The FEBS Journal

FEBS PRESS science publishing by scientists

STATE-OF-THE-ART REVIEW

Cannabinoid control of hippocampal functions: the *where* matters

Almudena Robledo-Menendez[1,2], Maria Vella[1,2], Pedro Grandes[1,2] and Edgar Soria-Gomez[1,2,3]

1 Department of Neurosciences, Faculty of Medicine and Nursing, University of the Basque Country UPV/EHU, Leioa, Spain
2 Achucarro Basque Center for Neuroscience, Leioa, Spain
3 IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

Keywords
astrocytes; cannabinoids; CB1 receptor; CB2 receptors; hippocampus; interneurons; memory; mitochondria

Correspondence
E. Soria-Gomez, Department of Neurosciences, Faculty of Medicine and Nursing. University of the Basque Country UPV/EHU; Achucarro Basque Center for Neuroscience, 48940 Leioa, Spain
Tel: +34(0)946015774
E-mails: edgarjesus.soria@ehu.eus; edgar.soria@achucarro.org

(Received 8 February 2021, revised 23 April 2021, accepted 30 April 2021)

doi:10.1111/febs.15907

In the brain, hippocampal circuits are crucial for cognitive performance (e.g., memory) and deeply affected in pathological conditions (e.g., epilepsy, Alzheimer). Specialized molecular mechanisms regulate different cell types underlying hippocampal circuitries functions. Among them, cannabinoid receptors exhibit various roles depending on the cell type (e.g., neuron, glial cell) or subcellular organelle (e.g., mitochondria). Determining the site of action and precise mechanisms triggered by cannabinoid receptor activation at a local cellular and subcellular level helps us understand hippocampal pathophysiological states. In doing so, past and current research have advanced our knowledge of cannabinoid functions and proposed novel routes for potential therapeutics. By outlining these data in this work, we aim to showcase current findings and highlight the pathophysiological impact of the cannabinoid receptor type 1 (CB1) localization/activation in hippocampal circuits.

Introduction

Higher-order brain functions depend on synchronizing a plethora of delicately balanced systems that, like an orchestra, carry out the rhythms that define our thought processes, influence our behavior, and compose what we remember. In the brain, few structures have received as much attention as the hippocampus. Indeed, the study of this multilayered brain region has revolutionized brain research [1]. Nowadays, it is considered a key part of the mnemonic systems in different species, including humans. Furthermore, several studies have proposed the hippocampal formation as a hub where the unconscious and conscious experience occurs [1,2]. Thus, the study of the molecular mechanisms regulating hippocampal circuitries is crucial in advancing brain research and understanding neural functions. In this context, molecular effectors are seen as modulators of brain networks, implying that their actions shape cognitive and behavioral

Abbreviations
2-AG, 2-arachidonoylglycerol; Ach, acetylcholine; AEA, N-arachidonoylethanolamide; BDNF, Brain-derived neurotrophic factor; CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; CCK, cholecystokinin; COX-2, Cyclooxygenase-2; D1R, dopamine receptor type 1; DSI, depolarization-induced supression of inhibition; ECS, endocannabinoid system; EtOH, ethanol; GCs, granule cells; GPCR, G protein-coupled receptor; I-LTD, inhibitory long-term depression; JNK, c-Jun N-terminal kinases; LTD, long-term depression; LTP, long-term potentiation; MAP/ERK, mitogen-activated protein kinases/extracellular signal-regulated kinase; MCs, Mossy hiliar cells; mtCB1, mitochondrial cannabinoid receptor type 1; mTOR, mammalian target of rapamycin; NMDAr, N-Methyl-D-Asperate receptor; NOR, Novel object recognition; PGE2, prostaglandin E2; PI3K/Akt, phosphatidylinositol 3-kinases; PKA, protein kinase A; PV, parvalbumin; SWM, spatial working memory; SWR, sharp wave ripples; THC, Δ9- tetrahydrocannabinol; VHPC, ventral hippocampus.

outcomes. Indeed, brain circuits under the control of cellular and subcellular systems can underlie specific behaviors. The endocannabinoid system (ECS) is a widely distributed neuromodulatory system whose endogenous activation occurs in a precise temporal and spatial manner throughout the body and brain [3]. By achieving this specificity, it fine-tunes many physiological processes that are of most interest to current neuroscience research, such as neuronal excitation, neuroplasticity, adult neurogenesis, and cognitive performance.

The endocannabinoid system

The ECS is comprised of at least two G protein-coupled receptors (GPCR), their endogenous ligands, and the enzymes needed for their synthesis and degradation [4]. The cannabinoid receptor type 1 (CB1) is considered the most abundant GPCR in the brain [3]. The CB1 receptor is expressed in a wide range of brain regions, including the prefrontal cortex, basal ganglia, and hippocampus, the latter, which will be the topic of this review. On the other hand, the cannabinoid receptor type 2 (CB2) is mainly localized in peripheral tissue and immune cells [4], making it an attractive therapeutic target [5]. Interestingly, it was recently proven that the CB2 receptor participates in neuromodulation [6,7], but whether or not CB2 receptors are anatomically present in neurons is still under constant scrutiny [6,8,9]. The most studied receptor is the CB1 receptor, and further work is needed to clarify CB2 receptor's location and mechanisms. In this review, we will focus mainly on the CB1 receptor modulation of hippocampal function.

The endogenous ligands that activate cannabinoid receptors are two lipid compounds known as anandamide (N-arachidonoylethanolamide, AEA) and 2-arachidonoylglycerol (2-AG) [3]. Endocannabinoids act as retrograde messengers; they are synthesized in a postsynaptic, activity-dependent manner, and stimulate Gi/o proteins coupled to presynaptic CB1 receptors. The activation of these receptors results in a reduction in neurotransmitter release [10]. The discovery of CB1 receptors in perisomatic and dendritic areas of neurons, glial cells (namely astrocytes), and mitochondria of both neurons and astrocytes suggests a wider collection of noncanonical mechanisms of action [3,11]. Additionally, CB1 receptors are known to activate different signaling pathways, most importantly the mammalian target of rapamycin (mTOR), the mitogen-activated protein kinases/extracellular signal-regulated kinase (MAPK/ ERK), c-Jun N-terminal kinases (JNK), and phosphatidylinositol 3-kinases (PI3K/Akt) pathways [12]. The location-dependent modulation of cannabinoid signaling is reinforced by the fact that CB1 receptors form homodimers and heterodimers with other GPCRs, including CB2 receptors and dopamine, opioid, serotoninergic, and orexin receptors [3,13–17]. Indeed, several heterodimers have been described as specific modulators of hippocampal function (e.g., adenosine receptor A_{2A}R-CB1 and orexin receptor OX1-CB1) [18,19]. Interestingly, activations of these heterodimers exert opposing effects to solitary CB1 receptor activation. The existence of these dimers could explain, at least in part, the heterogeneity in CB1 receptor's action, depending on its location and interactions with nearby proteins [3].

The phytocannabinoids commonly found in marijuana, including its main psychoactive component Δ-9-tetrahydrocannabinol (THC), and synthetic cannabinoid compounds, compose the exogenous ligands that activate cannabinoid receptors [4,20,21]. Unlike the endogenous ligands, exogenous cannabinoid agonists do not display the specificity and coordination of physiological activation. Consequently, the use of exogenous ligands leads to an unclear distinction that underlies current research between the endogenous functions of the ECS and the effects that result from a systemic pharmacological activation [22].

Cannabinoid action in hippocampal circuits

The ECS is widely distributed in the peripheral and central nervous systems and acts on different cell types and cellular compartments. Some of the most relevant characteristics of the ECS result from its presence in the hippocampus. As mentioned above, the hippocampus is a forebrain structure that participates in cognitive functions such as learning, memory, and sensory integration [7,20,23]. Additionally, it is a center of adult neurogenesis and is implicated in several neurological and psychiatric diseases [24–27]. Furthermore, it is particularly rich in CB1 receptors, whose activation is key to regulating pathophysiological processes [20,23]. In the hippocampus, the CB1 receptor is primarily localized in GABAergic neurons, but it can also be found in glutamatergic neurons and astrocytes as well as in subcellular compartments [3,28–30]. To ascertain the functions carried out by this extensive system, we need to distinguish its various roles at its different sites of action in cellular and subcellular levels. In this review, we examine the role of the CB1 receptor in hippocampal circuits (Fig. 1), discussing the findings that are shaping our current understanding of this relevant and complex system.

Canonical action: GABAergic transmission

CB1 receptors in the hippocampus are more densely expressed in GABAergic interneurons than in principal glutamatergic cells [31]. GABAergic inhibitory interneurons are widely distributed throughout the different hippocampal subregions. Although they represent only 10–15% of the neuronal population, they provide the inhibitory input necessary to regulate excitation and facilitate oscillatory activity. GABAergic cells are classified by their neuroanatomical characteristics, molecular expression profiles (parvalbumin (PV) or cholecystokinin (CCK) expressing cells), developmental origins, or their electrical activity (for an extensive review, see Pelkey et al.) [32]. Notably, most CCK-positive interneurons express CB1 receptors (approximately 90%), whereas only about 4% of PV interneurons do [33].

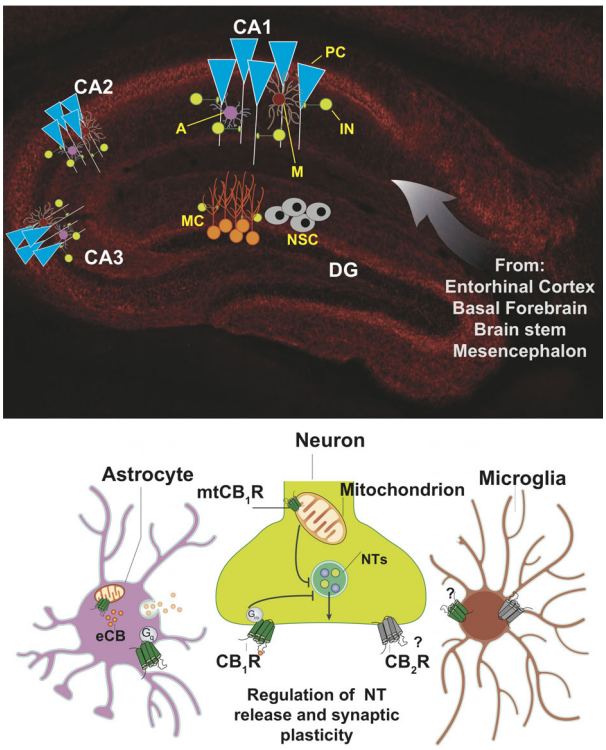

Fig. 1. Cannabinoid receptors localization in the hippocampus. Top, CB1 receptor immunofluorescence (red) in the hippocampal formation: CA1, CA2, and CA3 cell populations (pyramidal cells (PC), Interneurons (IN), Astrocytes (A), and microglia (M)). Dentate gyrus (DG) containing mossy hilar cells (MC) and neural stem cells (NSC). Besides internal control, cannabinoid signaling also modulates incoming information arriving from different brain regions such as the basal forebrain, mesencephalon, and brain stem. Bottom, graphical representation of cannabinoid receptors (CB_1R and CB_2R) expression in different cell types and cellular compartments. eCB, endocannabinoids; NTs, neurotransmitter.

The reduction in GABA transmission has been signaled as the primary cause of the amnesic effects produced by cannabinoids. The result of the inhibition of GABAergic transmission leads to an imbalance of neurotransmitter release and an increase in excitatory firing rates. Subsequently, the mTOR signaling pathway is activated due to the increased excitation via an NMDA receptor (NMDAr)-mediated mechanism. The activation of mTOR then provokes an upregulation of translation rates and an aberrant increase in protein synthesis, which has been associated with memory deficits [34].

In the dentate gyrus, exogenous cannabinoids acting on CB1 receptors present in GABAergic neurons could also result in a disruption of short-term memory [23]. In contrast, there is evidence that physiological activation of CB1 receptors in GABAergic neurons is essential for certain types of memory [20,35,36]. The inhibition produced by endocannabinoid signaling in hippocampal GABAergic neurons is necessary for complex learning processes such as incidental associations [35]. Incidental associations are those produced when low salience stimuli are associated with other stimuli paired with a meaningful event. In other words, these initially 'nonmeaningful' stimuli mediate behavior through their associations with other conditioned stimuli. Mice lacking CB1 receptors from GABAergic cells display deficits in mediated aversion while maintaining normal expression of direct learning [35]. Furthermore, a specific sub-population of hippocampal GABAergic interneurons that expresses both the dopamine receptor type 1 (D1R) and CB1 receptors is necessary for hippocampal long-term episodic memory. These neurons control the late stages of novel object recognition (NOR) memory by facilitating learning-induced long-term potentiation (LTP) [36]. The dopaminergic system participates in memory consolidation [37], and particularly, in the coding of novel experiences [38]. Intriguingly, the study of these neurons also suggests that there is a dopaminergic regulation that underlies cannabinergic signaling and memory consolidation. The exact phenotype of this subset of neurons also remains to be determined (e.g., PV or CCK). As stated above, most of the CB1 receptor-positive GABAergic neurons are also CCK-positive interneurons [39]. The ECS regulates working memory and aversive conditioning by influencing the communication between the ventral hippocampus (vHPC) and the prefrontal cortex by acting on CCK-positive GABAergic interneurons. By doing so, the ECS modulates excitation through a process of

synaptic plasticity known as depolarization-induced suppression of inhibition (DSI). CCK-positive interneurons hence mediate feedforward inhibition [40]. Thus, this subset of neurons is crucial in forming memory-relevant functional networks between hippocampal circuits and prefrontal cortex.

The cellular basis of memory relies on experience-dependent changes of synaptic transmission known as neuroplasticity. These changes can result in long-lasting effects that impact synaptic strength. The changes induced by experience can potentiate synaptic transmission through long-term potentiation (LTP) or weaken it through long-term depression (LTD). LTD is especially linked to novelty acquisition [41]. Hippocampal GABAergic regulation by the ECS has an important impact in different forms of neuroplasticity [7,35,36,42–44]. The ECS participates in controlling activity-dependent forms of plasticity by inducing long-term changes in GABAergic release. Excitatory activity in the hippocampus can induce the synthesis of 2-AG that then acts upon presynaptic GABAergic synapses, modulating excitability by regulating the inhibitory tone of hippocampal circuits [32]. Plasticity occurs as a result of an intracellular rise of calcium at the postsynaptic neuron and is mediated primarily by NMDAr [45]. Nevertheless, the ECS and CB1 receptor can modify inhibitory tone in a retrograde manner and provoke LTD through an NMDAr-independent mechanism. This LTD is observed in electrophysiological studies after applying a high frequency stimulation protocol to dendritic inhibitory synaptic terminals (I-LTD) on CA1 pyramidal neurons. Additionally, this type of LTD relies on the activation of the mGluR 1/5 [46].

The effects of the exogenous cannabinoid application on synaptic plasticity [21,45] depend on the dosage, procedure, and type of plasticity studied. The changes in synaptic plasticity that occur in the hippocampus can be further explained by the high sensitivity of the CB1 receptor [47]. The over-stimulation of CB1 receptors by even low doses of THC creates a functional tolerance that abolishes LTD [47]. Other observations indicate that the expression and function of hippocampal CB1 receptors are dynamically regulated. For example, several studies demonstrated phasic changes in CB1 receptor expression depending on the light-dark cycle, as well as a result of food ingestion, and physical activity [20,48–50]. Repeated administration of cannabinoid agents also produced changes in CB1 receptor efficacy in hippocampal GABAergic neurons [3]. Moreover, GABAergic CB1 receptors regulate inflammatory processes in the hippocampus, specifically through the regulation of hippocampal microglia. Although the mechanisms that underlie this regulation are unknown, the genetic deletion of CB1 receptors from GABAergic neurons resulted in a proinflammatory microglial phenotype and altered reactivity to bacterial infections in mice [51]. Thus, immunological regulation by hippocampal CB1 receptors represents a novel target for future research.

Glutamatergic transmission

In the hippocampus, there exist distinct types of excitatory neurons, including pyramidal neurons and granular and mossy cells present in the dentate gyrus and the hilus, respectively. All three types of neurons have been extensively studied given their implications in epilepsy, dementia, and neuroplasticity [52]. Considering that cannabinoids weaken the synaptic connections they act upon [3], CB1 receptors on glutamatergic terminals protect postsynaptic neurons from over-excitation. The correct balance of neuronal activation is necessary for normal brain function and to avoid the harsh effects of excitotoxicity. Indeed, the ECS is a neuromodulatory system that has been proposed as neuroprotective [53–55]. For example, following epileptiform producing injections of kainic acid, mice that did not express CB1 receptors in principal glutamatergic neurons (Glu-CB1-KO mice) suffered from more severe seizures than wild-type mice [53,54]. Furthermore, activation of the ECS alleviates disturbances in oscillatory activity, hippocampal alterations, and neuron loss in a kainic acid-induced excitotoxicity model in Guinea pigs [55]. The activation of CB1 receptors in principal glutamatergic neurons also induced intracellular signaling pathways that led to ERK phosphorylation and an increase in the transcription factor c-Fos and the brain-derived neurotrophic factor (BDNF). These changes, among others, underlie the neuroprotective shield against excessive neuronal activation [54,56,57].

The ECS also mediates neuroplastic changes in glutamatergic neurons. A cannabinoid-dependent form of LTD has been identified in the medial perforant path synapses of the dentate gyrus [58,59]. Interestingly, the expression and efficacy of these receptors could be altered by alcohol intake. Alcohol (EtOH) consumption has effects on glutamatergic CB1 receptors in the hippocampus. EtOH intake, both chronic and adolescent 'binge drinking', decreases the expression of CB1 receptors and endogenous cannabinoid levels. These changes result in a disruption of endocannabinoid-mediated LTD that entail long-lasting alterations of neuroplasticity and memory deficits [60]. It has also been demonstrated that the cognitive and motor

deficits provoked by EtOH during adolescence can be reversed by an enriched environment after alcohol withdrawal [61]. It is still to be determined whether the behavioral changes correlate with functional or structural changes in the CB1 receptor.

Recent data indicate that CB1 receptor control of glutamate release in the dentate gyrus mediates the communication between the entorhinal cortex and the hippocampus [62]. In particular, by regulating the synaptic connectivity between mossy (MC) and granular cells (GC). MCs are highly active and extensive excitatory cells that have the potential to elicit LTP. CB1 receptors stabilize MC-to-GC synaptic transmission by a tonic and phasic activation that suppresses glutamate release. The tonic CB1 receptor-dependent inhibition of glutamate release negatively regulates LTP in the absence of endocannabinoids. However, phasic activity is dependent on endocannabinoid release and causes a further suppression of LTP. The phasic activity observed in these receptors could serve a protective function to avoid runaway activity that could lead to epilepsy. This study sheds light on the differential activations of CB1 receptors and their specificity and functional outcomes.

Exogenous cannabinoids also activate CB1 receptors in glutamatergic neurons. This activation, which causes an imbalance in neurotransmitters, is yet another explanation for the hippocampal-dependent memory deficits related to cannabinoid use. The imbalance of neurotransmission that results from THC exposure is also provoked by an alteration of CB1 receptors in glutamatergic cells. THC causes a reduction in CB1 receptors and alterations in hippocampal CA1 morphology that affect not only GABAergic neurons but also glutamatergic neurons, astrocytes, and mitochondria [63]. It is also worth mentioning that some of the effects observed post-THC administration might not be specific to the CB1 receptor. THC, 2-AG, and AEA can all activate both CB1 and CB2 receptors. Activation of CB2 receptors in hippocampal slices increased both dendritic spine density in CA1 neurons and miniature excitatory postsynaptic currents. Consequently, CB2 receptors influence excitatory transmission. Curiously, these effects were only observed after chronic activation of CB2 receptors and were absent in acute activations [64].

Cholinergic and dopaminergic transmission

Besides the classic excitatory/inhibitory transmission regulation by CB1 receptors, cannabinoid signaling also controls cholinergic and dopaminergic neurotransmitter release [54,65]. In particular, the cholinergic terminals in the hippocampus establish mixed GABAergic synapses, where both acetylcholine (ACh) and GABA are released [66]. However, the potential functional impact of cannabinoid signaling on those synapses is still unknown. In this context, cognitive and emotional processes could be regulated by CB1 receptor-dependent modulation of dopaminergic and cholinergic transmission in the hippocampus. The use of cannabinoid antagonists has been related to enhanced cognitive functions [67]. This can be explained by elevated acetylcholine levels in the hippocampus that result from cholinergic disinhibition. THC has a dose-dependent biphasic effect on ACh neurotransmission. Thus, only high doses of THC (but not low or moderate) suppress ACh levels which are mediated by dopamine receptor activation. Hence, hippocampal D2 receptor activity leads to ACh suppression induced by high doses of THC, while septal D1 receptor signaling causes an increase in ACh efflux [68]. Intrahippocampal CB1 receptor activation produces an increase in ventral tegmental area dopaminergic terminal activity (firing frequency and bursting rates). A well-balanced regulation of these systems is crucial for optimal hippocampal function, especially under periods of stress, when variations in the levels of neurotransmitters could lead to maladaptive changes associated with neuropsychiatric diseases [7,65,69]. For example, dysregulation of the hippocampal ECS and overdrive of mesolimbic dopaminergic activity could be related to the pathogenesis of schizophrenia [70].

Beyond neurons: astrocytes

The importance of the role that astrocytes play in information processing has become increasingly clear during the past years. Previous research has highlighted some of the mechanisms by which astrocytes participate in synaptic signaling and defined the so-called 'tripartite synapse' [71]. With the ECS as one of the mediators in the neuron-astrocyte relationship [72], the CB1 receptor can modulate glutamatergic transmission indirectly through astrocyte-dependent mechanisms such as the release of gliotransmitters like glutamate and D-serine [42,71,73]. The release of glutamate by astrocytes has been described as a way that astrocytic CB1 receptor activation can potentiate synaptic transmission. This effect opposes synaptic CB1 receptor activation which depresses synaptic transmission. The latter has been proposed as a short-range effect of endocannabinoid release, whereas astrocytic CB1 receptor activation has a long-range effect that targets synapses further away from the

endocannabinoid releasing location [73]. Furthermore, the gliotransmitter D-serine is also modulated by CB1 receptor activation [42]. The release of D-serine involves a biochemical pathway known as the phosphorylated pathway which is defined as the cytosolic de novo synthesis of L-serine that stems from aerobic glycolysis and takes place primarily in astrocytes [74]. The product of this pathway allows the formation of D-serine, a coagonist of the NMDAr. The activation of astroglial CB1 receptors gives rise to an intracellular calcium increase that in turn activates the phosphorylated pathway and leads to the release of D-serine. Interestingly, recognition memory is dependent on astroglial CB1 receptor modulation of astrocytic D-Serine production [42]. Furthermore, astrocytic cannabinoid signaling is sensitive to environmental factors proven to impair memory performance. In particular, alcohol consumption during adolescence provokes a reduction in hippocampal astroglial CB1 receptors as well as astrocytic swelling [75].

Although the use of exogenous cannabinoids has been suggested as a potential therapeutic agent for certain forms of epilepsy, astroglial CB1 receptors might play an important role in the maintenance of epileptiform activity [76]. They react to glutamate and ATP produced during neuronal excitation by producing more glutamate and creating a positive feedback loop. The rise of glutamate provoked by astrocytes is also responsible for the impairment of spatial working memory (SWM) that results from cannabinoid exposure. The SWM deficits are generated by an astrocyte-dependent mechanism that is defined as hippocampal CB-LTD (LTD induced by cannabinoid exposure) [77]. Cannabinoids induce *in vivo* LTD of CA3-CA1 synapses as a result of an increase in glutamate following CB1 receptor activation. The excessive glutamate release activates NMDAr and provokes the internalization of AMPA receptors that lead to LTD.

There are differences in the signaling pathways activated by endogenous cannabinoids versus exogenous ones, in this case, THC. Depending on the ligand, CB1 receptor activation could lead to stimulation or inhibition of cyclooxygenase-2 (COX-2), an enzyme that converts arachidonic acid to prostanoids. The endogenous ligand 2-AG induces the suppression of COX-2 in response to inflammation or excitotoxicity. This differential cannabinoid modulation is determined by the activation of either the G-β- γ subunit (by THC) or the G-α-I subunit (by 2-AG) of the CB1 receptor-coupled G protein. The THC-induced rise in expression and activity of COX-2 is also accompanied by an increase in prostaglandin E2 (PGE2) levels. PGE2 in turn stimulates glutamate release and elevates extracellular glutamate levels. Notably, the THC-induced expression of COX-2 is particularly elevated in astrocytes. Persistent elevation of COX-2 will have a detrimental effect on synaptic integrity and plasticity and is associated with a reduced expression of glutamate receptor subunits GluA1, GluN2A, and GluN2B and a lower density of dendritic spines [78]. These observations reflect the main idea proposed by this review: Hippocampal CB1 receptor activity has complex consequences and sometimes opposing effects depending on its location [3,20] (Fig. 2).

Inside the cell: mitochondria

Mitochondrial CB1 receptor (mtCB1) activity has been connected to changes in bioenergetics and mitochondrial respiration that condition hippocampal synaptic transmission, and subsequently, memory consolidation [20,29,79]. The mtCB1 receptor reduces mitochondrial respiration by activating a subset of mitochondrial G_{α} proteins, namely $G_{\alpha i}$ proteins. This activation reduces protein kinase A (PKA) activity in the mitochondria and decreases the phosphorylation of proteins involved in oxidative phosphorylation (OXPHOS). OXPHOS is the biochemical process in which ATP is formed in the mitochondria by the transfer of electrons obtained from the citric acid cycle through the mitochondrial respiratory chain [3,80,81]. Considering that synaptic transmission has drastic energetic costs, any interruptions of mitochondrial respiration directly affect this activity. Activation of the mtCB1 receptor produces a reduction in ATP and interrupts excitatory synaptic transmission in the CA1- CA3 circuit, resulting in a disruption of long-term memory [29]. CB1 receptors have recently been described in hippocampal astroglial mitochondria [28], but their implications on astrocytic activity and potential modulation of hippocampal networks are only just starting to emerge. A recent study linked astroglial mtCB1 receptor activation to a disruption of glucose metabolism and lactate production [30]. The application of THC to cultured astrocytes leads to reduced phosphorylation of mitochondrial proteins, which in turn affects reactive oxygen species (ROS) levels, resulting in the downregulation of the transcription factor hypoxia-inducible factor 1 (HIF-1). Given that the HIF-1 pathway stimulates glycolysis, its downregulation has negative effects on glucose metabolism and lactate production. The authors link these metabolic changes to changes in social-associated behavioral responses in mice. In future studies, it would be interesting to see what impact these metabolic changes have in hippocampal astrocytes. Furthermore, other organelles such as endosomes have also

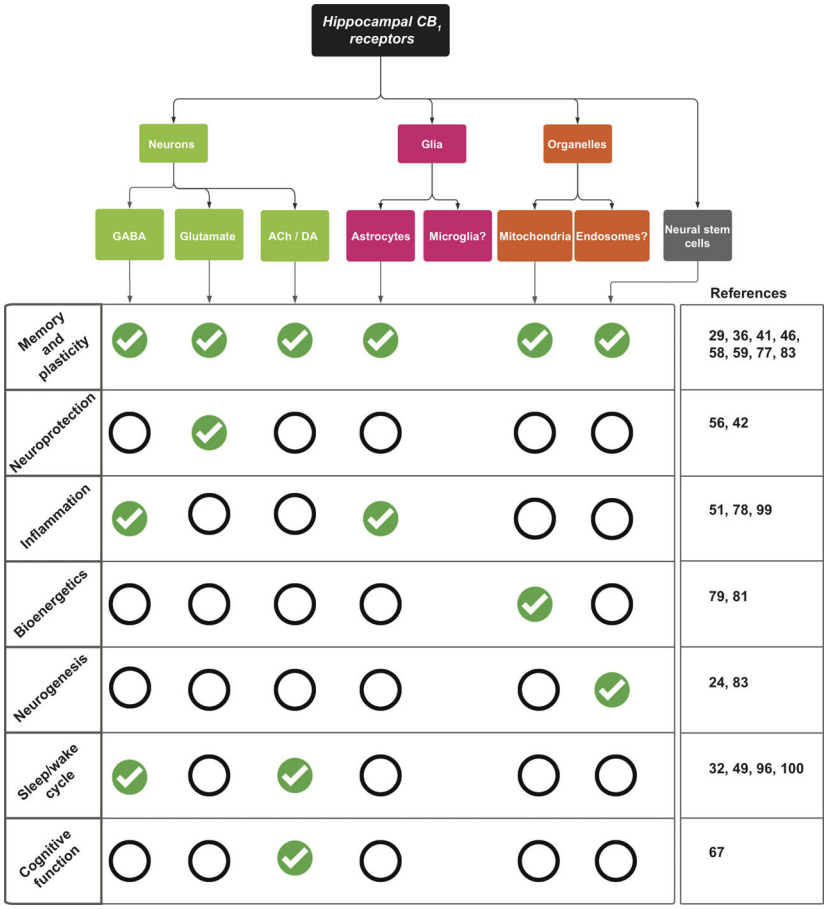

Fig. 2. Chart describing the subcellular specificity of CB1 receptors in hippocampal functions. Notably, there is an increasing amount of data studying the role of cannabinoid modulation of hippocampal circuits, and future studies will reveal the functional relevance of cannabinoid signaling in microglia, endosomes, and other cellular compartments.

been shown to express CB1 receptors [3]. Their presence in endosomal and lysosomal compartments has been related to the internalization postactivation of these receptors in the rat forebrain (including the hippocampus) [82]. Considering that there is limited data regarding this subclass of CB1 receptors, other studies must be done to fully characterize CB1 receptor expression in intracellular compartments.

Neurogenesis: neuronal stem cells

The subgranular zone of the dentate gyrus and the sub-ventricular area of the lateral ventricle are described as neurogenic niches in the adult mouse brain [25]. The newborn neurons produced in the dentate gyrus migrate to the granule cell layer. There, they are incorporated into local hippocampal circuitry, where they contribute to synaptic plasticity and hippocampal functions, like learning and memory. The ECS, particularly the CB1 receptor and its endogenous ligands have been involved in this process [24,83]. Previous studies demonstrate the presence of the CB1 receptor in neural stem cells (NSCs) and neural progenitor cells. Additionally, CB1 receptor deletion and inhibition of endocannabinoid synthesis result in a reduction in adult neurogenesis. The deletion of CB1 receptor from NSCs reduces the pool of astrocytes, neuroblasts, and neurons due to an altered proliferative capacity. CB1 receptor is also involved in the integration of adult-born neurons in hippocampal circuity; mice lacking this receptor have altered neuronal growth and dendritic morphology. These morphological changes translate to physiological alterations that affect LTP in the hippocampus, potentially leading to deficits in spatial memory and an enhancement of behavioral despair [83].

System-wide regulations: cannabinoid control of network oscillations

Brain functions are maintained by synchronous oscillatory activity of neuronal networks. Specifically,

hippocampal-dependent memories are supported by three types of network activity that aid the process of memory formation and consolidation. These are hippocampal theta (4–12 Hz), gamma (30–80 Hz), and ripple oscillations (100–200 Hz). The first two are hypothesized to be essential for the encoding of episodic memory, whereas ripple oscillations are associated with long-term memory consolidation [84]. By provoking variations in the firing patterns of hippocampal neurons, CB1 receptor agonists reduce cell assembly organization, and spike synchrony leading to changes in network oscillations. The lack of firing coordination as well as the reduction in power of local field potentials (especially theta power) correlated with memory impairments [20,84]. Apart from decreasing overall synchrony, the synergistic effects of systemic cannabinoid activation decrease the amplitude of the three types of network activity mentioned above, as well as reduce spiking correlation of the hippocampus and prefrontal cortex [85]. At a behavioral level, the activation of CB1 receptors by endogenous cannabinoids is thought to play a role in the downregulation of aversive memories and this could explain the physiological role for these changes [11,29,46].

The role of the hippocampus in the coding of spatial information is well characterized. Additionally, it also participates in the process of nonspatial information, like time [1]. In humans, a recent study concluded that THC administration leads to time overestimation, only in infrequent cannabis users [86]. Although the concept of specific time cells is in dispute, the neural substrate of the spatiotemporal construction probably lies within the hippocampus [1,87]. Regardless of whether it is time cells or more complex network activation patterns that dictate our perception of time, it is likely that these events are mediated by the ECS. Altogether, considering the well-known effect that THC has on the perception of time [86,88], the study of CB1 receptor activation and its impact on time perception is a complex, yet fascinating area of future research.

The lack of highly selective antibodies for CB2 as well as of a full CB2 knockout mouse have hindered the study of this receptor and led to controversy. Notwithstanding, evidence of a physiological role for CB2 receptors in neurons begins to emerge [4]. Stempel *et al*. have provided an in-depth description of CB2 receptor expression and characterized the effects of CB2 activation in hippocampal pyramidal neurons. Although the hyperpolarizing effects they observed in these neurons post-CB2 receptor activation were restricted to CA2/3, they acknowledge the possibility of similar effects extending to the whole of the hippocampal formation. The hyperpolarization of postsynaptic pyramidal neurons could serve a neuroprotective role complementary to that of CB1 and fine-tune hippocampal excitability. The modulation of excitability exerted by CB2 could also facilitate slow gamma oscillations in CA3 thus modifying local network rhythms and memory. Additionally, it is precisely in CA3 where the encoding of episodic memories proposed as the neural representation of consciousness takes place [2,89]. How, when, where, and to what extent CB2 activation modifies normal brain function will be a compelling topic in endocannabinoid research.

Sleep

Sleep can be described as a natural recurrent and reversible state of reduced wakefulness and responsiveness to external stimuli. It is composed by alternating transitions between rapid eye movement (REM) sleep and non-REM sleep. The latter includes slow wave sleep (SWS) and light sleep. REM sleep is more abundant in later parts of the night and is characterized by the desynchronization of neuronal networks, pontogeniculo-occipital (PGO) waves, and theta activity. REM sleep has been associated with both hippocampus-dependent and independent forms of memory consolidation. Additionally, REM sleep is characterized by a high cholinergic activity that has been proposed to support memory consolidation [90]. As reported by Rueda-Orozco *et al*., an injection of AEA directly in the hippocampus leads to an increased frequency (not duration) of REM sleep when administered in the dark phase (active phase) of a 12 h light/dark cycle. Given that they had previously found that the concentrations of endocannabinoids and the density of CB1 receptor varied depending on the light-dark cycle [48], they also studied the effect that endocannabinoids had on sleep at different times of the day. They hypothesize that the CB1 receptor could play a role in the generation of REM sleep and that the administration of endocannabinoids could favor REM sleep in an unfavorable context such as insomnia [91]. An overall reduction in REM sleep has also been observed after administration of various types of CB1 receptor agonists. The reduction in time spent in REM sleep can also be explained by reduced cholinergic activity provoked by high doses of CB1 receptor agonists. CB1 receptor activation also leads to an increase in total time spent sleeping. On the contrary, CB1 receptor antagonists have the opposite effect and lead to an increase in wakefulness [49].

The ECS is considered to act as a homeostatic modulator of plasticity and has the capacity to promote not

only potentiation of synaptic strength but also downscaling or inhibition of certain synapses [44,92]. Intriguingly, these processes also occur during REM sleep states [93,94]. Endocannabinoids have also been shown to disrupt sharp wave ripples (SWR). SWRs are fast oscillations (120–250 Hz) that occur during slow wave sleep and are associated with memory consolidation. The reduction in neurotransmitter release provoked by AEA in hippocampal slices reduced the frequency of SWR and reorganizes spiking patterns [95]. This finding could be linked to the memory deficits associated with the use of exogenous cannabinoids. Additionally, the ECS has also been proposed as an influence over the content of dreams, given its ability to modulate cognitive, emotional, and memory-related processes as well as consciousness and wakefulness [96].

Pathophysiology

ECS dysfunction is associated with a significant number of pathologies. As mentioned before, the ECS mediates inflammatory responses via GABAergic neurons and astrocytes and neuroprotective functions via glutamatergic neurons. Furthermore, alterations in the ECS could underlie age-related neurodegeneration [97]. A reduced expression of CB1 receptors in the hippocampus is linked to age-related cognitive decline and the deletion of CB1 receptors from GABAergic hippocampal neurons led to neuronal loss and increased inflammation [98]. Additionally, disrupted endocannabinoid signaling between GABAergic neurons and microglia is linked to age-related dysfunction and development of neurodegenerative disorders [99]. Given that the CB1 receptor is also involved in sleep regulation, these changes have also been proposed to contribute to sleep disturbances in the elderly [100]. Alterations in CB1 receptor signaling specific to glutamatergic neurons could also play a role in age-related cognitive decline and reduced synaptic integrity and plasticity. Given that CB1 receptor activation increased BDNF expression, a reduction in levels of this neurotrophic factor could contribute to age-related pathologies and excitotoxicity [97].

Interestingly, abnormalities in the ECS have been found in both animal models of Alzheimer's disease (AD) and human brain samples. As explained by Thompson 2020, these alterations depend on the stage of the disease. Early AD is characterized by an increase in CB1 receptor expression and activity while the opposite occurs in later stages of the disease. Accordingly, the deletion of CB1 receptors worsened AD progression and synaptic dysfunction [101]. On the other hand, components of the ECS have been proposed as biomarkers for mood and neuropsychiatric disorders [102]. Decreased endocannabinoid tone has been reported in rat models of chronic stress as well as in women with major depression [103]. Moreover, The ECS is also consistently disrupted in schizophrenia [104,105]. Animal models of schizophrenia have shown decreased levels of CB1 receptors in the hippocampus, and human postmortem brain samples have shown altered levels of endocannabinoids AEA and 2-AG [105,106].

Recent data seem to support the possibility that CB2 receptor signaling mediates microglial gene expression, influencing the fluctuations between proinflammatory (M1), neuroprotective (M2), and homeostatic (Mo) activations [87]. Considering that alterations in microglial activation have been associated with AD, schizophrenia, stress, and autism, further studies regarding these interactions could shed light on the involvement of the hippocampal ECS in these pathologies [99,107–109]. Preliminary data from our laboratory support CB1 receptor localization in microglial cells (Grandes *et al.*, personal communication). However, the precise role of both CB1 and CB2 receptors on microglia and the related inflammatory impact on hippocampal functions is still to be determined.

Alterations in endocannabinoid signaling and neuroinflammation are strongly linked to epileptic seizures. The ECS's role of maintaining a neuroprotective, anti-inflammatory environment and preventing excitotoxicity make it a major player in the pathophysiology of epilepsy [110]. Thus, cannabinoid activity acts as a break for recurrent excitatory signaling [62], making it an important drug target for epilepsy [111]. Despite this fact, the multiphasic cannabinoid action raises some issues, as we have also stated before in this review. A decreased activation of the ECS at inhibitory synapses will potentiate network excitation whereas the same decrease in excitatory synapses will have the opposite effect. The role of astrocytic CB1 receptors in maintaining epileptiform activity must also be considered (see section on astrocytes). Lastly, potentiating the ECS's anti-inflammatory actions requires a careful study of downstream signaling cascades and interactions. In summary, the functional dissection of hippocampal CB1 receptors is crucial to further understand brain disorders and age-related pathologies.

Conclusions

It is more and more evident that 'the *where* matters' when studying the influence of CB1 receptor activity in

pathophysiological processes. In the hippocampus, canonical activation of CB1 receptors in GABAergic cells participates in the consolidation of memories and downscaling of possibly aversive memories [11,35,36,46]. On the other hand, cannabinoid action on glutamatergic cells regulates LTP and serves a neuroprotective function by suppressing glutamate release and favoring the secretion of neurotrophic factors [53,54,62]. Meanwhile, actions in astrocytes stand out because they 'contradict' previously stated effects. CB1 receptors in astrocytes participate in a positive feedback loop activated by glutamate and produce the NMDAr coagonist D-Serine thus favoring glutamatergic activity [42,73,76]. Furthermore, mtCB1 receptors regulate mitochondrial respiration and neuronal energy metabolism, resulting in modulation of memory processes and social recognition [29,79]. Finally, CB1 receptors present in neural stem cells enhance adult neurogenesis and facilitate the integration of new neurons into hippocampal circuitry [12,24]. Thus, hippocampal cannabinoid signaling forms a key molecular mechanism that composes the cellular basis of cognitive functions and related pathological conditions.

Acknowledgements

This work was supported by Ikerbasque, MINECO (Ministerio de Economía y Competitividad) PGC2018-093990-A-I00 (MICIU/AEI/FEDER, UE), and Endeavour Scholarship Scheme (Malta-EU) (to E.S.-G.); Basque Government (IT1230-19), Red de Trastornos Adictivos, Instituto de Salud Carlos III (ISC-III) and European Regional Development Funds-European Union (ERDF-EU; RD16/0017/0012), Ministry of Science and Innovation (PID2019-107548RB-I00) (to P.G.).

Conflict of interest

The authors declare no conflict of interest.

Author contributions

All authors contributed to the writing of the manuscript.

References

1 Lisman J, Buzsáki G, Eichenbaum H, Nadel L, Rangananth C & Redish AD (2017) Viewpoints: how the hippocampus contributes to memory, navigation and cognition. *Nat Neurosci* **20**, 1434–1447.
2 Behrendt RP (2017) Hippocampus as a wormhole: gateway to consciousness. *Wiley Interdiscip Rev Cogn Sci* **8**, e1446.
3 Busquets-Garcia A, Bains J & Marsicano G. (2018) CB1 receptor signaling in the brain: extracting specificity from ubiquity. *Neuropsychopharmacology* **43**, 4–20.
4 Chen DJ, Gao M, Gao FF, Su QX & Wu J (2017) Brain cannabinoid receptor 2: expression, function and modulation. *Acta Pharmacol Sin* **38**, 312–316.
5 López A, Aparicio N, Pazos MR, Grande MT, Barreda-Manso MA, Benito-Cuesta I, Vázquez C, Amores M, Ruiz-Pérez G, García-García E *et al.* (2018) Cannabinoid CB 2 receptors in the mouse brain: relevance for Alzheimer's disease. *J Neuroinflammation* **15**, 1–11.
6 Jordan CJ & Xi ZX (2019) Progress in brain cannabinoid CB2 receptor research: from genes to behavior. *Neurosci Biobehav Rev* **98**, 208–220.
7 Scarante FF, Vila-Verde C, Detoni VL, Ferreira-Junior NC, Guimarães FS & Campos AC (2017) Cannabinoid modulation of the stressed hippocampus. *Front Mol Neurosci* **10**, 1–17.
8 Zhang H-Y, Shen H, Jordan CJ, Liu Q-R, Gardner EL, Bonci A & Xi Z-X (2019) CB 2 receptor antibody signal specificity: correlations with the use of partial CB 2 -knockout mice and anti-rat CB 2 receptor antibodies. *Acta Pharmacol Sin* **40**, 398–409.
9 Atwood BK & MacKie K (2010) CB2: a cannabinoid receptor with an identity crisis. *Br J Pharmacol* **160**, 467–479.
10 Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M & Watanabe M (2009) Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev* **89**, 309–380.
11 Younts TJ & Castillo PE (2014) Endogenous cannabinoid signaling at inhibitory interneurons. *Curr Opin Neurobiol* **26**, 42–50.
12 Galve-Roperh I, Chiurchiù V, Díaz-Alonso J, Bari M, Guzmán M & Maccarrone M (2013) Cannabinoid receptor signaling in progenitor/stem cell proliferation and differentiation. *Prog Lipid Res* **52**, 633–650.
13 Mackie K (2005) Cannabinoid receptor homo- and heterodimerization. *Life Sci* **77**, 1667–1673.
14 Hojo M, Sudo Y, Ando Y, Minami K, Takada M, Matsubara T, Kanaide M, Taniyama K, Sumikawa K & Uezono Y (2008) μ-opioid receptor forms a functional heterodimer with cannabinoid CB1 receptor: electrophysiological and FRET assay analysis. *J Pharmacol Sci* **108**, 308–319.
15 Kearn C, Blake-Palmer K, Scotter E, Mackie K & Glass M (2005) Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? *Mol Pharmacol* **67**, 1697–1704.
16 Ellis J, Pediani JD, Canals M, Milasta S & Milligan G (2006) Orexin-1 receptor-cannabinoid CB1 receptor heterodimerization results in both ligand-dependent

and -independent coordinated alterations of receptor localization and function*. *J Biol Chem* **281**, 38812–38824.
17 Maroteaux L, Béchade C & Roumier A (2019) Dimers of serotonin receptors: impact on ligand affinity and signaling. *Biochimie* **161**, 23–33.
18 Aso E, Fernández-Dueñas V, López-Cano M, Taura J, Watanabe M, Ferrer I, Luján R & Ciruela F (2019) Adenosine A2A-cannabinoid CB1 receptor heteromers in the hippocampus: cannabidiol blunts Δ9-tetrahydrocannabinol-induced cognitive impairment. *Mol Neurobiol* **56**, 5382–5391.
19 Zhu F, Wang X-Q, Chen Y-N, Yang N, Lang S-Y, Zuo P-P, Zhang J-T & Li R-S (2015) Changes and overlapping distribution in the expression of CB1/OX1-GPCRs in rat hippocampus by kainic acid-induced status epilepticus. *Brain Res* **1597**, 14–27.
20 Busquets-Garcia A, Desprez T, Metna-Laurent M, Bellocchio L, Marsicano G & Soria-Gomez E (2015) Dissecting the cannabinergic control of behavior: the *where* matters. *BioEssays* **37**, 1215–1225.
21 Hoffman AF, Hwang E-K & Lupica CR (2020) Impairment of synaptic plasticity by cannabis, Δ 9 -THC, and synthetic cannabinoids. *Cold Spring Harb Perspect Med* **11**, a039743.
22 Soria-Gomez E, Metna M, Bellocchio L, Busquets-Garcia A & Marsicano G (2017) The endocannabinoid system in the control of behavior. In Handbook of Neurobehavioral Genetics and Phenotyping (Tucci V, ed), pp. 323–355. John Willey & Sons, Inc., New York, NY.
23 Hampson RE & Deadwyler SA (1999) Cannabinoids, hippocampal function and memory. *Life Sci* **65**, 715–723.
24 Oddi S, Scipioni L & Maccarrone M (2020) Endocannabinoid system and adult neurogenesis: a focused review. Endocannabinoid system and adult neurogenesis: a focused review. *Curr Opin Pharmacol* **50**, 25–32.
25 Petrik D & Encinas JM (2019) Perspective: of mice and men – how widespread is adult neurogenesis? *Front Neurosci* **13**, 923.
26 Wu W, Brickman AM, Luchsinger J, Ferrazzano P, Pichiule P, Yoshita M, Brown T, Decarli C, Barnes CA, Mayeux R *et al.* (2008) The brain in the age of old: the hippocampal formation is targeted differentially by diseases of late life. *Ann Neurol* **64**, 698–706.
27 Li H, Liu Y, Gao X, Liu L, Amuti S, Wu D, Jiang F, Huang L, Wang G, Zeng J *et al.* (2019) Neuroplastin 65 modulates anxiety- and depression-like behavior likely through adult hippocampal neurogenesis and central 5- \textlessscp\textgreaterHT\textless/scp \textgreater activity. *FEBS J* **286**, 3401–3415.
28 Gutiérrez-Rodríguez A, Bonilla-Del Río I, Puente N, Gómez-Urquijo SM, Fontaine CJ, Egaña-Huguet J, Elezgarai I, Ruehle S, Lutz B, Robin LM *et al.* (2018) Localization of the cannabinoid type-1 receptor in subcellular astrocyte compartments of mutant mouse hippocampus. *Glia* **66**, 1417–1431.
29 Hebert-Chatelain E, Desprez T, Serrat R, Bellocchio L, Soria-Gomez E, Busquets-Garcia A, Pagano Zottola AC, Delamarre A, Cannich A, Vincent P *et al.* (2016) A cannabinoid link between mitochondria and memory. *Nature* **539**, 555–559.
30 Jimenez-Blasco D, Busquets-Garcia A, Hebert-Chatelain E, Serrat R, Vicente-Gutierrez C, Ioannidou C, Gómez-Sotres P, Lopez-Fabuel I, Resch-Beusher M, Resel E *et al.* (2020) Glucose metabolism links astroglial mitochondria to cannabinoid effects. *Nature* **583**, 603–608.
31 Gutiérrez-Rodríguez A, Puente N, Elezgarai I, Ruehle S, Lutz B, Reguero L, Gerrikagoitia I, Marsicano G & Grandes P (2017) Anatomical characterization of the cannabinoid CB1 receptor in cell-type–specific mutant mouse rescue models. *J Comp Neurol* **525**, 302–318.
32 Pelkey KA, Chittajallu R, Craig MT, Tricoire L, Wester JC & McBain CJ (2017) Hippocampal gabaergic inhibitory interneurons. *Physiol Rev* **97**, 1619–1747.
33 Katona I, Sperlágh B, Sík A, Käfalvi A, Vizi ES, Mackie K & Freund TF (1999) Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J Neurosci* **19**, 4544–4558.
34 Puighermanal E, Marsicano G, Busquets-Garcia A, Lutz B, Maldonado R & Ozaita A (2009) Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling. *Nat Neurosci* **12**, 1152–1158.
35 Busquets-Garcia A, Oliveira da Cruz JF, Terral G, Zottola ACP, Soria-Gómez E, Contini A, Martin H, Redon B, Varilh M, Ioannidou C *et al.* (2018) Hippocampal CB1 receptors control incidental associations. *Neuron* **99**, 1247–1259.e7.
36 Oliveira da Cruz JF, Busquets-Garcia A, Zhao Z, Varilh M, Lavanco G, Bellocchio L, Robin L, Cannich A, Julio-Kalajzić F, Lesté-Lasserre T *et al.* (2020) Specific hippocampal interneurons shape consolidation of recognition memory. *Cell Rep* **32**, 108046.
37 Espadas I, Ortiz O, García-Sanz P, Sanz-Magro A, Alberquilla S, Solis O, Delgado-García JM, Gruart A & Moratalla R (2020) Dopamine D2R is required for hippocampal-dependent memory and plasticity at the CA3-CA1 synapse. *Cereb Cortex* **31**: 1–18.
38 Duszkiewicz AJ, McNamara CG, Takeuchi T & Genzel L (2019) Novelty and dopaminergic modulation of memory persistence: a tale of two systems. *Trends Neurosci* **42**, 102–114.
39 Tsou K, Mackie K, Sañudo-Peña MC & Walker JM (1999) Cannabinoid CB1 receptors are localized

primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. *Neuroscience* **93**, 969–975.
40 Liu X, Dimidschstein J, Fishell G & Carter AG (2020) Hippocampal inputs engage CCK+ interneurons to mediate endocannabinoid-modulated feed-forward inhibition in the prefrontal cortex. *eLife* **9**. http://dx.doi.org/10.7554/elife.55267
41 Kemp A & Manahan-Vaughan D (2004) Hippocampal long-term depression and long-term potentiation encode different aspects of novelty accquisition. *Proc Natl Acad Sci USA* **101**, 8192–8197.
42 Robin LM, Oliveira da Cruz JF, Langlais VC, Martin-Fernandez M, Metna-Laurent M, Busquets-Garcia A, Bellocchio L, Soria-Gomez E, Papouin T, Varilh M *et al.* (2018) Astroglial CB1 receptors determine synaptic D-serine availability to enable recognition memory. *Neuron* **98**, 935–944.e5.
43 Silva-Cruz A, Carlström M, Ribeiro JA & Sebastião AM (2017) Dual influence of endocannabinoids on long-term potentiation of synaptic transmission. *Front Pharmacol* **8**, 921.
44 Mouro FM, Köfalvi A, André LA, Baqi Y, Müller CE, Ribeiro JA & Sebastião AM (2019) Memory deficits induced by chronic cannabinoid exposure are prevented by adenosine A2AR receptor antagonism. *Neuropharmacology* **155**, 10–21.
45 Hampson RE, Miller F, Palchik G & Deadwyler SA (2011) Cannabinoid receptor activation modifies NMDA receptor mediated release of intracellular calcium: implications for endocannabinoid control of hippocampal neural plasticity. *Neuropharmacology* **60**, 944–952.
46 Chevaleyre V & Castillo PE (2003) Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. *Neuron* **38**, 461–472.
47 Mato S, Chevaleyre V, Robbe D, Pazos A, Castillo PE & Manzoni OJ (2004) A single in-vivo exposure to Δ9THC blocks endocannabinoid-mediated synaptic plasticity. *Nat Neurosci* **7**, 585–586.
48 Rueda-Orozco PE, Soria-Gomez E, Montes-Rodriguez CJ, Martínez-Vargas M, Galicia O, Navarro L & Prospero-García O (2008) A potential function of endocannabinoids in the selection of a navigation strategy by rats. *Psychopharmacology* **198**, 565–576.
49 Goonawardena AV, Plano A, Robinson L, Ross R, Greig I, Pertwee RG, Hampson RE, Platt B, Riedel G & Author BP (2015) Modulation of food consumption and sleep-wake cycle in mice by the neutral CB 1 antagonist ABD459 HHS Public Access Author manuscript. *Behav Pharmacol* **26**, 289–303.
50 Hillard CJ (2018) Circulating endocannabinoids: from whence do they come and where are they going? *Neuropsychopharmacology* **43**, 155–172.
51 Ativie F, Komorowska JA, Beins E, Albayram Ö, Zimmer T, Zimmer A, Tejera D, Heneka M & Bilkei-Gorzo A (2018) Cannabinoid 1 receptor signaling on hippocampal GABAergic neurons influences microglial activity. *Front Mol Neurosci* **11**, 1–14.
52 Andersen P, Morris R, Amaral D, Bliss T & O'Keefe J (2009) The Hippocampus Book. Oxford University Press, Oxford.
53 Monory K, Massa F, Egertová M, Eder M, Blaudzun H, Westenbroek R, Kelsch W, Jacob W, Marsch R, Ekker M *et al.* (2006) The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron* **51**, 455–466.
54 Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, Azad SC, Cascio MG, Ortega-Gutiérrez S, Van der Stelt M *et al.* (2003) CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* **302**, 84–88.
55 Shubina L, Aliev R & Kitchigina V (2017) Endocannabinoid-dependent protection against kainic acid-induced long-term alteration of brain oscillations in guinea pigs. *Brain Res* **1661**, 1–14.
56 Calabrese F, Rossetti AC, Racagni G, Gass P, Riva MA & Molteni R (2014) Brain-derived neurotrophic factor: a bridge between inflammation and neuroplasticity. *Front Cell Neurosci* **8**, 1–7.
57 Kaczmarek L (2018) From c-Fos to MMP-9: in control of synaptic plasticity to produce healthy and diseased mind, a personal view. *Postepy Biochem* **64**, 101–109.
58 Peñasco S, Rico-Barrio I, Puente N, Gómez-Urquijo SM, Fontaine CJ, Egaña-Huguet J, Achicallende S, Ramos A, Reguero L, Elezgarai I *et al.* (2019) Endocannabinoid long-term depression revealed at medial perforant path excitatory synapses in the dentate gyrus. *Neuropharmacology* **153**, 32–40.
59 Fontaine CJ, Gräfe EL, Pinar C, Bonilla-Del Río I, Grandes P & Christie BR (2020) Endocannabinoid receptors contribute significantly to multiple forms of long-term depression in the rat dentate gyrus. *Learn Mem* **27**, 380–389.
60 Peñasco S, Rico-Barrio I, Puente N, Fontaine CJ, Ramos A, Reguero L, Gerrikagoitia I, de Fonseca FR, Suarez J, Barrondo S *et al.* (2020) Intermittent ethanol exposure during adolescence impairs cannabinoid type 1 receptor-dependent long-term depression and recognition memory in adult mice. *Neuropsychopharmacology* **45**, 309–318.
61 Rico-Barrio I, Peñasco S, Puente N, Ramos A, Fontaine CJ, Reguero L, Giordano ME, Buceta I, Terradillos I, Lekunberri L *et al.* (2019) Cognitive and neurobehavioral benefits of an enriched environment on young adult mice after chronic ethanol consumption during adolescence. *Addict Biol* **24**, 969–980.

62 Jensen KR, Berthoux C, Nasrallah K & Castillo PE (2021) Multiple cannabinoid signaling cascades powerfully suppress recurrent excitation in the hippocampus. *Proc Natl Acad Sci USA* **118**, e2017590118.
63 Bonilla-Del Río I, Puente N, Mimenza A, Ramos A, Serrano M, Lekunberri L, Gerrikagoitia I, Christie BR, Nahirney PC & Grandes P (2021) Acute Δ9 - tetrahydrocannabinol prompts rapid changes in cannabinoid CB 1 receptor immunolabeling and subcellular structure in CA1 hippocampus of young adult male mice. *J Comp Neurol* **529**, 2332–2346.
64 Li Y & Kim J (2015) Neuronal expression of CB2 cannabinoid receptor mRNAs in the mouse hippocampus. *Neuroscience* **311**, 253–267.
65 Degroot A, Köfalvi A, Wade MR, Davis RJ, Rodrigues RJ, Rebola N, Cunha RA & Nomikos GG (2006) CB1 receptor antagonism increases hippocampal acetylcholine release: site and mechanism of action. *Mol Pharmacol* **70**, 1236–1245.
66 Takács VT, Cserép C, Schlingloff D, Pósfai B, Szőnyi A, Sos KE, Környei Z, Dénes Á, Gulyás AI, Freund TF & *et al*. (2018) Co-transmission of acetylcholine and GABA regulates hippocampal states. *Nat Commun* **9**, 1–15.
67 Chaperon F & Thiébot MH (1999) Behavioral effects of cannabinoid agents in animals. *Crit Rev Neurobiol* **13**, 243–281.
68 Tzavara ET, Wade M & Nomikos GG (2003) Biphasic effects of cannabinoids on acetylcholine release in the hippocampus: site and mechanism of action. *J Neurosci* **23**, 9374–9384.
69 Wyrofsky RR, Reyes BAS, Zhang XY, Bhatnagar S, Kirby LG & VanBockstaele EJ (2019) Endocannabinoids, stress signaling, and the locus coeruleus-norepinephrine system. *Neurobiol Stress* **11**, 100176.
70 Loureiro M, Renard J, Zunder J & Laviolette SR (2015) Hippocampal cannabinoid transmission modulates dopamine neuron activity: impact on rewarding memory formation and social interaction. *Neuropsychopharmacology* **40**, 1436–1447.
71 Perea G, Navarrete M & Araque A (2009) Tripartite synapses: astrocytes process and control synaptic information. *Trends Neurosci* **32**, 421–431.
72 Navarrete M & Araque A (2008) Endocannabinoids mediate neuron-astrocyte communication. *Neuron* **57**, 883–893.
73 Navarrete M & Araque A (2010) Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. *Neuron* **68**, 113–126.
74 Murtas G, Marcone GL, Sacchi S & Pollegioni L (2020) L-serine synthesis via the phosphorylated pathway in humans. *Cell Mol Life Sci* **77**, 5131–5148.
75 Bonilla-Del Río I, Puente N, Peñasco S, Rico I, Gutiérrez-Rodríguez A, Elezgarai I, Ramos A, Reguero L, Gerrikagoitia I, Christie BR *et al*. (2019) Adolescent ethanol intake alters cannabinoid type-1 receptor localization in astrocytes of the adult mouse hippocampus. *Addict Biol* **24**, 182–192.
76 Coiret G, Ster J, Grewe B, Wendling F, Helmchen F, Gerber U & Benquet P (2012) Neuron to astrocyte communication via cannabinoid receptors is necessary for sustained epileptiform activity in rat hippocampus. *PLoS One* **7**, 1–8.
77 Han J, Kesner P, Metna-Laurent M, Duan T, Xu L, Georges F, Koehl M, Abrous DN, Mendizabal-Zubiaga J, Grandes P *et al*. (2012) Acute cannabinoids impair working memory through astroglial CB 1 receptor modulation of hippocampal LTD. *Cell* **148**, 1039–1050.
78 Chen R, Zhang J, Fan N, Teng ZQ, Wu Y, Yang H, Tang YP, Sun H, Song Y & Chen C (2013) XΔ9-THC-caused synaptic and memory impairments are mediated through COX-2 signaling. *Cell* **155**, 1154.
79 Bénard G, Massa F, Puente N, Lourenço J, Bellocchio L, Soria-Gómez E, Matias I, Delamarre A, Metna-Laurent M, Cannich A *et al*. (2012) Mitochondrial CB 1 receptors regulate neuronal energy metabolism. *Nat Neurosci* **15**, 558–564.
80 Hebert-Chatelain E, Reguero L, Puente N, Lutz B, Chaouloff F, Rossignol R, Piazza P-V, Benard G, Grandes P & Marsicano G (2014) Studying mitochondrial CB1 receptors: yes we can. *Mol Metab* **3**, 339.
81 Hebert-Chatelain E, Reguero L, Puente N, Lutz B, Chaouloff F, Rossignol R, Piazza PV, Benard G, Grandes P & Marsicano G (2014) Cannabinoid control of brain bioenergetics: exploring the subcellular localization of the CB1 receptor. *Mol Metab* **3**, 495–504.
82 Thibault K, Carrel D, Bonnard D, Gallatz K, Simon A, Biard M, Pezet S, Palkovits M & Lenkei Z (2013) Activation-dependent subcellular distribution patterns of CB1 cannabinoid receptors in the rat forebrain. *Cereb Cortex* **23**, 2581–2591.
83 Zimmermann T, Maroso M, Beer A, Baddenhausen S, Ludewig S, Fan W, Vennin C, Loch S, Berninger B, Hofmann C *et al*. (2018) Neural stem cell lineage-specific cannabinoid type-1 receptor regulates neurogenesis and plasticity in the adult mouse hippocampus. *Cereb Cortex* **28**, 4454–4471.
84 Robbe D, Montgomery SM, Thome A, Rueda-Orozco PE, McNaughton BL & Buzsaki G (2006) Cannabinoids reveal importance of spike timing coordination in hippocampal function. *Nat Neurosci* **9**, 1526–1533.
85 Sales-Carbonell C, Rueda-Orozco PE, Soria-Gómez E, Buzsáki G, Marsicano G & Robbe D (2013) Striatal

GABAergic and cortical glutamatergic neurons mediate contrasting effects of cannabinoids on cortical network synchrony. *Proc Natl Acad Sci USA* **110**, 719–724.
86 Sewell RA, Schnakenberg A, Elander J, Radhakrishnan R, Williams A, Skosnik PD, Pittman B, Ranganathan M & D'Souza DC (2013) Acute effects of THC on time perception in frequent and infrequent cannabis users. *Psychopharmacology* **226**, 401–413.
87 Umbach G, Kantak P, Jacobs J, Kahana M, Pfeiffer BE, Sperling M & Lega B (2020) Time cells in the human hippocampus and entorhinal cortex support episodic memory. *Proc Natl Acad Sci USA* **117**, 28463–28474.
88 Atakan Z, Morrison P, Bossong MG, Martin-Santos R & Crippa JA (2012) The effect of cannabis on perception of time: a critical review. *Curr Pharm Des* **18**, 4915–4922.
89 Faw M & Faw B (2017) Neurotypical subjective experience is caused by a hippocampal simulation. *Wiley Interdiscip Rev Cogn Sci* **8**, e1412.
90 Diekelmann S & Born J (2010) The memory function of sleep. *Nat Rev Neurosci* **11**, 114–126.
91 Rueda-Orozco PE, Soria-Gómez E, Montes-Rodríguez CJ, Pérez-Morales M & Prospéro-García O (2010) Intrahippocampal administration of anandamide increases REM sleep. *Neurosci Lett* **473**, 158–162.
92 Li W, Ma L, Yang G & Gan WB (2017) REM sleep selectively prunes and maintains new synapses in development and learning. *Nat Neurosci* **20**, 427–437.
93 Grosmark AD, Mizuseki K, Pastalkova E, Diba K & Buzsáki G (2012) REM sleep reorganizes hippocampal excitability. *Neuron* **75**, 1001–1007.
94 Niethard N & Born J (2019) Back to baseline: sleep recalibrates synapses. *Nat Neurosci* **22**, 149–151.
95 Sun Y, Norimoto H, Pu XP, Matsuki N & Ikegaya Y (2012) Cannabinoid receptor activation disrupts the internal structure of hippocampal sharp wave-ripple complexes. *J Pharmacol Sci* **118**, 288–294.
96 Murillo-Rodriguez E, Pastrana-Trejo JC, Salas-Crisóstomo M & De-la-Cruz M (2017) The endocannabinoid system modulating levels of consciousness, emotions and likely dream contents. *CNS Neurol Disord Drug Targets* **16**, 370–379.
97 Bilkei-Gorzo A (2012) The endocannabinoid system in normal and pathological brain ageing. *Philos Trans R Soc B Biol Sci* **367**, 3326–3341.
98 Albayram O, Alferink J, Pitsch J, Piyanova A, Neitzert K, Poppensieker K, Mauer D, Michel K, Legler A, Becker A *et al.* (2011) Role of CB1 cannabinoid receptors on GABAergic neurons in brain aging. *Proc Natl Acad Sci USA* **108**, 11256–11261.
99 Carrera J, Tomberlin J, Kurtz J, Karakaya E, Bostanciklioglu M & Albayram O (2020) Endocannabinoid signaling for GABAergic-microglia (mis)communication in the brain aging. *Front Neurosci* **14**, 606808.
100 Murillo-Rodríguez E, Budde H, Veras AB, Rocha NB, Telles-Correia D, Monteiro D, Cid L, Yamamoto T, Machado S & Torterolo P (2020) The endocannabinoid system may modulate sleep disorders in aging. *Curr Neuropharmacol* **18**, 97–108.
101 Aso E, Andrés-Benito P & Ferrer I (2018) Genetic deletion of CB1 cannabinoid receptors exacerbates the Alzheimer-like symptoms in a transgenic animal model. *Biochem Pharmacol* **157**, 210–216.
102 Navarrete F, García-Gutiérrez MS, Jurado-Barba R, Rubio G, Gasparyan A, Austrich-Olivares A & Manzanares J (2020) Endocannabinoid system components as potential biomarkers in psychiatry. *Front Psychiatry* **11**, 1–30.
103 Parolaro D, Realini N, Vigano D, Guidali C & Rubino T (2010) The endocannabinoid system and psychiatric disorders. *Exp Neurol* **224**, 3–14.
104 Fakhoury M (2016) Could cannabidiol be used as an alternative to antipsychotics? *J Psychiatr Res* **80**, 14–21.
105 Fakhoury M (2017) Role of the endocannabinoid system in the pathophysiology of schizophrenia. *Mol Neurobiol* **54**, 768–778.
106 Muguruza C, Morentin B, Meana JJ, Alexander SP & Callado LF (2019) Endocannabinoid system imbalance in the postmortem prefrontal cortex of subjects with schizophrenia. *J Psychopharmacol (Oxf)* **33**, 1132–1140.
107 Beins EC, Beiert T, Jenniches I, Hansen JN, Leidmaa E, Schrickel JW & Zimmer A (2021) Cannabinoid receptor 1 signalling modulates stress susceptibility and microglial responses to chronic social defeat stress. *Transl Psychiatry* **11**, 1–15.
108 Araujo DJ, Tjoa K & Saijo K (2019) The endocannabinoid system as a window into microglial biology and its relationship to autism. *Front Cell Neurosci* **13**, 1–7.
109 Tanaka M, Sackett S & Zhang Y (2020) Endocannabinoid modulation of microglial phenotypes in neuropathology. *Front Neurol* **11**, 1–17.
110 Kwan Cheung KA, Peiris H, Wallace G, Holland OJ & Mitchell MD (2019) The interplay between the endocannabinoid system, epilepsy and cannabinoids. *Int J Mol Sci* **20**, 1–21.
111 Morano A, Fanella M, Albini M, Cifelli P, Palma E, Giallonardo AT & Di Bonaventura C (2020) Cannabinoids in the treatment of epilepsy: current status and future prospects. *Neuropsychiatr Dis Treat* **16**, 381–396.