, enhancing endocannabinoid levels in the brain would modulate the motivational and conditioned aspects of cocaine-directed behaviors, while the consummatory behaviors are not under endocannabinoid control [246].

CONCLUSIONS
In the present manuscript, we have reviewed scientific evidence regarding how AEA works as a behavioral reinforcer under certain conditions and how it could elicit aversive behaviors or be anxiogenic under different conditions. Such opposite effects are also shown by many other drugs and very often depend on the dose of the drug under investigation. However, with cannabinoids, doses are not always the explanation for opposite outcomes. In several animal models, experimental conditions other than drug doses may have a more important impact test results for cannabinoids than for other drug classes [156]. For example, drug pre-exposure, timing between drug injections, and species differences are among the most likely candidates to explain some of the contradictory results of behavioral tests. For example, THC, similar to AEA and methAEA, is self-administered by squirrel monkeys but not by other monkeys or by rodents. Furthermore, similar to THC, AEA does not show a clear "drug of abuse" profile when tested in animal paradigms such as ICSS or conditioned place preference. To understand the neurobiology underlying these interesting discrepancies would certainly require many more years of study. On the other hand, AEA and drugs that modulate its circulating brain levels have been shown not only to be involved in mediating the effects of several drugs of abuse but also, in many circumstances, to create potential positive interference with the dependence-producing actions of several drugs of abuse. Indeed, blockers of AEA metabolism have been shown to efficiently counteract some of the behavioral/reinforcing effects of nicotine, alcohol, opiates, and psychostimulants, suggesting potential therapeutic activity against substance use disorders. For example, the effects of cannabinoids on nicotine response were studied initially by

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trying to block CB1Rs with antagonists such rimonabant. However, full antagonism of CB1Rs could severely compromise brain physiological functions, and indeed, significant psychiatric side effects of rimonabant use have led the European Medicines Agency to suspend the marketing of this drug [247]. In contrast to the potential therapeutic effects of rimonabant mediated by CB1R antagonism [248], FAAH blockade seems to exert its own potential therapeutic effects on nicotine use disorders by merely increasing the levels of AEA, which is a partial agonist of CB1Rs. This interesting discrepancy could be the result of inverse-agonist effects of rimonabant, in addition to its antagonist effects, or it could result from partial activation of CB1 receptors or from their activation exclusively in selected brain areas. Indeed, after FAAH blockade, AEA levels are enhanced only in those brain areas where AEA is released. Another explanation might come from potential interactions between CB1Rs and/or CB2Rs and PPARα, all of which are pharmacological targets of AEA. Moreover, OEA and PEA, which are substrates of the enzyme FAAH and whose circulating brain levels increase after FAAH blockade, might be a potential factor in the therapeutic actions of cannabinoid-level enhancers. Potential therapeutic intervention based on modulation of endocannabinoid levels has also been suggested for pain [42] and for several brain disorders [16, 249] other than substance use disorders, for example, depression and anxiety. To this end, cannabinoid drugs that are currently under investigation in clinical trials for psychiatric disorders might open new avenues for treatments related to substance use disorders [219, 249]. Translational preclinical research in the endocannabinoid field has greatly accelerated in recent years. We believe that the same acceleration should be facilitated for clinical studies aimed at the development of potential pharmacotherapies for substance use disorders based on alteration of brain endocannabinoids.

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All authors have contributed to the writing of the present manuscript.

ADDITIONAL INFORMATION
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