Anandamide and 2-arachidonoylglycerol: Pharmacological Properties, Functional Features, and Emerging Specificities of the Two Major Endocannabinoids

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Abstract Since the discovery of endocannabinoids and their receptors, two major members of the endocannabinoid family, anandamide (AEA) and 2-arachidonoylglycerol (2-AG), have been regarded almost as twin brothers. Pharmacological properties were initially considered to be similar, as these molecules were believed mutually exchangeable and almost indistinguishable in the regulation of synaptic functions, such as long- and short-term synaptic plasticity, and in behavioral aspects, such as learning and memory, reward and addiction, antinociception, and anxiety. In recent years, however, endocannabinoid signaling specificity began to emerge, in particular, due to the production of genetically engineered mice lacking key enzymes in endocannabinoid synthesis or degradation, together with the development of selective inhibitors of AEA or 2-AG catabolic enzymes. Evidence now suggests that AEA and 2-AG possess specific pharmacological properties, are engaged in different forms of synaptic plasticity, and take part in different behavioral functions. In this review, we provide an overview on similarities and specificities of the two endocannabinoids in the CNS and on the unresolved questions concerning their role in synaptic signaling.

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Introduction

Among recent advances in pharmacology, the isolation of arachidonoylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), which followed cloning and functional expression of the first type of cannabinoid receptors (CB1-R) [1], have provided a starting point to uncover the structure, chemistry, and functionality of the endocannabinoid (eCb) system. This system comprises a family of lipid molecules, receptors, and enzymes which role in the physiology and pathophysiology in the central and peripheral nervous systems has been spotlighted over the past 20 years.

As a consequence, the discovery of lipid mediators like AEA in 1992 [2] and 2-AG [3, 4] in 1995 has been the foremost evidence that the nervous system possessed its own endocannabinoid molecules, resembling in terms of the structure the main active principle of *Cannabis* plants, Δ^9-tetrahydrocannabinol (THC) [5, 6]. AEA and 2-AG are lipid molecules derived from arachidonic acid (AA), which are conjugated with ethanolamide and glycerol, respectively (Fig. 1). Although similar in structure and general function, these eCbs exhibit large differences in terms of biochemical steps, receptor affinity, and breakdown pathways. AEA can be generated via a specific N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD)-dependent mechanism [7], whereas 2-AG is mainly synthesized by the combined action of a phospholipase C and two isoforms (α and β) of diacylglycerol-lipase (DAGL) [8, 9], although different alternative pathways are involved (Fig. 1) (see [10] for a recent excellent review). In addition, although AEA and 2-AG both bind to CB1-R and CB2-R, they have different affinities [3, 11, 12] and it is now clear that some
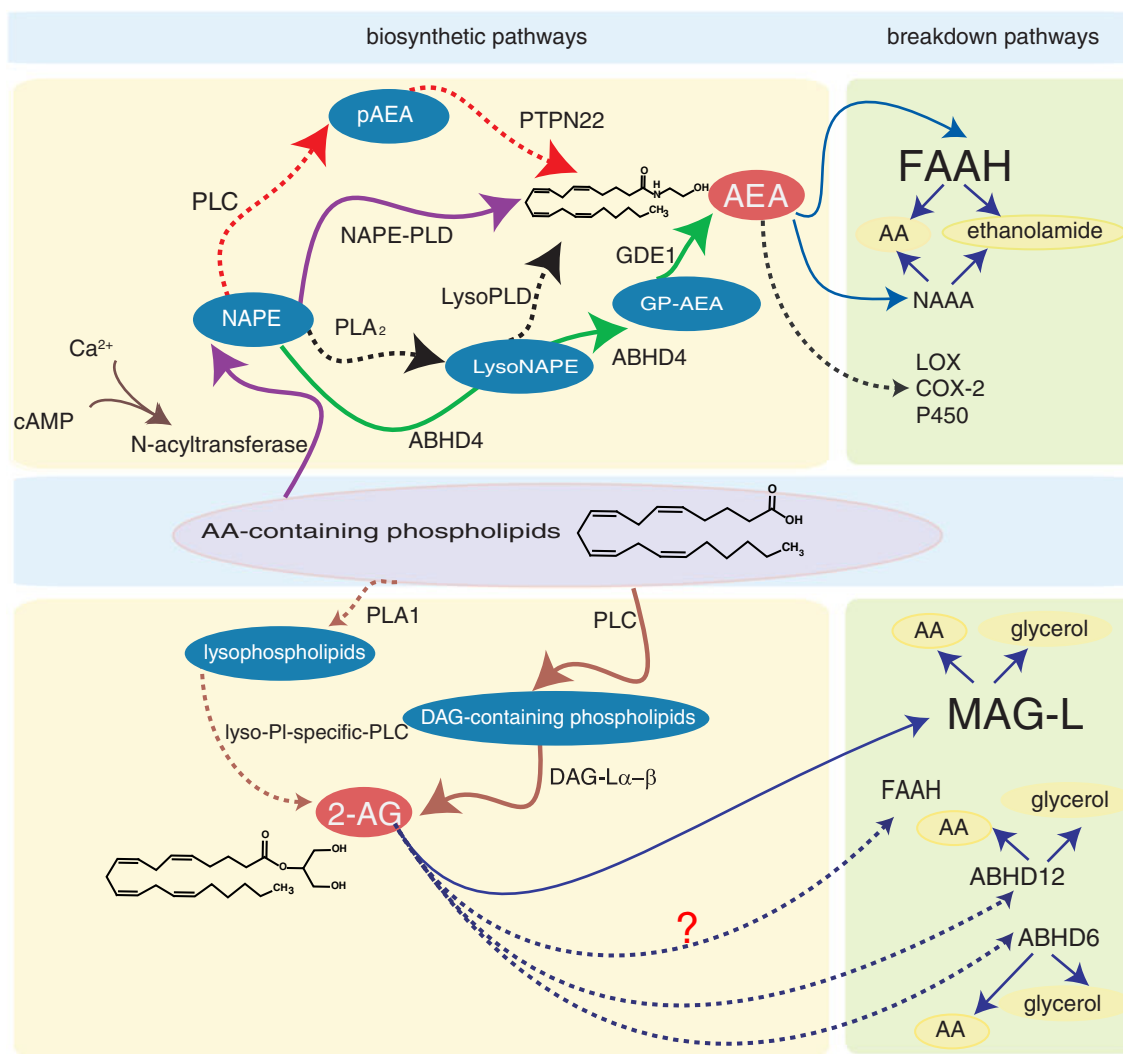
Fig. 1 Graphical depiction showing the main pathways involved in AEA and 2-AG formation and catabolism. Although both AEA and 2-AG are derivatives of AA-containing phospholipids, their production requires different enzymes and precursors. In fact, AEA is mainly synthesized by the activity of *N*-acyltransferase, which converts phospholipids into NAPE, and by the recruitment of several different enzymes. In fact, although the principal route of AEA biosynthesis involves a conversion form NAPE to AEA by a specific NAPE-PLD, recent studies have reported the existence of at least three other alternative pathways for AEA synthesis. In particular, it has been shown that: (**a**) NAPE can be converted into pAEA and, by PTPN22, into AEA; (**b**) a specific PLA2 is involved in lysoNAPE synthesis, which is then converted into AEA by a lysoPLD; and (**c**) ABHD4 hydrolyzes NAPE in lysoNAPE and the same enzyme yields GP-AEA. Ultimately by the activity of GDE1, GP-AEA is converted into AEA (*top left panel*). In line with multiple pathways for AEA production, 2-AG biosynthesis involves different routes, requiring the activity of either PLC or α and β isoforms of DAGL or PLA1 and PLC (*bottom left*

panel). The main pathways involved in AEA and 2-AG breakdown also differs, requiring the activity of FAAH (*top right panel*) and MAG-L (*bottom right panel*), respectively. Furthermore, AEA and 2-AG catabolism might occur by the activity of other enzymes (e.g., NAAA, COX-2, and several LOX isoenzymes). Among the catabolic enzymes involved in 2-AG degradation, recent studies have reported a role of two hydrolases named ABHD6 and ABHD12, which activity dissociate 2-AG in AA and glycerol. *AA* arachidonic acid, *ABHD4 6, 12*, alpha, beta hydrolase 4, 6 and 12, *COX-2* cyclooxygenase-2, *DAGL alpha and beta* diacylglycerol lipase alpha and beta, *FAAH* fatty acid amide hydrolase, *GDE1* glycerolphosphodiesterase 1, *GP-AEA* glycerol-phosphoanandamide, *LOX* lipoxygenase, *LysoNAPE* lyso N-acyl phosphatidylethanolamine, *LysoPLD* lyso phospholipase D, *MAG-L* monoacylglycerol lipase, *NAAA* N-acylethanolamine-hydrolyzing acid amidase, *NAPE* N-arachidonoylphosphatidylethanolamine, *NAPE-PLD* N-acyl phosphatidylethanolamine phospholipase D, *pAEA* phosphoanandamide, *PLA1* phospholipase A1, *PLC* phospholipase C, *PTPN22* protein tyrosine phosphatase N22

AEA actions are also driven by receptor activation other than CB1 or CB2 [13]. Finally, it has been demonstrated that baseline levels of AEA and 2-AG are regulated by two main families of hydrolyzing enzymes, the fatty acid amide

hydrolase (FAAH) and a monoacylglycerol lipase (MAG-L), respectively [14] (Fig. 1). As in their synthesis, the enzymatic machinery involved in AEA and 2-AG inactivation is far more complex, as summarized in Fig. 1. Besides

FAAH, AEA can be hydrolyzed by N-acylethanolamine-hydrolyzing acid amidase (NAAA) [15] and oxygenated by cyclooxygenase-2 (COX-2) [16], lipooxigenase isoenzymes (LOX) [17], and by the P-450 cytochrome [18]. In addition to MAG-L, 2-AG can be hydrolyzed by a series of serine hydrolase α-β-hydrolase domain 6 or 12 (ABHD6, ABHD12) [19, 20].

Although early studies have regarded AEA and 2-AG as congeners with mutually exchangeable properties in the modulation of synaptic signaling, this notion has been overcome by recent discoveries about their sophisticated specificity in the regulation of several brain functions. To clarify this differential aspect, a pivotal role has been played by both the development of mouse lines lacking genes encoding for either FAAH or MAG-L [21, 22] and drugs that inhibit AEA and 2-AG catabolic enzymes (e.g., URB597 and JZL184) [23–25]. By either genetic or pharmacological FAAH and MAG-L inhibition, we have clarified the presumed interchangeable roles of AEA and 2-AG in synaptic plasticity, learning, memory, and reward, and interesting cues have been provided to drive new therapeutic applications for specific brain disorders. For this reason, in this review we present evidence that specific eCb molecules, through diverse biochemical pathways and mechanisms, may differentially modulate or coregulate physiological or pathological brain functioning.

AEA and 2-AG in synaptic plasticity: toward and beyond 2-AG

Synaptic plasticity modulation by CB1-R activation and the upstream involvement of eCbs in this process have attracted the interest of several research groups. Two decades of observations in this field have provided evidence that AEA and 2-AG are not similar in their functioning and that they perform highly specific roles in synaptic remodeling regulation [26].

eCbs have been regarded as main characters in synaptic plasticity since their discovery. Pioneer studies carried out by different groups have strongly supported this notion. In neurons, the intracellular cascade triggered by cannabinoid agonist binding to CB1-Rs depresses neurotransmitter release by activating presynaptic K^+ channels [27] and inhibiting $N-$ and P/Q-type Ca^{2+} channels [28–31]. A form of synaptic plasticity attributed to eCbs was the so-called "depolarization-induced suppression of inhibition" (DSI) [32, 33], which, occurring in a Ca^{2+}-dependent fashion, was first observed in the hippocampus [33]. In 2001, eCbs were discovered as DSI mediators, being mobilized as needed (on-demand) by postsynaptic cells to inhibit GABA release from presynaptic neurons [34, 35] (Fig. 2). Stimuli leading to eCb synthesis are the activation of specific metabotropic

receptors (metabotropic glutamate receptors, mGluRs; D2 dopamine, muscarinic), depolarization of the postsynaptic cell, or stimulation of excitatory afferents (see [10, 26, 36] for excellent reviews). The discovery of eCb-mediated DSI was soon followed by the description of eCb involvement in "depolarization-induced suppression of excitation" (DSE) [37] (Fig. 2) and by findings that AEA and/or 2-AG mediated long-term forms of synaptic plasticity (e.g., long-term depression, LTD, and heterosynaptic long-term potentiation, LTP) [38–41].

### Differential role of AEA and 2-AG in synaptic plasticity

Evidence suggests that 2-AG is the main eCb involved in synaptic plasticity regulation [36, 42], whereas AEA plays a minor role. Hence, studies performed with selective DAGL (i.e., tetrahydrolipstatin, THL) or MAG-L (i.e., JZL184) inhibitors and genetic ablation of either enzyme has underscored the critical 2-AG involvement in short- and long-term modulation of synaptic plasticity. Among brain areas where 2-AG exerts its properties as a neuromodulator, much research has been dedicated to the hippocampus and cerebellum. For instance, 2-AG mediates LTD at inhibitory synapses (LTDi) in the hippocampus CA1 region [38] and mGlu-dependent synaptic plasticity in hippocampal slices [43]. By using MAG-L$^{-/-}$ mice, in which 2-AG brain levels are considerably elevated due to the genetic ablation of 2-AG-degrading enzymes, Pan et al. [22] reported an augmented DSI in the hippocampus CA1 region, which was accompanied by a potentiated LTP and an increase in learning performance in knockout mice when compared to wild-type littermates. Other evidence comes from Mackie's group, who demonstrated that 2-AG application and the employment of MAG-L inhibitors modulated both DSE [44, 45] and DSI [46, 47] in autaptic hippocampal neurons. Interestingly, the latter effects do not seem to involve AEA, since the blockade of both excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) by AEA [44, 46] could not be washed out, a profile inconsistent with AEA being a mediator of short-term synaptic depression.

Also synapses into Purkinje cells (PC) in the cerebellum have been identified as a specific site of action for 2-AG. For instance, studies in rodents have uncovered that 2-AG is required in mGluR-dependent plasticity [48] and LTD in PC [39]. Furthermore, separate subsets of experiments by Safo and Regehr [39] also showed that 2-AG is involved in DSI recorded in the cerebellar cortex, suggesting that this eCb might participate in the regulation of motor coordination and cognitive efficiency. Conversely, Szabo et al. [49] provided further evidence that AEA is not engaged in short-term forms of plasticity in the cerebellum, since FAAH

Anandamide
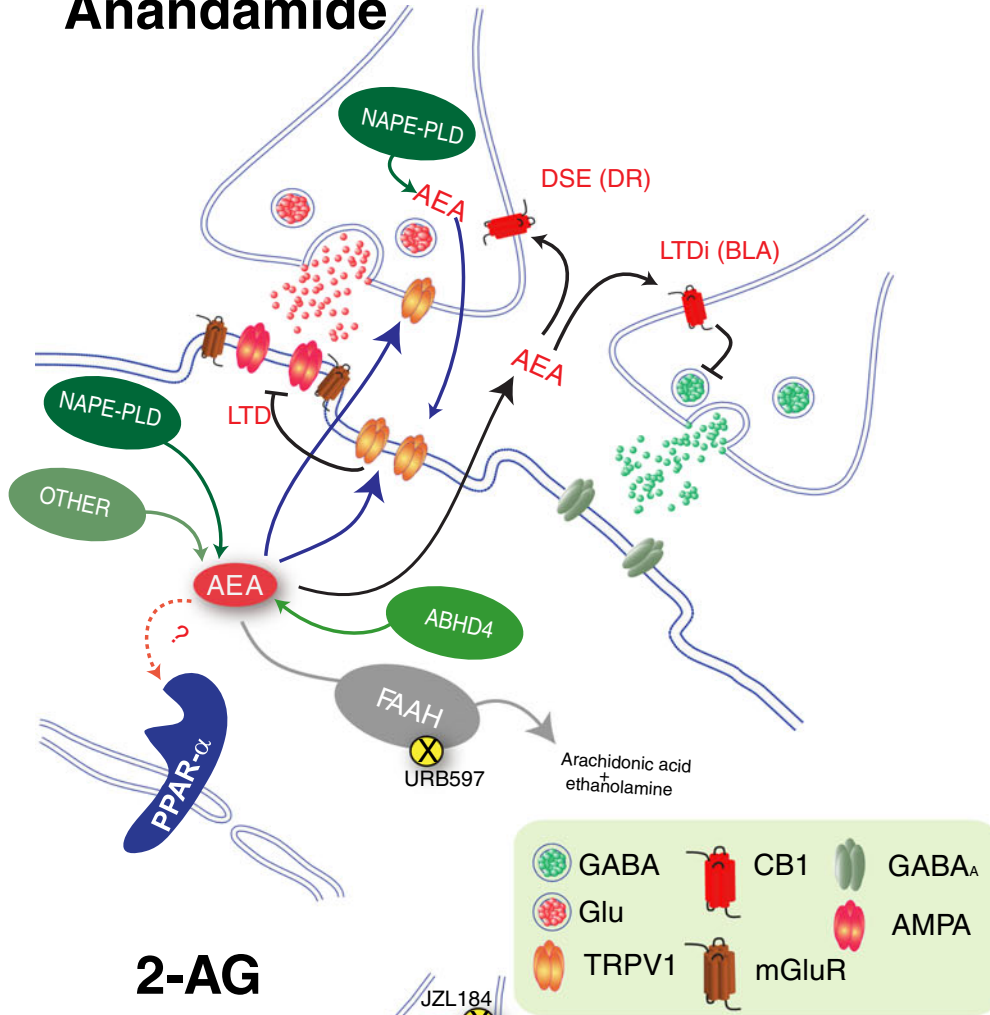
2-AG
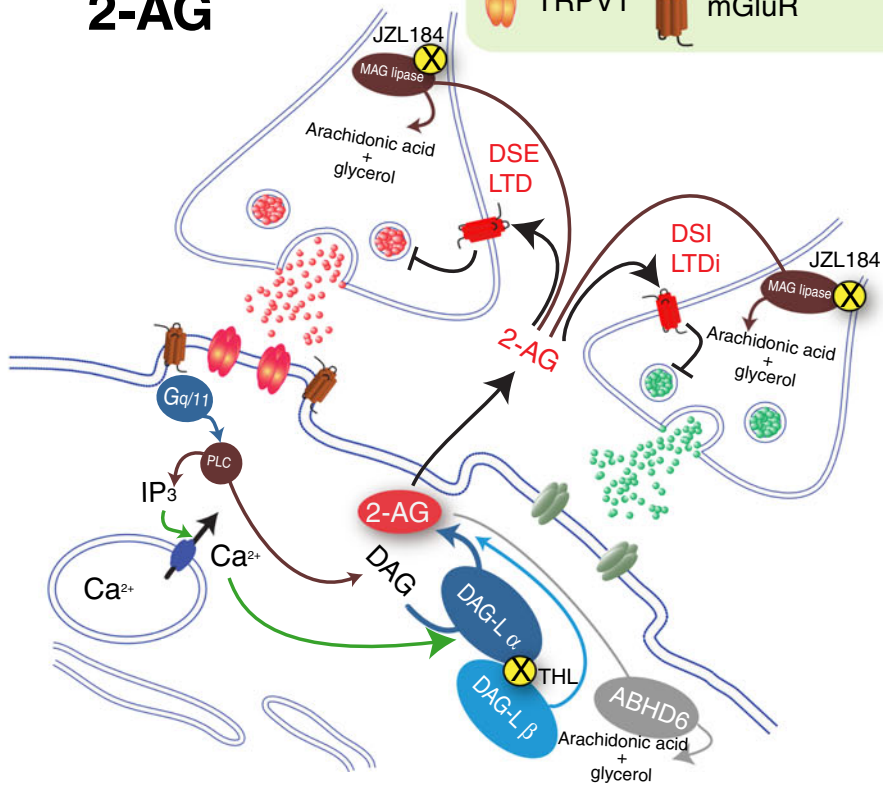
◀ **Fig. 2** Synaptic mechanisms of action for anandamide (AEA) (*top panel*) and 2-arachidonoylglycerol (2-AG) (*bottom panel*). An important part of the knowledge about AEA and 2-AG functioning derives from studies where FAAH or MAG-L were pharmacologically blocked by their selective inhibitors, such as URB597 and JZL184, respectively. *Top panel* AEA is synthesized by postsynaptic NAPE-PLD or ABDH4 (or other enzymes, see Fig. 1) and hydrolyzed by FAAH. NAPE-PLD is expressed also presynaptically and AEA might act at postsynaptic TRPV1. Beside this mechanism, it was shown that AEA might retrogradely activate presynaptic TRPV1, or in an autocrine-like fashion, postsynaptic TRPV1. In particular, postsynaptic activation of TRPV1 leads to LTD. In vitro investigations have also reported a putative binding affinity of AEA on PPARα (*dotted arrows*). AEA functions as a classical retrograde messenger at presynaptic CB1-R at specific synapses in the BLA and in the DR, where it evokes LTDi and DSE, respectively. *Bottom panel* 2-AG is synthesized mainly by DAGL α and β isoforms (inhibited by THL or other selective compounds, see [10]). Synthesis involves a rapid production of 2-AG which, in this example, is triggered by activation of mGluRs in the postsynaptic cell. mGluRs, via a $G_{q/11}$ protein, activate a phospholipase C (PLC) dependent increase in intracellular Ca^{2+} (mobilized from intracellular stores by IP_3) and DAG mobilization. This intracellular pathway leads to 2-AG-release. 2-AG activates CB1-R-expressed on axon terminals, which ultimately suppress either GABA or glutamate release and trigger short- and long-term forms of synaptic plasticity (DSI, DSE, LTDi, or LTD). 2-AG is then hydrolyzed by MAG-L expressed in the presynaptic terminals or ABDH6, expressed postsynaptically. *ABHD4* α,β-hydrolase-4, *ABHD6* serine hydrolase α-β-hydrolase-6, *BLA* basolateral amygdala, *CB1* cannabinoid type-1 receptor, *DAG* diacylglycerol, *DR* dorsal raphe, *DSE* depolarization-induced suppression of excitation, *DSI* depolarization-induced suppression of inhibition, *FAAH* fatty acid amide hydrolase, *Glu* glutamate, IP_3 inositol trisphosphate, *LTD* long-term depression at excitatory synapses, *LTDi* long-term depression at inhibitory synapses, *MAG* monoacylglycerol, *NAPE-PLD* N-acyl phosphatidylethanolamide phospholipase D, *PLC* phospholipase C, *PPARα* peroxisome proliferator-activated receptor type-α, *THL* tetrahydrolipstatin, *TRPV1* transient potential vanilloid receptor type-1

manipulation did not affect DSI on PC, indicating that 2-AG likely plays a pivotal role in these eCb-mediated functions.

Among other synaptic remodeling aspects involving 2-AG, several authors focused on cortical, striatal, and limbic circuits. Laforucade et al. [50] reported a 2-AG-mediated effect in LTD expression in V/VI layers of the prefrontal cortex (PFC), and Yoshino et al. [51] recently showed that 2-AG modulates DSI in the same region. Overall, acting on both short- and long-term forms of plasticity, 2-AG might be a suitable candidate to explain synaptic modifications underlying cognitive and motivational functions mediated by the PFC.

Electrophysiological and molecular results by our group uncovered a prominent role of 2-AG in regulation of excitatory afferents to dopamine (DA) neurons in the ventral tegmental area (VTA), a brain region primarily involved in response to rewarding stimuli [52]. Furthermore, Seif et al. [53] described that 2-AG enabled subthreshold doses of DA D1 and D2 receptor agonists to increase firing activity of nucleus accumbens (NAc) neurons [53]. Taken together, these findings point to 2-AG as a neuromodulator involved in synaptic plasticity in the mesoaccumbens pathway.

2-AG involvement in retrograde signaling modulation in the brain has been investigated by genetic ablation of specific DAGL isoforms. In fact, the DAGLα isoform plays a prominent role in 2-AG synthesis [54], whereas the β isoform has a minor involvement in this process. In line with this evidence, it has been shown that DAGLα$^{-/-}$ mice display a dramatic reduction in 2-AG levels and a complete abolishment of the main forms of eCb-mediated retrograde signaling [54, 55]. In light of these findings, 2-AG activity is sufficient to explain eCb-driven synaptic plasticity. It must be pointed out that AEA also decreases in DAGLα$^{-/-}$ mice, leaving open the possibility that this eCb could also mediate synaptic plasticity. A possible explanation of the parallel decrease in 2-AG and AEA brain levels is provided in [10]. Here the author hypothesizes that, brain 2-AG levels being ~200-fold higher than those of AEA, 2-AG catabolism is a source of free AA [56], which can be reesterified into phospholipids. Reduced AA-containing phospholipids result in reduced brain AEA levels [10]. Nonetheless, recent studies are now complicating the scenario by involving different DAGLα pools to explain different 2-AG-mediated effects in response to distinct stimuli [57] and reporting that DAGL is not recruited in hippocampal DSI [58], suggesting other synthetic pathways for 2-AG regulated forms of short-term adaptation.

Beside the broad 2-AG effect in neuronal plasticity and retrograde signaling, AEA appears as a character of secondary importance; nonetheless, it exerts apparently finer and more discrete actions at specific synapses. In this regards, it must be pointed out that AEA binds not only to CB1- and CB2-Rs, but also to vanilloid (e.g., TRPV1) [59] and nuclear receptors (e.g., PPARs) [60, 61]. In particular, since many effects of AEA are mediated via TRPV1 receptors and prevail over those mediated by CB1-Rs or CB2-Rs, this molecule is more an endovanilloid than an endocannabinoid [62]. Noteworthy, these multiple AEA receptor targets are emerging as additional modulators of synaptic functions (see below).

Studies performed by either genetic FAAH deletion or AEA administration showed that AEA in a CB1-dependent manner regulates LTDi in the basolateral amygdala (BLA) [63] and DSE evoked by glutamatergic afferent stimulation in serotonin neurons of the dorsal raphe [64]. In the former study, the possible coinvolvement of 2-AG in LTDi in the BLA was ruled out, and in the latter study, 2-AG contribution was not investigated. AEA might also modulate synaptic plasticity through activation of other receptor types, as demonstrated by Edwards et al. [65]. In fact, the authors showed that in a specific CA1 layer, the stratum radiatum, AEA triggered LTD at excitatory synapses on interneurons in a TRPV1-dependent fashion [65]. In 2010, Chavez et al. [66] and Grueter et al. [67] discovered that AEA can act in an autocrine-like fashion by activating postsynaptic TRPV1

in the hippocampus and NAc. Activated TRPV1 trigger a postsynaptically mediated LTD resulting from endocytosis of AMPA receptors. These results broaden the scenario about eCb modulation of synaptic plasticity in cognitive/subcortical areas, where the control by 2-AG had never been questioned before.

Overall, findings about AEA and 2-AG reported here support the notion that these molecules play highly specific roles in different forms of synaptic plasticity within the central nervous system (Fig. 2). Intriguingly, the possibility exists that AEA modulates synaptic transmission with mechanisms different from the classical retrograde action. This hypothesis is supported by studies showing that in hippocampal glutamatergic axon terminals, NAPE-PLD, the enzyme that synthesizes AEA, is expressed presynaptically rather than postsynaptically [68]. This localization, coupled with the absence of CB1-Rs on the same terminals and the presence of FAAH in somatodendritic domain of postsynaptic principal cells, suggests that this eCb, but possibly also other N-acylethanolamides synthesized by the same enzyme, might mediate both short- and long-term forms of synaptic plasticity through an anterograde mechanism. The targets of anterograde AEA were not identified by Nyilas at al., but postsynaptic TRPV1 receptors are likely candidates. Another mechanism, suggested by Cristino et al. [69], is that the postsynaptic NAPE-PLD localization in the cerebellum is compatible with an autocrine action of AEA and other N-acylethanolamides on synaptic plasticity via TRPV1 receptors [70].

Hence, this AEA feature might support and integrate 2-AG-mediated effects in forms of synaptic plasticity that require interactions between both eCbs. In line with this possibility, studies have shown that in the extended amygdala 2-AG and AEA modulate short- and long-term forms of synaptic plasticity through retrograde and autocrine-like mechanisms, respectively [71]. In fact, these authors showed that while 2-AG mediates short-term depression (STD) through CB1-R-dependent retrograde signaling, AEA released by a postsynaptic mGluR5-dependent mechanism modulates LTD through postsynaptic TRPV1 activation. In light of these findings, it appears that AEA and 2-AG cooperate by using different strategies to modulate synaptic plasticity in brain regions that govern highly dynamic processes [72]. Other examples of AEA and 2-AG interactions in the same form of synaptic plasticity within the same brain region have also been reported. For instance, in vitro studies by Sheinin et al. [73] demonstrated that both AEA and 2-AG are involved in STD on pyramidal neurons of the CA1, through a mechanism that requires postsynaptic mGluR5 activation leading to eCb release and a reversible decrease in IPSC amplitude form apposing GABA axons. Overall, recent literature supports the notion that AEA and 2-AG play mostly specialized roles to modulate complex

forms of synaptic remodeling, which ultimately takes part in adaptive responses to environmental requests.

Endocannabinoids in neuroprotection: from physiology to injury

Neuronal damage caused by toxic or ischemic insults, such as energy or oxygen deprivation, or traumatic injury, is worsened by excitatory amino acid release, depolarization of cell membranes, and intracellular Ca^{2+} increases. The same stimuli are able to trigger release of eCbs from a variety of neurons. eCbs might reduce potential excitotoxic damage by depressing excitation strength, and therefore, they can be envisaged as neuroprotective agents [74]. Consistently, mice lacking the CB1 receptor gene are more susceptible to injury after stroke [75] or kainic-induced epileptic seizures [76]. Accordingly, exogenous cannabinoids exert neuroprotection in a variety of in vitro and in vivo neuronal injury models [77]. When specific eCbs are concerned, a number of in vivo and in vitro studies suggest that 2-AG prevails over AEA in neuronal repair and adaptation mechanisms induced by injury.

AEA and 2-AG in neuroprotection

The overwhelming interest in 2-AG, which has dominated the debate on AEA and 2-AG involvement in neuronal repair, is supported by a number of findings on its protective role in different injury models. Studies over the last 10 years have provided evidence that 2-AG is recruited as protective neuromodulator against traumatic brain injuries [78], cerebral focal ischemia in mice [79], and β-amyloid insults [80]. Electrophysiological studies carried out in our laboratory highlighted that 2-AG is the main eCb enrolled to protect DA neurons against hypoxia/ischemia [81]. Additionally, studies on underlying 2-AG mechanisms in neuroprotection revealed that not only CB1-Rs are involved. Experimental observations from different groups suggested multiple 2-AG-activated pathways, which include CB2-Rs and other atypical receptors. This is consistent with the fact that 2-AG is a full agonist at CB2-Rs, whereas AEA is a partial agonist and might even behave as a functional antagonist during elevated tonic activation of CB2-Rs [82, 83].

Based on these studies, 2-AG might induce neuroprotection through either a neuronal CB1-R-dependent mechanism [76, 81] or CB2-R activation [84–86]. CB2-R are expressed by immune cells and microglia and upregulated in activated microglia [87], and stimulation by CB2-R ligands, such as 2-AG, increases microglial cell migration and decreases the release of several factors, such as proinflammatory cytokines [85, 86]. These molecules are cytotoxic and can secondarily activate astrocytes leading to a further induction of

the expression of inflammatory factors. These proinflammatory mediators promote influx into the CNS of immunocytes from peripheral non-neuronal sites that also express CB2-Rs [87, 88]. A recent paper by Kreutz et al. found that through the activation of an abnormal cannabidiol receptor, 2-AG was able to induce neuroprotection by modulating migration and proliferation of microglial cells after brain lesion in the hippocampal dentate gyrus [89].

Most of these studies did not take into account the possible synergy between 2-AG and AEA. Indeed, other studies revealed that AEA also mediates neuronal protection. In agreement with the latter consideration, molecular studies have reported that AEA levels vary significantly when the brain is exposed to a number of insults. For instance, early studies by Hansen et al. [90], who reported changes in eCb levels after mild to moderate brain injuries, observed augmented AEA levels, but not 2-AG. Moreover, other molecular findings extended the neuroprotective AEA effect to other brain damage models, such as the controlled in vivo blood flow interruption [91] and middle cerebral artery occlusion (MCAO) [92]. Interestingly, the latter effect might be likely due to FAAH activity reduction and increased NAPE-PLD levels [92], suggesting AEA as a protective agent in acute degeneration phases. Amantea et al. [92] reported that AEA levels in MCAO mice were threefold higher in the striatum compared to controls, with substantially no changes in the cortex, which indicated that AEA might be protective in specific brain areas. Additionally, in vitro assays in transfected cells by Iuvone et al. [93] showed that elevated AEA levels were protective against proliferation of activated glial cells in neural damage. In vivo studies have reported that AEA provided support to white matter during neurodevelopment, which protected the neonatal brain in a model of excitotoxic lesion induced by several agents (i.e., S-bromo-willardiine and AMPA-kainate receptor agonists) [94].

Studies carried out with FAAH inhibitors like URB597 and AM374 have broadened the range of AEA effects in neuroprotection. For instance, AEA catabolism inhibition appears to be protective in distinct injury models, such as retinal damage in ischemic/reperfusion studies [95], kainic acid-induced seizures in the hippocampus [96], and multiple sclerosis-induced spasticity [97]. This might represent an interesting frontier for the development of new pharmacological approaches against neuronal damage based on therapeutic efficacy of FAAH inactivation. However, beside this number of findings on protective roles of enhanced AEA levels, other studies have underscored pathological conditions where AEA might not be involved. In line with this possibility, De Lago et al. [98] demonstrated that UCM709, an AEA uptake inhibitor, does not prevent neurodegeneration nor improve neurological recovery in Huntington's disease (HD), autoimmune encephalitis (EAE), and Parkinson's

disease models. On the other hand, UCM709 does improve hyperkinesia and spasticity of the hind limb in HD and EAE models, suggesting a strong involvement of AEA in some, but not all, symptoms of these degenerative disorders.

Interplay between AEA and 2-AG as protective agents

In contrast to studies that described AEA and 2-AG as separate neuromodulators involved in neural protection, the literature lacks observations about synergistic/cooperative effects between these eCbs in the same topic. One of the few studies that investigated this interplay showed that in a cerebral focal ischemia model, electroacupuncture pretreatment induced neuroprotection, upregulated the neuronal expression of rat brain CB1-Rs, and elevated the brain tissue content of AEA and 2-AG [99]. The same study also showed that pretreatment with both AEA and 2-AG reduced infarct size and improved neurological outcome in rodents through CB1-Rs.

Role of eCbs in learning and memory: toward and beyond AEA

THC, an exogenous counterpart of eCbs, has been consistently regarded as a molecule with detrimental effects on memory and learning. In contrast to studies focused on synaptic plasticity, those on learning and memory have traditionally spotlighted AEA as a principal eCb. However, recent findings have also taken into account the role of 2-AG in specific plasticity forms associated with mnemonic and learning functions.

AEA and 2-AG in learning and memory

Among compartments in which memory can be divided, AEA has been often regarded as a strong modulator of acquisition phases. In fact, early observations by Murillo-Rodriguez et al. [100] reported that AEA regulates several processes that ultimately drive memory acquisition. In this study, by taking advantage of inhibitory avoidance paradigms, the authors showed that AEA interacts in memory acquisition only in late phases of testing (24 h after training), being ineffective in such modulation shortly after (15 min) the training session. This result is even more interesting, since they reported AA effects in both parameters (15 min and 24 h). Phospholipids containing AA participate in biochemical pathways to produce both AEA and 2-AG; thus, eCbs might play a role in the early stages of memory acquisition by switching competencies in modulation of cognitive functioning. However, further studies should better characterize 2-AG involvement in acquisition of memory traces.

A wealth of preclinical data underscored that AEA micro-infusions in the BLA or the administration of the methylated, metabolically stable form of AEA (methAEA) prevent memory retrieval (24 h after training) during aversive memory acquisition tasks [101] and in encoding phases of memory acquisition under delayed nonmatch-to-sample tasks [102], respectively. These studies focused on specific brain areas that encode for emotional learning and memory such as the BLA and hippocampus and provided evidence on AEA involvement in specific phases of memory formation. The latter study also ruled out TRPV1 in AEA-induced effects on hippocampal neuron inhibition, indicating that CB1-Rs are likely main players [102]. Moreover, by performing experiments using FAAH$^{-/-}$ mice, Wise et al. [103] showed that AEA plays a critical role in the improved acquisition of specific subsets of emotional memory. In this study, the authors highlighted that this eCb only modulates aversively motivated memory formation, being ineffective on the appetitive counterpart and broadened the scenario on AEA participation in early memory performance.

Other observations have provided evidence that AEA effects on memory might be multidirectional. For instance, a study reported that FAAH$^{-/-}$ mice have faster acquisition during the first session of working memory tasks compared to wild-type littermates [104]. Nonetheless, no difference between FAAH$^{-/-}$ and FAAH$^{+/+}$ have been reported in subsequent sessions of the same task. The same authors showed that AEA exogenous application disrupted working memory performance only in FAAH$^{-/-}$ mice and was ineffective in controls. Further, methAEA impaired performance both in knockout and wild-type littermates. These latter results suggested that elevated AEA levels were responsible for worsening or improvement in different phases of memory performance depending on brain concentrations of this eCb. Finally, among other possible explanations of paradoxical AEA effects in memory performance modulation, studies have taken into account the possibility that receptors other than CB1-R might be involved. For example, Mazzola et al. [105] have uncovered an URB597-induced effect in memory acquisition that appeared to be mediated by the stimulation of a family of nuclear receptor transcription factors, known as α-type peroxisome proliferator-activated receptors (PPARα). However, since this study did not clarify whether AEA or other FAAH substrates, such as noncannabinoid N-acylethanolamides, were recruited in this effect, further studies should better analyze this possibility.

Other than memory acquisition, AEA is apparently involved in memory consolidation and extinction, expanding the range of action of this eCb in different phases of mnemonic mechanisms. In fact, recent studies have found that FAAH inactivation by URB597 worsened memory consolidation by producing amnesia-like effects during the object recognition test [106] and enhanced the extinction of aversive memories [107]. Interestingly, the first observation was not replicated using JZL184; thus, this specific task most likely does not involve 2-AG [106]. These data have shed some light on the precise AEA and 2-AG functions in memory-relevant brain regions. Besides AEA, the role of 2-AG in the regulation of mnemonic functions has been investigated. In fact, since 2-AG plays a prominent role as a retrograde messenger in the hippocampus, where it modulates short- and long-term forms of plasticity (see above), it is highly likely that it is involved in memory-related synaptic mechanisms. Studies are now taking advantage of new selective MAG-L inhibitors and genetic tools. Among them, Pan et al. [22] showed an improved performance in Morris' water maze and novel object recognition test in MAG-L$^{-/-}$ mice compared to MAG-L$^{+/+}$ littermates, providing new cues to evaluate the role of 2-AG in learning and memory tasks. On the contrary, other studies have found that augmented PFC 2-AG levels might explain memory and cognitive disruptions in an animal schizophrenia-like model [108]. Therefore, the role of 2-AG in these complex behavioral functions remains controversial. Finally, a recent work by Yoshida et al. [109] assessed 2-AG-regulated synaptic plasticity underlying extinction of fear memory. In fact, in this study, results from morphological and electrophysiological experiments suggested that biosynthetic pathways involved in 2-AG formation play a prominent role in the DSI of afferents from cholecystokinin-positive GABA interneurons to pyramidal cells in the BLA basal nucleus. To conclude, it appears that AEA and 2-AG have segregated roles in memory and learning regulation, with the former exerting broader effects, but with growing evidence that 2-AG is recruited to some functional subcompartments.

Interplay between 2-AG and AEA in learning and memory

Evidence is lacking in the literature that 2-AG and AEA cooperate in memory modulation. To our knowledge, only Cuellar and Isokawa [110] provided data about an interplay between AEA and 2-AG in memory and learning. They described an involvement of both eCbs in the ghrelin-induced inhibition of CREB activity and NR1 function in the hippocampus. According to this study, AEA and 2-AG might cooperate at different levels to produce neurobiological modifications relevant for cognitive functions through direct action on NMDA receptors and CB1-Rs, respectively. Further studies are needed to assess differential involvement of the two major eCbs in memory-related functions.

Role of AEA and 2-AG in nociception: crosstalk and specificities

THC is antinociceptive when administered to humans and animals and the employment of *Cannabis* derivatives as therapeutic analgesics is still debated [111, 112]. Early studies have proposed spinal and supraspinal mechanisms through which THC exerts analgesic effects in rodents [113], and this effect is now well characterized. In addition to THC, eCbs possess analgesic properties and exerts differential and similar effects in specific aspects of pain perception.

Differential involvement of AEA and 2-AG in nociception

Although interactions between AEA and 2-AG in pain modulation have been proposed, several studies have highlighted a differential involvement of these eCbs in specific nociception aspects. For instance, Starowicz et al. [114] reported that local infusion of AEA or URB597 produced antiallodynic and antihyperalgesic effects in an animal model of spinal neuropathic pain. This study underscored that spinal AEA produces antihyperalgesic effects by acting either at CB1 or TRPV1 receptors depending on the local concentration. In particular, CB1-mediated effects prevailed at low concentrations, whereas the TRPV1-mediated ones at high concentrations.

On the other hand, 2-AG likely played a role in pain modulation. According to Guindon et al. [115], local injection of 2-AG dose-dependently reduced nociception in the late phase of the formalin-induced pain test. These results have been replicated by the same group by administering the MAG-L inhibitor URB602. They observed an additive antinociceptive effect when both 2-AG and URB602 were coinfused at their EC_{50}, suggesting that 2-AG might be a potential substrate for novel pharmacological approaches against nociception. Additionally, recent investigations described AEA and 2-AG effects in distinct regions of the dorsolateral (dl) and the ventrolateral (vl) periaqueductal gray (PAG). In fact, Olango et al. [116] used formalin nociception tests and reported that physiological enhancements of AEA levels in dlPAG was involved in fear-conditioning induced by nociception, whereas Liao et al. [117] described antinociceptive properties of 2-AG in vlPAG. Notably, the latter results provided a logic of action of 2-AG. The authors propose that 2-AG formation was triggered by TRPV1 activation on glutamatergic terminals in vlPAG. Released glutamate, in turn, activated postsynaptic mGluR5 receptors, which, via Gq protein, stimulated phospholipase C to yield DAG which is then deacylated by DAGL to 2-AG [117].

Further studies have evaluated the dual, yet specific, involvement of AEA and 2-AG in pain perception. Among

them, Spradley et al. [118] recently reported a nonoverlapping effect of enhanced levels of AEA and 2-AG, induced by respective catabolism inhibitors in the modulation of capsaicin-induced nociception. Here, the authors found that either AEA or 2-AG regulated specific nociceptive features such as nocifensive behavior, thermal hyperalgesia, and mechanical allodynia, and reported that FAAH inhibition was only able to counteract the latter parameter, whereas JZL184 (the MAG-L inhibitor) affected the others. These results underscored that AEA and 2-AG may be separately recruited to exert antinociception in different brain subregions involved in pain.

Interplay between AEA and 2-AG in nociception

AEA and 2-AG synergy in modulation of pain have been reported in several studies. Early results showed a coordinated effect of AEA and 2-AG in prolonged footshock modulation in stress-induced analgesia [119] and indicated that both eCbs have augmented levels in the ipsilateral lumbar V dorsal root ganglion in an animal model of spinal nerve ligation [120]. The employment of specific FAAH and MAG-L inhibitors facilitated the investigation of eCb effects on pain. In fact, pharmacological studies carried out by Kinsey et al. [121] have shown that FAAH and MAG-L inhibition might be suitable targets to develop new pharmacological strategies to treat nociception. In this study, they showed that enhanced levels of AEA or 2-AG attenuates mechanical and acetone-induced cold allodynia but through different receptor-dependent mechanism. In fact, FAAH inactivation was effective through either CB1-R- or CB2-R-dependent mechanisms, whereas effects due to augmented 2-AG levels were counteracted only by CB1-R antagonism [121]. Another example of interplay has been shown by other studies where either AEA or 2-AG local injection or the administration of FAAH and MAG-L inhibitors were reported to reduce mechanical hyperalgesia in a mouse model of bone cancer [122, 123]. These studies are more interesting in light of the finding that AEA and 2-AG act through clearly separate mechanisms, involving dorsal root ganglion CB1-R and peripheral CB2-Rs, respectively [122, 123]. This would suggest that besides a switch in molecular pathways involved in pain, AEA and 2-AG may synergistically strengthen their role in nociception. Petrosino et al. [124] provided evidence of an interesting cooperation between AEA and 2-AG, which levels were enhanced after 3 and 7 days of chronic constriction injury of the sciatic nerve in the rat. Interestingly, after 3 days, AEA and 2-AG levels were increased only in the spinal cord and PAG, whereas after 7 days, augmented concentrations were detected also in the rostral ventral medulla.

An example of how AEA and 2-AG can cause different effects in the vl-PAG was provided also by Maione et al.

[125]. These authors suggested that while AEA can produce antinociceptive effects via TRPV1 activation, 2-AG, like the synthetic agonist WIN55,212-2, may cause both antinociceptive effects by acting at CB1 on presynaptic GABAergic terminals and pronociceptive effects by acting at CB1 on presynaptic excitatory axons innervating vl-PAG output neurons. These results suggest that combined modulation induced by eCbs is present in both spinal and supraspinal levels and that AEA and 2-AG participate in chronic pain modulation.

AEA and 2-AG in anxiety

The role of cannabinoids in anxiety is highly debated. Exogenous cannabinoids display both anxiolytic and anxiogenic properties [126]. A likely explanation is that this bidirectional effect might depend on the dose administered (e.g., high doses of synthetic cannabinoids are anxiogenic and low doses are anxiolytic) [127] and by other parameters [128]. Nonetheless, a number of studies have reported an anxiolytic eCb effect. At first glance, AEA was studied in more detail in anxiety, when compared with other eCbs.

Do AEA and 2-AG differentially counteract anxiety?

AEA involvement in anxiety has been studied for a long time, and interest has not yet faded. In 2003, Kathuria et al. provided the first evidence on URB597 anxiolytic properties [24, 129], likely mediated by AEA through CB1-R activation. Subsequent studies have confirmed this idea using FAAH inhibitors or FAAH$^{-/-}$ mice. For instance, it was shown that anxiety-like behavioral responses were reduced by URB597 or by FAAH knockout through a CB1-R-dependent mechanism [130]. Additionally, among possible mechanisms by which both genetic and pharmacological FAAH blockade counteract anxiety, Rossi et al. [131] showed that enhanced AEA levels might act on striatal CB1-Rs, which levels in anxious phenotypes were dramatically downregulated. Finally, a recent paper by Cippitelli et al. [132] has proposed that AEA may be implicated in the modulation of nicotine withdrawal-induced anxiety responses, as measured by elevated plus maze and shock-probe defensive burying paradigms [132]. These authors observed AEA fluctuations with unaltered 2-AG concentrations in brain regions including amygdala, PFC, hippocampus, and hypothalamus during nicotine withdrawal in dependent rats. Further, they showed a protective effect of URB597 in the expression of withdrawal syndrome in these animals but not in the reduction of withdrawal-induced somatic signs.

Other studies have focused on AEA in anxiety by taking advantage of different tools, such as membrane transport inhibitors or local AEA injections. AM404, an AEA transport blocker, reportedly enhanced AEA levels to mediate anxiolytic responses in three animal anxiety models through PFC CB1-Rs [133] and low doses augmented the time spent in open arms of the elevated plus maze test [134]. Studies with AEA transport blockers have the limitation that these drugs might also interfere with 2-AG membrane transport [135, 136]; therefore, interpretation of results is difficult.

Local administration of AEA catabolism inhibitors has also confirmed the role of this eCb in anxiety. For example, the ventral hippocampus (vHIP) was investigated in a study where in situ URB597 administration improved mice responses using the elevated plus maze paradigm [137]. Nonetheless, AEA as antianxiety agent is questioned by conflicting evidence, which reported that AEA-induced effects may vary according to different parameters. For instance, studies have proposed that the AEA effects as an antianxiety molecule strongly depended on environmental conditions, since a change in environmental aversiveness in the experimental setup allowed a rapid AEA shift from anxiolytic to ineffective [138]. Further observations by Scherma et al. [139] reported that low AEA and URB597 administered together more likely exerted anxiolytic effects in rats under place-conditioning procedures and that, conversely, relatively high doses of AEA facilitated anxiety.

In accordance with these latter results, intriguing observations by Rubino et al. [140] reported that while microinjection of low URB597 doses in the PFC exerted anxiolytic effects, the administration of the same compound at high doses did not produce any effect or even increased anxiety in animal models [140]. The same authors also showed that virally mediated overexpression of FAAH in the PFC was anxiogenic. Finally, a study by Campos et al. [141] demonstrated that AEA microinjections in the vHIP in nonstressed animals induced anxiogenic behavior as measured by the elevated plus maze test, but under restrained conditions before the test (24 h), animals that received a local injection of the same eCb in the vHIP showed reduced anxiety behaviors. It is important to point out that in these studies, TRPV1 have a role in AEA effects on anxiety, to the extent that AEA act as endocannabinoid or endovanilloid depending on its concentrations. This dual function of AEA, together with environmental conditions, might indeed explain paradoxical results in animal models of anxiety, as well as in fear and compulsive behaviors [142–144].

Recent studies have also investigated the role of 2-AG in anxiety. In fact, 2-AG effects apparently converge into specific aspects of anxious experiences, such as those emerging from complex and unpredictable environmental requests. In line with this consideration, Sumislawski et al. [145] showed 2-AG enhancement during chronic, stress-induced, anxiety-like behavior in the BLA. The authors showed that 2-AG acts by potentiating LTD on GABAergic synapses

and that this effect was likely due to reduced synthesis of MAG-L. Finally, additional results have also demonstrated a specific effect of MAG-L inactivation in anxiety response modulation under high, but not low, levels of environmental aversiveness [146]. Overall, AEA and 2-AG effects described here indicated a prominent involvement of AEA in such processes, which can be implemented by 2-AG under specific conditions.

Interplay between AEA and 2-AG in anxiety

Tools to deactivate catabolic enzymes for AEA and 2-AG did not only provide evidence of differential involvement of these molecules in anxiety modulation, but also interesting interactions between the two eCbs. A recent paper by Kinsey et al. [147] demonstrated that FAAH and MAG-L inhibitors prevented obsessive–compulsive (OC) behavior during a marble burying test. OC behavior represents an interesting aspect of anxiety disorders and this finding may drive future research on pharmacology for OC disorders, where therapeutic approaches remain poorly effective. Kinsey's results are even more intriguing, since he showed that the effects of FAAH and MAG-L inhibitors were due to the CB1-R activation. This finding was not unique though, since other studies have reported that anxiolytic properties of FAAH and MAG-L inhibitors in the elevated plus maze test and other paradigms were caused by the activation of CB1- and CB2-Rs, respectively [106], which complicated the scenario concerning receptors involved in anxiety modulation. In light of these findings, it is possible that synergies between AEA and 2-AG recruit a similar/different mechanism according to specific subtypes of anxiety manifestations and specific brain areas.

AEA and 2-AG in reward and drug addiction: the paradox of addictive molecules counteracting addiction

Since the discovery of AEA and 2-AG, much interest has focused on whether these molecules are involved in neurobiological processes underlying drug abuse and addiction. Similar with the psychoactive component of *Cannabis*, THC, both eCbs are self-administered by nonhuman primates [148–150]. This effect is also related to AEA and 2-AG involvement in synaptic plasticity in reward-related areas. In fact, neurobiological processes that ultimately drive drug addiction require an array of short- and long-term modifications in synaptic plasticity in motivational and emotional brain areas [151], where AEA and 2-AG might take part.

Studies over the last two decades have substantially confirmed this hypothesis. Nonetheless, some recent finding have complicated this scenario by providing evidence that FAAH inhibitors, i.e., URB597 or PF-3845, are not self-administered by nonhuman primates [149], do not produce generalization to the discriminative effects of THC in rats [152], do not cause cross-tolerance with cannabinoid agonists, and do not desensitize CB1-Rs upon chronic administration [153]. Inhibition of MAG-L, on the other hand, causes behavioral effects not observed following chronic FAAH blockade, such as hypomotility and hyperreflexia [25, 154], physical dependence, tolerance to CB1-R agonists, CB1-Rs desensitization, and downregulation [153], which suggest a broader impact on the brain cannabinoid system [153]. Although neither FAAH nor MAG-L inhibitors showed abuse liability, experiments with the recently developed dual FAAH/MAG-L blocker (JZL195) reported THC-like effects in drug discrimination tests [154].

Specificity of 2-AG and AEA in rewarding and addictive properties of drugs of abuse

Among the vast number of studies on the possible interaction between the eCb system and addictive drugs in the brain reward circuitry, one of the most explored areas is the role of AEA in nicotine addiction. For instance, using the AEA catabolism inhibitor, URB597, Scherma et al. [155] reported that FAAH inhibition prevents nicotine-induced behavioral and neurochemical effects. Consistently, our studies performed with electrophysiological techniques [156, 157] demonstrated that FAAH inhibition blocks nicotine-induced electrophysiological effects predictive of its rewarding properties, i.e., excitation of VTA DA neurons' firing rate [156] and inhibition of medium spiny neurons in the shell of the NAc (ShNAc) [157]. However, our evidence suggested that AEA was probably not involved. In fact, URB597, by blocking FAAH, does not only raise AEA levels, but also concentrations of other noncannabinoid *N*-acylethanolamines, i.e., oleoylethanolamide (OEA) and palmitoylethanolamide (PEA). These molecules are endogenous PPARα ligands and most URB597-mediated effects were blocked by the PPARα antagonist, MK886 [156, 157]. In a separate subset of experiments, we showed that both in vivo and in vitro administration of AEA and its hydrolysis-resistant analogue, methAEA, did not mimic URB597-mediated action on VTA DA neurons [156]. These results do not completely rule out AEA in nicotine-induced effects in the brain reward circuitry, since our further observations have discerned that URB597 effects on nicotine action in the ShNAc were prevented by both CB1-R and PPARα antagonists (e.g., rimonabant and MK886, respectively) [157]. Thus, this latter result might suggest interplay between AEA and other *N*-acylethanolamines.

In line with this possibility, recent studies by Scherma et al. [158] and Gamaleddin et al. [159] have clarified the role

of AEA in nicotine rewarding and addictive behaviors. They found that the AEA transport inhibitors, AM404 and VDM11, which enhance AEA levels, but not those of OEA and PEA, block nicotine-induced conditioned place preferences, nicotine-induced elevation of DA levels to the ShNAc [158] and attenuate the reinstatement of seeking behavior induced by nicotine-associated cues and nicotine priming [159]. In neither study, however, the mechanism by which AEA transport inhibitors exerted their effects was investigated by using selective CB1 or TRPV1 antagonists. Furthermore, parallel observations by Cippitelli et al. [132] showed that AEA levels in brain areas involved in nicotine rewarding responses are physiologically altered in nicotine-abstinent mice, providing evidence that FAAH inhibition may also counteract withdrawal signs in animal models of nicotine addiction. No information on receptors involved is provided by this study. FAAH inhibitors represent suitable candidates for antismoking medications but other studies have reported contrasting results. For instance Merritt et al. [160] showed that FAAH$^{-/-}$ mice developed an augmented nicotine-induced condition place preference, enhanced withdrawal signs in a model of nicotine abstinence, and condition–place aversion in mecamylamine-induced nicotine withdrawal.

In contrast to the evidence on AEA and nicotine interactions in the brain reward circuitry, no studies have yet taken into account 2-AG involvement in the modulation of nicotine-rewarding and nicotine-addictive properties, with the exception of González et al. [161] who showed that chronic nicotine exposure elevates AEA and 2-AG levels in the brain stem and midbrain and decreases their levels in the hippocampus, striatum, and cortex. Regarding other addicting drugs, AEA is involved in several cocaine-, ethanol-, and opioid-induced rewarding effects. Among them, the role of the eCb system in cocaine reward and addiction is still questioned. In fact, Adamczyk et al. [162] reported that FAAH inhibition blocks cocaine-seeking behavior, but induced no changes in cocaine self-administration. Additionally, no changes in the AEA NAc levels after repeated administration of cocaine were found [163]. Our studies demonstrated that URB597 prevented cocaine-induced ShNAc responses, an effect not reverted by CB1-R antagonists, but by the PPARα antagonist MK886 [157], suggesting that OEA and PEA, rather than AEA, were responsible for this effect. On the other hand, this scenario is further complicated by the finding that AEA levels increase in the striatum after cocaine administration [164].

The possibility that AEA may modulate alcohol-induced effects is less controversial. Studies by our group showed that FAAH inhibition counteracted ethanol-induced ShNAc responses [165], and Serrano et al. [166] reported altered FAAH mRNA expression in the amygdala after ethanol withdrawal in animals exposed to either acute or intermittent administration. Furthermore, in the latter study, altered

MAG-L mRNA expression was described and its levels were more pronounced only during intermittent alcohol exposure, suggesting a prominent role of AEA, rather than 2-AG, in the modulation of ethanol-induced responses. AEA might reduce ethanol intake by activating TRPV1 receptors, since a study illustrated that TRPV1$^{-/-}$ mice showed significantly higher preference for ethanol and consumed more ethanol in a two-bottle choice test as compared with wild type littermates [167]. This study and that by Adamczyk et al. [168] for cocaine addictive behavior underscore the emerging role of TRPV1 in addiction.

2-AG involvement in the modulation of rewarding and addictive responses is less clear. Studies with alcohol have reported that dialysate levels of 2-AG in the NAc were increased after ethanol self-administration [163], supporting the role of 2-AG in early steps of alcohol dependence. This notion has also been supported by other studies, which showed that alcohol intake and preference was increased by chronic treatment with neurotoxic doses of the psychostimulant, methamphetamine [169]. In this case, the authors showed that 7 days after methamphetamine administration, 2-AG levels were enhanced, whereas MAG-L activity was reduced in the limbic forebrain and that MAG-L inhibitors enhanced ethanol intake in treated and naïve mice [169].

Recent evidence pointed toward a possible modulatory effect of 2-AG in opiate reward and addiction. In fact, it has been recently shown that MAG-L inhibitors suppress withdrawal symptoms in a model of naloxone-precipitated abstinence [170], suggesting that 2-AG might play a prominent role in opiate withdrawal. Conversely, FAAH inhibition only partially reduced some measures of withdrawal score, suggesting that AEA has modest beneficial effects under these circumstances. It has been demonstrated that 2-AG levels are strongly reduced in many limbic areas during morphine tolerance [171]. However, beside this intriguing role of 2-AG on chronic opiate effects, other studies have highlighted interactions between the latter eCb and AEA (see below), which suggested coinvolvement of 2-AG in the mechanisms of early and late phases of morphine and heroin addiction.

Interactions between endocannabinoids in rewarding and addictive properties of drugs of abuse

Few studies have specifically investigated the interactions between 2-AG and AEA. eCb levels were measured in brain tissue during drug intake and often found elevations/decreases of both AEA and 2-AG levels. For instance, Malinen et al. [172] found increased AEA and 2-AG levels in specific brain regions of alcohol-preferring male and female rats (AA rats) compared with nonpreferring counterparts. AEA and 2-AG changes were nonoverlapping and showed differential occurrences in accordance with gender

and alcohol exposure sessions, suggesting that AEA and 2-AG might have a distinct modulatory role. The findings by Caillè et al. [163] provided further support to the notion that AEA and 2-AG display different profiles during drug intake. In this paper, the authors showed that in heroin self-administration, an increase in NAc AEA levels was accompanied by a slight, but significant, decrease in 2-AG concentrations in this brain area. Others reported that under chronic administration of drugs of abuse, changes in eCb contents may involve AEA and 2-AG [161]. For example, studies highlighted that AEA and 2-AG levels were decreased in the midbrain after chronic alcohol exposure and increased in the brain stem and decreased in hippocampus and cortex after chronic administration of nicotine [161]. In an interesting study, Viganò et al. [173] showed AEA and 2-AG interactions during different phases of morphine sensitization that might suggest a homeostatic adaptation of the whole eCb system induced by chronic opiate exposure. Here, the authors reported changes in AEA and 2-AG levels in opposite directions after acute morphine administration, prolonged withdrawal, and expression phase of sensitization, with increase in AEA and decrease in 2-AG concentrations in most of the areas analyzed (i.e., NAc, caudate–putamen, and hippocampus). These results are in line with the study by Caillè et al. [163] with heroin self-administration and might underscore AEA and 2-AG interactions to balance their contents during opiate sensitization, thereby likely preserving the integrity of this circuitry.

Concluding remarks

The present literature review draws a complex picture about AEA and 2-AG, which may interact or differentially compete in the modulation of specific CNS functions. It is now well established that AEA and 2-AG are not fully interchangeable, since they definitely take part in highly specialized compartments of physiological and pathophysiological functioning. Even when they cooperate, AEA and 2-AG often partake in distinct steps of the same function. Overall, it appears that 2-AG is more involved in diffuse physiological functions, such as synaptic plasticity and neuroprotection, whereas AEA might intervene to fine-tune those processes. Accordingly, 2-AG might be regarded as providing functional support and orientating physiological modifications necessary in homeostasis, development, and adaptive behavior. On the other hand, AEA apparently plays a prominent role in modulation of superior functions such as learning and memory. Interestingly, even when interacting, AEA and 2-AG preserve their specific functionality, for instance, by recruiting different mechanisms and acting through different pathways.

In spite of this reductive view, which does not account for circumstances where AEA and 2-AG act through the same mechanism, some issues need to be discussed. First of all, recent observations demonstrated that AEA catabolism inhibitors, while promoting increases of AEA levels in nervous tissue, may also reduce the 2-AG biosynthesis in specific brain regions, such as the striatum [72, 174]. In light of these findings, it becomes less clear whether effects observed in some functions by application of URB597 and congeners were actually mediated by increased AEA levels, or by reduced 2-AG levels. Moreover, the findings that FAAH hydrolyzes both AEA and other N-acylethanolamides (i.e., OEA and PEA) [24] make it difficult to isolate the AEA-mediated effects from those mediated by OEA and PEA. Additionally, it has been reported that AEA may have an affinity for PPARα ([175] but see [156, 176, 177]). In line with the multifaceted AEA profile, which goes beyond the affinity for classical CB1-Rs, studies have demonstrated that effects produced by this eCb are likely due to TRPV1 activation [59, 178] (see above). Interestingly, this effect may strictly link AEA activity with that mediated by 2-AG, since it has been observed that TRPV1 receptor activation may also trigger the 2-AG biosynthesis [117], or, conversely, reduce 2-AG levels [174, 179]. In conclusion, these combined circumstances make the scenario on AEA and 2-AG interaction as neuromodulators more complex and provide a challenge that is worth addressing in the future to specifically isolate the role of eCb-like molecules in brain physiology and pathophysiology.

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