

**Cellular and intracellular mechanisms involved in the cognitive impairment of cannabinoids**

Emma Puighermanal<sup>§</sup>, Arnau Busquets-Garcia, Rafael Maldonado, Andrés Ozaita<sup>#</sup>.

Departament de Ciències Experimentals i de la Salut, Facultat de Ciències de la Salut i de la Vida. Universitat Pompeu Fabra. Barcelona, Spain.

<sup>§</sup>Current address: Institut de Génomique Fonctionnelle, Inserm U661, CNRS UMR 5203, 34094 Montpellier Cedex 05, France

Keywords: memory, intracellular signalling, endocannabinoid system, cannabinoid receptor, CB1

Running title: Signalling in cannabinoid-mediated memory impairment

<sup>#</sup> Corresponding author: Andrés Ozaita, Departament de Ciències Experimentals i de la Salut, Facultat de Ciències de la Salut i de la Vida, Universitat Pompeu Fabra, Parc de Recerca Biomèdica de Barcelona, C/ Doctor Aiguader 88, 08003 Barcelona, Spain. Phone: +34-93-3160823; Fax: + 34-93-3160901; E-mail: andres.ozaita@upf.edu

**Abstract**

Exogenous cannabinoids, such as delta9-tetrahydrocannabinol (THC), as well as the modulation of endogenous cannabinoids, affect cognitive function through the activation of cannabinoid receptors. Indeed, these compounds modulate in the brain the activity of a number of intracellular signalling pathways, some critically implicated in the deleterious effect of cannabinoids on learning and memory. Thus, the involvement of the mammalian target of rapamycin (mTOR) pathway and extracellular signal-regulated kinases (ERKs), together with their consequent regulation of cellular processes such as protein translation, play a critical role in the amnesic-like effects of cannabinoids. In the present article we summarize the cellular and molecular mechanisms reported in the modulation of cognitive function by the endocannabinoid system.

## 1. Natural and synthetic cannabinoids affecting cognition

Marijuana and other derivatives of the plant *Cannabis sativa* have been used for recreational and medical purposes for thousands of years. To date, more than 70 unique compounds derived from the hemp plant named phytocannabinoids have been identified [1]. The main psychoactive ingredient of cannabis is delta9-tetrahydrocannabinol (THC) [2]. Since its discovery, the pharmacological effects of THC have been extensively characterized in animal models as well as in humans. These pharmacological effects are well known in humans and include mood-altering properties, sedation, impairments of memory and motor function, analgesia, anti-emesis and appetite stimulation, among others [3]. Studies that linked the structure of phytocannabinoids with their pharmacological activity, together with the cloning of cannabinoid receptors, allowed the development of new molecules displaying different intrinsic activity and selectivity for cannabinoid receptors. A number of biologically active analogs of THC have been synthesized [4]. These compounds are collectively called cannabinoids for their cannabimimetic properties and share most characteristics of THC, presenting slightly different pharmacological profiles. According to their chemical structure, synthetic cannabinoid agonists can be classified as classical, non-classical and aminoalkylindoles [4]. The classical group consists of dibenzopyran derivatives of THC that include HU-210, HU-243 and nabilone [4]. The non-classical group consists of bicyclic and tricyclic analogs of THC that lack the pyran ring. CP55,940 would be the most representative compound for this group [4]. The aminoalkylindole group shows a completely different structure to THC, and the best known member in this group is WIN55,212-2 [4]. Interestingly, when cognitive performance was tested, most cannabinoids demonstrated certain impairing effects on a diverse array of learning and memory tests [5, 6]. On the other hand, the generation of selective antagonists for the different cannabinoid

receptors, such as SR141716A (rimonabant) [7] and AM251 [8] for the CB1 cannabinoid receptor (CB1R) subtype, and SR144528 [9] and AM630 [10] for the CB2 cannabinoid receptor (CB2R) subtype, represent excellent tools to characterize the role of specific components of the endocannabinoid system (ECS) in cognition. In this regard, several of these antagonists have shown memory improving capabilities in spatial and operant paradigms, further supporting the role of the ECS in cognitive function [11, 12].

## **2. The endocannabinoid system**

The ECS is composed by the cannabinoid receptors, their endogenous ligands (endocannabinoids), and the enzymes involved in the synthesis and degradation of these endocannabinoids. The ubiquitous presence of the ECS correlates with its role as a modulator of multiple physiological processes, being a homeostatic mechanism that guarantees a fine adjustment of information processed in the brain and multiple peripheral tissues, and providing counter-regulatory mechanisms aimed at preserving the structure and function of organs [13, 14].

### *2.1. Cannabinoid receptors: structure and distribution*

Cannabinoids exert their pharmacological actions through the activation of at least two distinct cannabinoid receptors: CB1R and CB2R, although compelling evidences supports the existence of other receptors that bind cannabinoid ligands, such as GPR55 [15, 16]. CB1R was cloned in 1990 [17] and three years later CB2R was also cloned [18]. Both receptors are G-protein-coupled receptors with seven-transmembrane domains, and there are considerable differences regarding their body distribution [4]. Although recent studies have reported a role for CB2R in the central nervous system

[19, 20, 21, 22, 23, 24], the abundance of CB1R and scarcity of CB2R at the central level entail that CB1R is the primary responsible for the psychoactive effects of exogenous and endogenous cannabinoids. Indeed, CB1R is one of the most abundant G protein-coupled receptors in the brain and its distribution has been well characterized both in rodents [25, 26] and humans [27]. CB1R are mainly confined at the presynaptic terminals of central and peripheral neurons, where they modulate the release of different excitatory and inhibitory neurotransmitters that include glutamate, gamma-aminobutyric acid (GABA), acetylcholine, noradrenaline, dopamine, serotonin and cholecystokinin (CCK), among others [28, 29, 30]. Indeed, the ability of CB1R agonists to inhibit neurotransmitter release seems to be responsible for their main effects when administered *in vivo*. More recently, CB1R have been localized in astrocytes [31] and mitochondria [32].

## 2.2. Major endocannabinoids

Endocannabinoids are neuromodulatory lipids finely regulated by the balance between their synthesis and inactivation. The most studied endocannabinoids are N-arachidonylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) [33, 34, 35], both are synthesized on demand in response to elevations of intracellular calcium [36]. Similar to THC, AEA behaves as a partial agonist at both CB1R and CB2R, and also as an endogenous ligand for the vanilloid receptor TRPV1 [37]. 2-AG is the most prevalent endocannabinoid in the brain, and acts as a full agonist for both cannabinoid receptors, indicating that 2-AG is a true natural ligand for the cannabinoid receptors [38]. Endocannabinoids are considered to act as retrograde messengers in the central nervous system [39] behaving as neuromodulators in a wide variety of physiological processes, thus preventing the presence of excessive neuronal activity in a manner that maintains homeostasis in physiological and pathological conditions [40].

### *2.3. Enzymes involved in the biosynthesis and degradation of endocannabinoids*

Both 2-AG and AEA are produced from cell membrane lipids through several biosynthetic pathways. AEA is synthesized from the phosphatidylethanolamine present on the cell membrane by the activation of two enzymes: the N-acyltransferase and phospholipase D (NAPE-PLD) [41]. 2-AG is generated when calcium stimulates phospholipase C, which transforms membrane phosphoinositides into a diacylglycerol, from which 2-AG is synthesized by diacylglycerol lipase (DAGL) [13]. However, other pathways might also be involved in the synthesis of these endocannabinoids [42].

The identification of the enzymes involved in the degradation of endocannabinoids prompted a search for inhibitory compounds that target these enzymes [43]. AEA is mainly degraded by fatty-acid amide hydrolase (FAAH) [44], while 2-AG is primarily metabolized by monoacylglycerol lipase (MAGL) [45]. Therefore, the action of AEA can be prolonged by inhibiting its degradation through FAAH enzyme inhibitors, such as URB532, URB597 [46], OL-135, OL-92 [47] and PF-3845 [48]. On the other hand, endogenous 2-AG concentrations can be enhanced by the administration of the selective MAGL inhibitor JZL184 [49]. Therefore, the use of these specific inhibitors of endocannabinoid metabolism allows modulating specifically AEA or 2-AG accumulation at their specific site of action.

### **3. Mechanisms underlying memory modulation by the ECS**

The ECS is distributed at the pre- and postsynaptic side of the nerve terminals in brain areas involved in learning and memory, such as the hippocampus, modulating synaptic function [50].

### *3.1. Role of the ECS in synaptic plasticity in the hippocampus*

Neuronal activity is a potent stimulus for endocannabinoid synthesis and release [13]. Once released by the postsynaptic neurons, endocannabinoids travel retrogradely across the synapse to bind presynaptic CB1R, suppressing neurotransmitter release at both excitatory and inhibitory synapses in a short- and long-term manner [51, 52, 53]. Activation of CB1R and subsequent long-term inhibition of transmitter release defines endocannabinoid-mediated long-term depression (eCB-LTD). When eCB-LTD occurs at inhibitory terminals (I-LTD), it can facilitate the induction of long-term potentiation (LTP) at excitatory inputs [54, 55]. Nevertheless, CB1R also mediates short-term plasticity, as in the case of depolarization-induced suppression of inhibition or excitation (DSI or DSE, respectively). In the same target cell, the difference between eCB-LTD and eCB-DSI/DSE relies on the duration of CB1R activity, which engages distinct signalling events in the neuron leading to a short or long suppression of neurotransmitter release [53]. A role for intracellular CB1R and mitochondrial mechanisms has been recently reported for eCB-DSI in the hippocampus [32]. On the other hand, the ECS can be directly modulated by exogenous cannabinoids. In this regard, the exposure to a single administration of THC abolished eCB-LTD and I-LTD when measured in hippocampal slices obtained the next day after cannabinoid administration, an effect that was reversed to control conditions when the electrophysiological recordings were performed three days after THC administration [56]. More recently, a critical role for astroglial CB1R was revealed using *in vivo* recordings of cannabinoid-induced LTD (CB-LTD) at hippocampal CA3-CA1

synapses, since this specific modulation of synaptic plasticity was not observed in mice that did not express CB1R in astrocytes [57]. Indeed, the CB-LTD detected after THC or HU-210 administration, correlated with the impairment of spatial working memory, an effect that depended on astroglial CB1R [57]. When THC was administered chronically for seven days, but not after a single administration, Schaffer collateral-CA1 LTP generated by theta burst stimulation or high-frequency stimulation in hippocampal slices was abolished [58]. A similar result was obtained after chronic THC in hippocampal perforant path LTP induced by theta-burst stimulation [59]. The blockade of LTP as result of the chronic exposure to THC persisted for three days after its last administration, and did not fully recover until fourteen days of the last THC injection [58]. On the other hand, hippocampal slices of mice lacking CB1R showed an increase of LTP in Schaffer collateral-CA1 synapses [60], as well as in the dentate gyrus at perforant path-granule cell layer synapses [61].

Therefore, the modulation of the ECS in the hippocampus shapes different forms of synaptic plasticity in ways that influence hippocampal function and therefore may affect cognition.

### *3.2. Role of the ECS in cognition*

The physiological role of the ECS in cognition has been widely investigated. It was reported that the ECS has a specific role in facilitating extinction and/or forgetting processes [62, 63]. In this sense, CB1R knockout mice showed impaired short-term and long-term extinction in auditory fear conditioning tests, with unaffected memory acquisition and consolidation. Treatment of control mice with rimonabant mimicked the phenotype of CB1R-deficient mice, revealing that CB1R is required for memory extinction. Consistently, tone presentation during extinction trials resulted in elevated



levels of endocannabinoids in the basolateral amygdala complex, a region known to control extinction of aversive memories [62].

Both acute and chronic exposure to cannabis are associated with dose-related cognitive impairments, most consistently in attention, working memory, verbal learning, and memory functions in animals [5, 6] and humans [64, 65]. In addition to reduced learning, heavy cannabis use is also associated with a decreased mental flexibility, increased perseveration and reduced ability to sustain attention [66]. Long-term heavy cannabis users show impairments in memory and attention that, depending on the task analyzed, might be reversible [67], although in some cases they persist beyond the period of intoxication and get worse with increasing years of regular cannabis use [68].

Multiple animal models have been used to assess the effects of the ECS on various stages of memory (acquisition, consolidation, retrieval, and extinction) and using a wide range of behavioural paradigms [69, 70, 63, 71, 6]. Evidences indicate that the activation of the ECS interferes with working memory and the acquisition of long-term memory, whereas inhibiting the ECS can enhance similar phases of memory. On the other hand, other stages of memory, such as memory retrieval could be resistant to cannabinoid alteration [72, 73]. A detailed review of the literature on the different conditions of pharmacological treatment and behavioural tasks analyzed has been previously reported [6].

### *3.3. Neuroanatomical basis for the effects of cannabinoids in cognition*

In rodents, activation of cannabinoid receptors by endogenous or exogenous agonists impaired learning and memory by a mechanism that involves the hippocampus. As mentioned above, synapses at different levels in the hippocampus respond to cannabinoid exposure by increasing or decreasing their functional connectivity.

Multiple studies to reveal memory impairment produced by cannabinoids have been performed in paradigms involving spatial tasks known to be hippocampus-dependent, including the 8-arm radial maze, the spatial alternation in a T-shaped maze, the context-recognition test and the open-field water maze, among others [50, 71, 6]. However, in the majority of the studies, cannabinoid agonists are administered systemically and the contribution of hippocampus is not directly confirmed.

Interestingly, intrahippocampal infusion of rimonabant completely blocked the memory impairment produced by the systemic administration of THC or CP55,940 in the radial arm maze task, without affecting other pharmacological properties of cannabinoids, as assessed in the tetrad assay [74]. In agreement, intrahippocampal CP55,940 administration produced similar working memory deficits to those found after systemic cannabinoid administration [75]. Moreover, intrahippocampal administration of WIN55,212-2, disrupted memory in the radial and T-shaped maze delayed alternation tasks [76], and in the spontaneous object- and place-recognition paradigms [77].

Electrophysiological evidences also suggest a predominant role of the hippocampus in the memory disruptive effects of cannabinoids. Thus, systemic administration of THC or WIN55,212-2 disrupted memory in a delayed non-match-to-sample operant task that was related to depressed hippocampal cell firing [78]. Accordingly, exogenous cannabinoid agonists [58] and endocannabinoids [79] decrease LTP in hippocampal slices. In addition, THC and HU-210 induced LTD in CA3-CA1 synapses in anesthetized and freely moving rats, an effect that was directly related to an impairment in spatial working memory [57]. Interestingly, both THC and CP55,940 decreased the power of theta, gamma, and ripple oscillations in the rat hippocampus, which correlated with memory impairment on the hippocampus-dependent delayed alternation memory paradigm [80]. Most of these electrophysiological and cognitive effects of cannabinoid

agonists were attenuated by the administration of rimonabant [81, 82, 83, 50, 71] or the use of CB1R knockout mice [57]. On the other hand, rimonabant facilitated olfactory memory in the social recognition test [11] and working memory in the radial arm maze [12]. In agreement with these pharmacological data, mice lacking CB1R showed an increase of LTP in the hippocampus [60], an improvement in memory retention in the object-recognition paradigm [11, 84] and an increased number of conditional changes in the active avoidance task [85].

Taken together, these findings are consistent with the notion that CB1R located in the hippocampus contribute to the amnesic-like effects produced by cannabinoid agonists. However, the involvement of CB1R in other brain regions cannot be excluded. As an example, THC infusion into the prefrontal cortex disrupted memory on a radial arm maze procedure of short delay [86], but not on the standard radial arm task [87]. Therefore, the type of cognitive task can determine the neural substrates underlying the memory impairment produced by cannabinoids [6].

#### *3.4. Cellular and subcellular localization of CB1R and its implication in cognition*

The widespread anatomical localization of CB1R in the brain may explain its involvement in multiple memory stages that might require different neural substrates. In the hippocampus, CB1R are highly expressed in interneurons, mainly in CCK-positive basket cells, which axon terminals surround the soma of pyramidal neurons [88, 89]. However, CB1R are not detected in parvalbumin-positive fast-spiking basket cells. This differential distribution of CB1R in inhibitory terminals in the hippocampus has implications for the differential control of inhibitory inputs to the principal neurons [90]. In 2006, the development of a high-titer CB1R antibody allowed the localization of CB1R in the terminals of glutamatergic neurons [91, 92]. However, the density of

CB1R on excitatory terminals is much lower than that on inhibitory terminals [92, 93]. On the other hand, CB1R is also expressed in the cortex, which participates in certain types of memory, as well as in the amygdala, a structure involved in emotional memory processes. More recently, CB1R have been also detected in astrocytes [31] and mitochondria [32] where they can also participate in the control of cognitive processes.

Several studies point to the deregulation of the excitatory/inhibitory neurotransmission in the hippocampus as a putative mechanism underlying the deleterious effects of cannabinoids on memory formation. Indeed, CB1R are much more densely expressed on GABAergic than glutamatergic terminals in the hippocampus [92, 93] and THC has been shown to act as a full agonist at CB1R located on those GABAergic terminals, while it acts as a partial agonist at CB1R present on glutamatergic terminals [94]. Therefore, the activation of CB1R located in GABAergic terminals, leading to a suppression of GABA release [89], would produce a concomitant unspecific increase in excitatory firing contributing to the miss-encoding of memory traces. In this regard, a selective GABA reuptake blocker has been reported to enhance spatial learning [95]. Furthermore, the amnesic-like effects of THC are sensitive to pre-treatment with N-methyl-D-aspartate receptors (NMDAR) antagonists [71, 57] also pointing for a role of glutamate transmission in the cannabinoid-mediated cognitive deficiency. In addition, THC administration decreases GABA levels and increases glutamate concentrations in the rat prefrontal cortex [96].

An alternative or complementary explanation could come from the presence of CB1R in astroglia, since CB1R promote the release of glutamate from astroglia, which could then act on perisynaptic NMDARs turning on long-term plastic changes [31, 57].

The specific enhancement of AEA levels with the FAAH inhibitor URB597 also affected object-recognition memory consolidation through the activation of NMDARs, since this effect was abolished by the pre-treatment with the NMDAR antagonist MK801 [97]. In agreement, enhanced NMDAR-mediated synaptic transmission in a particular line of knockout mice that exhibits a marked increase in LTP at Schaffer collateral-CA1 pyramidal synapses, the IRSp53 knockout mice, is associated with impaired memory in Morris water maze and object-recognition tasks [98]. In the same line, mice lacking dystrophin protein, which is enriched in the postsynaptic densities of pyramidal neurons, exhibit enhancement of CA1 hippocampal LTP and impaired long-term memory in the object-recognition task, probably due to a decrease in the threshold for NMDAR activation [99]. Several studies support the idea that enhanced LTP is not often correlated with enhanced memory, and thus, numerous mutant mice showing increased LTP display memory impairments [100].

The use of CB1R conditional knockout mice that lack CB1R either in glutamatergic, GABAergic [101], or astrocytic cells [57] have provided new insights into the role of CB1R on memory regulation. Thus, most of the pharmacological effects of THC, such as catalepsy, hypothermia, hypolocomotion, and antinociception (cannabinoid tetrad), have been linked to the activation of CB1R expressed in principal glutamatergic neurons since they were mostly abolished in mice lacking CB1R in forebrain glutamatergic neurons [102]. In agreement, THC produces full tetrad effects in the conditional knockout mice lacking CB1R in GABAergic terminals [102]. Likewise, the GABA-A receptor antagonist bicuculline, does not block THC-induced tetrad effects [103]. Interestingly, the effects of cannabinoids on long-term memory and working memory have been associated to CB1R in GABAergic terminals [71] or astrocytes [57], respectively. CB1R located in GABAergic neurons in the hippocampus are more

abundant [91, 92, 93] and more sensitive [104, 94] to cannabinoid agonists than CB1R expressed in glutamatergic neurons. Since THC would preferentially decrease GABA release and has less effect on glutamate release, memory impairment could be a consequence of a disruption of hippocampal network activity, which is mediated by synchronized GABAergic discharges that are disrupted by cannabinoids [105, 80]. In agreement, mutant mice overexpressing the GABA transporter type 1, which removes GABA from the synaptic cleft, displayed impaired object-recognition [106], indicating that decreased GABAergic tone, as a consequence of increased clearance of GABA from the synaptic cleft, alters memory in the object-recognition task. Electrophysiological studies show that repetitive low-frequency synaptic stimulation promotes persistent up-regulation of endocannabinoid signalling at CA1 GABAergic synapses. In this way, LTD would be induced at inhibitory synapses while LTP is facilitated at glutamatergic synapses [107]. Altogether, these studies suggest that enhanced NMDAR-mediated LTP, through a possible unbalance between excitatory and inhibitory transmission produced by cannabinoids could lead to memory impairment.

Lastly, the modulatory effect of CB1R on other neurotransmitters has been also proposed to explain the control of cognitive function by the ECS. Indeed, memory impairment produced by cannabinoids has been related to an inhibition of cholinergic activity in the CNS [108]. In agreement, both *in vitro* [109] and *in vivo* [110] studies have shown that cannabinoid agonists induce an inhibition of acetylcholine release in rat hippocampus. Moreover, the inhibition of CCK release from CCK-positive interneurons has been also suggested as a mechanism since the blockade of CCK receptors impairs learning in a radial arm maze [111].

### *3.5. Intracellular signalling cascades activated by the ECS affecting cognition*

The activation of cannabinoid receptors leads to the engagement of numerous signal transduction pathways [14]. However, the precise molecular signalling cascades underlying the disruptive memory effects of cannabinoids have not been fully characterized. As members of the G protein-coupled receptor superfamily, cannabinoid receptors were initially reported to mediate their biological effects by activating heterotrimeric Gi/o type G proteins, although they can also couple to other G proteins [112]. One of the most characterized CB1R-mediated effects through Gi/o proteins is the inhibition of adenylyl cyclase activity and reduction in cyclic AMP production, accompanied by a subsequent decrease in protein kinase A (PKA) activity. This particular signalling cascade was found relevant at the presynaptic level in the modulation by endocannabinoids of the inhibitory LTD (I-LTD) in hippocampal slices through the presynaptic active zone protein Rab3-interacting molecule-1 alpha (RIM1alpha) [113].

In addition, other signalling cascades are modulated in the brain through the stimulation of CB1R. Thus, the phosphorylation of focal adhesion kinase (FAK) was modulated by THC and the endocannabinoids AEA and 2-AG in a CB1R-dependent manner [114, 115]. Interestingly, FAK is critically involved in the regulation of integrins and their association with the actin cytoskeleton, a key regulator of synaptic plasticity [116].

Furthermore, CB1R coupling to G proteins can lead to the phosphorylation and activation of multiple members of the mitogen-activated protein kinase (MAPK) family, including extracellular signal-regulated kinase 1 and 2 (ERK1/2), p38 and c-Jun N-terminal kinase [112]. The relevance of the changes in ERK activity in the hippocampus for the amnesic-like effects of cannabinoids has not been clarified, although it has been

hypothesized a role for ERK activation in the molecular adaptations related to cannabinoid abuse liability [117, 118].

THC also modulates the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt)/glycogen synthase kinase-3 (GSK-3) in the hippocampus after acute exposure [119]. This would be an independent event from ERK activity since the ERK inhibitor SL327 did not affect THC-induced Akt phosphorylation [119]. Instead, another downstream transduction pathway from Akt, the mammalian target of rapamycin (mTOR)/p70-S6 kinase (p70S6K) pathway, was associated to the impairing effects of THC in two cognitive test involving the hippocampus, the object-recognition test and the context-recognition test [71]. In this sense, inhibition of the mTOR/p70S6K pathway with systemic rapamycin, a specific inhibitor of mTOR, prevented the phosphorylation of p70S6K after THC administration, as well as the memory deficits produced by the cannabinoid agonist. In agreement, an increase in the activity of the hippocampal mTOR pathway was observed when the endogenous AEA levels were enhanced by the FAAH inhibitor URB597 that was correlated with a memory deficit in both memory tasks. Interestingly, systemic inhibition of mTOR prevented both the enhanced signalling through this molecular pathway and the cognitive deficit [97]. Indeed, 2-AG enhanced levels with the MAGL inhibitor JZL184 did not induce mTOR activation in the hippocampus nor memory impairment in the object-recognition and the context-recognition memory tasks [97], indicating a dichotomy in the physiological role of both endocannabinoids in memory modulation.

mTOR is a serine/threonine kinase involved in synaptic plasticity as well as in memory processes [120, 121] and through the formation of mTOR complex 1 exerts a crucial role in the regulation of protein synthesis [122]. mTOR contributes to overall cap-dependent translation by phosphorylating the initiation factor 4E binding protein (4E-



BP), and in combination with the activation of the other target p70S6K, might further enhance the translation efficiency, by up-regulating ribosomal proteins and translational factors [122]. In this regard, acute systemic THC administration promotes in the hippocampus the phosphorylation of both effectors, p70S6K and 4E-BP as well as some components of the translational apparatus and factors that participate in the initiation step of translation, such as the ribosomal protein S6 and the eukaryotic initiation factors eIF4E, eIF4G, and eIF4B [71]. Interestingly, non-amnesic doses of the protein translation inhibitor anisomycin prevented the disruptive effects that THC produces in long-term memory in the object-recognition task, indicating that mRNA translation is required for the long-term amnesic-like effects of THC and AEA [71, 97].

The intact function of mTOR and the precise control of translation are required for proper memory storage. Thus, either an enhanced or reduced level of activity of the mTOR signalling cascade has been recently associated to memory disruption [123]. Upon CB1R stimulation by THC or AEA, the mTOR signalling pathway is over-activated and memory consolidation is distorted. In this sense, several lines of mutant mice that show an activation of the hippocampal mTOR pathway also display memory deficits. This is the case of tuberous sclerosis complex 1 (TSC1) and tuberous sclerosis complex 2 (TSC2) heterozygous mice (TSC1<sup>+/-</sup> and TSC2<sup>+/-</sup>) [124, 125], and the fragile X mental retardation protein (FMRP) knockout mouse, an animal model for fragile X syndrome [126]. Furthermore, the FK506 binding protein 12 (FKBP12) knockout mice, which showed enhanced mTOR and p70S6K phosphorylation in the hippocampus, display enhanced associative contextual fear memory and an anomalous performance in the object recognition task probably due to perseveration [127]. However, it is unknown in all these mutant mice whether mTOR-driven translational control leads to an increase of translation of a specific subset of mRNAs or promotes unspecific general translation.

#### 4. Concluding remarks

The ECS has been proposed as a critical neuromodulatory system that affects learning and memory. This is due to its neuromodulatory effects induced in specific brain areas involved in cognitive function such as the hippocampus.

Recent reports have shown how cannabinoid receptors expressed in particular neuronal populations are crucial to regulate the amnesic-like effects of cannabinoids. It has also been proposed a certain degree of functional specificity for the main endocannabinoids, AEA and 2-AG. The subcellular localization of the ECS components in synaptic terminals readily affects synaptic plasticity processes that lie behind cognitive performance. In addition, the intracellular signalling pathways associated to the activation of the ECS overlaps to those involved in the physiological mechanisms for memory formation. Among those signalling pathways, the mTOR pathway has been considered crucial for the effects of cannabinoids in cognition based on biochemical and behavioural evidences. This pathway, modulated *in vivo* by cannabinoids, plays a key role in preventing or promoting memory in physiological and pathological conditions.

Additional studies would be required to clarify the complexity of the cannabinoid signal transduction in the brain areas involved in the control of cognitive functions. In addition, it would be of interest to identify the potential role that the ECS may play in specific pathological conditions running with cognitive deficits, where pharmacological modulation by cannabinoid receptor ligands might be beneficial. The new evidences on the signalling pathways involved in the cognitive deficits produced by cannabinoids open new opportunities to design therapeutic strategies to minimize the deleterious effects of cannabinoids while preventing other therapeutic actions such as analgesic,

anti-emetic, anti-epileptic, anti-ischemic or anti-tumoral effects. Moreover, uncovering the specific role of the ECS in the physiological processes regulating cognition may serve as a tool to modulate specific memory traces or to regulate cognitive competence.

**Acknowledgements**

EP is a recipient of an EMBO Fellowship with the support of European Commission (EMBOCOFUND2010, GA-2010-267146) and Marie Curie Actions. AB-G is a recipient of a predoctoral fellowship, Spanish Ministry of Education and Culture. This study was supported by grants from La Marató de TV3 (#090910 to AO), Grants from the Spanish Ministry of Science and Innovation (#SAF2009-07309 to AO and #SAF2011-29864 to RM); Instituto de Salud Carlos III (RD06/0001/0001 to RM); PLAN E (Plan Español para el Estímulo de la Economía y el Empleo); Generalitat de Catalunya (SGR-2009-00731 to RM and SGR-2009-00718 to RdIT); ICREA (Institució Catalana de Recerca i Estudis Avançats) Academia to RM.

## References

1. Elsohly, M.A. & Slade, D. 2005 Chemical constituents of marijuana: the complex mixture of natural cannabinoids. *Life Sci* **78**, 539-548. (DOI 10.1016/j.lfs.2005.09.011)
2. Gaoni, Y. & Mechoulam, R. 1964 Isolation, structure and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc.* **86**, 1646-1647. (DOI 10.1021/ja01333a025)
3. Adams, I.B. & Martin, B.R. 1996 Cannabis: pharmacology and toxicology in animals and humans. *Addiction* **91**, 1585-1614. (DOI: 10.1046/j.1360-443.1996.911115852.x)
4. Pertwee, R.G., *et al.* 2010 International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB and CB<sub>2</sub>. *Pharmacol Rev* **62**, 588-631. (DOI 10.1124/pr.110.003004)
5. Castellano, C., Rossi-Arnaud, C., Cestari, V. & Costanzi, M. 2003 Cannabinoids and memory: animal studies. *Curr Drug Targets CNS Neurol Disord* **2**, 389-402. (DOI 10.2174/1568007033482670)
6. Zanettini, C., Panlilio, L.V., Alicki, M., Goldberg, S.R., Haller, J., Yasar, S. 2011 Effects of endocannabinoid system modulation on cognitive and emotional behavior. *Front Behav Neurosci* **5**, 57. (DOI 10.3389/fnbeh.2011.00057)
7. Rinaldi-Carmona, M., *et al.* 1994 SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett* **350**, 240-244. (DOI 10.1016/0014-5793(94)00773-X)
8. Gately, S.J., Gifford, A.N., Volkow, N.D., Lan, R. & Makriyannis, A. 1996 123I-labeled AM251: a radioiodinated ligand which binds in vivo to mouse brain cannabinoid CB<sub>1</sub> receptors. *Eur J Pharmacol* **307**, 331-338. (DOI 10.1016/S0014-2999(96)00279-8)
9. Rinaldi-Carmona, M., *et al.* 1998 SR 144528, the first potent and selective antagonist of the CB<sub>2</sub> cannabinoid receptor. *J Pharmacol Exp Ther* **284**, 644-650.
10. Pertwee, R., Griffin, G., Fernando, S., Li, X., Hill, A. & Makriyannis, A. 1995 AM630, a competitive cannabinoid receptor antagonist. *Life Sci* **56**, 1949-1955. (DOI 10.1016/0024-3205(95)00175-6)
11. Terranova, J.P., Storme, J.J., Lafon, N., Perio, A., Rinaldi-Carmona, M., Le Fur, G. & Soubrie, P. 1996 Improvement of memory in rodents by the selective CB<sub>1</sub> cannabinoid receptor antagonist, SR 141716. *Psychopharmacology (Berl)* **126**, 165-172. (DOI 10.1007/BF02246352)
12. Lichtman, A.H. 2000 SR 141716A enhances spatial memory as assessed in a radial-arm maze task in rats. *Eur J Pharmacol* **404**, 175-179. (DOI 10.1016/S0014-2999(00)00615-4)
13. Kano, M., Ohno-Shosaku, T., Hashimoto, Y., Uchigashima, M., Watanabe, M. 2009 Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev* **89**, 309-380. (DOI 10.1152/physrev.00019.2008)
14. Bosier, B., Muccioli, G.G., Hermans, E. & Lambert, D.M. 2010 Functionally selective cannabinoid receptor signalling: therapeutic implications and opportunities. *Biochem Pharmacol* **80**, 1-12. (DOI 10.1016/j.bcp.2010.02.013)
15. Baker, D., Pryce, G., Davies, W.L. & Hiley, C.R. 2006 In silico patent searching reveals a new cannabinoid receptor. *Trends Pharmacol Sci* **27**, 1-4. (DOI 10.1016/j.tips.2005.11.003)
16. Ryberg, E., *et al.* 2007 The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* **152**, 1092-1101. (DOI 10.1038/sj.bjp.0707460)

17. Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C. & Bonner, T.I. 1990 Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346**, 561-564. (DOI 10.1038/346561a0)
18. Munro, S., Thomas, K.L. & Abu-Shaar, M. 1993 Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365**, 61-65. (DOI 10.1038/365061a0)
19. Morgan, N.H., Stanford, I.M. & Woodhall, G.L. 2009 Functional CB2 type cannabinoid receptors at CNS synapses. *Neuropharmacology* **57**, 356-368. (DOI 10.1016/j.neuropharm.2009.07.017)
20. Aracil-Fernández, A., Trigo, J.M., García-Gutiérrez, M.S., Ortega-Álvaro, A., Ternianov, A., Navarro, D., Robledo, P., Berbel, P., Maldonado, R., Manzanares, J. 2012 Decreased Cocaine Motor Sensitization and Self-Administration in Mice Overexpressing Cannabinoid CB(2) Receptors. *Neuropsychopharmacology* **37**, 1749-1763. (DOI 10.1038/npp.2012.22)
21. García-Gutiérrez MS, Pérez-Ortiz JM, Gutiérrez-Adán A, Manzanares J. 2010 Depression-resistant endophenotype in mice overexpressing cannabinoid CB(2) receptors. *Br J Pharmacol.* 160:1773-84. (DOI 10.1111/j.1476-5381.2010.00819.x)
22. García-Gutiérrez MA, Manzanares J. 2011 Overexpression of CB2 cannabinoid receptors decreased vulnerability to anxiety and impaired anxiolytic action of alprazolam in mice. *J Psychopharmacol.* **25**, 111-120. (DOI 10.1177/0269881110379507)
23. Onaivi E.S., Ishiguro, H., Gong, J.P., Patel, S., Meozzi, P.A., Myers, L., Perchuk, A., Mora, Z., Tagliaferro, P.A., Gardner, E., Brusco, A., Akinshola, B.E., Liu, Q.R., Chirwa, S.S., Hope, B., Lujilde, J., Inada, T., Iwasaki, S., Macharia, D., Teasenfitz, L., Arinami, T., Uhl, G.R. 2008 Functional expression of brain neuronal CB2 cannabinoid receptors are involved in the effects of drugs of abuse and in depression. *Ann N Y Acad Sci* **1139**, 434-449. (DOI 10.1196/annals.1432.036)
24. Onaivi, E.S., Ishiguro, H., Gu, S., Liu, Q.R. 2012 CNS effects of CB2 cannabinoid receptors: beyond neuro-immuno-cannabinoid activity. *J Psychopharmacol.* **26**, 92-103. (DOI 10.1177/0269881111400652)
25. Herkenham, M., Lynn, A.B., Johnson, M.R., Melvin, L.S., de Costa, B.R. & Rice, K.C. 1991 Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J Neurosci* **11**, 563-583.
26. Tsou, K., Brown, S., Sanudo-Pena, M.C., Mackie, K. & Walker, J.M. 1998 Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* **83**, 393-411. (DOI 10.1016/S0306-4522(97)00436-3)
27. Westlake, T.M., Howlett, A.C., Bonner, T.I., Matsuda, L.A. & Herkenham, M. 1994 Cannabinoid receptor binding and messenger RNA expression in human brain: an in vitro receptor autoradiography and in situ hybridization histochemistry study of normal aged and Alzheimer's brains. *Neuroscience* **63**, 637-652. (DOI 10.1016/0306-4522(94)90511-8)
28. Howlett, A.C. 2002 The cannabinoid receptors. *Prostaglandins Other Lipid Mediat* **68-69**, 619-631. (DOI 10.1016/S0090-6980(02)00060-6)
29. Pertwee, R.G. & Ross, R.A. 2002 Cannabinoid receptors and their ligands. *Prostaglandins Leukot Essent Fatty Acids* **66**, 101-121.
30. Szabo, B. & Schlicker, E. 2005 Effects of cannabinoids on neurotransmission. *Handb Exp Pharmacol*, 327-365.

31. Navarrete, M. & Araque, A. 2010 Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. *Neuron* **68**, 113-126. (DOI 10.1016/j.neuron.2010.08.043)
32. Bènard, G., *et al.* 2012 Mitochondrial CB(1) receptors regulate neuronal energy metabolism. *Nat Neurosci* **15**, 558-564. (DOI 10.1038/nn.3053.)
33. Devane, W.A., *et al.* 1992 Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946-1949. (DOI 10.1126/science.1470919)
34. Mechoulam, R., *et al.* 1995 Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* **50**, 83-90. (DOI 10.1016/0006-2952(95)00109-D)
35. Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A. & Waku, K. 1995 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun* **215**, 89-97.
36. Di Marzo, V. 2008 Endocannabinoids: synthesis and degradation. *Rev Physiol Biochem Pharmacol* **160**, 1-24. (DOI 10.1007/112\_0505)
37. van der Stelt, M. & Di Marzo, V. 2005 Anandamide as an intracellular messenger regulating ion channel activity. *Prostaglandins Other Lipid Mediat* **77**, 111-122. (DOI 10.1016/j.prostaglandins.2004.09.007)
38. Sugiura, T., Kobayashi, Y., Oka, S. & Waku, K. 2002 Biosynthesis and degradation of anandamide and 2-arachidonoylglycerol and their possible physiological significance. *Prostaglandins Leukot Essent Fatty Acids* **66**, 173-192. (DOI 10.1054/plef.2001.0356)
39. Wilson, R.I. & Nicoll, R.A. 2002 Endocannabinoid signaling in the brain. *Science* **296**, 678-682. (DOI 10.1126/science.1063545)
40. Di Marzo, V. & Petrosino, S. 2007 Endocannabinoids and the regulation of their levels in health and disease. *Curr Opin Lipidol* **18**, 129-140. (DOI 10.1097/MOL.0b013e32803dbdec)
41. Di Marzo, V., Fontana, A., Cadas, H., Schinelli, S., Cimino, G., Schwartz, J.C. & Piomelli, D. 1994 Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature* **372**, 686-691. (DOI 10.1038/372686a0)
42. Muccioli, G.G. 2010 Endocannabinoid biosynthesis and inactivation, from simple to complex. *Drug Discov Today* **15**, 474-483. (DOI 10.1016/j.drudis.2010.03.007)
43. Minkkilä, A., Saario, S. & Nevalainen, T. 2010 Discovery and development of endocannabinoid-hydrolyzing enzyme inhibitors. *Curr Top Med Chem* **10**, 828-858. (DOI 10.2174/156802610791164238)
44. Cravatt, B.F., Giang, D.K., Mayfield, S.P., Boger, D.L., Lerner, R.A. & Gilula, N.B. 1996 Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**, 83-87. (DOI 10.1038/384083a0)
45. Dinh, T.P., Carpenter, D., Leslie, F.M., Freund, T.F., Katona, I., Sensi, S.L., Kathuria, S. & Piomelli, D. 2002 Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci U S A* **99**, 10819-10824. (DOI 10.1073/pnas.152334899)
46. Tarzia, G., *et al.* 2003 Design, synthesis, and structure-activity relationships of alkylcarbamic acid aryl esters, a new class of fatty acid amide hydrolase inhibitors. *J Med Chem* **46**, 2352-2360. (DOI 10.1021/jm021119g)
47. Boger D.L., *et al.* 2005 Discovery of a potent, selective, and efficacious class of reversible alpha-ketoheterocycle inhibitors of fatty acid amide hydrolase effective as analgesics. *J Med Chem* **48**, 1849-1856. (DOI 10.1021/jm049614v)

48. Ahn, K., *et al.* 2009 Discovery and characterization of a highly selective FAAH inhibitor that reduces inflammatory pain. *Chem Biol* **16**, 411-420. (DOI 10.1016/j.chembiol.2009.02.013)
49. Long, J.Z., *et al.* 2009 Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. *Nat Chem Biol* **5**, 37-44. (DOI 10.1038/nchembio.129)
50. Davies, S.N., Pertwee, R.G. & Riedel, G. 2002 Functions of cannabinoid receptors in the hippocampus. *Neuropharmacology* **42**, 993-1007. (DOI 10.1016/S0028-3908(02)00060-6)
51. Alger, B.E. 2002 Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. *Prog Neurobiol* **68**, 247-286. (DOI 10.1016/S0301-0082(02)00080-1)
52. Freund, T.F., Katona, I. & Piomelli, D. 2003 Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev* **83**, 1017-1066. (DOI 10.1152/physrev.00004.2003)
53. Heifets, B.D. & Castillo, P.E. 2009 Endocannabinoid signaling and long-term synaptic plasticity. *Annu Rev Physiol* **71**, 283-306. (DOI 10.1146/annurev.physiol.010908.163149)
54. Carlson, G., Wang, Y. & Alger, B.E. 2002 Endocannabinoids facilitate the induction of LTP in the hippocampus. *Nat Neurosci* **5**, 723-724. (DOI 10.1038/nn879)
55. Chevaleyre, V. & Castillo, P.E. 2004 Endocannabinoid-mediated metaplasticity in the hippocampus. *Neuron* **43**, 871-881. (DOI 10.1016/j.neuron.2004.08.036)
56. Mato, S., Chevaleyre, V., Robbe, D., Pazos, A., Castillo, P.E., Manzoni, O.J. 2004 A single in-vivo exposure to delta 9THC blocks endocannabinoid-mediated synaptic plasticity. *Nat Neurosci* **7**, 585-586. (DOI 10.1038/nn1251)
57. Han, J., *et al.* 2012 Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. *Cell* **148**, 1039-1050. (DOI 10.1016/j.cell.2012.01.037)
58. Hoffman, A.F., Oz, M., Yang, R., Lichtman, A.H. & Lupica, C.R. 2007 Opposing actions of chronic Delta9-tetrahydrocannabinol and cannabinoid antagonists on hippocampal long-term potentiation. *Learn Mem* **14**, 63-74. (DOI 10.1101/lm.439007)
59. Fan, N., Yang, H., Zhang, J. & Chen, C. 2010 Reduced expression of glutamate receptors and phosphorylation of CREB are responsible for in vivo Delta9-THC exposure-impaired hippocampal synaptic plasticity. *J Neurochem* **112**, 691-702. (DOI 10.1111/j.1471-4159.2009.06489.x)
60. Bohme, G.A., Laville, M., Ledent, C., Parmentier, M. & Imperato, A. 2000 Enhanced long-term potentiation in mice lacking cannabinoid CB1 receptors. *Neuroscience* **95**, 5-7. (DOI 10.1016/S0306-4522(99)00483-2)
61. Jacob, W., Marsch, R., Marsicano, G., Lutz, B. & Wotjak, C.T. 2012 Cannabinoid CB1 receptor deficiency increases contextual fear memory under highly aversive conditions and long-term potentiation in vivo. *Neurobiol Learn Mem.* **98**, 47-55. (DOI 10.1016/j.nlm.2012.04.008)
62. Marsicano, G., *et al.* 2002 The endogenous cannabinoid system controls extinction of aversive memories. *Nature* **418**, 530-534. (DOI 10.1038/nature00839)
63. Varvel, S.A. & Lichtman, A.H. 2002 Evaluation of CB1 receptor knockout mice in the Morris water maze. *J Pharmacol Exp Ther* **301**, 915-924. (DOI 10.1124/jpet.301.3.915)
64. Solowij N, Battisti R. 2008 The chronic effects of cannabis on memory in humans: a review. *Curr Drug Abuse Rev* **1**, 81-98.



65. Bolla, K.I., Brown, K., Eldreth, D., Tate, K., Cadet, J.L. 2002 Dose-related neurocognitive effects of marijuana use. *Neurology* **59**, 1337-1343. (DOI 10.1212/01.WNL.0000031422.66442.49)
66. Lundqvist, T. 2005 Cognitive consequences of cannabis use: comparison with abuse of stimulants and heroin with regard to attention, memory and executive functions. *Pharmacol Biochem Behav* **81**, 319-330. (DOI 10.1016/j.pbb.2005.02.017)
67. Crean, R.D., Crane, N.A., Mason, B.J. 2011 An evidence based review of acute and long-term effects of cannabis use on executive cognitive functions. *J Addict Med* **5**, 1-8. (DOI 10.1097/ADM.0b013e31820c23fa)
68. Solowij, N., Stephens, R.S., Roffman, R.A., Babor, T., Kadden, R., Miller, M., Christiansen, K., McRee, B. & Vendetti, J. 2002 Cognitive functioning of long-term heavy cannabis users seeking treatment. *Jama* **287**, 1123-1131. (DOI 10-1001/pubs)
69. Lichtman, A.H., Varvel, S.A. & Martin, B.R. 2002 Endocannabinoids in cognition and dependence. *Prostaglandins Leukot Essent Fatty Acids* **66**, 269-285. (DOI 10.1054/plf.2001.0351)
70. Riedel, G. & Davies, S.N. 2005 Cannabinoid function in learning, memory and plasticity. *Handb Exp Pharmacol*, 445-477. (DOI 10.1007/3-540-26573-2\_15)
71. 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. (DOI 10.1038/nn.2369)
72. De Oliveira Alvares, L., Genro, B.P., Diehl, F. & Quillfeldt, J.A. 2008 Differential role of the hippocampal endocannabinoid system in the memory consolidation and retrieval mechanisms. *Neurobiol Learn Mem* **90**, 1-9. (DOI 10.1016/j.nlm.2008.01.009)
73. Pamplona, F.A., Prediger, R.D., Pandolfo, P. & Takahashi, R.N. 2006 The cannabinoid receptor agonist WIN 55,212-2 facilitates the extinction of contextual fear memory and spatial memory in rats. *Psychopharmacology (Berl)* **188**, 641-649. (DOI 10.1007/s00213-006-0514-0)
74. Wise, L.E., Thorpe, A.J. & Lichtman, A.H. 2009 Hippocampal CB(1) receptors mediate the memory impairing effects of Delta(9)-tetrahydrocannabinol. *Neuropsychopharmacology* **34**, 2072-2080. (DOI 10.1038/npp.2009.31)
75. Lichtman, A.H., Dimen, K.R. & Martin, B.R. 1995 Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. *Psychopharmacology (Berl)* **119**, 282-290. (DOI 10.1007/BF02246292)
76. Suenaga, T., Kaku, M. & Ichitani, Y. 2008 Effects of intrahippocampal cannabinoid receptor agonist and antagonist on radial maze and T-maze delayed alternation performance in rats. *Pharmacol Biochem Behav* **91**, 91-96. (DOI 10.1016/j.pbb.2008.06.015)
77. Suenaga, T. & Ichitani, Y. 2008 Effects of hippocampal administration of a cannabinoid receptor agonist WIN 55,212-2 on spontaneous object and place recognition in rats. *Behav Brain Res* **190**, 248-252. (DOI 10.1016/j.bbr.2008.02.029)
78. Hampson, R.E. & Deadwyler, S.A. 2000 Cannabinoids reveal the necessity of hippocampal neural encoding for short-term memory in rats. *J Neurosci* **20**, 8932-8942.

79. Stella, N., Schweitzer, P. & Piomelli, D. 1997 A second endogenous cannabinoid that modulates long-term potentiation. *Nature* **388**, 773-778. (DOI 10.1038/42015)
80. Robbe, D., Montgomery, S.M., Thome, A., Rueda-Orozco, P.E., McNaughton, B.L. & Buzsaki, G. 2006 Cannabinoids reveal importance of spike timing coordination in hippocampal function. *Nat Neurosci* **9**, 1526-1533. (DOI 10.1038/nn1801)
81. Collins, D.R., Pertwee, R.G. & Davies, S.N. 1995 Prevention by the cannabinoid antagonist, SR141716A, of cannabinoid-mediated blockade of long-term potentiation in the rat hippocampal slice. *Br J Pharmacol* **115**, 869-870.
82. Mallet, P.E. & Beninger, R.J. 1998 The cannabinoid CB1 receptor antagonist SR141716A attenuates the memory impairment produced by delta9-tetrahydrocannabinol or anandamide. *Psychopharmacology (Berl)* **140**, 11-19. (DOI 10.1007/s002130050733)
83. Terranova, J.P., Michaud, J.C., Le Fur, G. & Soubrie, P. 1995 Inhibition of long-term potentiation in rat hippocampal slices by anandamide and WIN55212-2: reversal by SR141716 A, a selective antagonist of CB1 cannabinoid receptors. *Naunyn Schmiedebergs Arch Pharmacol* **352**, 576-579. (DOI 10.1007/BF00169393)
84. Reibaud, M., Obinu, M.C., Ledent, C., Parmentier, M., Bohme, G.A. & Imperato, A. 1999 Enhancement of memory in cannabinoid CB1 receptor knock-out mice. *Eur J Pharmacol* **379**, R1-2. (DOI 10.1016/S0014-2999(99)00496-3)
85. Martin, M., Ledent, C., Parmentier, M., Maldonado, R. & Valverde, O. 2002 Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology (Berl)* **159**, 379-387. (DOI 10.1007/s00213-001-0946-5)
86. Silva de Melo, L.C., Cruz, A.P., Rios Valentim, S.J., Jr., Marinho, A.R., Mendonca, J.B. & Nakamura-Palacios, E.M. 2005 Delta(9)-THC administered into the medial prefrontal cortex disrupts the spatial working memory. *Psychopharmacology (Berl)* **183**, 54-64. (DOI 10.1007/s00213-005-0141-1)
87. Egashira, N., Mishima, K., Iwasaki, K. & Fujiwara, M. 2002 Intracerebral microinjections of delta 9-tetrahydrocannabinol: search for the impairment of spatial memory in the eight-arm radial maze in rats. *Brain Res* **952**, 239-245. (DOI 10.1016/S0006-8993(02)03247-X)
88. Katona, I., Sperlagh, B., Magloczky, Z., Santha, E., Kofalvi, A., Czirjak, S., Mackie, K., Vizi, E.S. & Freund, T.F. 2000 GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. *Neuroscience* **100**, 797-804. (DOI 10.1016/S0306-4522(00)00286-4)
89. Katona, I., Sperlagh, B., Sik, A., Kafalvi, A., Vizi, E.S., Mackie, K. & Freund, T.F. 1999 Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J Neurosci* **19**, 4544-4558.
90. Freund, T.F. & Katona, I. 2007 Perisomatic inhibition. *Neuron* **56**, 33-42. (DOI 10.1016/j.neuron.2007.09.012)
91. Katona, I., Urban, G.M., Wallace, M., Ledent, C., Jung, K.M., Piomelli, D., Mackie, K. & Freund, T.F. 2006 Molecular composition of the endocannabinoid system at glutamatergic synapses. *J Neurosci* **26**, 5628-5637. (DOI 10.1523/JNEUROSCI.0309-06.2006)
92. Kawamura, Y., Fukaya, M., Maejima, T., Yoshida, T., Miura, E., Watanabe, M., Ohno-Shosaku, T. & Kano, M. 2006 The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and

- cerebellum. *J Neurosci* **26**, 2991-3001. (DOI 10.1523/JNEUROSCI.4872-05.2006)
93. Bellocchio, L., *et al.* 2010 Bimodal control of stimulated food intake by the endocannabinoid system. *Nat Neurosci* **13**, 281-283. (DOI 10.1038/nn.2494)
  94. Laaris, N., Good, C.H. & Lupica, C.R. 2010 Delta9-tetrahydrocannabinol is a full agonist at CB1 receptors on GABA neuron axon terminals in the hippocampus. *Neuropharmacology* **59**, 121-127. (DOI 10.1016/j.neuropharm.2010.04.013)
  95. O'Connell, A.W., Fox, G.B., Kjoller, C., Gallagher, H.C., Murphy, K.J., Kelly, J. & Regan, C.M. 2001 Anti-ischemic and cognition-enhancing properties of NNC-711, a gamma-aminobutyric acid reuptake inhibitor. *Eur J Pharmacol* **424**, 37-44. (DOI 10.1016/S0014-2999(01)01116-5)
  96. Pistis, M., Ferraro, L., Pira, L., Flore, G., Tanganelli, S., Gessa, G.L. & Devoto, P. 2002 Delta(9)-tetrahydrocannabinol decreases extracellular GABA and increases extracellular glutamate and dopamine levels in the rat prefrontal cortex: an in vivo microdialysis study. *Brain Res* **948**, 155-158. (DOI 10.1016/S0006-8993(02)03055-X)
  97. Busquets-Garcia, A., Puighermanal, E., Pastor, A., de la Torre, R., Maldonado, R. & Ozaita, A. 2011 Differential Role of Anandamide and 2-Arachidonoylglycerol in Memory and Anxiety-like Responses. *Biol Psychiatry* **70**, 479-486. (DOI 10.1016/j.biopsych.2011.04.022)
  98. Kim, M.H., *et al.* 2009 Enhanced NMDA receptor-mediated synaptic transmission, enhanced long-term potentiation, and impaired learning and memory in mice lacking IRSp53. *J Neurosci* **29**, 1586-1595. (DOI 10.1523/JNEUROSCI.4306-08.2009)
  99. Vaillend, C., Billard, J.M. & Laroche, S. 2004 Impaired long-term spatial and recognition memory and enhanced CA1 hippocampal LTP in the dystrophin-deficient Dmd(mdx) mouse. *Neurobiol Dis* **17**, 10-20. (DOI 10.1016/j.nbd.2004.05.004)
  100. Lee, Y.S. & Silva, A.J. 2009 The molecular and cellular biology of enhanced cognition. *Nat Rev Neurosci* **10**, 126-140. (DOI 10.1038/nrn2572)
  101. Monory, K., *et al.* 2006 The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron* **51**, 455-466. (DOI 10.1016/j.neuron.2006.07.006)
  102. Monory, K., Blaudzun, H., Massa, F., Kaiser, N., Lemberger, T., Schutz, G., Wotjak, C.T., Lutz, B. & Marsicano, G. 2007 Genetic dissection of behavioural and autonomic effects of Delta(9)-tetrahydrocannabinol in mice. *PLoS Biol* **5**, e269. (DOI 10.1371/journal.pbio.0050269)
  103. Varvel, S.A., Anum, E., Niyuhire, F., Wise, L.E. & Lichtman, A.H. 2005 Delta(9)-THC-induced cognitive deficits in mice are reversed by the GABA(A) antagonist bicuculline. *Psychopharmacology (Berl)* **178**, 317-327. (DOI 10.1007/s00213-004-1988-2)
  104. Ohno-Shosaku, T., Tsubokawa, H., Mizushima, I., Yoneda, N., Zimmer, A. & Kano, M. 2002 Presynaptic cannabinoid sensitivity is a major determinant of depolarization-induced retrograde suppression at hippocampal synapses. *J Neurosci* **22**, 3864-3872. (DOI 20026387)
  105. Hajos, N., Katona, I., Naiem, S.S., MacKie, K., Ledent, C., Mody, I. & Freund, T.F. 2000 Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. *Eur J Neurosci* **12**, 3239-3249. (DOI 10.1046/j.1460-9568.2000.00217.x)

106. Hu, J.H., Ma, Y.H., Jiang, J., Yang, N., Duan, S.H., Jiang, Z.H., Mei, Z.T., Fei, J. & Guo, L.H. 2004 Cognitive impairment in mice over-expressing gamma-aminobutyric acid transporter 1 (GAT1). *Neuroreport* **15**, 9-12.
107. Zhu, P.J. & Lovinger, D.M. 2007 Persistent synaptic activity produces long-lasting enhancement of endocannabinoid modulation and alters long-term synaptic plasticity. *J Neurophysiol* **97**, 4386-4389. (DOI 10.1152/jn.01228.2006)
108. Braida, D. & Sala, M. 2000 Cannabinoid-induced working memory impairment is reversed by a second generation cholinesterase inhibitor in rats. *Neuroreport* **11**, 2025-2029. (DOI 10.1097/00001756-200006260-00044)
109. Gifford, A.N. & Ashby, C.R., Jr. 1996 Electrically evoked acetylcholine release from hippocampal slices is inhibited by the cannabinoid receptor agonist, WIN 55212-2, and is potentiated by the cannabinoid antagonist, SR 141716A. *J Pharmacol Exp Ther* **277**, 1431-1436.
110. Gessa, G.L., Mascia, M.S., Casu, M.A. & Carta, G. 1997 Inhibition of hippocampal acetylcholine release by cannabinoids: reversal by SR 141716A. *Eur J Pharmacol* **327**, R1-2. (DOI 10.1016/S0014-2999(97)89683-5)
111. Harro, J. & Orelund, L. 1993 Cholecystikinin receptors and memory: a radial maze study. *Pharmacol Biochem Behav* **44**, 509-517. (DOI 10.1016/0091-3057(93)90160-U)
112. Howlett, A.C. 2005 Cannabinoid receptor signaling. *Handb Exp Pharmacol* **168**, 53-79.
113. Chevaleyre, V., Heifets, B.D., Kaeser, P.S., Sudhof, T.C. & Castillo, P.E. 2007 Endocannabinoid-mediated long-term plasticity requires cAMP/PKA signaling and RIM1alpha. *Neuron* **54**, 801-812. (DOI 10.1016/j.neuron.2007.05.020)
114. Derkinderen, P., Toutant, M., Burgaya, F., Le Bert, M., Siciliano, J.C., de Franciscis, V., Gelman, M. & Girault, J.A. 1996 Regulation of a neuronal form of focal adhesion kinase by anandamide. *Science* **273**, 1719-1722. (10.1126/science.273.5282.1719)
115. Derkinderen, P., Toutant, M., Kadaré, G., Ledent, C., Parmentier, M., Girault, J.A. 2001 Dual role of Fyn in the regulation of FAK+6,7 by cannabinoids in hippocampus. *J Biol Chem* **276**, 38289-38296. (DOI 10.1074/jbc.M105630200)
116. McGeachie, A.B., Cingolani, L.A. & Goda, Y. 2011 Stabilising influence: integrins in regulation of synaptic plasticity. *Neurosci Res* **70**, 24-29.
117. Derkinderen, P., Valjent, E., Toutant, M., Corvol, J.C., Enslin, H., Ledent, C., Trzaskos, J., Caboche, J. & Girault, J.A. 2003 Regulation of extracellular signal-regulated kinase by cannabinoids in hippocampus. *J Neurosci* **23**, 2371-2382.
118. Rubino, T., Viganò, D., Premoli, F., Castiglioni, C., Bianchessi, S., Zippel, R., Parolaro, D. 2006 Changes in the expression of G protein-coupled receptor kinases and beta-arrestins in mouse brain during cannabinoid tolerance: a role for RAS-ERK cascade. *Mol Neurobiol* **33**, 199-213. (DOI 10.1385/MN:33:3:199)
119. Ozaita, A., Puighermanal, E. & Maldonado, R. 2007 Regulation of PI3K/Akt/GSK-3 pathway by cannabinoids in the brain. *J Neurochem* **102**, 1105-1114. (DOI 10.1111/j.1471-4159.2007.04642.x)
120. Costa-Mattioli, M., Sossin, W.S., Klann, E. & Sonenberg, N. 2009 Translational control of long-lasting synaptic plasticity and memory. *Neuron* **61**, 10-26. (DOI 10.1016/j.neuron.2008.10.055)

121. Hoeffler, C.A. & Klann, E. 2010 mTOR signaling: at the crossroads of plasticity, memory and disease. *Trends Neurosci* **33**, 67-75. (DOI 10.1016/j.tins.2009.11.003)
122. Ma, X.M. & Blenis, J. 2009 Molecular mechanisms of mTOR-mediated translational control. *Nat Rev Mol Cell Biol* **10**, 307-318. (DOI 10.1038/nrm2672)
123. Troca-Marin, J.A., Alves-Sampaio, A. & Montesinos, M.L. 2012 Deregulated mTOR-mediated translation in intellectual disability. *Prog Neurobiol* **96**, 268-282. (DOI 10.1016/j.pneurobio.2012.01.005)
124. Goorden, S.M., van Woerden, G.M., van der Weerd, L., Cheadle, J.P. & Elgersma, Y. 2007 Cognitive deficits in Tsc1<sup>+/-</sup> mice in the absence of cerebral lesions and seizures. *Ann Neurol* **62**, 648-655. (DOI 10.1002/ana.21317)
125. Ehninger, D., Han, S., Shilyansky, C., Zhou, Y., Li, W., Kwiatkowski, D.J., Ramesh, V. & Silva, A.J. 2008 Reversal of learning deficits in a Tsc2<sup>+/-</sup> mouse model of tuberous sclerosis. *Nat Med* **14**, 843-848. (DOI 10.1038/nm1788)
126. Sharma, A., Hoeffler, C.A., Takayasu, Y., Miyawaki, T., McBride, S.M., Klann, E. & Zukin, R.S. 2010 Dysregulation of mTOR signaling in fragile X syndrome. *J Neurosci* **30**, 694-702. (DOI 10.1523/JNEUROSCI.3696-09.2010)
127. Hoeffler, C.A., *et al.* 2008 Removal of FKBP12 enhances mTOR-Raptor interactions, LTP, memory, and perseverative/repetitive behavior. *Neuron* **60**, 832-845. (DOI 10.1016/j.neuron.2008.09.037)