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Hippocampal Cb₂ receptors: an untold story

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Abstract: The field of cannabinoid research has been receiving ever-growing interest. Ongoing debates worldwide about the legislation of medical cannabis further motivates research into cannabinoid function within the central nervous system (CNS). To date, two well-characterized cannabinoid receptors exist. While most research has investigated Cb₁ receptors (Cb₁Rs), Cb₂ receptors (Cb₂Rs) in the brain have started to attract considerable interest in recent years. With indisputable evidence showing the wide-distribution of Cb₂Rs in the brain of different species, they are no longer considered just peripheral receptors. However, in contrast to Cb₁Rs, the functionality of central Cb₂Rs remains largely unexplored. Here we review recent studies on hippocampal Cb₂Rs. While conflicting results about their function have been reported, we have made significant progress in understanding the involvement of Cb₂Rs in modulating cellular properties and network excitability. Moreover, Cb₂Rs have been shown to be expressed in different subregions of the hippocampus, challenging our prior understanding of the endocannabinoid system. Although more insight into their functional roles is necessary, we propose that targeting hippocampal Cb₂Rs may offer novel therapies for diseases related to memory and adult neurogenesis deficits.

Keywords: adult neurogenesis; cannabinoids; endocannabinoid system; hippocampal Cb₂ receptors; hippocampus; memory.

Introduction

Peripheral Cb₂ receptors – a half-truth

Our understanding of the endocannabinoid system (ECS) is continuously being shaped by novel discoveries of complex interactions and intricate processes. Since the initial description of the cannabinoid (Cb) receptors, namely the Cb₁ receptors (Cb₁Rs) (Matsuda et al. 1990) and the Cb₂ receptors (Cb₂Rs) (Munro et al. 1993), to the discoveries of the “blissful” substances anandamide (Devane et al. 1992) and 2-arachidonylglycerol (Mechoulam et al. 1995; Stella et al. 1997), emerging roles of the ECS in several brain processes have been recognized. Interestingly, the ECS encompasses both the central nervous system (CNS) and peripheral tissues (Mechoulam and Parker 2013). The Cb₁R is widely distributed in the CNS, with abundant expression in the basal ganglia, cortex, cerebellum, and hippocampus, as shown by radiography (Herkenham et al. 1991), *in situ* hybridization (Mailleux and Vanderhaeghen 1992; Matsuda et al. 1993) and immunohistochemistry (Egertová and Elphick 2000; Tsou et al. 1998). These receptors are typically expressed on axon terminals and mediate retrograde signaling by endocannabinoids (Castillo et al. 2012). Much less is known about Cb₂R-mediated signaling. In fact, for several years central Cb₂Rs were largely overlooked and more attention was given to the peripheral Cb₂Rs (Atwood and Mackie 2010; Kopach et al. 2012). Most cannabinoid research in the CNS to date has thus been focused on Cb₁R. Recently, however, Cb₂Rs have attracted considerable interest as potential modulators in drug-seeking behavior, pain, depression, anxiety, memory, neuroinflammation, and neurodegenerative diseases (Chen et al. 2017). During the last two decades, numerous studies have helped disperse the myth of Cb₂Rs being exclusively expressed in the periphery. *In situ* hybridization and quantitative real-time PCR detected Cb₂R mRNA in the hippocampus, cortex, cerebellum, brainstem, and midbrain of both rodents and nonhuman primates (García-Gutiérrez et al. 2012; Li and Kim 2015; Liu et al. 2009; Navarrete et al. 2012; Zhang et al. 2014). Moreover, Cb₂R protein expression has been demonstrated for various brain regions (Ashton et al. 2006; Baek et al. 2008; Brusco et al. 2008; Gong et al. 2006; Van Sickle et al. 2005). Cannabinoid receptor expression has also been investigated in *postmortem* human brain tissue: Cb₂R mRNA was found in the human prefrontal cortex

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while the expression of both Cb₁R and Cb₂R proteins was demonstrated in the cerebellum (Rodríguez-Cueto et al. 2014a,b).

Brain Cb₂Rs are attractive therapeutic targets. Despite the low physiological expression, they are highly inducible under some pathological conditions, and their expression is quickly enhanced in the brain. The upregulation of Cb₂Rs has been described in several disorders, including neurodegenerative diseases, brain injuries, and neuroinflammation. Among them, Parkinson's disease (Gómez-Gálvez et al. 2016; Grünblatt et al. 2007; Navarrete et al. 2018), Alzheimer's disease (AD) (López et al. 2018), post-traumatic stress disorder (PTSD) (Lisboa et al. 2019; Morena et al. 2018), traumatic brain injury (Tchantchou and Zhang 2013), vascular dementia (Luo et al. 2018), stroke (Zarruk et al. 2012), and neuroinflammation (Torres et al. 2011). The inducibility of Cb₂R expression in pathological conditions was found mainly in the hippocampus, but there is also evidence for its occurrence across the CNS, including the cerebral cortex and the cerebellum (Figure 1A). The Cb₂Rs are also induced in the spinal cord, in *in vitro* preparations for studying multiple sclerosis and in regions remotely connected to a primary site of focal brain damage during remote cell death (Figure 1B) (Askari and Shafiee-Nick 2019; Viscomi et al. 2009; Wen et al. 2015). Pathology-induced Cb₂Rs were found in microglia, astrocytes, oligodendrocytes, and neurons. The activation of Cb₂Rs in these cells modulates the release of several cytokines, which regulate immune function and inflammatory responses (Figure 1C).

Regional increases in Cb receptor expression have been shown to modulate the potency and efficacy of exogenous agonists at disease sites, theoretically allowing for targeted activation at the local of injury (Miller and Devi 2011). However, there has only been limited success in transitioning Cb₂R agonists from preclinical studies to clinical trials (Dhopeshwarkar and Mackie 2014). But it should be noted that even for the case of Cb₁Rs, which have been extensively studied through decades, there is a paucity of well-established clinical applications. This reflects the complex role of the ECS in modulating brain function and could indicate the need for improvement in preclinical models (An et al. 2020). Notwithstanding, Cb₂Rs have remarkable advantages over Cb₁Rs as potential therapeutic targets (Chen et al. 2017). In contrast to Cb₁Rs, Cb₂Rs are less prone to produce psychotropic effects, which have been a key point in the ongoing worldwide debates regarding the legislation of medical marijuana (Dhopeshwarkar and Mackie 2014). Moreover, Cb₂Rs have lower expression levels and a more specific distribution than Cb₁Rs in the brain. Thus, Cb₂R ligands

could offer therapeutic treatments without the adverse effects often seen with Cb₁R-ligands (Onaivi et al. 2011). Furthermore, Cb₂Rs can balance Cb₁R activation effects since the former are expressed in neuronal somatodendritic areas, and the latter are predominantly expressed on neuronal terminals (An et al. 2020). Considering these characteristics, studies aiming to unveil the Cb₂R role in the neurophysiology of specific brain regions may foster the development of clinically effective Cb₂R-modulators, which will likely offer novel strategies for treating neuropsychiatric and neurological diseases.

In this review, we discuss recent developments in hippocampal Cb₂R research. We selected the hippocampus as a major area of interest since it plays a crucial role in cognition, learning, and memory, which are important functions disrupted in neurodegenerative and neuroinflammatory diseases (Leuner and Gould 2010). Moreover, the layers, subregional differences, and cell populations within this structure are well-known, facilitating inferences about potential Cb₂R roles in hippocampal activity. Although the function of hippocampal Cb₂R is still largely unexplored, recent reports show that they modulate cellular and network excitability, suggesting a meaningful role in regulating hippocampal output. As will be argued below, further exploring the function of hippocampal Cb₂R is an important step to understand the cognitive effects of both exogenous and endogenous cannabinoids.

Different cell populations express Cb₂ receptors in the hippocampus

In a series of experiments, Onaivi and colleagues explored the distribution of Cb₂Rs in the hippocampus, and found them to be broadly distributed among its different subregions (Brusco et al. 2008; Gong et al. 2006; Onaivi 2006; Onaivi et al. 2006) (Figure 2). Together, these experiments also provided the first evidence for the expression of hippocampal Cb₂Rs in microglia, principal neurons and interneurons, which encouraged further research. Below we discuss the findings for each cell type individually.

Cb₂ receptors in microglia

The function of peripheral Cb₂Rs as potent immune modulators has been clearly demonstrated (Basu and Dittel 2011; Cabral and Griffin-Thomas 2009; Racz et al. 2008; Turcotte et al. 2016). Accordingly, the fact that the CNS microglial cells express Cb₂Rs is not surprising since they

Cb₂R expression inducibility in pathophysiological conditions

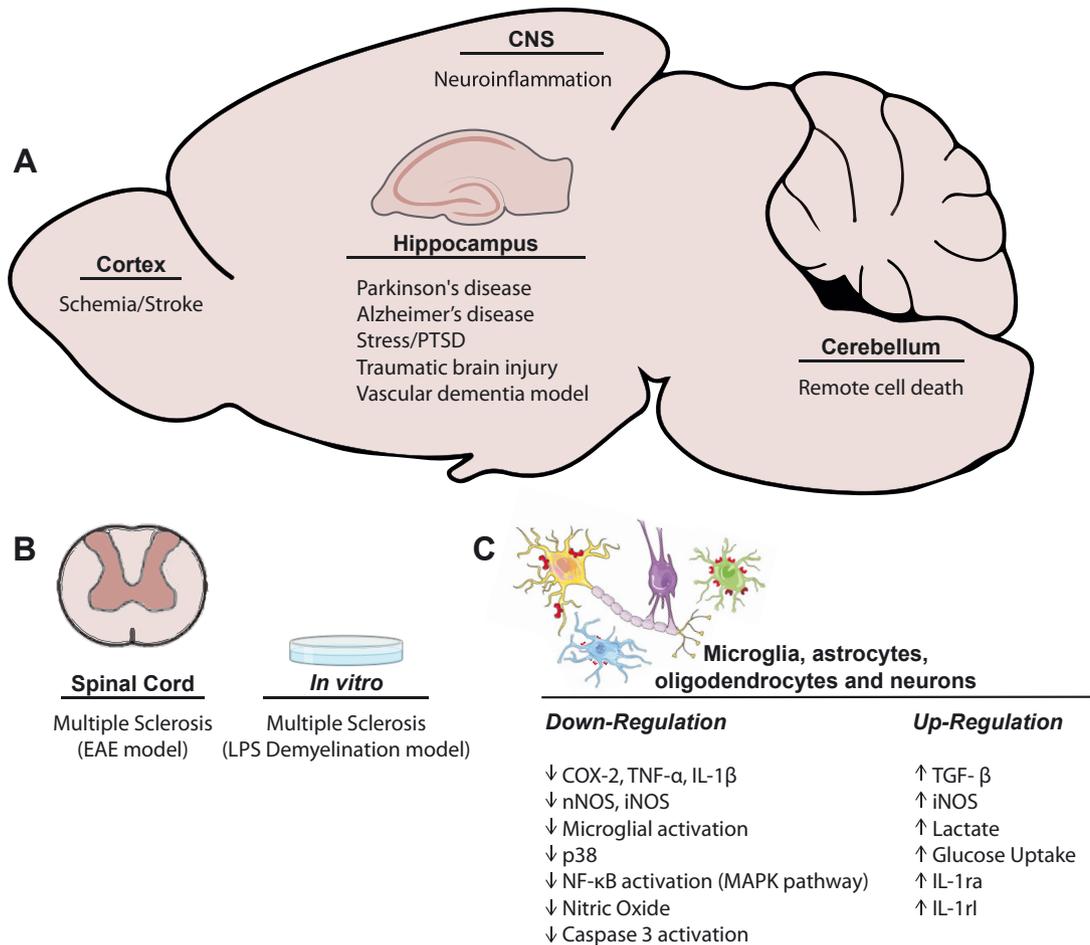


Figure 1: Cb₂R expression inducibility under pathological conditions.

(A) Neuroinflammation (Torres et al. 2011), ischemia and stroke (Zarruk et al. 2012), Parkinson's (Gómez-Gálvez et al. 2016; Grünblatt et al. 2007; Navarrete et al. 2018) and Alzheimer's disease (AD) (López et al. 2018), post-traumatic stress disorder (PTSD) (Lisboa et al. 2019; Morena et al. 2018), traumatic brain injury (Tchantchou and Zhang 2013), vascular dementia (Luo et al. 2018), and remote cell death are related to the inducibility of Cb₂Rs over the central nervous system (CNS), including neocortical, hippocampal and cerebellar regions. (B) Multiple sclerosis is associated with Cb₂R upregulation in the spinal cord and *in vitro* models (Askari and Shafiee-Nick 2019; Wen et al. 2015). (C) Pathology-induced Cb₂R modulates immunological response by regulating metabolic enzymes, cytokines release, and downstream functional molecules in microglia, astrocytes, oligodendrocytes, and neurons (Brusco et al. 2008; Campos et al. 2017; Gong et al. 2006). (COX-2) cyclooxygenase-2; (IL-1 β) interleukin-1 β ; (IL-1ra) interleukin-1 receptor antagonist; (IL-1rl) interleukin-1 receptor, type I; (iNOS) inducible nitric oxide synthases; (LPS) lipopolysaccharide; (nNOS) neuronal nitric oxide synthases; (NF- κ B) nuclear factor kappa-light-chain-enhancer of activated B cells; (TGF- β) transforming growth factor- β ; (TNF- α) tumor necrosis factor- α .

are the resident macrophages (Perry and Teeling 2013). Microglial Cb₂R expression in mouse and rat hippocampi was first demonstrated using different Cb₂R-specific antibodies (Brusco et al. 2008; Gong et al. 2006). More recent studies challenge some of the results previously shown by Onaivi and colleagues. For instance, when combining RNAscope, an ultrasensitive *in situ* hybridization technique, with immunostaining against the microglial marker

Iba1, no overlap could be detected in the CA1 region of healthy rat hippocampi (Li and Kim 2015). Some publications also challenge the specificity of Cb₂R antibodies, and overall results indicate that currently available antibodies may lack specificity and may lead to conflicting outcomes (Li and Kim 2015). Considering these results, more validating research needs to be conducted. It is worth noting that, usually, the experiments that failed in finding Cb₂R

Summary of Cb₂R expression studies in the hippocampus

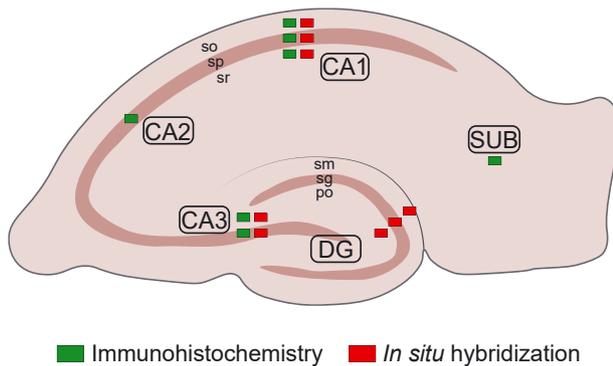


Figure 2: Cb₂R expression in the hippocampus. Several studies have attempted to quantify the expression of Cb₂R protein in the hippocampus. *In situ* hybridization (red rectangles) and immunohistochemistry (green rectangles) revealed Cb₂R expression in CA1 (Schmöle et al. 2015; Wu and Wang 2018), CA2 (Gong et al. 2006), CA3 (Stempel et al. 2016), dentate gyrus (DG) (Schmöle et al. 2015), and subiculum (SUB) (Gong et al. 2006). (so) *stratum oriens*; (sp) *stratum pyramidale*; (sr) *stratum radiatum*; (sm) *stratum moleculare*; (sg) *stratum granulosum*; (po) polymorphic layer.

expression in hippocampal cells (microglia, principal neurons, and interneurons) were conducted using healthy tissue, while inflammatory responses in the brain have been suggested to increase Cb₂R and its mRNA expression (Guida et al. 2017; Luongo et al. 2010; Palazuelos et al. 2009; Walter et al. 2003). Hence, the absence of microglial Cb₂R expression in healthy hippocampal tissue would not exclude a potential functional relevance of this receptor during pathological conditions.

Cb₂ receptors in principal neurons

Most Cb₂Rs in the CA1 region of the hippocampus are expressed in the principal neurons, which are the excitatory pyramidal cells (Li and Kim 2015; Onaivi 2006). The localization of Cb₂Rs in these cells is primarily postsynaptic. Still, they can also be observed in the rough endoplasmic reticulum, Golgi apparatus, neuronal cytoplasm, and in dendrites near the plasma membrane (Brusco et al. 2008). These findings support that Cb₂Rs are synthesized in the soma and subsequently transported to target dendrites. Interestingly, no expression occurs in axon terminals (Brusco et al. 2008), and since hippocampal Cb₁Rs are mainly expressed presynaptically (Castillo et al. 2012; Kano et al. 2009; Katona et al. 1999; Monory et al. 2015), this indicates a functional difference between Cb₁ and Cb₂ receptors. Moreover, this also

suggests that the notion of the ECS as a retrograde signaling system might not be complete (Castillo et al. 2012). The postsynaptic localization of Cb₂Rs in principal cells thus reveals a more complex role for the ECS in the hippocampus than previously thought.

Cb₂ receptors in interneurons

Hippocampal interneurons consist of a morphologically diverse group of cell types. It has been suggested that there are at least 21 different classes of interneurons in the CA1 region alone (Klausberger and Somogyi 2008). In this region, the Cb₁Rs are found primarily in GABAergic interneurons (Tsou et al. 1999). In contrast, Cb₂R mRNA is found in about 20% of both glutamatergic and non-glutamatergic cells in the CA1 (Li and Kim 2015). Whether there exists an overlap of Cb₁R and Cb₂R expression within specific interneuron types is still open for exploration. Further understanding the localization of Cb₂R expression might add a new perspective on the action of cannabinoids in the CA1 region, as some of the functions previously thought to be mediated by Cb₁R could potentially be due to overlooked Cb₂ intra- and/or interneuronal signaling cascades.

Moving from expression to functionality

Correlation does not imply causation, a mantra for science. Similarly, expression does not denote function. Therefore, albeit evident that both Cb₂R mRNA and protein can be found in the hippocampus, whether this has functional relevance is debatable, as we revisit below.

Synaptic function of hippocampal Cb₂ receptors

Although the levels of Cb₂R mRNA are significantly lower in the CNS than in the periphery (Onaivi 2006; Van Sickle et al. 2005), there is enough evidence to support a functional importance of Cb₂R for hippocampal activity. Recently, Stempel et al. (2016) reported a Cb₂R-dependent long-lasting hyperpolarization in CA2 and CA3 pyramidal cells (Stempel et al. 2016). The hyperpolarization depended on the sodium-bicarbonate co-transporter, illustrating an important mechanism of Cb₂R activity. By directly affecting the membrane potential, Cb₂R may thus

act complementary to Cb₁R as modulators of network excitability.

Traditionally, the functional effects of the ECS in the hippocampus have been credited to Cb₁R, which modulate presynaptic neurotransmitter release (Monory et al. 2015). Depending on the neuronal cell type and brain region, the activation of Cb₁R can have opposite effects, either increasing or decreasing excitability (Chevaleyre and Castillo 2003; Miraucourt et al. 2016; Winters et al. 2012). Whether this is also the case for Cb₂R is an open question. In layers II and V of the medial entorhinal cortex, activation of Cb₂R decreases the amplitude of spontaneous inhibitory postsynaptic currents through suppression of GABAergic transmission (Morgan et al. 2009). On the other hand, a recent study reported that inhibitory synaptic transmission is not affected by acute activation of Cb₂R in CA1 but rather that chronic activation of Cb₂R results in increased excitatory transmission (Kim and Li 2015). The regional or cell-specific factors underlying the different actions of Cb₂R in the hippocampus and medial entorhinal cortex have yet to be identified.

As stated above, whether the low levels of Cb₂R in CA1 are relevant to synaptic function under physiological conditions is still an open question (Kim and Li 2015; Li and Kim 2016a; Stempel et al. 2016). To further complicate matters, the postsynaptic localization of Cb₂R has also been challenged. Namely, Morgan et al. (2009) observed no changes in the kinetics of miniature inhibitory postsynaptic currents in the presence of a selective Cb₂R agonist, which would be expected if Cb₂R were postsynaptically located (Morgan et al. 2009). Therefore, there are important unanswered questions regarding the synaptic function and localization of Cb₂R, but still enough pieces of evidence suggesting a functional Cb₂R role in the hippocampus, which could lead to new interpretations of the effects of exogenous and endogenous cannabinoids in this region.

Cb₂ receptors and memory consolidation

As discussed above, there is evidence of upregulation of Cb₂R in microglia as a response to neuroinflammation, but the functional consequences are not yet clear. In addition, Cb₂R mRNA and protein expression are upregulated in AD, for which one of the hallmark features is hippocampal-dependent memory impairment. However, how do Cb₂R relate to memory consolidation? Köfalvi et al. (2016) observed in hippocampal slices of both young and old healthy mice that Cb₂R activation increases glucose transporters (GLUT) in hippocampal astrocytes

and neurons. In contrast, these authors reported that the glucose uptake induced by Cb₂R activation is impaired in a mouse model of AD (TgAPP mice). TgAPP mice present β -amyloid-burden and object recognition memory impairment. Interestingly, prolonged oral administration of JWH-133, a selective agonist of Cb₂R, rescued hippocampal glucose uptake, diminished β -amyloid levels, and prevented the memory deficit in TgAPP mice (Köfalvi et al. 2016; Martín-Moreno et al. 2012). Dagon et al. (2007) induced hepatic encephalopathy in wild-type and Cb₂R knockout mice. Hepatic encephalopathy is a neuropsychiatric syndrome caused by liver dysfunction and characterized by impaired glucose oxidative pathways in the brain, amnesia, and confusion. The authors found that treatment with Δ^9 -tetrahydrocannabinol (THC) increased AMP-activated protein kinase, which in turn stimulated GLUT expression and transport efficiency in the hippocampus. Interestingly, THC also prevented spatial working memory deficit assessed by the eight-arm maze in wild-type mice but not in Cb₂R knockout animals (Dagon et al. 2007). Given that brain glucose availability controls cognition and memory in humans (Messier 2004) and that central metabolic boosting alleviates the cognitive symptoms of brain disorders (Branconnier 1983), the studies mentioned above support a role of hippocampal Cb₂R in counteracting cognitive impairment via regulation of glucose uptake.

In Cb₂R knockout mice, hippocampal-dependent long-term contextual fear memory is impaired while hippocampal-independent cued fear memory is not affected (Li and Kim 2016a). A follow-up study showed that knocking out the Cb₂R gene decreases hippocampal excitatory synaptic transmission, long-term potentiation, and dendritic spine density, indicating that the endogenous activity of Cb₂R contributes to the maintenance of synaptic function and regulates cognitive functions such as long-term memory (Li and Kim 2016b). These results suggest that the loss of Cb₂R may lead to hippocampal-dependent memory deficits, though they should be interpreted with caution since compensatory mechanisms may occur in developmental knockout mice. Furthermore, these results were obtained using a general Cb₂R knockout mouse line and it is therefore not possible to infer whether memory impairment was specifically due to the loss of Cb₂R in the hippocampus. Subsequently, Li and Kim (2017) used either Cre-dependent overexpression of Cb₂R or CRISPR-Cas9 genome-editing techniques to delete Cb₂R gene in combination with the injection of adeno-associated viruses into the dorsal hippocampus of transgenic mouse lines. With this approach, they were able to investigate the role of Cb₂R in specific cell

populations (i.e., pyramidal cells, interneurons, and microglia) and found that increasing or decreasing the expression of Cb₂Rs in microglia respectively enhances or impairs contextual fear memory (Li and Kim 2017). They also showed that disruption of Cb₂R expression in CA1 pyramidal neurons enhances spatial working memory, while overexpression reduces anxiety levels as tested by the open field test (Li and Kim 2017). Noteworthy, in studies that genetically modulate Cb₂R expression it is impossible to disentangle if the memory impairments are due to hindered consolidation, acquisition or retrieval. In this regard, despite the limitations in the current available Cb₂R agonists/antagonists, pharmacological studies are more advantageous to investigate the functions of Cb₂R in specific memory phases.

Nasehi et al. (2017, 2018) reported that microinjection of a Cb₂R agonist (Gp1a) into CA1 impairs aversive memory consolidation in rats and mice. Moreover, aversive memory consolidation was further impaired when simultaneously injecting muscimol (an ionotropic GABA receptor agonist), suggesting an interactive effect between Cb₂ and GABA_A signaling (Nasehi et al. 2017, 2018). This notion is also supported by Garcia-Gutiérrez and Manzanares (2011), who reported an upregulation of GABA_A protein expression after chronic activation of Cb₂Rs in the cortex. A more recent study showed that Gp1a infusion into CA3 also impairs aversive memory consolidation. This effect was increased by coinfusion of scopolamine (a nonselective antagonist of muscarinic acetylcholine receptors), suggesting that Cb₂Rs can also interact with cholinergic signaling (Nasehi et al. 2020). The suggestion of an interaction between cannabinoid and cholinergic signaling during memory processing was made previously by Robinson et al. (2010), although without a direct mention of Cb₂Rs. They reported that intraperitoneal administration of WIN55,212-2 (a nonselective cannabinoid receptor agonist) before spatial memory acquisition caused memory impairment through a mechanism that was independent of Cb₁R, and that this impairment was reversed by coinfusion of a cholinesterase inhibitor (Robinson et al. 2010). Similarly, studies in rats showed that cannabidiol disrupts consolidation of (specific and generalized) fear memories via Cb₂R localized in the dorsal hippocampus (Raymundi et al. 2020; Stern et al. 2017).

It should be noted, however, that in addition to the studies showing that activating Cb₂R signaling has disruptive effects on memory, there is also evidence for heightened Cb₂R activity improving spatial and fear memory. Chronic treatment with Cb₂R agonists and Cb₂R upregulation could rescue spatial memory deficits in mouse models of vascular dementia or AD (Çakır et al. 2019; Lou et al. 2017; Wu et al.

2017). Moreover, Ratano et al. (2018) showed that the endocannabinoid 2-arachidonoylglycerol (2-AG) enhanced memory consolidation in an inhibitory avoidance task through a Cb₂R-dependent modulation of mTOR signaling. In a previous study of the same group, they also showed that blocking Cb₂R signaling tended to impair fear memory retention (Ratano et al. 2017). Consistently, chronic treatment with systemic Cb₂R antagonist aggravated fear memory loss caused by orthopedic surgery (Sun et al. 2017). Nevertheless, downregulation of Cb₂R expression in hippocampal cells has been associated with impaired spatial memory, object recognition, and fear conditioning acquisition and retention (Tang et al. 2017).

To summarize, it is clear that hippocampal Cb₂Rs do influence memory, but contradictory results have been reported regarding the exact role of these receptors on memory consolidation and disruption. While Cb₂R gene overexpression in the dorsal hippocampus enhanced fear conditioning, acute Cb₂R agonism in the same region impaired fear conditioning consolidation (Li and Kim 2017; Raymundi et al. 2020). Nevertheless, increasing Cb₂R activity in CA1 and CA3 impaired memory consolidation in inhibitory avoidance, but systemic Cb₂R agonism enhanced memory retention in this same task (Nasehi et al. 2017, 2018, 2020; Ratano et al. 2018). In the Morris water maze, one study reported impaired spatial memory after treatment with a potent cannabinoid receptor agonist (Robinson et al. 2010), but several others showed improvement of spatial learning and prevention of memory deficits with selective Cb₂R agonism (Çakır et al. 2019; Lou et al. 2017; Sun et al. 2017; Wu et al. 2017) (for an overview, see Table 1). These results suggest that Cb₂R activation differently modulates aversive and neutral memories. Consistently, Cb₂R mRNA transcripts in the DG and CA1 regions of the dorsal hippocampus were shown to be increased in stressed and anxious mice, further supporting the inducibility of Cb₂R expression and indicating a role in coping mechanisms (Robertson et al. 2017). On the other hand, most studies using Cb₂R knockout or antagonism showed impaired neutral, aversive, and spatial memory (see Table 2). But there is also evidence for contrasting promnesic and amnesic effects of Cb₂R knockout on the Y-maze and fear conditioning, respectively (Li and Kim 2016b). Therefore, Cb₂Rs modulate memory and behavior, as well as anxiety and stress, but less is known about their specific role and the exact mechanisms underlying their function (Figure 3). With increasing research interests and emerging techniques such as optogenetics and DREADDs, we hope that these questions will be addressed soon.

Table 1: Cb₂R overexpression and agonism effects on memory and behavior.

Model	Behavior test	Effect	Reference
JWH-133 (0.2 mg/kg), intraperitoneal (IP) injections for 13 days, Okadaic acid (OKA) Alzheimer's Disease (AD) model Sprague–Dawley rats, male	Morris Water Maze	Reduced neurodegeneration, neuro-inflammation, and spatial memory impairment in the OKA-induced AD model	Çakır et al. (2019)
Δ9-tetrahydrocannabinol (THC) (0.1 mg/kg/day for five days), IP injection, Sabra mice, adult, female	Eight-arm Maze	Prevented spatial work memory deficit caused by liver failure	Dagon et al. (2007)
JWH-133 (0.2 mg/kg/day in the drinking water for four months), TgAPP transgenic mice, adult, male	Object Recognition	Rescued memory deficit	Köfalvi et al. (2016) and Martín-Moreno et al. (2012)
Overexpression in interneurons (CA1), Gad2-Cas9 mice, 2–3 months	Y-Maze, Fear Conditioning, Tail Suspension, Open Field	No effect	Li and Kim (2017)
Overexpression in pyramidal neurons (CA1), Camk2a-Cas9 mice, 2–3 months	Open Field	Reduced anxiety levels	Li and Kim (2017)
Overexpression in microglia (CA1), Cx3cr1-Cas9 mice, 2–3 months	Fear Conditioning	Enhanced contextual fear memory	Li and Kim (2017)
β-caryophyllene (BCP) (16, 48, and 144 mg/kg), IP injection, Sprague-Dawley rats, adult, male	Morris Water Maze (50 days after bilateral carotid artery clamping)	Improved spatial learning and memory	Lou et al. (2017)
GP1a (150 ng/rat), post-training, intra-CA1 microinjection, Wistar rats, adult, male	Inhibitory Avoidance	Impaired memory consolidation	Nasehi et al., (2017)
GP1a (100 μg/mouse), post-training intra-CA1 microinjection, NMRI mice, adult, male	Inhibitory Avoidance	Impaired memory consolidation	Nasehi et al. (2018)
GP1a (10 and 100 μg/mouse), post-training intra-CA3 microinjection, NMRI mice, adult, male	Inhibitory Avoidance	Impaired memory consolidation	Nasehi et al. (2020)
2-arachidonoylglycerol (2-AG) (5 mg/kg), IP injection, Sprague–Dawley rats, adult, male	Inhibitory Avoidance	Enhanced memory retention	Ratano et al. (2018)
CBD (10–30 pmol), intradorsal hippocampus injection, immediately, 1 or 3 h after fear conditioning, Wistar rats, adult male	Fear Conditioning	Impaired contextual fear memory consolidation	Raymundi et al. (2020)
WIN55,212-2 (1 and 3 mg/kg), IP injection 30 min prior to each daily training session, lister Hooded rats, adult, male	Morris Water Maze	Impaired spatial memory	Robinson et al. (2010)
JWH-133 (2 mg/kg, every 24 h post orthopedic surgery), IP injections, C57BL/6 mice, adult	Training preoperative, Fear Conditioning (30 min after injection)	Attenuated surgery-induced memory loss	Sun et al. (2017)
MDA7 (14 mg/kg every second day for five months), IP injection, APP/PS1 mice, adult, female	Morris Water Maze	Improved spatial memory	Wu et al. (2017)

Cb₂ receptors and hippocampal adult neurogenesis

Previously we discussed the expression and some of the functions of Cb₂R present in fully differentiated hippocampal cells. However, what about the hippocampal neural progenitor cells? For decades, scientists believed that the adult brain did not generate new neurons. This belief persisted until Altman and Das (1965) first reported neurogenesis in the DG of adult rodents, a finding that was later confirmed by several studies (Gonçalves et al. 2016;

Jorgensen 2018). Currently, it is widely accepted that hippocampal adult neurogenesis happens in the subgranular zone of the DG of humans and several other vertebrates (Gonçalves et al. 2016; Jorgensen 2018). This process has been associated with memory and learning (Cameron and Glover 2015; Deng et al. 2010), mood disorders (Jorgensen 2018; Snyder et al. 2011), and neurological diseases (Horgusluoglu et al. 2017).

In the last decades, several studies have shown an important role of Cb₂R in hippocampal adult neurogenesis. First, Palazuelos et al. (2006) used both *in vitro* and *in vivo*

Table 2: Cb₂R knockout and antagonism effects on memory and behavior.

Model	Behavior test	Effect	Reference
Cb₂R knockout mice (C57BL/6) background), 2–4 months	Fear Conditioning, Y-Maze	Impaired contextual long-term memory, enhanced spatial working memory	Li and Kim (2016)
Disruption of <i>Cnr2</i> gene expression in interneurons (CA1), Gad2-Cas9 mice, 2–3 months	Y-Maze, Fear Conditioning, Tail Suspension, Open Field	No effect	Li and Kim (2017)
Disruption of <i>Cnr2</i> gene expression in pyramidal neurons (CA1), Camk2a-Cas9, 2–3 months	Y-Maze	Enhanced spatial working memory	Li and Kim (2017)
Disruption of <i>Cnr2</i> gene expression in microglia (CA1), Cx3cr1-Cas9, 2–3 months	Fear Conditioning	Decreased contextual fear memory	Li and Kim (2017)
AM630 (75 and 100 ng/rat), post-training, intra-CA1 microinjection, Wistar rats, adult, male	Inhibitory Avoidance	Impaired memory consolidation	Nasehi et al. (2017)
AM630 (1, 10, and 100 µg), post-training, intra-CA3 microinjection, NMRI mice, adult, male	Inhibitory Avoidance	No effect	Nasehi et al. (2020)
SR144528 (0.1 mg/kg), IP injection, Sprague–Dawley rats, adult, male	Inhibitory Avoidance	Tended to impair fear memory retention	Ratano et al. (2017)
AM630 (0.3 mg/kg), IP or dorsal hippocampus injection, Wistar rats, 13–15 weeks, male	Fear Conditioning	Prevented the disrupting effects of cannabidiol on fear memory consolidation	Stern et al. (2017)
AM630 (3 mg/kg, every 24 h post orthopedic surgery), IP injections, C57BL/6 mice, adult	Training preoperative, Fear Conditioning (30 min after injection)	Aggravated surgery-induced memory loss	Sun et al. (2017)
miR-139 (3 mM), intradentate gyrus microinjection, SAMP8 Alzheimer’s Disease mouse model, six months old	Morris Water Maze, Novel Object Recognition, Contextual Fear Conditioning	Impaired spatial memory acquisition and retention, impaired novel object recognition, impaired fear conditioning	Tang et al. (2017)

approaches to show that (1) Cb₂R are expressed in both developmental and adult neural progenitor cells, and this expression is reduced after cell differentiation; (2) ablation of Cb₂R signaling through knockout impairs adult neurogenesis; and (3) Cb₂R activation increases hippocampal adult neurogenesis (Palazuelos et al. 2006). Then, in a follow-up study, Palazuelos et al. (2012) reproduced their previous results and extended them by showing that Cb₂R promotes adult neurogenesis through the activation of the PI3K/Akt/mTORC1 pathway. This results in the inhibition of the cyclin-dependent kinase inhibitor p27Kip1, a protein that inhibits the G₁-S phase transition in neural progenitor cells (Palazuelos et al. 2012). Later, Avraham et al. (2014) showed that Cb₂R activation could reverse the deficits in hippocampal adult neurogenesis caused by the human immunodeficiency virus (HIV) glycoprotein 120 (Gp120) and could thus be a potential mechanism to treat HIV-associated neurocognitive disorders (Avraham et al. 2014). On the other hand, a recent study by Rodrigues et al. (2017) partially contradicted the previously mentioned results by showing that activation of Cb₂Rs alone is not enough to induce the proliferation of DG neural precursor

cells. Instead, activation of both Cb₂Rs and Cb₁Rs was necessary to induce this proliferation. Furthermore, they showed that, although Cb₂R agonism is enough to induce differentiation of DG neural precursor cells, Cb₁R signaling is still needed since its blockade prevents the effect of Cb₂R agonism. Finally, they suggest the formation of Cb₂R-Cb₁R heteromers in these cells, which could be controlling neuronal differentiation (Rodrigues et al. 2017). Moreover, another recent study showed that Cb₂R-deficient mice have normal hippocampal adult neurogenesis, suggesting that Cb₂R signaling might not be necessary for basal hippocampal adult neurogenesis (Mensching et al. 2019). However, since the animals used in this study were constitutive knockouts, compensatory mechanisms might have influenced the results.

In sum, these studies strongly suggest that Cb₂R signaling is important to control/modulate hippocampal adult neurogenesis. However, it is still not fully clear if it acts alone or in conjunction with Cb₁R signaling or the specific mechanisms involved. Thus, further experiments are necessary to address these questions and whether Cb₂R

Cellular Mechanisms of Cb₂R function

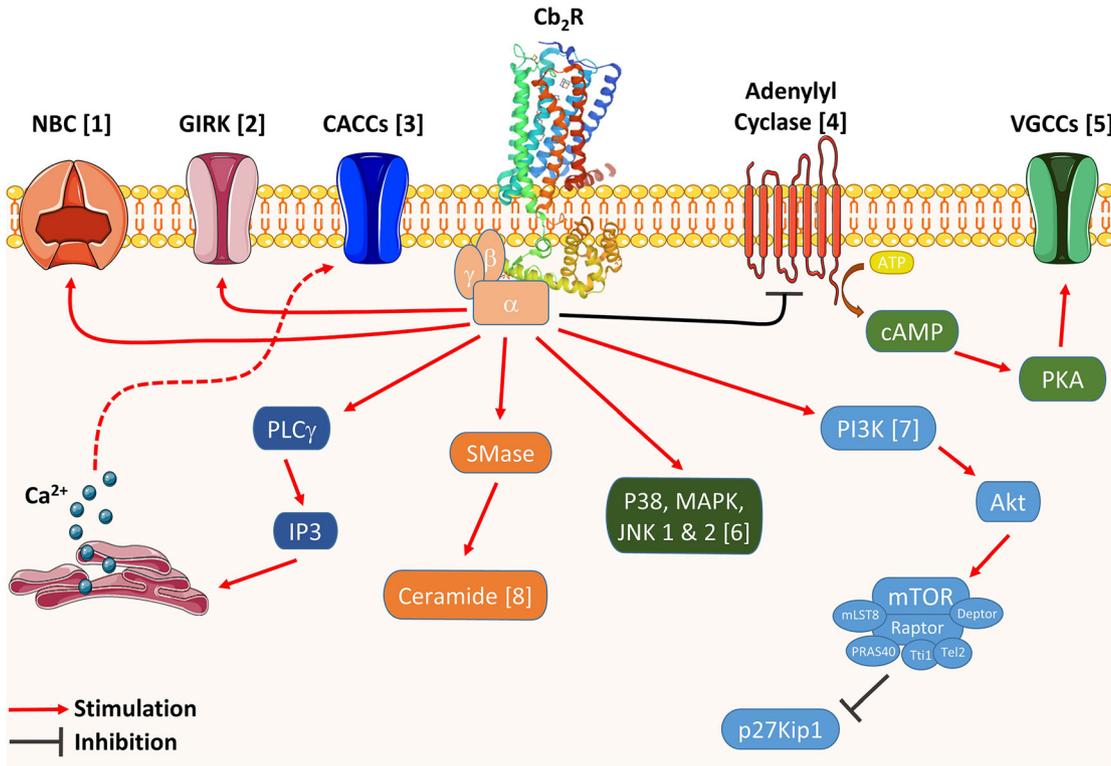


Figure 3: Cellular mechanisms of Cb₂R function in a variety of expression systems and animal species. Activation of Cb₂Rs has a variety of downstream effects via different pathways. These effects are mostly mediated by the activation of the coupled G-protein (represented by its α , β , and γ subunits). Cb₂R activation can: (1) mediate plasticity in hippocampal CA3 principal neurons via a G-protein and Na⁺-dependent modulation of the sodium/bicarbonate co-transporter (NBC) (Stempel et al. 2016); (2) lead to a G-protein gated inward rectifying potassium channel (GIRK) mediated cell-autonomous hyperpolarization (Stumpf et al. 2018); (3) through phospholipase C (PLC) production, lead to Ca²⁺ release via IP₃ which in turn leads to the opening of Ca²⁺-activated Cl⁻ channels (CACCs) in layer II/III pyramidal neurons of the rat medial prefrontal cortex (den Boon et al. 2012); (4) negatively affect the production of adenylyl cyclase, which, through decreased cyclic adenosine monophosphate (cAMP) production results in reduced activation of protein kinase A (PKA) (Dhopeshwarkar and Mackie 2014); (5) mediate suppression of voltage-gated calcium channels (VGCCs) via reduced activation of PKA (Qian et al. 2017); (6) cause increased phosphorylation of p38 MAPK, JNK1 and JNK2 (Neves et al. 2018); (7) mediate upregulation of the PI3K/Akt pathway, increasing the activity of mTORC1 (composed by mTOR, Raptor, Deptor, mLST8, PRAS40, Tti1, and Tel2), which in turn inhibits the cyclin-dependent kinase inhibitor p27Kip1 (Cao et al. 2018; Palazuelos et al. 2012); (8) through activation of sphingomyelin phosphodiesterase (SMase), can result in increased ceramide production (Askari and Shafiee-Nick 2019). For full reviews of Cb₂R function mechanisms and therapeutic potentials, see Aghazadeh Tabrizi et al. (2016) and Cassano et al. (2017). Cb₂R crystal structure taken from RCSB Protein Data Bank (<https://www.rcsb.org/structure/5ZTY>) (Li et al. 2019).

signaling is necessary for basal hippocampal adult neurogenesis or is only recruited in specific situations.

Concluding remarks and future perspectives

There is now enough data to support not only the existence but also the functional relevance of hippocampal Cb₂Rs, which challenges our prior understanding of ECS action in

the CNS and warrants further exploration. Hopefully, modern techniques will offer more robust approaches to answering some of the outstanding questions and shed light on the contradictory results in the literature. Among them, we should address the suggested postsynaptic localization of hippocampal Cb₂Rs, which would crucially differentiate them from presynaptic Cb₁Rs. Both light- and electron microscopy may help in this regard, and even more advanced techniques such as super-resolution microscopy could produce robust results (Cristino et al. 2017). Moreover, it is worth noting that the studies reviewed by us

reported the discovery and active presence of Cb₂Rs in the mammalian brain. Still, more quantitative approaches are needed to provide information to support differences in Cb₂R expression along the hippocampal subregions and between their ventral and dorsal portions. This would improve the discussion of the functions of hippocampal Cb₂Rs since the dorsal and ventral parts differently contribute to memory, anxiety, neurogenesis, and related pathologies (Fanselow and Dong 2010; Nadel et al. 2013). Until now, the most common approach for studying Cb receptor activity has been through pharmacology, with a large degree of uncertainty regarding the specificity of the compounds used (Console-Bram et al. 2012). Novel Cb₂R-specific compounds and emerging transgenic tools now offer more targeted methods (Bickle 2016; Nevalainen 2014), and further combining these tools with current knowledge of regional and neuronal diversity should generate significant new insights.

The Cb₂Rs may play a crucial role in regulating hippocampal-dependent memory formation and adult hippocampal neurogenesis, particularly during neuro-inflammatory conditions where Cb₂Rs are upregulated. The properties of low expression but high-inducibility during pathological conditions should incite more research on therapeutic strategies with selective Cb₂R-modulators, especially considering their lower psychoactive effects than those of Cb₁R-ligands (Chen et al. 2017). Further exploring hippocampal Cb₂Rs should not only increase our understanding of the ECS but also contribute to the debate on the legislation of medical cannabis that currently concerns several countries worldwide. The emerging field of hippocampal Cb₂Rs provides avenues for exploration and discovery; insightful times lie ahead.

Outstanding questions

- (1) Is there a functional relationship between central Cb₁ and Cb₂ receptors? Can they form heteromers? If so, are they present in adult cells and what would be the functional implications?
- (2) Are Cb₂Rs mostly expressed postsynaptically? If so, should we replace the retrograde signaling view of the ECS in the CNS by a more complex signaling dynamics?
- (3) How are Cb₂Rs expressed in different hippocampal subregions and neuronal cell types? Would they mark a specific subset of interneurons? And what is the balance of expression between immune and nonimmune cells?
- (4) What are the mechanisms of action of Cb₂Rs in the hippocampus? Would the activity of Cb₂Rs in microglia also influence network states?
- (5) Is there a therapeutic role for Cb₂R ligands, through orthosteric or allosteric binding, in neurodegenerative or neuroinflammatory diseases?

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References

- Aghazadeh Tabrizi, M., Baraldi, P.G., Borea, P.A., and Varani, K. (2016). Medicinal chemistry, pharmacology, and potential therapeutic benefits of cannabinoid CB2 receptor agonists. *Chem. Rev.* 116: 519–560.
- Altman, J. and Das, G.D. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J. Comp. Neurol.* 124: 319–335.
- An, D., Peigneur, S., Hendrickx, L.A., and Tytgat, J. (2020). Targeting cannabinoid receptors: current status and prospects of natural products. *Int. J. Mol. Sci.* 21: 5064.
- Ashton, J.C., Friberg, D., Darlington, C.L., and Smith, P.F. (2006). Expression of the cannabinoid CB2 receptor in the rat cerebellum: an immunohistochemical study. *Neurosci. Lett.* 396: 113–116.
- Askari, V.R. and Shafiee-Nick, R. (2019). Promising neuroprotective effects of β-caryophyllene against LPS-induced oligodendrocyte toxicity: a mechanistic study. *Biochem. Pharmacol.* 159: 154–171.
- Atwood, B.K. and Mackie, K. (2010). CB2: a cannabinoid receptor with an identity crisis. *Br. J. Pharmacol.* 160: 467–479.
- Avraham, H.K., Jiang, S., Fu, Y., Rockenstein, E., Makriyannis, A., Zvonok, A., Masliah, E., and Avraham, S. (2014). The cannabinoid CB2 receptor agonist AM1241 enhances neurogenesis in GFAP/Gp120 transgenic mice displaying deficits in neurogenesis. *Br. J. Pharmacol.* 171: 468–479.
- Baek, J.-H., Zheng, Y., Darlington, C.L., and Smith, P.F. (2008). Cannabinoid CB2 receptor expression in the rat brainstem cochlear and vestibular nuclei. *Acta Otolaryngol.* 128: 961–967.

- Basu, S. and Dittel, B.N. (2011). Unraveling the complexities of cannabinoid receptor 2 (CB2) immune regulation in health and disease. *Immunol. Res.* 51: 26–38.
- Bickel, J. (2016). Revolutions in neuroscience: tool development. *Front. Syst. Neurosci.* 10: 24.
- den Boon, F.S., Chameau, P., Schaafsma-Zhao, Q., van Aken, W., Bari, M., Oddi, S., Kruse, C.G., Maccarrone, M., Wadman, W.J., and Werkman, T.R. (2012). Excitability of prefrontal cortical pyramidal neurons is modulated by activation of intracellular type-2 cannabinoid receptors. *Proc. Natl. Acad. Sci. U.S.A.* 109: 3534.
- Branconnier, R.J. (1983). The efficacy of the cerebral metabolic enhancers in the treatment of senile dementia. *Psychopharmacol. Bull.* 19: 212–219.
- Brusco, A., Tagliaferro, P., Saez, T., and Onaivi, E.S. (2008). Postsynaptic localization of CB2 cannabinoid receptors in the rat hippocampus. *Synapse* 62: 944–949.
- Cabral, G.A. and Griffin-Thomas, L. (2009). Emerging role of the CB2 cannabinoid receptor in immune regulation and therapeutic prospects. *Expet Rev. Mol. Med.* 11: e3.
- Çakır, M., Tekin, S., Doğanyığıt, Z., Erden, Y., Soytürk, M., Çiğremiş, Y., and Sandal, S. (2019). Cannabinoid type 2 receptor agonist JWH-133, attenuates Okadaic acid induced spatial memory impairment and neurodegeneration in rats. *Life Sci.* 217: 25–33.
- Cameron, H.A. and Glover, L.R. (2015). Adult neurogenesis: beyond learning and memory. *Annu. Rev. Psychol.* 66: 53–81.
- Campos, A.C., Paraíso-Luna, J., Fogaça, M.V., Guimarães, F.S., and Galve-Roperh, I. (2017). Cannabinoids as regulators of neural development and adult neurogenesis. In: Pébay, A., and Wong, R.C. (Eds.), *Lipidomics of stem cells*. Springer, Cham, Switzerland, pp. 117–136.
- Cao, Q., Liu, X., Yang, F., and Wang, H. (2018). CB2R induces a protective response for epileptic seizure via the PI3K 110 α -AKT signaling pathway. *Exp. Ther. Med.* 16: 4784–4790.
- Cassano, T., Calcagnini, S., Pace, L., De Marco, F., Romano, A., and Gaetani, S. (2017). Cannabinoid receptor 2 signaling in neurodegenerative disorders: from pathogenesis to a promising therapeutic target. *Front. Neurosci.* 11: 30.
- Castillo, P.E., Younts, T.J., Chávez, A.E., and Hashimoto-dani, Y. (2012). Endocannabinoid signaling and synaptic function. *Neuron* 76: 70–81.
- Chen, D., Gao, M., Gao, F., Su, Q., and Wu, J. (2017). Brain cannabinoid receptor 2: expression, function and modulation. *Acta Pharmacol. Sin.* 38: 312–316.
- Chevaleyre, V. and Castillo, P.E. (2003). Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. *Neuron* 38: 461–472.
- Console-Bram, L., Marcu, J., and Abood, M.E. (2012). Cannabinoid receptors: nomenclature and pharmacological principles. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 38: 4–15.
- Cristino, L., Imperatore, R., and Di Marzo, V. (2017). Chapter four - techniques for the cellular and subcellular localization of endocannabinoid receptors and enzymes in the mammalian brain. In: Reggio, P.H. (Ed.), *Methods in enzymology*. Academic Press, San Diego, USA, pp. 61–98.
- Dagon, Y., Avraham, Y., Ilan, Y., Mechoulam, R., and Berry, E.M. (2007). Cannabinoids ameliorate cerebral dysfunction following liver failure via AMP-activated protein kinase. *FASEB J* 21: 2431–2441.
- Deng, W., Aimone, J.B., and Gage, F.H. (2010). New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? *Nat. Rev. Neurosci.* 11: 339–350.
- Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., and Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258: 1946–1949.
- Dhopeswarkar, A. and Mackie, K. (2014). CB2 cannabinoid receptors as a therapeutic target—what does the future hold? *Mol. Pharmacol.* 86: 430–437.
- Egertová, M. and Elphick, M.R. (2000). Localisation of cannabinoid receptors in the rat brain using antibodies to the intracellular C-terminal tail of CB1. *J. Comp. Neurol.* 422: 159–171.
- Fanselow, M.S. and Dong, H.-W. (2010). Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron* 65: 7–19.
- García-Gutiérrez, M.S. and 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.
- García-Gutiérrez, M.S., García-Bueno, B., Zoppi, S., Leza, J.C., and Manzanares, J. (2012). Chronic blockade of cannabinoid CB2 receptors induces anxiolytic-like actions associated with alterations in GABA(A) receptors. *Br. J. Pharmacol.* 165: 951–964.
- Gómez-Gálvez, Y., Palomo-Garo, C., Fernández-Ruiz, J., and García, C. (2016). Potential of the cannabinoid CB(2) receptor as a pharmacological target against inflammation in Parkinson's disease. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 64: 200–208.
- Gonçalves, J.T., Schafer, S.T., and Gage, F.H. (2016). Adult neurogenesis in the hippocampus: from stem cells to behavior. *Cell* 167: 897–914.
- Gong, J.-P., Onaivi, E.S., Ishiguro, H., Liu, Q.-R., Tagliaferro, P.A., Brusco, A., and Uhl, G.R. (2006). Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Res.* 1071: 10–23.
- Grünblatt, E., Zander, N., Bartl, J., Jie, L., Monoranu, C.-M., Arzberger, T., Ravid, R., Roggendorf, W., Gerlach, M., and Riederer, P. (2007). Comparison analysis of gene expression patterns between sporadic Alzheimer's and Parkinson's disease. *J. Alzheim. Dis.* 12: 291–311.
- Guida, F., Luongo, L., Boccella, S., Giordano, M.E., Romano, R., Bellini, G., Manzo, I., Furiano, A., Rizzo, A., Imperatore, R., et al. (2017). Palmitoylethanolamide induces microglia changes associated with increased migration and phagocytic activity: involvement of the CB2 receptor. *Sci. Rep.* 7: 375.
- Herkenham, M., Lynn, A., Johnson, M., Melvin, L., de Costa, B., and Rice, K. (1991). Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J. Neurosci.* 11: 563–583.
- Horgusluoglu, E., Nudelman, K., Nho, K., and Saykin, A.J. (2017). Adult neurogenesis and neurodegenerative diseases: a systems biology perspective. *Am. J. Med. Genet. Part B Neuropsychiatr. Genet.* 174: 93–112.
- Jorgensen, C. (2018). Adult mammalian neurogenesis and motivated behaviors. *Integr. Zool.* 13: 655–672.
- Kano, M., Ohno-Shosaku, T., Hashimoto-dani, Y., Uchigashima, M., and Watanabe, M. (2009). Endocannabinoid-mediated control of synaptic transmission. *Physiol. Rev.* 89: 309–380.

- Katona, I., Sperlágh, B., Sík, A., Käfalvi, A., Vizi, E.S., Mackie, K., and Freund, T.F. (1999). Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J. Neurosci.* 19: 4544–4558.
- Kim, J. and Li, Y. (2015). Chronic activation of CB2 cannabinoid receptors in the hippocampus increases excitatory synaptic transmission. *J. Physiol.* 593: 871–886.
- Klausberger, T. and Somogyi, P. (2008). Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science* 321: 53–57.
- Köfalvi, A., Lemos, C., Martín-Moreno, A.M., Pinheiro, B.S., García-García, L., Pozo, M.A., Valério-Fernandes, Â., Beleza, R.O., Agostinho, P., Rodrigues, R.J., et al. (2016). Stimulation of brain glucose uptake by cannabinoid CB2 receptors and its therapeutic potential in Alzheimer's disease. *Neuropharmacology* 110: 519–529.
- Kopach, O., Vats, J., Netsyk, O., Voitenko, N., Irving, A., and Fedirko, N. (2012). Cannabinoid receptors in submandibular acinar cells: functional coupling between saliva fluid and electrolytes secretion and Ca²⁺ signalling. *J. Cell Sci.* 125: 1884–1895.
- Leuner, B. and Gould, E. (2010). Structural plasticity and hippocampal function. *Annu. Rev. Psychol.* 61: 111–140.
- Li, Y. and Kim, J. (2015). Neuronal expression of CB2 cannabinoid receptor mRNAs in the mouse hippocampus. *Neuroscience* 311: 253–267.
- Li, Y. and Kim, J. (2016a). CB2 cannabinoid receptor knockout in mice impairs contextual long-term memory and enhances spatial working memory. *Neural Plast.* 2016: 9817089.
- Li, Y. and Kim, J. (2016b). Deletion of CB2 cannabinoid receptors reduces synaptic transmission and long-term potentiation in the mouse hippocampus. *Hippocampus* 26: 275–281.
- Li, Y. and Kim, J. (2017). Distinct roles of neuronal and microglial CB2 cannabinoid receptors in the mouse hippocampus. *Neuroscience* 363: 11–25.
- Li, X., Hua, T., Vemuri, K., Ho, J.-H., Wu, Y., Wu, L., Popov, P., Benchama, O., Zvonok, N., Locke, K., et al. (2019). Crystal structure of the human cannabinoid receptor CB2. *Cell* 176: 459–467.e13.
- Lisboa, S.F., Vila-Verde, C., Rosa, J., Uliana, D.L., Stern, C.A. J., Bertoglio, L.J., Resstel, L.B., and Guimaraes, F.S. (2019). Tempering aversive/traumatic memories with cannabinoids: a review of evidence from animal and human studies. *Psychopharmacology (Berlin)* 236: 201–226.
- Liu, Q.-R., Pan, C.-H., Hishimoto, A., Li, C.-Y., Xi, Z.-X., Llorente-Berzal, A., Viveros, M.-P., Ishiguro, H., Arinami, T., Onaivi, E.S., et al. (2009). Species differences in cannabinoid receptor 2 (CNR2 gene): identification of novel human and rodent CB2 isoforms, differential tissue expression and regulation by cannabinoid receptor ligands. *Genes Brain Behav.* 8: 519–530.
- López, A., Aparicio, N., Pazos, M.R., Grande, M.T., Barreda-Manso, M.A., Benito-Cuesta, I., Vázquez, C., Amores, M., Ruiz-Pérez, G., García-García, E., et al. (2018). Cannabinoid CB2 receptors in the mouse brain: relevance for Alzheimer's disease. *J. Neuroinflammation* 15: 158.
- Lou, J., Teng, Z., Zhang, L., Yang, J., Ma, L., Wang, F., Tian, X., An, R., Yang, M., Zhang, Q., et al. (2017). β -Caryophyllene/hydroxypropyl- β -cyclodextrin inclusion complex improves cognitive deficits in rats with vascular dementia through the cannabinoid receptor type 2-mediated pathway. *Front. Pharmacol.* 8, <https://doi.org/10.3389/fphar.2017.00002>.
- Luo, X.-Q., Li, A., Yang, X., Xiao, X., Hu, R., Wang, T.-W., Dou, X.-Y., Yang, D.-J., and Dong, Z. (2018). Paeoniflorin exerts neuroprotective effects by modulating the M1/M2 subset polarization of microglia/macrophages in the hippocampal CA1 region of vascular dementia rats via cannabinoid receptor 2. *Chin. Med.* 13: 14.
- Luongo, L., Palazzo, E., Tambaro, S., Giordano, C., Gatta, L., Scafuro, M.A., Rossi, F.S., Lazzari, P., Pani, L., de Novellis, V., et al. (2010). 1-(2',4'-dichlorophenyl)-6-methyl-N-cyclohexylamine-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide, a novel CB2 agonist, alleviates neuropathic pain through functional microglial changes in mice. *Neurobiol. Dis.* 37: 177–185.
- Mailleux, P. and Vanderhaeghen, J.-J. (1992). Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and in situ hybridization histochemistry. *Neuroscience* 48: 655–668.
- Martín-Moreno, A.M., Brera, B., Spuch, C., Carro, E., García-García, L., Delgado, M., Pozo, M.A., Innamorato, N.G., Cuadrado, A., and de Ceballos, M.L. (2012). Prolonged oral cannabinoid administration prevents neuroinflammation, lowers β -amyloid levels and improves cognitive performance in Tg APP 2576 mice. *J. Neuroinflammation* 9: 1–15.
- Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., and Bonner, T.I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346: 561–564.
- Matsuda, L.A., Bonner, T.I., and Lolait, S.J. (1993). Localization of cannabinoid receptor mRNA in rat brain. *J. Comp. Neurol.* 327: 535–550.
- Mechoulam, R. and Parker, L.A. (2013). The endocannabinoid system and the brain. *Annu. Rev. Psychol.* 64: 21–47.
- Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N.E., Schatz, A.R., Gopher, A., Almog, S., Martin, B.R., and Compton, D.R. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* 50: 83–90.
- Mensching, L., Djogo, N., Keller, C., Rading, S., and Karsak, M. (2019). Stable adult hippocampal neurogenesis in cannabinoid receptor CB2 deficient mice. *Int. J. Mol. Sci.* 20, <https://doi.org/10.3390/ijms20153759>.
- Messier, C. (2004). Glucose improvement of memory: a review. *Eur. J. Pharmacol.* 490: 33–57.
- Miller, L.K. and Devi, L.A. (2011). The highs and lows of cannabinoid receptor expression in disease: mechanisms and their therapeutic implications. *Pharmacol. Rev.* 63: 461–470.
- Miracourt, L.S., Tsui, J., Gobert, D., Desjardins, J.-F., Schohl, A., Sild, M., Spratt, P., Castonguay, A., De Koninck, Y., Marsh-Armstrong, N., et al. (2016). Endocannabinoid signaling enhances visual responses through modulation of intracellular chloride levels in retinal ganglion cells. *eLife* 5, <https://doi.org/10.7554/eLife.15932>.
- Monory, K., Polack, M., Remus, A., Lutz, B., and Korte, M. (2015). Cannabinoid CB1 receptor calibrates excitatory synaptic balance in the mouse hippocampus. *J. Neurosci.* 35: 3842–3850.
- Morena, M., Berardi, A., Colucci, P., Palmery, M., Trezza, V., Hill, M.N., and Campolongo, P. (2018). Enhancing endocannabinoid neurotransmission augments the efficacy of extinction training and ameliorates traumatic stress-induced behavioral alterations in rats. *Neuropsychopharmacology* 43: 1284–1296.

- Morgan, N.H., Stanford, I.M., and Woodhall, G.L. (2009). Functional CB₂ type cannabinoid receptors at CNS synapses. *Neuropharmacology* 57: 356–368.
- Munro, S., Thomas, K.L., and Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365: 61–65.
- Nadel, L., Hoscheidt, S., and Ryan, L.R. (2013). Spatial cognition and the hippocampus: the anterior-posterior axis. *J. Cognit. Neurosci.* 25: 22–28.
- Nasehi, M., Hajikhani, M., Ebrahimi-Ghiri, M., and Zarrindast, M.-R. (2017). Interaction between NMDA and CB₂ function in the dorsal hippocampus on memory consolidation impairment: an isobologram analysis. *Psychopharmacology (Berlin)* 234: 507–514.
- Nasehi, M., Gerami-Majd, F., Khakpai, F., and Zarrindast, M.-R. (2018). Dorsal hippocampal cannabinergic and GABAergic systems modulate memory consolidation in passive avoidance task. *Brain Res. Bull.* 137: 197–203.
- Nasehi, M., Forouzanmehr, E., Khakpai, F., and Zarrindast, M.-R. (2020). Possible interaction between the ventral hippocampal cannabinoid CB₂ and muscarinic acetylcholine receptors on the modulation of memory consolidation in mice. *NeuroReport* 31: 174–183.
- Navarrete, F., Pérez-Ortiz, J.M., and Manzanares, J. (2012). Cannabinoid CB₂ receptor-mediated regulation of impulsive-like behaviour in DBA/2 mice. *Br. J. Pharmacol.* 165: 260–273.
- Navarrete, F., García-Gutiérrez, M.S., Aracil-Fernández, A., Lanciego, J.L., and Manzanares, J. (2018). Cannabinoid CB₁ and CB₂ receptors, and monoacylglycerol lipase gene expression alterations in the basal ganglia of patients with Parkinson's disease. *Neurotherapeutics* 15: 459–469.
- Nevalainen, T. (2014). Recent development of CB₂ selective and peripheral CB₁/CB₂ cannabinoid receptor ligands. *Curr. Med. Chem.* 21: 187–203.
- Neves, L.M.S., Gonçalves, E.C.D., Cavalli, J., Vieira, G., Laurindo, L.R., Simões, R.R., Coelho, I.S., Santos, A.R.S., Marcolino, A.M., Cola, M., et al. (2018). Photobiomodulation therapy improves acute inflammatory response in mice: the role of cannabinoid receptors/ATP-sensitive K⁺ channel/p38-MAPK signalling pathway. *Mol. Neurobiol.* 55: 5580–5593.
- Onaivi, E.S. (2006). Neuropsychobiological evidence for the functional presence and expression of cannabinoid CB₂ receptors in the brain. *Neuropsychobiology* 54: 231–246.
- Onaivi, E.S., Ishiguro, H., Gong, J.-P., Patel, S., Perchuk, A., Meozzi, P.A., Myers, L., Mora, Z., Tagliaferro, P., Gardner, E., et al. (2006). Discovery of the presence and functional expression of cannabinoid CB₂ receptors in brain. *Ann. N. Y. Acad. Sci.* 1074: 514–536.
- Onaivi, E.S., Ishiguro, H., Gu, S., and Liu, Q.-R. (2011). CNS effects of CB₂ cannabinoid receptors: beyond neuro-immuno-cannabinoid activity. *J. Psychopharmacol.* 26: 92–103.
- Palazuelos, J., Aguado, T., Egia, A., Mechoulam, R., Guzmán, M., and Galve-Roperh, I. (2006). Non-psychoactive CB₂ cannabinoid agonists stimulate neural progenitor proliferation. *FASEB J* 20: 2405–2407.
- Palazuelos, J., Aguado, T., Pazos, M.R., Julien, B., Carrasco, C., Resel, E., Sagredo, O., Benito, C., Romero, J., Azcoitia, I., et al. (2009). Microglial CB₂ cannabinoid receptors are neuroprotective in Huntington's disease excitotoxicity. *Brain* 132: 3152–3164.
- Palazuelos, J., Ortega, Z., Díaz-Alonso, J., Guzmán, M., and Galve-Roperh, I. (2012). CB₂ cannabinoid receptors promote neural progenitor cell proliferation via mTORC1 signaling. *J. Biol. Chem.* 287: 1198–1209.
- Perry, V.H. and Teeling, J. (2013). Microglia and macrophages of the central nervous system: the contribution of microglia priming and systemic inflammation to chronic neurodegeneration. *Semin. Immunopathol.* 35: 601–612.
- Qian, W.-J., Yin, N., Gao, F., Miao, Y., Li, Q., Li, F., Sun, X.-H., Yang, X.-L., and Wang, Z. (2017). Cannabinoid CB₁ and CB₂ receptors differentially modulate L- and T-type Ca²⁺ channels in rat retinal ganglion cells. *Neuropharmacology* 124: 143–156.
- Racz, I., Nadal, X., Alferink, J., Baños, J.E., Rehnelt, J., Martín, M., Pintado, B., Gutierrez-Adan, A., Sanguino, E., Manzanares, J., et al. (2008). Crucial role of CB₂ cannabinoid receptor in the regulation of central immune responses during neuropathic pain. *J. Neurosci.* 28: 12125–12135.
- Ratano, P., Palmery, M., Trezza, V., and Campolongo, P. (2017). Cannabinoid modulation of memory consolidation in rats: beyond the role of cannabinoid receptor subtype 1. *Front. Pharmacol.* 8: 200.
- Ratano, P., Petrella, C., Forti, F., Passeri, P.P., Morena, M., Palmery, M., Trezza, V., Severini, C., and Campolongo, P. (2018). Pharmacological inhibition of 2-arachidonoylglycerol hydrolysis enhances memory consolidation in rats through CB₂ receptor activation and mTOR signaling modulation. *Neuropharmacology* 138: 210–218.
- Raymundi, A.M., da Silva, T.R., Zamprônio, A.R., Guimarães, F.S., Bertoglio, L.J., and Stern, C.A.J. (2020). A time-dependent contribution of hippocampal CB₁, CB₂ and PPAR γ receptors to cannabidiol-induced disruption of fear memory consolidation. *Br. J. Pharmacol.* 177: 945–957.
- Robertson, J.M., Achua, J.K., Smith, J.P., Prince, M.A., Staton, C.D., Ronan, P.J., Summers, T.R., and Summers, C.H. (2017). Anxious behavior induces elevated hippocampal CB₂ receptor gene expression. *Neuroscience* 352: 273–284.
- Robinson, L., Goonawardena, A.V., Pertwee, R., Hampson, R.E., Platt, B., and Riedel, G. (2010). WIN55,212-2 induced deficits in spatial learning are mediated by cholinergic hypofunction. *Behav. Brain Res.* 208: 584–592.
- Rodrigues, R.S., Ribeiro, F.F., Ferreira, F., Vaz, S.H., Sebastião, A.M., and Xapelli, S. (2017). Interaction between cannabinoid type 1 and type 2 receptors in the modulation of subventricular zone and dentate gyrus neurogenesis. *Front. Pharmacol.* 8, <https://doi.org/10.3389/fphar.2017.00516>.
- Rodríguez-Cueto, C., Benito, C., Romero, J., Hernández-Gálvez, M., Gómez-Ruiz, M., and Fernández-Ruiz, J. (2014a). Endocannabinoid-hydrolysing enzymes in the post-mortem cerebellum of humans affected by hereditary autosomal dominant ataxias. *Pathobiol. J. Immunopathol. Mol. Cell. Biol.* 81: 149–159.
- Rodríguez-Cueto, C., Benito, C., Fernández-Ruiz, J., Romero, J., Hernández-Gálvez, M., and Gómez-Ruiz, M. (2014b). Changes in CB₁ and CB₂ receptors in the post-mortem cerebellum of humans affected by spinocerebellar ataxias. *Br. J. Pharmacol.* 171: 1472–1489.
- Schmöle, A.-C., Lundt, R., Gennequin, B., Schrage, H., Beins, E., Krämer, A., Zimmer, T., Limmer, A., Zimmer, A., and Otte, D.-M. (2015). Expression analysis of CB₂-GFP BAC transgenic mice. *PLOS ONE* 10: e0138986.

- Snyder, J.S., Soumier, A., Brewer, M., Pickel, J., and Cameron, H.A. (2011). Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature* 476: 458–461.
- Stella, N., Schweitzer, P., and Piomelli, D. (1997). A second endogenous cannabinoid that modulates long-term potentiation. *Nature* 388: 773–778.
- Stempel, A.V., Stumpf, A., Zhang, H.-Y., Özdoğan, T., Pannasch, U., Theis, A.-K., Otte, D.-M., Wojtalla, A., Rácz, I., Ponomarenko, A., et al. (2016). Cannabinoid type 2 receptors mediate a cell type-specific plasticity in the hippocampus. *Neuron* 90: 795–809.
- Stern, C.A.J., da Silva, T.R., Raymundi, A.M., de Souza, C.P., Hiroaki-Sato, V.A., Kato, L., Guimarães, F.S., Andreatini, R., Takahashi, R.N., and Bertoglio, L.J. (2017). Cannabidiol disrupts the consolidation of specific and generalized fear memories via dorsal hippocampus CB1 and CB2 receptors. *Neuropharmacology* 125: 220–230.
- Stumpf, A., Parthier, D., Sammons, R.P., Stempel, A.V., Breustedt, J., Rost, B.R., and Schmitz, D. (2018). Cannabinoid type 2 receptors mediate a cell type-specific self-inhibition in cortical neurons. *Neuropharmacology* 139: 217–225.
- Sun, L., Dong, R., Xu, X., Yang, X., and Peng, M. (2017). Activation of cannabinoid receptor type 2 attenuates surgery-induced cognitive impairment in mice through anti-inflammatory activity. *J. Neuroinflammation* 14, <https://doi.org/10.1186/s12974-017-0913-7>.
- Tang, Y., Bao, J.S., Su, J.H., and Huang, W. (2017). MicroRNA-139 modulates Alzheimer's-associated pathogenesis in SAMP8 mice by targeting cannabinoid receptor type 2. *Genet. Mol. Res.* 16, <https://doi.org/10.4238/gmr16019166>.
- Tchantchou, F. and Zhang, Y. (2013). Selective inhibition of alpha/beta-hydrolase domain 6 attenuates neurodegeneration, alleviates blood brain barrier breakdown, and improves functional recovery in a mouse model of traumatic brain injury. *J. Neurotrauma* 30: 565–579.
- Torres, E., Gutierrez-Lopez, M.D., Mayado, A., Rubio, A., O'Shea, E., and Colado, M.I. (2011). Changes in interleukin-1 signal modulators induced by 3,4-methylenedioxymethamphetamine (MDMA): regulation by CB2 receptors and implications for neurotoxicity. *J. Neuroinflammation* 8: 53.
- Tsou, K., Brown, S., Sañudo-Peña, M.C., Mackie, K., and Walker, J.M. (1998). Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* 83: 393–411.
- Tsou, K., Mackie, K., Sañudo-Peña, M.C., and Walker, J.M. (1999). Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. *Neuroscience* 93: 969–975.
- Turcotte, C., Blanchet, M.-R., Laviolette, M., and Flaman, N. (2016). The CB2 receptor and its role as a regulator of inflammation. *Cell. Mol. Life Sci.* 73: 4449–4470.
- Van Sickle, M.D., Duncan, M., Kingsley, P.J., Mouihate, A., Urbani, P., Mackie, K., Stella, N., Makriyannis, A., Piomelli, D., Davison, J.S., et al. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* 310: 329–332.
- Viscomi, M.T., Oddi, S., Latini, L., Pasquariello, N., Florenzano, F., Bernardi, G., Molinari, M., and Maccarrone, M. (2009). Selective CB2 receptor agonism protects central neurons from remote axotomy-induced apoptosis through the PI3k/Akt pathway. *J. Neurosci.* 29: 4564–4570.
- Walter, L., Franklin, A., Witting, A., Wade, C., Xie, Y., Kunos, G., Mackie, K., and Stella, N. (2003). Nonpsychotropic cannabinoid receptors regulate microglial cell migration. *J. Neurosci.* 23: 1398–1405.
- Wen, J., Ribeiro, R., Tanaka, M., and Zhang, Y. (2015). Activation of CB2 receptor is required for the therapeutic effect of ABHD6 inhibition in experimental autoimmune encephalomyelitis. *Neuropharmacology* 99: 196–209.
- Winters, B.D., Krüger, J.M., Huang, X., Gallaher, Z.R., Ishikawa, M., Czaja, K., Krueger, J.M., Huang, Y.H., Schlüter, O.M., and Dong, Y. (2012). Cannabinoid receptor 1-expressing neurons in the nucleus accumbens. *Proc. Natl. Acad. Sci. U.S.A.* 109: E2717–E2725.
- Wu, Q. and Wang, H. (2018). The spatiotemporal expression changes of CB2R in the hippocampus of rats following pilocarpine-induced status epilepticus. *Epilepsy Res.* 148: 8–16.
- Wu, J., Hocevar, M., Foss, J.F., Bie, B., and Naguib, M. (2017). Activation of CB2 receptor system restores cognitive capacity and hippocampal Sox2 expression in a transgenic mouse model of Alzheimer's disease. *Eur. J. Pharmacol.* 811: 12–20.
- Zarruk, J.G., Fernández-López, D., García-Yébenes, I., García-Gutiérrez, M.S., Vivancos, J., Nombela, F., Torres, M., Burguete, M.C., Manzanares, J., Lizasoain, I., et al. (2012). Cannabinoid type 2 receptor activation downregulates stroke-induced classic and alternative brain macrophage/microglial activation concomitant to neuroprotection. *Stroke* 43: 211–219.
- Zhang, H.-Y., Gao, M., Liu, Q.-R., Bi, G.-H., Li, X., Yang, H.-J., Gardner, E.L., Wu, J., and Xi, Z.-X. (2014). Cannabinoid CB2 receptors modulate midbrain dopamine neuronal activity and dopamine-related behavior in mice. *Proc. Natl. Acad. Sci. U.S.A.* 111: E5007.