Check for updates

Received: 1 December 2021 | Revised: 15 February 2022 | Accepted: 17 March 2022

DOI: 10.1002/glia.24172

GLIA

WILEY

SPECIAL ISSUE ARTICLE

Endocannabinoid signaling in brain diseases: Emerging relevance of glial cells

Ana Bernal-Chico[1,2,3,4] | Vanja Tepavcevic[2] | Andrea Manterola[1,2,3] | Carmen Utrilla[2] | Carlos Matute[1,2,3] | Susana Mato[1,2,3,4]

[1]Department of Neurosciences, University of the Basque Country UPV/EHU, Leioa, Spain

[2]Achucarro Basque Center for Neuroscience, Leioa, Spain

[3]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain

[4]Neuroimmunology Unit, Biocruces Bizkaia, Barakaldo, Spain

Correspondence
Susana Mato, Department of Neurosciences, University of the Basque Country UPV/EHU, E-48940, Leioa, Spain.
Email: susana.mato@ehu.es

Present address
Andrea Manterola, Parque Científico y Tecnológico de Guipuzkoa, Viralgen, San Sebastian, Spain

Funding information
CIBERNED, Grant/Award Number: CB06/05/0076; ARSEP Foundation; European Regional Development Fund (FEDER) and Instituto de Salud Carlos III (ISCIII), Grant/Award Number: PI18/00513; Basque Government, Grant/Award Numbers: PIBA19-0059, IT1203-19; Spanish Ministry of Science and Innovation, Grant/Award Number: SAF2016-75292-R

Abstract

The discovery of cannabinoid receptors as the primary molecular targets of psychotropic cannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC) in late 1980s paved the way for investigations on the effects of cannabis-based therapeutics in brain pathology. Ever since, a wealth of results obtained from studies on human tissue samples and animal models have highlighted a promising therapeutic potential of cannabinoids and endocannabinoids in a variety of neurological disorders. However, clinical success has been limited and major questions concerning endocannabinoid signaling need to be satisfactorily addressed, particularly with regard to their role as modulators of glial cells in neurodegenerative diseases. Indeed, recent studies have brought into the limelight diverse, often unexpected functions of astrocytes, oligodendrocytes, and microglia in brain injury and disease, thus providing scientific basis for targeting glial cells to treat brain disorders. This Review summarizes the current knowledge on the molecular and cellular hallmarks of endocannabinoid signaling in glial cells and its clinical relevance in neurodegenerative and chronic inflammatory disorders.

KEYWORDS
CB_1, CB_2, endocannabinoids, glial cells, neurodegenerative diseases

The medical uses of *Cannabis sativa* derivatives have been documented across the globe for centuries (Alexander, 2016). The discovery of cannabinoids, the bioactive products derived from cannabis extracts, in the 1960's inspired research on the mechanisms underlying the effects of cannabis consumption and paved the way for the subsequent identification of cannabinoid receptors (Matsuda et al., 1990; Munro et al., 1993) and their endogenous ligands, endocannabinoids (Devane et al., 1992; Mechoulam et al., 1995). It now appears clear that the endogenous cannabinoid system is centrally involved in maintaining and restoring brain homeostasis in health and disease. Because initial studies reported protective effects of neuronal cannabinoid receptors, extensive research on the role of endocannabinoid signaling in brain pathology has been performed in the past few decades, with the ultimate goal of targeting the

Ana Bernal-Chico and Vanja Tepavcevic contributed equally to this study.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2022 The Authors. *GLIA* published by Wiley Periodicals LLC.

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

endocannabinoid system as a therapeutic approach for various neurological disorders. These efforts led to the approval of nabiximols—a combination of the psychoactive cannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC) and the non-psychotropic cannabidiol (CBD)—for the treatment of spasticity and pain in multiple sclerosis (MS) in 2005 (Novotna et al., 2011). CBD displays protective activity in a wide range of experimental disease settings through a variety of mechanisms that include multiple non-cannabinoid receptor targets and antioxidant properties, and its use has been recently approved for otherwise unmanageable pediatric epilepsy (Billakota et al., 2019). However, human studies performed so far have demonstrated limited clinical efficacy, suggesting that better understanding of endocannabinoid-related networks in neurological disorders is needed for successful therapeutic application.

Neurodegenerative diseases cause progressive loss of cognitive and/or motor skills and often overlapping clinical syndromes. Despite diverse clinical manifestations that reflect region- and type-specific loss of neurons and synapses, it has become clear that different neurodegenerative diseases share certain features and underlying mechanisms. One such feature is the phenotypic transformation of astrocytes, microglia, and oligodendroglia to different flavors that contribute, both positively and negatively, to disease symptomatology and progression. Importantly, glial cells are nowadays recognized as both targets and sources of endocannabinoid signaling in the brain, and the pathological significance of glial endocannabinoidome in brain diseases has been recently highlighted, suggesting this system as a potential therapeutic target in neurodegenerative disorders. This article first describes a cellularly-diverse endocannabinoid system in the brain. We then discuss the mechanistic basis and contribution of endocannabinoid dysfunction to brain pathology. Finally, we focus on the therapeutic potential of endocannabinoid-based drugs in neurodegenerative diseases with a particular emphasis on glial cells as targets.

1 | GLIAL CELLS IN NEURODEGENERATION: CURRENT PERSPECTIVES

Advances in modern neuroscience have revealed a landscape of functions that astrocytes, microglia, and oligodendroglia perform in the brain. Recently developed methods for imaging and isolation of adult glial cells have enabled transcriptomic analyses at a single cell level, which has provided the unprecedented insights into the heterogeneity within each cell population that likely reflects diverse biological activities throughout the lifespan (Clarke et al., 2018; Hickman et al., 2013; Marisca et al., 2020). Astrocytes serve prominent roles in the maintenance of brain function that include ion and neurotransmitter homeostasis, modulation of synaptogenesis, synaptic maturation and myelination, gliotransmitter release and fine-tuning of synaptic networks, control of the blood brain barrier and regulation of neuronal metabolism (Verkhratsky & Nedergaard, 2018). Microglia are regarded as the principal resident immune cells of the brain. These cells perform important housekeeping functions that include synaptic remodeling and phagocytosis of damaged cells or myelin debris, and orchestration of neuroinflammatory responses to pathogenic stimuli through the production of cytokines and chemokines (Hickman et al., 2018). Finally, oligodendrocytes and their precursors mediate the generation, maintenance, and repair of the myelin sheath. Myelin acts as an insulator to accelerate the conduction velocity of the axons and provides these with trophic support, mediated by specific channel and transporter systems the function of which is critical for axonal integrity (Saab & Nave, 2017). Importantly, myelin plasticity during adult life is a determinant of neuronal function and cognitive performance (Saab & Nave, 2017).

Many homeostatic functions of glial cells become altered in neuropathological states, and this neuroglial dysfunction is nowadays regarded as the core of disease onset and progression. Despite substantial advancements in understanding glial alterations in the disease context, the underlying mechanisms remain insufficiently characterized. Disease signatures in glial cells have only recently been addressed at the transcriptomic, proteomic and metabolomic levels in combination with cell-specific rescue strategies, and the results evidence that astrocytic, microglial and oligodendroglial responses to pathology are diverse, context-specific, and therapeutically exploitable. Some of these studies highlight profound differences between the glial cells in the grey versus those in the white matter (Hasel et al., 2021; van der Poel et al., 2019). Importantly, recent neuroimaging studies have demonstrated myelin alterations in various neurological diseases even before the onset of clinical symptoms (Dean et al., 2017). Because endocannabinoids modulate many aspects of glial cell biology, understanding how these lipid mediators fine-tune the complex glial responses to neurodegeneration may provide new avenues to develop disease-modifying therapies aimed at restoring neuro-glial-function.

2 | ENDOCANNABINOID SIGNALING AND FUNCTION IN PHYSIOLOGICAL CONDITIONS: FOCUS ON GLIAL CELLS

The endocannabinoid system has been classically defined as a pleiotropic lipid signaling system that consists of the endocannabinoids, mainly anandamide (*N*-arachidonoylethanolamine, AEA) and 2-arachidonoylglycerol (2-AG), the corresponding anabolic/catabolic enzymes and transporter molecules, and the cannabinoid CB_1 and CB_2 receptors. AEA and 2-AG are known to activate a number of non-cannabinoid receptors and share metabolic pathways with other endocannabinoid-like lipid mediators, such as palmitoylethanolamide (PEA) and oleoylethanolamide (OEA), which lack affinity for cannabinoid receptors and act on their own target molecules. Endocannabinoids and their structurally related lipid molecules encompass a complex signaling network known as the endocannabinoidome (for comprehensive review [Cristino et al., 2020]). Below, we outline the main elements of endocannabinoid signaling in neurons and glial cells.

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

2.1 | Cannabinoid receptors

The isolation of Δ^9-THC in 1964 from confiscated hashish (Mechoulam, 1970) led to generation of many synthetic compounds structurally similar to phytocannabinoids, which finally resulted in the identification of the first receptor shared by these molecules, named cannabinoid receptor 1 (CB_1) (Devane et al., 1988). CB_1 was successfully cloned from the rodent and human brain (Matsuda et al., 1990) and shown to mediate the psychoactive effects of cannabis. Shortly after, a second cannabinoid receptor (CB_2) was identified by homology cloning (Munro et al., 1993). Initial studies showed that CB_1 is predominantly expressed in the brain whereas CB_2 receptors are localized at highest levels in peripheral immune cells and tissues and regarded as the main mediators of cannabinoid immunomodulatory activity (Matsuda et al., 1990; Munro et al., 1993). Although each receptor is associated to specific signaling pathways, both share the ability to couple G_i proteins and inhibit adenylyl cyclase (AC) and protein kinase A (PKA) activities (Pertwee et al., 2010). Beyond CB_1 and CB_2 receptors, endocannabinoids and endocannabinoid-related mediators can interact with a wider range of receptor proteins with complex consequences on brain physiology. In particular, AEA and some of its congeners also activate postsynaptic type-1 transient receptor potential vanilloid receptor channels (TRPV1), peroxisome proliferator-activated receptors (PPARs) and other G protein-coupled receptors (Godlewski et al., 2009). In addition, 2-AG can stimulate postsynaptic $GABA_A$ receptors under certain conditions (Naydenov et al., 2014; Sigel et al., 2011). The role and therapeutic implications of these non-classical endocannabinoid-signaling elements in neurological disorders have been recently reviewed in detail (Cristino et al., 2020; Di Marzo, 2018).

2.1.1 | CB_1 receptors

Early autoradiography studies indicated CB_1 as one of the most abundant G-protein coupled receptors in the mammalian brain, with protein levels comparable to those of the major excitatory and inhibitory neurotransmitter receptor channels (Herkenham et al., 1990). CB_1 receptor is widely and heterogeneously distributed in the brain, with the highest expression detected in the regions involved in memory/learning, regulation of motor activity, emotional behavior, or sensory and pain perceptions, among others. A number of studies combining different experimental approaches (e.g., imaging and electrophysiological techniques) have demonstrated the presence of CB_1 receptors in different neuronal types including GABAergic, glutamatergic, and serotonergic neurons (Häring et al., 2015; Kawamura et al., 2006; Lutz et al., 2015). Electron microscopy and super-resolution imaging studies show that the majority of neuronal CB_1 accumulate presynaptically on axonal terminals where they control neurotransmitter release, with higher expression levels in GABAergic neurons compared to glutamatergic cells of the forebrain (Dudok et al., 2015; Gutiérrez-Rodríguez et al., 2017; Kawamura et al., 2006). Using anatomical and functional techniques, the localization of CB_1 has also been demonstrated at postsynaptic sites (somatodendritic) in specific brain areas such as the neocortex, suggesting that CB_1 participate in cell-autonomous regulation processes (Marinelli et al., 2008; Maroso et al., 2016; Morello et al., 2016). The location of CB_1 in post-synaptic sites has been mostly associated with receptor presence in mitochondria or other intracellular organelles (see below) rather than on the plasma membrane (Rodriguez et al., 2001).

2.1.2 | CB_2 receptors

CB_2 has been classically considered as the peripheral cannabinoid receptor based on early in situ hybridization studies that showed high expression levels in the spleen but no evidence for receptor presence in brain tissue (Munro et al., 1993). Since then, many studies using different experimental settings have strengthened the conclusion that CB_2 expression is negligible in neurons (Atwood & Mackie, 2010), which is further supported by the recent analysis and characterization of CB_2-EYFP reporter mice (López et al., 2018). In contrast, substantial evidence also suggests presence of functional CB_2 in scarce neuronal populations in the healthy brain (Stempel et al., 2016; Stumpf et al., 2018; Van Sickle et al., 2005; Zhang, Gao, et al., 2014). Moreover, in vivo pharmacological studies combined with the analysis of CB_2 mutants indicate a role for CB_2 in a number of brain functions classically ascribed to neurons such as memory consolidation, social behavior and reward (García-Gutiérrez et al., 2013; Navarrete et al., 2013; Rodríguez-Arias et al., 2015). The extent of CB_2 receptor expression in neurons remains nowadays under scrutiny and solid anatomical evidence using immunoelectron microscopy with appropriate negative controls (e.g. CB_2 receptor knockout mice), is needed to corroborate previous observations. Regardless of whether CB_2 is constitutively expressed in neurons, it appears clear that this receptor is inducible both in neurons and glial cells under specific pathological conditions (Atwood & Mackie, 2010), thus providing a foundation for the therapeutic potential of CB_2 targeting in brain diseases.

Besides their expression in neurons, CB_1 and CB_2 have been also localized to adult neural stem and progenitor cells (NSCs/NPCs) where they positively modulate the proliferation, migration and differentiation processes that drive adult neurogenesis in a context-specific manner (Maccarrone et al., 2014).

2.1.3 | Glial localization of cannabinoid receptors

Astrocytes

Early electron microscopy analyses evidenced perivascular and perisynaptic expressions of astrocytic CB_1 receptors through the brain (Moldrich & Wenger, 2000; Rodriguez et al., 2001). More recently, combined pre-embedding immunogold and immunoperoxidase methods applied to transgenic mice have allowed quantitative analysis of astrocytic CB_1 topography in the mouse brain (Gutiérrez-Rodríguez et al., 2017; Gutiérrez-Rodríguez et al., 2018). This advanced methodology shows that around 60% of hippocampal astrocyte processes express CB_1 on their

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

plasma membrane at a density similar to that measured in excitatory synapses within the same brain area. Conversely, only anecdotal pharmacological and molecular evidence supports the localization of CB_2 in astrocytes and the prevailing view in the field is that astroglia in the intact brain express negligible levels of the receptor protein (López et al., 2018; Molina-Holgado et al., 2002). As for microglia (see below), the expression of CB_2 receptors in astrocytes is inducible in certain inflammatory conditions (Rodríguez-Cueto, et al., 2021).

Microglia

The localization of cannabinoid receptors in this innate immune cell population remains controversial. While transcriptomic databases support CB_1 expression in microglia, pharmacological studies in culture systems show conflicting results and bona-fide anatomical evidence of receptor localization in microglial processes in situ is still lacking (Mecha et al., 2016; Stella, 2010). The recent generation of a conditional mouse model of microglia-specific CB_1 receptor deletion may help untangle the role of this receptor in microglial function in health and disease (De Meij et al., 2021). A number of in vitro studies have suggested the expression of CB_2 in microglia of different origins even though only trace amount of CB_2 mRNA were detected (Stella, 2010). Anatomical examination of CB_2-EYFP reporter mice failed to demonstrate CB_2 expression in the healthy CNS (López et al., 2018). Based on these observations, the consensus, at the moment, appears to be that microglial cells in the resting state, found in the intact healthy nervous tissue, express only very low amounts cannabinoid receptors (Duffy et al., 2021; Mecha et al., 2016). Further supporting the concept that cannabinoid receptor expression in microglia is tightly related to their activation state, studies performed in culture systems show that microglial cells in a pro-inflammatory state downregulate CB_1 and CB_2 whereas acquisition of the repair-promoting phenotype is associated to increased cannabinoid receptor levels (Mecha et al., 2015).

Oligodendroglia

Early autoradiography studies reported very low CB_1 receptor levels in adult white matter tracts suggesting that this protein is virtually absent in oligodendrocytes and myelin (Glass et al., 1997; Herkenham et al., 1991). Yet, studies on rodent and human tissue during fetal development suggested transitional CB_1 mRNA and protein expression in oligodendrocyte precursor cells (OPCs) (Berrendero et al., 1998; Mato et al., 2003) and immunohistochemical analysis detected CB_1 receptor expression in adult human OPCs and oligodendrocytes (Benito et al., 2007). More recently, RNA sequencing of CNS cell types purified by immunopanning has demonstrated CB_1 receptor gene (*Cnr1*) expression in oligodendrocyte lineage cells of the mouse cerebral cortex at early postnatal stages, with higher transcript levels corresponding to OPCs (Zhang, Gao, et al., 2014). These observations have been refined by single-cell RNA sequencing of oligodendrocyte lineage cells isolated from the mouse juvenile and adult CNS (Marques et al., 2016). This study reported the presence of *Cnr1* transcripts in 11 out of 12 identified oligodendroglial populations thus suggesting that CB_1 receptors regulate cell function at multiple stages of oligodendrocyte lineage progression. This hypothesis is further supported by recent electron microscopy evidence from our laboratory showing that CB_1 receptors are expressed and quantifiable in white matter oligodendrocytes (Figure 1). Altogether, the above mentioned evidence warrants further investigation on the precise topography of CB_1 density and distribution in OPCs, oligodendrocytes, and myelin. CB_2 receptor expression is more controversial in terms of endocannabinoid-mediated effects and protein expression in oligodendroglia. Pharmacological studies in cultured cells suggest biological effects associated with CB_2 receptors expressed in oligodendrocytes and/or OPCs (Gomez et al., 2010; Gomez et al., 2011; Gomez et al., 2015; Sanchez-Rodriguez et al., 2018). However, the CB_2 localization in oligodendroglia has not been corroborated in situ by anatomical studies and seems unlikely based on characterization of the CB_2-EYFP reporter mouse (López et al., 2018). Furthermore, single-cell transcriptome analysis indicates that *Cnr2* expression is negligible or very low in oligodendrocyte populations of the mouse brain (Marques et al., 2016).

2.1.4 | Subcellular localization of cannabinoid receptors

Recently, it has been shown that functional CB_1 receptors are not restricted to the plasma membrane, being also present in the endosomal and lysosomal compartments in the context of receptor trafficking (Lutz, 2020). A number of anatomical and functional studies have reported presence of mitochondrial membrane-associated CB_1 ($mtCB_1$) receptors in neurons and astrocytes, where they modulate energy metabolism (Bénard et al., 2012; Gutiérrez-Rodríguez et al., 2018; Jimenez-Blasco et al., 2020). Although further studies are needed to decipher the relative contribution of the $mtCB_1$ to endocannabinoid regulation of brain functions, classically ascribed to the plasma membrane pool, it is possible that this receptor population mediates important activities in healthy and disease conditions.

2.2 | Endocannabinoid synthesis and inactivation

Endocannabinoids are diffusible bioactive lipids with a short half-life that appear to act in both autocrine and paracrine fashions. The initiation of endocannabinoid production is thought to occur "on demand" following intracellular Ca^{2+} elevations associated to increased neuronal activity, although tonic endocannabinoid signaling has also been proposed (Alger & Kim, 2011; Kano et al., 2009). As for other neuromodulatory systems, the duration and intensity of endocannabinoid signaling is fine-tuned by the relative activity of the enzymes involved in the biosynthesis and catabolism of these compounds. Importantly, modulation of endocannabinoid inactivation is considered as a promising therapeutic strategy in brain diseases.

2.2.1 | Endocannabinoid synthesis

The synthesis of AEA and other *N*-acylethanolamines involves the catalytic activity of an *N*-acylphosphatidylethanolamine (NAPE)-specific

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

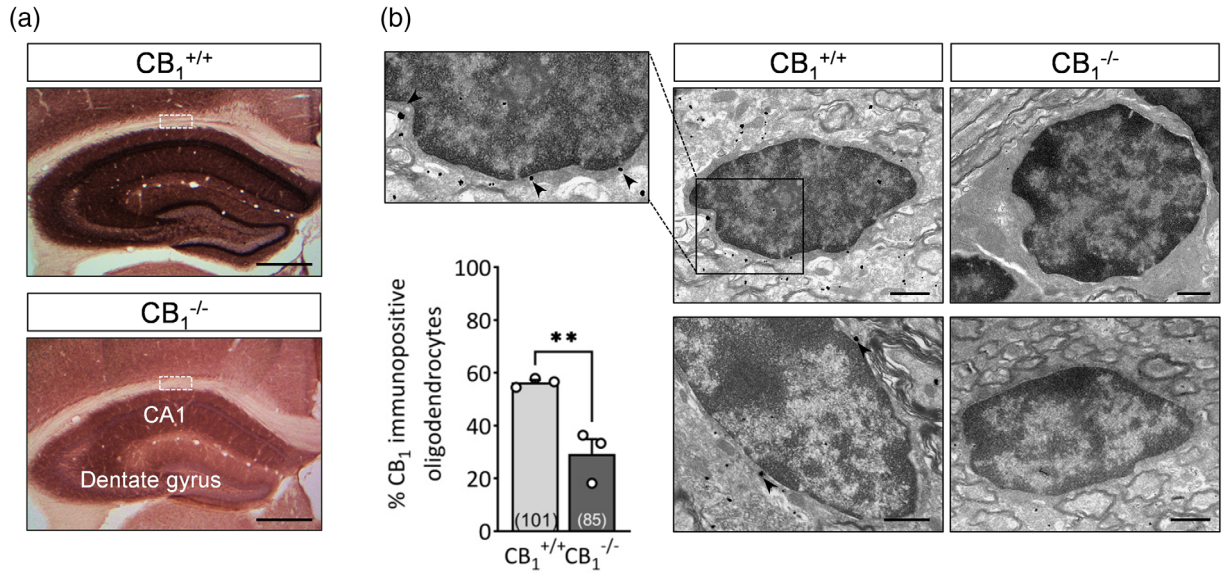

FIGURE 1 Localization of CB_1 receptors in myelinating oligodendrocytes of the mouse brain. (a) Specificity of CB_1 receptor immunostaining procedures for electron microscopy (EM) visualization of mature oligodendrocytes in the subcortical white matter. A pre-embedding silver intensified immunogold method for the detection of CB_1 receptors was applied to coronal brain sections from wild-type ($CB_1^{+/+}$) and CB_1 receptor knockout ($CB_1^{-/-}$) mice at postnatal day 60. Images depict the characteristic CB_1 receptor immunolabeling pattern in the mouse hippocampus that disappears in $CB_1^{-/-}$ mice. Dotted squares show the subcortical white matter area selected for EM analysis. Scale bars: 200 μm. (b) CB_1 receptor localization in identified oligodendrocytes within the subcortical white matter. CB_1 receptor gold particles were localized on plasma membranes of oligodendrocyte somata (black arrowheads). The proportion of CB_1 receptor gold particles in oligodendrocytes was significantly lower in $CB_1^{-/-}$ mice ($n = 3$ mice per group). The numbers of oligodendrocytes analyzed are indicated in parentheses at the bottom of each column. $^{**}p < 0.01$; Student's *t*-test. Scale bars: 1 μm

phospholipase D-like hydrolase (NAPE-PLD) (Okamoto et al., 2004). Alternatively, NAPE can be hydrolyzed by phospholipase C (PLC) to phospho-AEA, which, in turn, is dephosphorylated by phosphatases (Liu et al., 2006). The 2-AG biosynthesis, on the other hand, is thought to rely on the hydrolysis of membrane phosphatidylinositols by PLC, leading to the production of 1,2-diacylglycerol (DAG) which is converted to 2-AG by the action of postsynaptic integral membrane protein diacylglycerol lipase (DAGLα/β) (Bisogno et al., 2003). Characterization studies using DAGLα and DAGLβ deficient mice have suggested that α isoform of the enzyme is the primary mediator of 2-AG synthesis in neurons and astroglia whereas DAGLβ activity is predominant in microglia/macrophages (Gao et al., 2010; Hsu et al., 2012; Tanimura et al., 2010; Viader et al., 2016). Neuronal endocannabinoid biosynthesis can be induced by membrane depolarization or by activation of G_q coupled GPCRs, such as dopamine D2, glutamate $mGluR_{1/5}$ and muscarinic acetylcholine M_1/M_3 (Kano et al., 2009; Piomelli, 2003).

2.2.2 | Endocannabinoid catabolism

The catabolism of AEA and 2-AG and the subsequent termination of their signaling activity take place intracellularly. Endocannabinoids seem to be delivered into the intracellular compartment through a facilitated transporter across the plasma membrane, which can be modulated pharmacologically despite the fact that specific transporters have not been cloned yet (Chicca et al., 2017). A family of fatty acid binding proteins (FABPs) have been identified as intracellular carriers that deliver endocannabinoids, mostly AEA, to their catabolic enzymes (Kaczocha et al., 2009). Consistent with the role of these carriers in endocannabinoid inactivation, inhibition of FABP5 and FABP7 increases brain levels of AEA and the related *N*-acylethanolamines leading to analgesic and anti-inflammatory effects (Kaczocha et al., 2014; Kaczocha et al., 2015). In addition to their canonical role in intracellular endocannabinoid transport, recent studies have reported that FABP5 facilitates the extracellular delivery of 2-AG to the synaptic cleft thus modulating retrograde endocannabinoid signaling in specific brain areas (Haj-Dahmane et al., 2018). Fatty acid amide hydrolase (FAAH) is the main enzyme responsible for the hydrolysis of AEA (Cravatt et al., 1996) into free arachidonic acid (AA) and ethanolamine. AEA can be alternatively hydrolyzed by *N*-acylethanolamine-hydrolyzing acid amidase (NAAA). The most important difference between FAAH and NAAA is the pH range at which they are catalytically active, with FAAH active in a wide range of pHs while NAAA shows optimal activity at a pH of 4.5–5, consistent with its localization in lysosomes (Tsuboi et al., 2007). On the other hand, 2-AG is preferentially metabolized by monoacylglycerol lipase (MAGL) (Dinh et al., 2002). MAGL is widely expressed through the nervous system and mediates approximately 85% of 2-AG metabolism into AA and glycerol (Savinainen et al., 2012). These hydrolytic enzymes exhibit a differential localization in the synaptic cleft, with FAAH present mainly at the postsynaptic compartment and MAGL localized in the vicinity of CB_1 receptors

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

at the presynaptic site (Gulyas et al., 2004). Consistent with this anatomical localization, MAGL activity controls different forms of CB_1 receptor-mediated synaptic plasticity, and is regarded as the principal enzyme responsible for the termination of endocannabinoid signaling at the synaptic level. In addition to their classical localization at the plasma membrane, MAGL and FAAH have also been detected at the intracellular membrane constituents, which suggests a link between intracellular CB_1 signaling and endocannabinoid catabolism (Blankman et al., 2007; Morozov et al., 2004).

Apart from MAGL, two other serine hydrolases that break down 2-AG have been recently identified, namely α/β-hydrolase domain containing 6 and 12 (ABHD6 and ABHD12) (Blankman et al., 2007). ABHD6 is primarily expressed at the postsynaptic compartment of principal glutamatergic neurons, with some expression on GABAergic cells (Marrs et al., 2010). The highest ABHD6 enzymatic activity in nervous tissue has been measured in the frontal cortex, hippocampus, striatum and cerebellum (Baggelaar et al., 2017). In physiological conditions, this enzyme accounts for only ~4% of brain 2-AG, and several alternative substrates can be also hydrolyzed by ABHD6 (Navia-Paldanius et al., 2012). ABHD6 specifically controls long-term forms of endocannabinoid mediated synaptic plasticity (Cao et al., 2019). The importance of ABHD6 in the regulation of 2-AG metabolism during neuroinflammation has been recently highlighted by studies showing increased expression levels of this enzyme in rodent models of CNS damage (Poursharifi et al., 2017), which suggested that down-regulating its activity may have therapeutic benefits. On the other hand, ABHD12 is an integral membrane protein that accounts for approximately 9% of total 2-AG hydrolysis (Blankman et al., 2007). The essential role of this serine hydrolase in brain physiology is reflected by the fact that mutations in the *Abhd12* gene that severely compromise its expression and/or function underlie a neurodegenerative disease called PHARC (polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract) (Blankman et al., 2013; Chen et al., 2013; Fiskerstrand et al., 2010). Consequently, this piece of evidence has discouraged the evaluation of ABHD12 inhibitors for therapeutic benefit in neuroinflammatory conditions.

The products of endocannabinoid catabolism are important substrates for biosynthesis of inflammatory mediators. Thus, during the last decade, a number of studies have demonstrated the relevance of 2-AG enzymatic hydrolysis in the generation of the AA pool available for cycloxygenase-2 (COX-2) mediated prostaglandin (PGs) biosynthesis in certain neurodegenerative, inflammatory conditions (Nomura et al., 2011; Piro et al., 2012). In this scenario, the anti-inflammatory benefits of MAGL and ABHD6 inhibitors in rodent models of neuroinflammation may actually result from reduced PG production rather than enhanced receptor-mediated endocannabinoid signaling.

On the other hand, 2-AG and AEA can also be metabolized oxidatively via COX-2, leading to the formation of PG glycerol esters and ethanolamides that bind to a variety of receptors (Kozak et al., 2004; Valdeolivas et al., 2013). To complicate things further, 2-AG can be phosphorylated to the corresponding arachidonic acid-containing lysophosphatidic acid (LPA) with biological activity at LPA receptors (Nakane et al., 2002). Although the biological relevance of these newly-identified pathways remains to be fully elucidated, the bioactive lipid prostaglandin D2-glycerol ester has been implicated in the control of macrophage activation and inflammation by ABHD6 (Alhouayek et al., 2013). Furthermore, based on the above-mentioned observations, it is hypothesized that the anti-inflammatory effects of COX-2 inhibitors could be more complex than simply the inhibition of PG synthesis via reduced AA availability. Instead, these may be mediated by increased endocannabinoid levels acting through canonical or non-canonical cannabinoid receptors.

The above-mentioned observations illustrate the complexity of the networks that regulate endocannabinoid signaling in the brain. Inactivation of AEA and 2-AG hydrolytic enzymes and FABPs augments CNS endocannabinoids levels, engages cannabinoid receptor-dependent effects in vivo (Cravatt et al., 2001; Kaczocha et al., 2015; Long et al., 2009) and elicits therapeutic benefits in experimental models of neurodegeneration (see below). However, targeting endocannabinoid enzymatic hydrolysis increases the availability of these compounds for other metabolic pathways that lead to the activation of additional receptor proteins with complex biological consequences both in healthy and disease conditions. Chronic MAGL inactivation also leads to CB_1 desensitization and loss-of-function as unwanted side effects that further put into question the utility of targeting 2-AG hydrolysis as strategy in human therapy (Bernal-Chico et al., 2015; Schlosburg et al., 2010).

2.2.3 | Glial production and catabolism of endocannabinoids

The biosynthesis and catabolism of AEA and 2-AG by glial cells has been demonstrated by a combination of anatomical studies, biochemical determinations in culture systems and transgenic mouse models. However, the precise topography of endocannabinoid production and hydrolysis machinery and the spatio-temporal dynamics of glial endocannabinoid metabolism in situ have not yet been fully elucidated.

Astrocytes and microglia

Cultured astrocytes and microglial cells produce AEA and 2-AG in response to stimulus that trigger a sustained rise in intracellular Ca^{2+} such as endothelin-1 or ATP (Stella, 2009; Walter et al., 2002; Walter et al., 2004; Walter & Stella, 2003). These early in vitro studies also showed that microglia produce ~20-fold more endocannabinoids than neurons and astrocytes, suggesting a relevant role of these cells as source of these lipid compounds during neuroinflammation. Recent proteomic and functional analyses show that DAGLβ drives the production of 2-AG and downstream metabolic products PGs in microglia/macrophages and that the activity of this enzyme promotes pro-inflammatory signaling in neuroinflammation and pain (Hsu et al., 2012; Viader et al., 2016; Wilkerson et al., 2016). DAGLβ also promotes the secretion of TNFα in dendritic cells crucially regulating crosstalk between innate and adaptive immune pathways, with potential pro-inflammatory effects in neurodegenerative conditions (Shin et al., 2019). With regard to endocannabinoid inactivation, astrocytes

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

and microglia express MAGL and ABHD6 proteins and enzyme activity but only marginal FAAH expression has been ascribed to either cell type in vitro or in situ (Stella, 2004). More recently, the characterization of conditional genetic mouse models lacking MAGL specifically in neurons, astrocytes and microglia has shown that astrocytic MAGL is crucially involved in the termination of neuronal 2-AG signaling as well as chiefly responsible for the generation of pro-inflammatory PGs from this endocannabinoid ligand (Chen et al., 2016; Viader et al., 2015). On the other hand, astroglial cells also express significant levels of FABP7 whose role in the control of reactive astrogliosis is under investigation (Cheng et al., 2021; Kamizato et al., 2019; Kipp et al., 2011).

A relevant concept in the field of glial pathophysiology is that the acquisition of specific functional phenotypes by astrocytes and microglial cells may be associated with changes in the expression of cannabinoid receptors and metabolic enzymes that alter the efficacy of endocannabinoid signaling in these cells. This possibility has been addressed for microglia using in vitro settings as well as for astrocytes and microglia ex vivo (Mecha et al., 2015; Moreno-García et al., 2020). On the other hand, the extent to which deregulated endocannabinoid production by innate immune cells under pathological settings tunes acquisition of specific functional phenotypes and its impact on neighboring cells requires further analysis. This research would benefit from the use of novel tools allowing spatiotemporal resolution of endocannabinoid release and dynamics in vitro and in vivo (Dong et al., 2021).

Oligodendroglia

Oligodendrocyte lineage cells express gene transcripts of endocannabinoid biosynthetic and catabolic machinery at all differentiation stages with the most prominent levels corresponding to 2-AG related DAGLβ and MAGL (Marques et al., 2016). Oligodendrocytes and OPCs in culture exhibit constitutive 2-AG and AEA production, and pharmacological inactivation of MAGL potentiates $CB_{1/2}$ receptor mediated effects in these cells (Bernal-Chico et al., 2015; Gomez et al., 2010; Gomez et al., 2015). Noteworthy, the expression levels of endocannabinoid production and hydrolytic enzymes in oligodendroglia vary throughout the differentiation process suggesting that autocrine or paracrine endocannabinoid signaling may fine-tune oligodendrocyte lineage progression and myelination (Gomez et al., 2010). Also related to endocannabinoid inactivation, knockdown of FABP7 impairs OPC differentiation in vitro and delays developmental myelination (Foerster et al., 2020). However, the biological signals and specific features of endocannabinoid metabolism in oligodendroglia in situ remain to be unveiled.

2.3 | Endocannabinoid function in brain physiology

Endocannabinoid signaling homeostatically modulates a wide range of biological functions including learning and memory, anxiety and stress response, mood, sleep, feeding, movement and development, among others. Endocannabinoid-mediated control of brain functions has been mostly ascribed to the activation of CB_1 receptors in the presynaptic neuronal compartment where they suppress Ca^{2+} influx and subsequent neurotransmitter release. The role of endocannabinoids as inhibitory retrograde messengers has been demonstrated for glutamatergic and GABAergic transmission and underlies activity-dependent plasticity through the CNS (Araque et al., 2017). Although ubiquitous, brain endocannabinoid biological activity is highly versatile and context-specific. This specificity relies on the neuroanatomical configuration of endocannabinoid signaling machinery, the availability of intracellular signaling counterparts (e.g. G proteins), and the specific ligand that activates the receptor. AEA is a high affinity, low-efficacy cannabinoid receptor agonist whereas 2-AG behaves as a fully effective agonist at both CB_1 and CB_2 (Howlett, 2002). Nowadays it is well established that these compounds play differential roles in the regulation of biological responses with 2-AG regarded as the main ligand for presynaptic CB_1 receptor mediated control of synaptic function (Araque et al., 2017).

During the last years, studies in genetic mouse models bearing cell-specific and brain-region conditional CB_1 inactivation have allowed to dissect endocannabinoid-mediated modulation of brain functions. An initially puzzling observation but nowadays accepted dogma in the field is that relatively low amounts of CB_1 receptors in specific cellular and subcellular locations tightly regulate a number of relevant physiological processes in vivo. This concept was initially put forward for the CB_1 population at the presynaptic compartment of principal glutamatergic neurons, where low receptor levels prevent overexcitation and attenuate seizure severity following epileptogenic injections of kainic acid while favoring the secretion of neurotrophic factors (Marsicano et al., 2003; Monory et al., 2006). These landmark observations provide mechanistic foundations for the well-established neuroprotective properties of endocannabinoids against excessive neuronal activation. In the same line, the restricted population of CB_1 receptors localized in NSCs enhance adult neurogenesis and facilitate the integration of new neurons into hippocampal circuitry (Oddi et al., 2020). This concept is supported by the observations that mice bearing specific genetic loss of the CB_1 receptor in NSCs display decreased proliferation and reduced dendritic branches and spine numbers in the differentiating neurons along with impaired long-term potentiation and short-term spatial memory, and increased depression-like behavior (Zimmermann et al., 2018). Conversely, the CB_2 receptor may be particularly important under damaging conditions of reduced neurogenesis, following acute insults or chronic inflammation (Oddi et al., 2020). Altogether, these observations point to an important role of endocannabinoid signaling expressed by NSCs in the regulation of cellular plasticity in the adult healthy and diseased CNS.

2.3.1 | Role of $mtCB_1$ receptors in energy metabolism

There is now compelling evidence that $mtCB_1$ receptors are crucial mediators of endocannabinoid effects on brain function. This concept emerged upon demonstration that $mtCB_1$ inhibit complex I of the

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

respiratory chain by modulation of soluble AC and mitochondrial PKA activities, thereby decreasing neuronal energy production (Hebert-Chatelain et al., 2016). Moreover, this same study showed that mtCB$_1$ receptors in hippocampal neurons modulate mitochondrial motility, synaptic transmission and memory formation thus providing a link between cellular bioenergetics and cognitive behaviors. More recently, mtCB$_1$ located at striatonigral terminals have been put forward as mediators of cannabinoid-induced catalepsy through the decrease in cellular respiration and synaptic transmission (Soria-Gomez et al., 2021). These observations have unveiled a novel mechanism underlying endocannabinoid modulation of brain tasks and paved the way for in depth analysis of the metabolic face of endocannabinoid signaling in different cell types. At this point, it is important to emphasize that mitochondria have a central role in neurological disorders and the relevance of the metabolic pathways housed by these organelle in the pathogenesis of neuroinflammation and neurodegeneration is currently in the spotlight (Chan, 2020; Garabadu et al., 2019; Lin & Beal, 2006). Although very little is known about endocannabinoid modulation of mitochondrial basic processes in the context of neurodegeneration, the above mentioned evidence suggest that mtCB$_1$ are prone to play specific and relevant roles in CNS disease.

2.3.2 | Endocannabinoid modulation of glial cell function

The neurocentric view of brain endocannabinoid signaling changed with initial studies pinpointing expression of functional receptors in glial cells (Stella, 2004). The occurrence of endocannabinoid signaling onto glial cells is nowadays sustained by results from in vitro systems as well as from the in vivo analysis of the effects of endocannabinoid modulating drugs in physiological and disease conditions (Figures 2 and 3). However, in most cases, the question whether cannabinoid receptors expressed in glial cells are directly involved in endocannabinoid-mediated effects on brain function remains unsatisfactorily addressed.

Astrocytes

Among the best-studied biological effects of endocannabinoids in glial cell function is the modulation of astrocyte responses by CB$_1$ receptors. A number of studies addressing the impact of astrocyte-neuron communication on synaptic physiology show that astrocytic CB$_1$ respond to endocannabinoids produced during neuronal activity by inducing Ca^{2+} elevations mediated by the activation of G_q proteins (Araque et al., 2017). Astrocyte Ca^{2+} mobilization by CB$_1$ receptors occurs throughout the rodent brain as well as in cortical and hippocampal human tissue and is regarded as a general and relevant mechanism of endocannabinoid signaling in the regulation of astrocyte function. Cytosolic Ca^{2+} rises upon activation of astrocytic CB$_1$ promote the release of gliotransmitters such as glutamate and D-serine and indirectly favor excitatory transmission (Han et al., 2012; Min & Nevian, 2012; Navarrete & Araque, 2010; Robin et al., 2018). Remarkably, the modulation of glutamatergic transmission upon activation of astrocytic CB$_1$ has a direct impact on synaptic plasticity and cognitive performance and is responsible for the disrupting effects of Δ^9-THC in working memory (Han et al., 2012; Robin et al., 2018). This observation supports the relevance of CB$_1$ receptors in astrocytic regulation of synaptic activity while pointing to the specific involvement of astroglial CB$_1$ in the effects of cannabis-based medicines in humans. Altogether, these data also highlight that astrocytic CB$_1$ signaling opposes the inhibitory activity of neural CB$_1$ on synaptic function and may be relevant to brain pathology. Indeed, despite the well-established protective effects associated to neuronal CB$_1$ in epilepsy models (Marsicano et al., 2003; Monory et al., 2006) the receptor population localized to astrocytes might sustain epileptic activity through the potentiation of glutamatergic transmission (Coiret et al., 2012).

The recent identification of mtCB$_1$ receptors in astrocytes (Gutiérrez-Rodríguez et al., 2018) has fueled research on the role of these proteins as modulators of astrocyte energy metabolism. Specifically, activation of mtCB$_1$ receptors hampers astrocytic glucose metabolism through a signaling cascade that involves inhibition of mitochondrial complex I, leading to reduced production of reactive oxygen species (ROS) and downregulation of the transcription factor hypoxia-inducible factor 1 (HIF-1) (Jimenez-Blasco et al., 2020). The

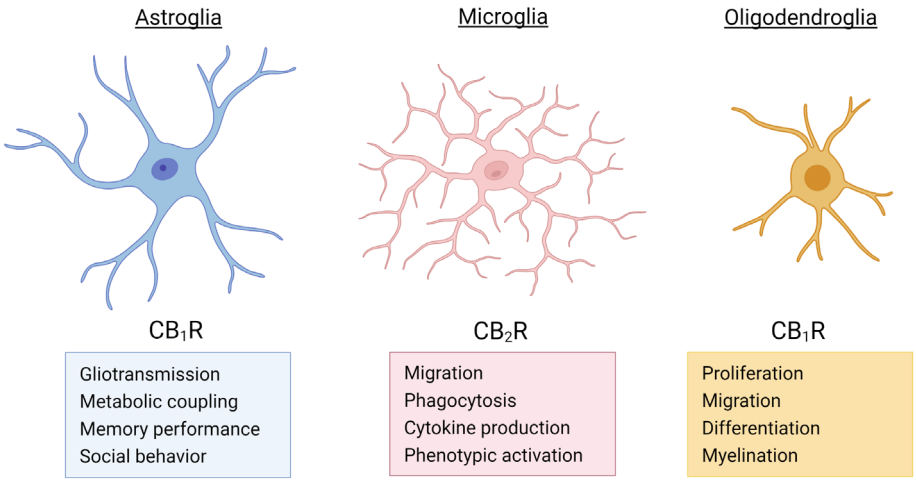

FIGURE 2 Biological functions of cannabinoid receptors in glial cells. (a) Astrocytic CB$_1$ receptors modulate neuroglial communication, metabolic coupling to neurons (mtCB$_1$), working memory and social behaviors. (b) CB$_2$ receptors in microglia modulate proliferation, migration, phagocytosis, cytokine production and the acquisition of pro- and anti-inflammatory phenotypes during activation. (c) CB$_1$ receptors in oligodendroglia drive the proliferation, migration and maturation of precursor cells, facilitating myelination and myelin maintenance. Created with BioRender.com

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

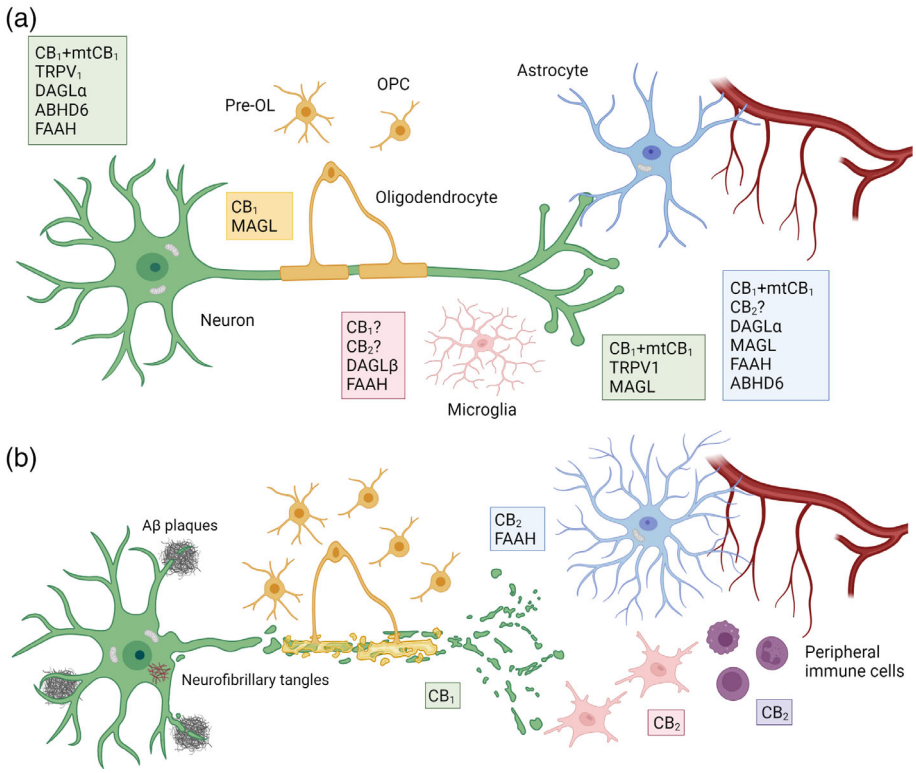

FIGURE 3 Neuronal and glial endocannabinoid signaling in brain physiology and pathology. (a) Cannabinoid CB_1 receptors present at presynaptic terminal and coupled to $G_{i/o}$ proteins inhibit neurotransmitter release upon activation by 2-arachidonoylglycerol (2-AG) or *N*-arachidonoylethanolamine (AEA). AEA can also activate neuronal type-1 transient receptor potential vanilloid receptor channels (TRPV1) to modulate synaptic transmission. Perisynaptic and perivascular astrocyte profiles display G_q-coupled CB_1 that engage increases in intracellular Ca^{2+} concentration with the subsequent release of gliotransmitters. Microglial cells display very low levels of CB_1 and CB_2 receptors in physiological conditions. CB_1 are also expressed by mitochondria ($mtCB_1$) at pre-and postsynaptic sites as well as in astrocytes where they inhibit oxidative phosphorylation and ATP production. Diacylglycerol lipase α (DAGLα) is the primary enzyme synthesizing 2-AG in neurons and astrocytes whereas DAGLβ activity is enriched in microglial cells. The major 2-AG degrading enzyme monoacylglycerol lipase (MAGL) is principally located at presynaptic terminals and in astrocytes. In contrast, α/β-hydrolase domain containing 6 (ABHD6) regulates 2-AG levels at the site of production. The enzyme responsible for AEA hydrolysis (FAAH) is present at the postsynaptic terminal as well as in glial cells. (b) Brain pathology associated to neurodegenerative diseases exemplified by the presence of Aβ plaques, neurofibrillary tangles and oligodendrocyte and myelin disturbances is associated to deregulated endocannabinoid signaling. Increased levels of CB_2 receptors in microglia and astrocytes is a hallmark of Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS). Loss of CB_1 receptor expression has been reported in several neurodegenerative conditions and emerges as pathogenic mechanism in HD. Reactive astrocytes in AD and MS upregulate FAAH. The activity of DAGLβ drives the production of 2-AG and downstream metabolic products prostaglandins in reactive microglia. The effects of (endo)cannabinoids in neurodegeneration are mainly mediated by neuronal CB_1 receptors, CB_2 populations in microglia, astrocytes and peripheral immune cells, and CB_1 receptors in oligodendrocyte populations. Created with BioRender.com

resulting reduction in the astrocytic glycolytic rate and lactate production ensues neuronal redox stress and impaired social interaction, which supports the notion that astrocytes play a crucial role in neuronal energy homeostasis and may have profound implications in neurological conditions (Finsterwald et al., 2015).

Microglia

Research into the role of endocannabinoids in the control of microglia physiological functions has been hindered by the lack of bona-fide evidence supporting microglia cannabinoid receptor expression in the normal brain. The concept that microglial cells respond to endocannabinoids is supported by pharmacological assessments in cultured cells showing modulation of cell motility, phagocytosis, proliferation and migration mainly ascribed to CB_2 receptors (Carrier et al., 2004; Ehrhart et al., 2005; Walter et al., 2003). However, in vivo support for direct endocannabinoid-mediated control of microglial biological activities in brain homeostasis - synapse pruning, modulation of synaptic activity and neuroprotection - is still lacking. Studies of CB_2 expression in pathological contexts consistently indicate receptor upregulation in activated microglia and show that the beneficial effects of cannabinoids in rodent models of neurodegeneration are associated to an attenuated microglial reactivity, reduced production of neurotoxic factors and pro-inflammatory mediators, and enhanced release of anti-inflammatory molecules (Cristino et al., 2020; Mecha et al., 2016). From mechanistic perspective, pharmacological and genetic studies in culture settings showed that CB_2 signaling

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

promotes the acquisition of homeostatic and regenerative phenotype-associated gene expression signatures (Mecha et al., 2015; Tanaka et al., 2020). Collectively, these data suggest that microglial CB_2 promotes the acquisition of repair-promoting state leading to protective effects in neuroinflammatory and neurodegenerative disease settings. However, most of the research on this topic is restricted to expression changes in a limited number of genes. Moreover, functional readouts of microglial activation states in response to endocannabinoid signaling have not been provided. Therefore, the concept that endocannabinoids modulate the acquisition of a specific functional phenotype by microglia should be validated in vivo by combining advanced techniques (i.e. single-cell/nuclei RNAseq) and transgenic mouse models to characterize the changes in microglial gene expression and function following microglia-specific manipulation of CB_2 (and CB_1) signaling.

Oligodendroglia

Research on the implications of endocannabinoid signaling in oligodendrocyte biology is still at its early stages. Initial studies of CB_1 receptor-mediated effects in oligodendroglia reported modulation of Ca^{2+} and K^+ conductances at the basis of oligodendrogliogenesis and myelination (Mato et al., 2009). Later on, a number of studies showed that oligodendroglial cells in culture produce endocannabinoids, which engages protective effects and drives the proliferation, migration and maturation of precursor cells through the activation of CB_1 and CB_2 (Bernal-Chico et al., 2015; Gomez et al., 2010; Gomez et al., 2011; Gomez et al., 2015; Sanchez-Rodriguez et al., 2018). The signaling mechanisms proposed as mediators of cannabinoid receptor-induced oligodendrocyte differentiation include PI_3K/Akt and the mammalian target of rapamycin (mTOR) pathways (Gomez et al., 2010; Gomez et al., 2015). In parallel, a number of in vivo pharmacological studies have shown that cannabinoids accelerate developmental myelination and facilitate myelin repair through mechanisms that involve activation of the mTOR cascade (Aguado et al., 2021; Huerga-Gómez et al., 2021). Based on these findings, direct endocannabinoid signaling onto OPCs and oligodendrocytes is hypothesized to enhance oligodendroglial survival and remyelination in rodent models of myelin damage and repair (Aguado et al., 2021; Bernal-Chico et al., 2015; Manterola et al., 2018). However, cannabinoid receptor expression in oligodendroglia in situ is low according to anatomical studies and whether activation of receptor subsets in these cells promotes OPC differentiation to oligodendrocytes in the context of myelination and myelin repair remains to be addressed using transgenic mouse models.

Taken together, these data highlight the existence of diverse cellular and subcellular endocannabinoid-related networks that mediate differential effects on cell function. This expanded signaling system implies several paradigms for endocannabinoid-based therapies, with inhibitors of endocannabinoid hydrolysis being most actively investigated in the context of neurological disorders. However, the redundancy and promiscuity of the network poses a challenge for the development of safe endocannabinoid-targeting compounds, and the current knowledge on the cellular/molecular mechanisms engaged by endocannabinoidome-modulating strategies is still limited.

3 | ENDOCANNABINOID SIGNALING IN NEUROLOGICAL DISORDERS

A large number of studies support the relevance of endocannabinoid signaling in the development, progression, and treatment of neurological disorders. Although most data have been acquired using experimental models of neurodegeneration, deregulation of endocannabinoid activity is also apparent in *postmortem* brain tissue and biological samples from patients affected by a variety of neurological disorders (Cristino et al., 2020). Such alterations often include changes in the expression of CB_1 and/or CB_2 receptors as well as in the levels of endocannabinoids, the impact of which on disease onset and progression remains unclear due to the multiplicity to endocannabinoid signaling networks and the complex and heterogeneous pathophysiology of neurodegenerative diseases. Importantly, preclinical studies in animal models of neurological disorders suggest that deregulation of endocannabinoid signaling takes place in a disease-stage and cell-type specific manner (Figure 3).

There is also a robust literature focused on the effects of modulating endocannabinoid signaling in neurological disorders (Cristino et al., 2020; Estrada & Contreras, 2020). Constitutive and, more recently, cell-type specific genetic manipulation of the system exerts symptom control and influences disease severity in experimental models of neurological conditions with drugs aimed at promoting endocannabinoid signaling often ameliorating the extent of inflammation and neurodegeneration (Chiarlone et al., 2014; Maresz et al., 2007; Rodríguez-Cueto et al., 2021). Mechanistically, the protective effects of cannabinoid compounds such as Δ^9-THC (and CBD) may involve CB_1 and CB_2 receptor independent mechanisms that will not be specifically addressed in this review. During the last few years, it has also become clear that both enhancers and blockers of cannabinoid signaling may produce beneficial effects in rodent models of neurological conditions (Cao et al., 2007; Nomura et al., 2011; Wilkerson et al., 2016). These observations likely reflect the complex biology of endocannabinoidome mediators targeting multiple receptors in different cell types during pathological states, which may limit their therapeutic potential. Indeed, clinical translation of the findings obtained using experimental models has proven less effective than initially expected, as the limited number of clinical studies conducted so far failed to confirm the neuroprotective, disease-modifying potential of (endo)cannabinoids observed in animal models of neurological disorders (Maas et al., 2006; Zajicek et al., 2013).

Below, we outline the principal alterations in the activity of cannabinoid receptors and classical endocannabinoids described in several neurodegenerative diseases, as well as preclinical and clinical evidence addressing the therapeutic opportunities of the system. Alterations in other components of the endocannabinoidome is beyond the scope of this manuscript, but detailed information on this

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

subject can be found in other reviews (Cristino et al., 2020; Di Marzo, 2018).

1. **Alzheimer's disease (AD)** is the most prevalent neurodegenerative disease affecting 40 million people worldwide and represents the main cause of dementia. While the exact causes/triggers of AD are not entirely clear, some of the mechanisms underlying progressive cognitive decline and disability have been characterized. These include deficient acetylcholine signaling, synapse loss, extracellular amyloid-β42 (Aβ) deposition in neuritic plaques, and intraneuronal accumulation of hyperphosphorilated tau protein in neurofibrillary tangles (Chen & Mobley, 2019). A chronic inflammatory state appears maintained by reactive astrocytes and activated microglia whose implications in the course of AD have been the focus of intense investigation (Leng & Edison, 2021). Assessments in rodent models (Bedse et al., 2014; Kalifa et al., 2011) and human *postmortem* studies (Berry et al., 2020) have reported discordant observations concerning deregulation of CB_1 in AD, and the results include both early increases as well as progressive reductions in the expression levels. Altered distribution and inhibitory activity of CB_1 receptors have been recently put forward in presymptomatic mouse AD brains, which suggests that disease-associated deregulation of receptor trafficking may have been overlooked (Maccarrone et al., 2018). Conversely, elevated CB_2 expression in astroglial, and predominantly, microglial cells is a consistent finding in advanced rodent (López et al., 2018) and human AD (Benito et al., 2003; Solas et al., 2013) with receptor levels positively correlating with Aβ concentration and plaque deposition. These observations support the hypothesis that CB_2 receptors modulate the activation state of glial cells surrounding plaque and tangle pathology in AD. Concerning endocannabinoids, *postmortem* studies show reduced AEA concentration and increased FAAH expression in neuritic plaque-associated astrocytes, suggesting deregulated endocannabinoid hydrolysis in AD pathology (Benito et al., 2003; Jung et al., 2012). Remarkably, AEA levels were inversely correlated to cognitive scores and accumulation of Aβ peptide in *postmortem* AD brains, and enhancing Aβ levels directly reduced AEA concentration in cell lysates (Jung et al., 2012). Collectively, these observations led to the hypothesis that impaired AEA mobilization in response to Aβ contributes to cognitive dysfunction in AD. In the brains of patients with AD, increased DAGLβ expression in microglial cells and reduced neuronal MAGL and ABHD6 levels have been detected, pointing to unbalanced 2-AG metabolism (Mulder et al., 2011). Partially mirroring these observations, enhanced 2-AG levels have been reported in a rodent model of Aβ toxicity, in association with β-amyloid protein-induced hippocampal degeneration and gliosis (van der Stelt et al., 2006). However, analysis in AD transgenic mice have failed to detect changes in the levels of AEA or 2-AG (Vázquez et al., 2015). Findings in experimental models of AD show that enhancing endocannabinoid signaling ameliorates the AD phenotype. Thus, both CB_1- and CB_2-selective agonists attenuate cognitive deficits associated with the pathology (Aso et al., 2012; Aso et al., 2013; Wu et al., 2017). Studies addressing the outcome of CB_2 receptor knockdown in transgenic AD mice report conflicting results regarding the effect on amyloid pathology (Koppel et al., 2014; López et al., 2018). Nevertheless, a number of in vitro and in vivo assessments suggest that CB_2 protects against memory impairments in AD by decreasing the production of neurotoxic factors and pro-inflammatory mediators by astrocytes and microglial cells (Aso & Ferrer, 2016). In addition, recent data suggest that this receptor population might tune the appearance of disease-associated microglia in AD towards repair-promoting phenotypes (Duffy et al., 2021). Regarding the role of CB_1 signaling in AD models, receptor antagonism elicited both protective and disease-exacerbating effects in a model of Aβ toxicity and neuroinflammation, the bases of which are not fully understood (Mazzola et al., 2003; Vázquez et al., 2015). Finally, it is also worth mentioning that several studies support beneficial effects of CBD against Aβ pathology with the potential contribution of PPARγ receptors (Esposito et al., 2007; Esposito et al., 2011). Concerning pharmacological targeting of endocannabinoid hydrolysis, MAGL inactivation was reported to suppress Aβ production, alleviate neuropathology, and attenuate memory deficits in AD mice through CB_1/CB_2 receptor-independent mechanisms that may involve PPARγ receptor-mediated signaling and/or attenuated PG production (Chen et al., 2012; Hashem et al., 2021; Piro et al., 2012; Zhang & Chen, 2018; Zhang, Hu, et al., 2014). From mechanistic perspective, in vitro assessments also show direct anti-inflammatory effects of MAGL inhibition in astrocytes and microglia challenged with Aβ peptides (Pihlaja et al., 2015). Pharmacological targeting of FAAH has led to more controversial results. While in vitro studies in microglial cells showed that FAAH blockade drives Aβ induced-microglial polarization towards an anti-inflammatory phenotype (Grieco et al., 2021), enzyme inhibitors had no impact on cognitive impairment, plaque deposition and gliosis in AD mice (Vázquez et al., 2015). Remarkably, genetic inactivation of FAAH in a mouse model of AD diminished soluble amyloid levels, neuritic plaques, and gliosis while engaging CB_1 receptor-independent behavioral improvements in spatial memory despite increased expression of inflammatory cytokines (Vázquez et al., 2015). A recent follow-up study showed a reversal of AD neuronal phenotype by FAAH knockdown associated with increased microglial activation and phagocytosis of Aβ peptide (Ruiz-Pérez et al., 2021), thus supporting the therapeutic potential of endocannabinoid-mediated modulation of neuroinflammation in AD. Despite encouraging preclinical evidence mentioned above, very few clinical trials addressing the efficacy of endocannabinoid targeting in AD have been completed. The synthetic Δ^9-THC analog nabilone reduced agitation and aggression in patients with AD, but cognitive outcomes were not different from placebo (Herrmann et al., 2019). An important limitation when designing studies to evaluate cannabinoid-modulating drugs as a therapeutic option in AD, as in other neurodegenerative conditions, is that disease-associated molecular changes take place several years before the symptoms manifest. Modulation of endocannabinoid signaling for

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

neuroprotective and immunomodulatory purposes when disease is already exacerbated may thus lead to negative results. In addition, long-term heavy cannabis use has negative effects on cognitive functions (Kuhns et al., 2021) that may complicate the interpretation of results and discourage long-term clinical studies.

2. **Parkinson's disease (PD)** is the second most common neurodegenerative disorder characterized by the loss of dopaminergic neurons in motor circuits leading to dyskinesia, bradykinesia, rigidity and constant muscle tremors as well as a range of non-motor symptoms (cognitive deficits, mood disorders hallucinations, pain, sleep disorders). The main pathological hallmarks of PD are the accumulation of α-synuclein aggregates and the degeneration of dopaminergic neurons in the substantia nigra pars compacta projecting to the basal ganglia. The majority of data gathered from PD animal models (Di Marzo et al., 2000; van der Stelt et al., 2005) and human patients (Pisani et al., 2005) demonstrate increased endocannabinoid levels that respond to dopamine replacement therapy, hence suggesting that alterations are associated to disease symptoms. Enhanced AEA levels in experimental parkinsonism have been associated with decreased FAAH activity (Gubellini et al., 2002). CB_1 receptor expression in the basal ganglia is reduced at early disease stages but increase later on, as supported by animal (García-Arencibia et al., 2009; Rojo-Bustamante et al., 2018) and human (Van Laere et al., 2012) studies. Assessments of changes in CB_2 receptor expression show enhanced levels in activated microglia and astrocytes of PD patients (Navarrete et al., 2018) and rodent disease models (Gómez-Gálvez et al., 2016). While the above-mentioned expression data supports a role of endocannabinoid signaling in PD progression, the relevance of the system in the pathogenesis of the disease and its potential as a therapeutic target remain obscure. On one hand, non-selective cannabinoids and CB_2 agonists have been reported to attenuate neuronal loss and inflammation in rodent models of PD, and these improvements appear mediated by neuroglial cells involved in disease pathogenesis (García-Arencibia et al., 2007; Gómez-Gálvez et al., 2016; Lastres-Becker et al., 2005). However, pharmacological modulation of CB_1 receptors has rendered conflicting results as application of both agonists and antagonists led to disease-ameliorating effects in animal models of PD (Cao et al., 2007; Fernandez-Espejo et al., 2005). With regard to endocannabinoid hydrolysis, breakthrough observations demonstrated that pharmacological inhibition of MAGL activity protects against dopaminergic degeneration via CB_1 and CB_2 receptor-independent mechanisms that involve attenuated PG production (Nomura et al., 2011). More recent studies have suggested that blocking enzymatic activity of MAGL and FAAH results in protection and/or amelioration of motor PD symptomatology, likely via activation of CB_1 and CB_2 receptors and restoration of astrocytic and microglial homeostatic functions (Celorrio et al., 2016; Fernández-Suárez et al., 2014). At the clinical level, early studies showed a reduction in levodopa-induced dyskinesia in PD patients treated with Δ^9-THC and nabilone and no amelioration following administration of the CB_1 receptor antagonist SR141716A (Mesnage et al., 2004; Sieradzan et al., 2001). However, a randomized, double-blind, placebo-controlled crossover trial showed that orally-administered cannabis extracts resulted in no objective or subjective improvement in dyskinesias or parkinsonism (Carroll et al., 2004). More recently, nabilone was reported to ameliorate non-motors symptoms in PD patients (Peball et al., 2020).
3. **Huntington's disease (HD)** is a devastating neurodegenerative disorder caused by a CAG repeat expansion (>39 CAG repeats) in the gene that encodes the huntingtin (HTT) protein that leads to progressive motor, mood, and cognitive mental dysfunction. The neuropathological basis of the disease is the specific degeneration of striatal GABAergic medium spiny neurons (MSNs) and, to a lesser extent, of glutamatergic pyramidal neurons in the cerebral cortex. A number of studies have suggested that deficient endocannabinoid signaling contributes to HD severity and progression. Early and progressive loss of CB_1 receptors has been reported in *postmortem* brain tissue from HD patients (Glass et al., 1993) and murine models (Denovan-Wright & Robertson, 2000; Dowie et al., 2009) of the disease. Downregulation of CB_1 receptors in HD affects mainly the lateral striatum but not the cortex, and is associated with transcriptional repression of the *Cnr1* gene by mutant HTT (Blázquez et al., 2011; Laprairie et al., 2014). Further supporting endocannabinoid hypofunction in HD, reduced striatal levels of AEA have been reported in animal models, although findings regarding 2-AG are controversial (Bari et al., 2013; Bisogno et al., 2008; Dowie et al., 2009). Accordingly, striatal expression of the AEA-degrading enzyme FAAH is upregulated in mouse models and patients with HD whereas MAGL levels remain unchanged (Blázquez et al., 2011). Studies using genetic, pharmacogenetic, and pharmacological approaches show a neuroprotective effect of CB_1 receptors, particularly those located at corticostriatal terminals, on MSNs through mechanisms that include the production of BDNF and the attenuation of glutamate excitotoxicity (Blázquez et al., 2011; Chiarlone et al., 2014). On the other hand, increased levels of CB_2 receptors associated with microglial cells have been reported in transgenic mouse models and *postmortem* tissue from HD patients (Palazuelos et al., 2009) as well as after intrastriatal malonate injection (Sagredo et al., 2009). As reported for CB_1 receptors (Blázquez et al., 2011), genetic CB_2 receptor deficiency accelerates the onset of motor deficits and increases disease severity in HD transgenic mouse models (Bouchard et al., 2012). Consistently, stimulation of endocannabinoid signaling by administration of cannabinoid agonists such as Δ^9-THC and inhibitors of 2-AG hydrolysis protects striatal neurons and attenuates motor impairments in rodent models of HD (Blázquez et al., 2011; Ruiz-Calvo et al., 2019). Of interest, the protection against striatal damage observed after MAGL blockade appears dependent on the inhibition of this 2-AG inactivating enzyme specifically in astrocytes (Ruiz-Calvo et al., 2019). Altogether, these results support the notion that loss of CB_1 receptors is a major pathogenic event in HD and that pharmacological strategies aimed at promoting CB_1 (and CB_2) receptor signaling may result in therapeutic benefits, the extent of which will most likely depend on the degree of disease

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

severity at the time of drug administration. Based on encouraging findings in preclinical models, a number of clinical trials assessing the effect of cannabinoid (nabiximols, CBD, nabilone) administration in HD have been performed with contrasting results (Consroe et al., 1991; Curtis et al., 2009; López-Sendón Moreno et al., 2016). In most cases, the drugs were reported as safe and well tolerated, but the only significant improvement observed was the attenuation of disease-associated dystonia (Saft et al., 2018).

4. **Amyotrophic lateral sclerosis (ALS)** is a rare and devastating neurodegenerative disease that affects motor neurons and leads to progressive muscle denervation and paralysis. The main clinical manifestations of the disease are impaired speaking, swallowing, walking, and breathing, with 15% of the patients also displaying frontotemporal (FTD) dementia. ALS is usually sporadic, but ~10% of the cases are caused by specific mutations, the inheritance of which is dependent on the specific gene. Most commonly, the mutations affect superoxide dismutase 1 (SOD1) and TAR-DNA binding protein (TDP-43), among other genes, and are inherited as an autosomal dominant trait (Renton et al., 2014). Highlighting the complexity and intermingled pathogenic mechanisms of neurodegeneration, recent observations have demonstrated pathogenic expansions of the HTT gene in both ALS and FTD (Dewan et al., 2021). Studies in SOD1 and TDP-43 mouse models of ALS show increased levels of endocannabinoids in diseased mice, likely associated with altered NAPE-PLD and FAAH activities (Bilsland et al., 2006; Espejo-Porras et al., 2015; Witting et al., 2004). Yet, these observations that have not been recapitulated in human samples (Espejo-Porras et al., 2018). A consistent finding in experimental ALS (Espejo-Porras et al., 2015) and *postmortem* human tissue (Espejo-Porras et al., 2018; Yiangou et al., 2006) is the upregulation of CB_2 receptors, particularly in astrocytes and microglia, interpreted as a protective adaptive response of glial populations (see below). Conversely, the expression of CB_1 in animal models of ALS has been reported either as unchanged (Espejo-Porras et al., 2015; Moreno-Martet et al., 2014) or as altered early during disease progression (Rossi et al., 2010; Zhao et al., 2008). The latter observation suggests that deficient endocannabinoid signaling may contribute to ALS etiopathology. Cumulative evidence from studies using pharmacological and genetic approaches suggests that targeting endocannabinoid signaling may have neuroprotective effects in ALS. Early research using Δ^9-THC and non-selective synthetic cannabinoid agonists showed delayed disease progression and/or increased life-span in rodent ALS models (Bilsland et al., 2006; Raman et al., 2004). These findings have been recapitulated using selective CB_2 agonists, and associated with attenuated astroglial and microglial immunoreactivity (Espejo-Porras et al., 2019). Further supporting the therapeutic potential of promoting CB_2 signaling in ALS, genetic inactivation of the receptor has been reported to accelerate neurological deterioration and glial reaction while shortening the life span of TDP-43 mice (Rodríguez-Cueto et al., 2021). Conversely, genetic ablation of the CB_1 receptor had no effect on disease onset in SOD1 mice but significantly extended life span (Bilsland et al., 2006). Increasing endocannabinoid levels by genetic and pharmacological inactivation of MAGL and/or FAAH was shown to delay disease progression and attenuate neuropathology in experimental ALS, thus recapitulating the effects of cannabinoid receptor agonists (Bilsland et al., 2006). Collectively, preclinical studies in ALS show protective effects of targeting CB_2 signaling via modulation of glial cell function. These observations encourage further research aimed at elucidating specific cellular mechanisms and molecular pathways engaged by endocannabinoid signaling in ALS-associated astrocytes and microglia. The above-mentioned potential of cannabinoids to attenuate ALS symptomatology in preclinical models has been addressed by a limited number of controlled clinical trials. Δ^9-THC was well-tolerated by ALS patients but the only improvement observed following its administration was a modest attenuation of cramps and fasciculations (Weber et al., 2010). More recently, a proof-of-concept trial using nabiximols demonstrated a reduction in spasticity in patients with ALS (Riva et al., 2019). A clinical trial aimed at evaluating the efficacy of a standardized cannabis extract with high CBD/low Δ^9-THC ratio is currently in progress (Urbi et al., 2019).
5. **Multiple sclerosis (MS)** is a chronic inflammatory demyelinating disease of the CNS that represents the leading cause of acquired non-traumatic disability in young and middle-aged adults in the developed world (Lassmann, 2014). MS is thought to arise due to both environmental and genetic factors, and recent landmark studies point to infection with Epstein Bar virus as a crucial pathogenic mechanism (Bjornevik et al., 2022; Lanz et al., 2022). In most patients, MS starts as so-called relapsing remitting (RR) disease in which periods of neurological symptoms alternate with those of recovery. The predominant pathological feature in this form of the disease is the appearance of focal demyelinating lesions disseminated throughout the CNS characterized by the heterogeneous presence of peripheral immune cells, astrocytes and microglia, and variable amount of axonal injury/loss (Lassmann, 2014). RRMS eventually evolves into a progressive form characterized by lower extent of inflammation, expanding demyelination, and axonal/neuronal loss. A subset of patients directly enters the progressive phase. While immunomodulatory agents are successfully used to alleviate symptoms in RRMS, these largely fail to prevent the transition into the progressive phase, characterized by the development of irreversible neurological deficits. Thus, preventing MS progression and development of permanent neurological handicap is an unmet clinical need for patients with MS. Importantly, the process of myelin regeneration, also called remyelination, occurs in MS lesions at variable extent, and lesions in which repair is successful show less axonal degeneration than the chronically demyelinated ones (Kornek & Lassmann, 2003). Thus, stimulating remyelination is considered as a potential neuroprotective strategy to prevent axonal damage and halt/reduce MS progression. The role of the endocannabinoid system in MS became the focus of many studies due to anecdotal reports that street cannabis can improve symptoms such as limb spasticity in self-medicating patients with MS. Concerning alterations of the

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

endocannabinoidome, increased concentrations of AEA but not 2-AG have been reported in patients with MS and animal models of the disease, and correlated to imbalanced NAPE-PLD/FAAH activities (Centonze et al., 2007; Eljaschewitsch et al., 2006; Jean-Gilles et al., 2009). Although the cell specificity of the changes observed has not been thoroughly investigated, these may reflect alterations in peripheral immune cells as well as in astrocytes and microglia activated early during disease progression (Benito et al., 2003; Centonze et al., 2007; Moreno-García et al., 2020). In contrast to the above mentioned evidence, early evaluations of *postmortem* MS lesions suggested FAAH upregulation in hypertrophic astrocytes (Benito et al., 2003), and preclinical and clinical assessments have also revealed unaltered or reduced levels of both endocannabinoids in MS (Di Filippo et al., 2008) and its models (Manterola, Bernal-Chico, Cipriani, Canedo-Antelo et al., 2018). Finally, deregulated expression of astrocytic FABP7 has been shown during experimental demyelination as well as in human MS lesions (Kipp et al., 2011). In particular, demyelinating lesions with impaired remyelination capacity show weak FABP7 expression and astrocytic levels of the carrier protein seem to correlate with the presence of OPCs, suggesting an association between astrocyte FABP7 activity and myelin repair. However, FABP7 knockout mice displayed a slightly earlier onset of inflammation and clinical signs but lower clinical scores in the chronic phase of the experimental autoimmune demyelination model, in a later study (Kamizato et al., 2019). Because FABP7 is also expressed in OPCs (Foerster et al., 2020), it could be that the phenotype of FABP7 knockout mice is also OPC-dependent. Indeed, FABP7 deficiency in OPCs was associated with defects in developmental myelination, but not remyelination in a focal remyelination model (Foerster et al., 2020). In all, currently there is no consensus on whether endocannabinoid system is overactive or impaired in MS in terms of endocannabinoid availability, and the prevailing hypothesis is that local changes take place in a disease-stage and lesion type-dependent manner. Regarding cannabinoid receptors, CB_2 levels have been consistently reported as upregulated in activated microglia and immune cells within MS lesions (Benito et al., 2003; Loría et al., 2008; Maresz et al., 2007) whereas results regarding the modulation of CB_1 receptor expression are controversial (Berrendero et al., 2001; Cabranes et al., 2006; Manterola, Bernal-Chico, Cipriani, Canedo-Antelo, et al., 2018). Studies on the effects of endocannabinoid-modulating drugs in MS and its preclinical models has led to consistent and encouraging observations supporting the clinical potential of targeting the system for therapeutic purposes. Cannabinoid agonists and MAGL/FAAH inhibitors engage multiple beneficial effects that include neuroprotection and amelioration of inflammatory responses (Bernal-Chico et al., 2015; Ortega-Gutiérrez et al., 2005; Pryce et al., 2013), while pharmacological ABHD6 blockade engages only modest protection in MS animal models (Manterola, Bernal-Chico, Cipriani, Canedo-Antelo, et al., 2018; Manterola, Bernal-Chico, Cipriani, Ruiz, et al., 2018). A number of studies also show that deficiency or blockade of FABP5 and FABP7 confers protection in MS models via immune inhibition and oligodendrocyte protection but whether these effects involve an enhanced endocannabinoid tone remains to be addressed (Cheng et al., 2021; Kamizato et al., 2019; Reynolds et al., 2007). The alleviation of MS symptoms by (endo)cannabinoids appears mediated by the neuronal population of CB_1 receptors as well as CB_2 receptors in hematopoietic cells and microglia, as suggested by both pharmacological and genetic approaches in animal models of the disease (Maresz et al., 2007; Palazuelos et al., 2008; Pryce et al., 2003). As in other pathologies, CB_2 receptors have been implicated in the acquisition of a pro-regenerative phenotype by microglial cells, likely contributing to a reduction in the tissue damage (Mecha et al., 2018). The hypothesis that endocannabinoid signaling promotes myelin repair has also been recently highlighted (Aguado et al., 2021; Feliú et al., 2017). *In vivo* observations on the pro-remyelinating effects of (endo)cannabinoids are further supported by the *in vitro* results showing protective and differentiation-promoting effects on oligodendroglia, associated with the activation of CB_1 and CB_2 receptors (Bernal-Chico et al., 2015; Gomez et al., 2010). However, few data are available on the mechanisms underlying the effect of cannabinoids on oligodendroglia in models of MS, and the implication of oligodendrocyte CB_1 (and/or CB_2) receptors in the protective and regenerative effects of (endo)cannabinoids remains to be clearly demonstrated. Similarly, bona-fide evidence that cannabinoid receptor populations in astrocytes modulate the onset and progression of MS is still lacking. Clinical studies in patients with MS show that administration of Δ^9-THC, alone or in combination with CBD and its analogs, modestly attenuates spasticity (Chiurchiù et al., 2018). Following approval of nabiximols for the treatment of pain and refractory spasticity in MS patients (Novotna et al., 2011) clinical experience has corroborated the anti-spastic profile of this endocannabinoid-targeting medication (Giacoppo et al., 2017; Markovà et al., 2019). Systematic analyses have not provided clear conclusions for other MS symptoms (Nielsen et al., 2018). Research on the mechanisms engaged by nabiximols in MS patients is ongoing, and the immunomodulatory and disease-modifying efficacy is under debate (Sorosina et al., 2018). Clinical experience with cannabis-based medicines in MS has also demonstrated that the therapeutic efficacy of Δ^9-THC is restricted to is use at high doses and usually accompanied by undesired effects including psychoactivity or memory impairments (Baker et al., 2012). Current research on the therapeutic potential of the endocannabinoid system in MS, thus, aims at developing strategies to target CB_1 and CB_2, whilst limiting such adverse responses associated with Δ^9-THC administration.

4 | CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The studies outlined in this review demonstrate that endocannabinoid system plays a role in the onset, progression, and/or symptomatology of major neurological disorders and provides several, often multifaceted substrates for therapeutic targeting. Because endocannabinoids are intimately involved in the regulation of glial

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

responses during brain pathology and glial cells are crucial determinants of neuropathological states, endocannabinoid control of glial function represents an attractive target for the development of novel therapeutic agents for neurodegenerative diseases. However, the redundancy and promiscuity of the endocannabinoid network poses a significant challenge. Thus, to ensure therapeutic progress, several questions remain to be addressed regarding endocannabinoid-mediated modulation of glial cell function as well as the nature of glial dysfunction in neurodegeneration. These questions include:

Is the expression of cannabinoid receptors homogeneous within the diverse populations of astrocytes, microglia, and oligodendrocytes? Which is the topography of endocannabinoid signaling machinery in glial cells and its relation to the neuronal compartment in health and disease? Does the phenotypic transformation of neuroglial cells in pathological context imply alterations in autocrine and paracrine endocannabinoid signaling? Which are the implications of endocannabinoid signaling in the metabolic activity of astrocytes, microglia, and oligodendroglia? Do endocannabinoids modulate glial bioenergetics failure during neurodegeneration? Do these lipid mediators play a role in white matter alterations associated with neurological diseases?

All these points should be explored by combining transgenic models and rescue strategies that allow cell-type specific manipulation of endocannabinoid signaling *in vivo* with the analysis of transcriptomic and epigenetic profiles of glial cells at various disease states. Correlation of changes at the molecular level with functional readouts and corroborations using reliable, patient-derived human *in vitro* models are also necessary to minimize the translation gap. Finally, assessing the potential of endocannabinoidome-targeting drugs as disease-modifying therapies in patients remains a major challenge for future research.

5 | MATERIALS AND METHODS

For the analysis of CB_1 receptor localization in oligodendrocytes we used C57BL6N male mice deficient in the cannabinoid CB_1 receptor (Marsicano et al., 2002) and their corresponding wild-type littermates, herein referred to as $CB_1^{-/-}$ and $CB_1^{+/+}$, respectively (colony founders kindly provided by Dr. Beat Lutz, Institute of Molecular Biology, Mainz, Germany). Animals were housed under standard conditions (12 h light/dark cycles) with access to food and water ad libitum. All experiments were conducted under the supervision and with the approval of the Animal Welfare Committee of the University of the Basque Country UPV/EHU (CEEA395. All efforts were made to minimize animal suffering and to reduce the number of mice used, in compliance with the European Communities Council Directive of 22 September 2010 on the protection of animals used for scientific purposes (Directive 2010/63/EU).

5.1 | Mice sacrifice and tissue processing

Male mice with a C57BL6N background were intraperitoneally (i.p.) anesthetized with ketamine/xylazine (80/10 mg/Kg; Imalgene®, Mérial, France/Rompun®, Bayer, Germany) and transcardially perfused with saline solution (0.9% NaCl; pH 7.4) to clear blood vessels followed by fixative solution containing 4% paraformaldehyde, 0.1% glutaraldehyde and 0.2% picric acid in 0.1 M phosphate buffer (PB; pH 7.4), using a peristaltic pump. After extraction, brains were post-fixed overnight in 4% paraformaldehyde at 4°C. Coronal sections (40 μm-thick) containing corpus callosum and hippocampus were obtained on a vibratome (VT1000S, Leica, Wetzlar, Germany) and stored in 0.1 M PB containing 0.02% sodium azide until use.

5.2 | Preembedding immunogold method for electron microscopy

Tissue sections were pre-incubated in a blocking solution containing 10% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO), 0.1% sodium azide and 0.02% saponin in Tris–HCl buffered saline (TBS; pH 7.4) for 30 min at room temperature (RT). Subsequently, sections were incubated with a polyclonal rabbit anti-CB_1 receptor antibody (1:500; ImmunoGenes, Budapest, Hungary) prepared in blocking solution with 0.004% saponin for 2 days at 4°C. After several washes in 1% BSA/TBS, tissue sections were incubated in a secondary 1.4 nm gold-labeled goat anti-rabbit IgG (1:200; Nanoprobes Inc., Yaphank, NY) prepared in the washing solution with 0.1% sodium azide and 0.004% saponin for 4 h at RT. Tissue was washed overnight in 1% BSA/TBS at 4°C, postfixed in 1% glutaraldehyde in TBS for 10 min at RT and washed in double-distilled water (dd$H_2$0). Gold particles were silver-intensified with a HQ Silver kit (Nanoprobes) in the dark for 12 min and tissue was washed with ddH20 followed by 0.1 M PB. The day after, sections were osmicated (1% OsO_4 in 0.1 M PB; pH 7.4) for 30 min. After 3 × 10 min washes in 0.1 M PB, tissue sections were dehydrated in graded ethanol concentrations (50%–100%) to propylene oxide and embedded in epoxy resin (Sigma-Aldrich) by immersion in decreasing concentration of propylene oxide (1:3 for 30 min, 1:1 for 1 h and 3:1 for 2 h). Tissue was then embedded in fresh resin overnight and allowed to polymerize at 60°C for 2 days. Following visualization at the light microscope, selected tissue portions were trimmed and glued onto epoxy resin capsules. Semi-thin sections (500 nm-thick) were cut from epoxy blocks using a Power Tome ultramicrotome (RMC Boeckeler, Tucson, AZ) and stained with 1% toluidine blue. Ultrathin (50–60 nm-thick) sections were then cut with a diamond knife (Diatome, Hatfield PA), collected on nickel mesh grids and stained with 4% uranyl acetate for 30 min and 2.5% lead citrate for electron microscope visualization.

5.3 | Semi-quantification of the CB_1 receptor immunogold staining

Immunogold labeling was visualized on the tissue slices with a light microscope and portions of the corpus callosum and with consistent immunolabeling of CB_1 receptors identified and trimmed down for

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

ultrathin sectioning. To standardize conditions and avoid false negatives, only the first 20 ultrathin sections were collected onto the grids and photographed for analysis. Ultrathin sections were examined with a Jeol JEM 1400 Plus electron microscope (Jeol, Tokio, Japan) at the Service of Analytical and High-Resolution Microscopy in Biomedicine of University of the Basque Country UPV/EHU. For the analysis of CB_1 receptor localization in mature oligodendrocytes, the electron micrographs were taken with a digital sCMOS camera (Hamamatsu Photonics France, Cerdañola, Spain) at magnification 4000–8000×. Sampling was always carefully and accurately carried out in the same way for all the animals studied. Mature oligodendrocytes in the corpus callosum were identified by their distinctive morphological features, such as electron-dense nuclei with pronounced aggregates of heterochromatin and the usual presence of thin rims of perinuclear cytoplasm (Peters & Folger, 2013). Image-J software (NIH, Bethesda, MD) was used to measure the membrane length (perimeter) of oligodendrocyte somata. Positive labeling was considered if at least one immunoparticle was found within approximately 30 nm from the membrane. Percentages of CB_1 receptor positive somata were analyzed and displayed as mean ± *SEM* using a GraphPad Prism. Results correspond to the analysis of 22–38 oligodendrocytes per animal.

ACKNOWLEDGMENTS

The authors are grateful to Dr. JC Chara for his assistance during sample preparation for EM analysis. Research of the authors is supported by grants from FEDER and ISCIII (AES 2021 - PI21/00629 to Susana Mato), the Basque Government (PIBA19-0059 to Susana Mato and IT1203-19 to Carlos Matute), the Spanish Ministry of Science and Innovation (SAF2016-75292-R to Carlos Matute), CIBERNED (CB06/05/0076), and ARSEP Foundation (to Susana Mato).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ORCID

Ana Bernal-Chico https://orcid.org/0000-0002-7913-2350
Vanja Tepavcevic https://orcid.org/0000-0003-3230-4888
Andrea Manterola https://orcid.org/0000-0001-6989-3167
Carmen Utrilla https://orcid.org/0000-0001-9860-4520
Carlos Matute https://orcid.org/0000-0001-8672-711X
Susana Mato https://orcid.org/0000-0002-2005-9793

REFERENCES

Aguado, T., Huerga-Gómez, A., Sánchez-de la Torre, A., Resel, E., Chara, J. C., Matute, C., & Palazuelos, J. (2021). Δ9-tetrahydrocannabinol promotes functional remyelination in the mouse brain. *Brain Journal de Pharmacologie*, *178*(20), 4176–4192.

Alexander, S. P. (2016). Therapeutic potential of cannabis-related drugs. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *64*, 157–166.

Alger, B. E., & Kim, J. (2011). Supply and demand for endocannabinoids. *Trends in Neurosciences*, *34*(6), 304–315.

Alhouayek, M., Masquelier, J., Cani, P. D., Lambert, D. M., & Muccioli, G. G. (2013). Implication of the anti-inflammatory bioactive lipid prostaglandin D2-glycerol ester in the control of macrophage activation and inflammation by ABHD6. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(43), 17558–17563.

Araque, A., Castillo, P. E., Manzoni, O. J., & Tonini, R. (2017). Synaptic functions of endocannabinoid signaling in health and disease. *Neuropharmacology*, *124*, 13–24.

Aso, E., & Ferrer, I. (2016). CB2 cannabinoid receptor as potential target against Alzheimer's disease. *Frontiers in Neuroscience*, *10*, 243.

Aso, E., Juvés, S., Maldonado, R., & Ferrer, I. (2013). CB2 cannabinoid receptor agonist ameliorates Alzheimer-like phenotype in AβPP/PS1 mice. *Journal of Alzheimer's Disease*, *35*(4), 847–858.

Aso, E., Palomer, E., Juvés, S., Maldonado, R., Muñoz, F. J., & Ferrer, I. (2012). CB1 agonist ACEA protects neurons and reduces the cognitive impairment of AβPP/PS1 mice. *Journal of Alzheimer's Disease*, *30*(2), 439–459.

Atwood, B. K., & Mackie, K. (2010). CB2: A cannabinoid receptor with an identity crisis. *British Journal of Pharmacology*, *160*(3), 467–479.

Baggelaar, M. P., van Esbroeck, A. C., van Rooden, E. J., Florea, B. I., Overkleeft, H. S., Marsicano, G., & van der Stelt, M. (2017). Chemical proteomics maps brain region specific activity of endocannabinoid hydrolases. *ACS Chemical Biology*, *12*(3), 852–861.

Baker, D., Pryce, G., Jackson, S. J., Bolton, C., & Giovannoni, G. (2012). The biology that underpins the therapeutic potential of cannabis-based medicines for the control of spasticity in multiple sclerosis. *Multiple Sclerosis and Related Disorders*, *1*(12), 64–75.

Bari, M., Battista, N., Valenza, M., Mastrangelo, N., Malaponti, M., Catanzaro, G., & Maccarrone, M. (2013). In vitro and in vivo models of Huntington's disease show alterations in the endocannabinoid system. *The FEBS Journal*, *280*(14), 3376–3388.

Bedse, G., Romano, A., Cianci, S., Lavecchia, A. M., Lorenzo, P., Elphick, M. R., & Gaetani, S. (2014). Altered expression of the CB1 cannabinoid receptor in the triple transgenic mouse model of Alzheimer's disease. *Journal of Alzheimer's Disease*, *40*(3), 701–712.

Bénard, G., Massa, F., Puente, N., Lourenço, J., Bellocchio, L., Soria-Gómez, E., & Marsicano, G. (2012). Mitochondrial CB_1 receptors regulate neuronal energy metabolism. *Nature Neuroscience*, *15*(4), 558–564.

Benito, C., Núñez, E., Tolón, R. M., Carrier, E. J., Rábano, A., Hillard, C. J., & Romero, J. (2003). Cannabinoid CB2 receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *The Journal of Neuroscience*, *23*(35), 11136–11141.

Bernal-Chico, A., Canedo, M., Manterola, A., Victoria Sánchez-Gómez, M., Pérez-Samartín, A., Rodríguez-Puertas, R., & Mato, S. (2015). Blockade of monoacylglycerol lipase inhibits oligodendrocyte excitotoxicity and prevents demyelination in vivo. *Glia*, *63*(1), 163–176.

Berrendero, F., García-Gil, L., Hernández, M. L., Romero, J., Cebeira, M., de Miguel, R., & Fernández-Ruiz, J. J. (1998). Localization of mRNA expression and activation of signal transduction mechanisms for cannabinoid receptor in rat brain during fetal development. *Development*, *125*(16), 3179–3188.

Berrendero, F., Sánchez, A., Cabranes, A., Puerta, C., Ramos, J. A., García-Merino, A., & Fernández-Ruiz, J. (2001). Changes in cannabinoid CB(1) receptors in striatal and cortical regions of rats with experimental allergic encephalomyelitis, an animal model of multiple sclerosis. *Synapse*, *41*(3), 195–202.

Berry, A. J., Zubko, O., Reeves, S. J., & Howard, R. J. (2020). Endocannabinoid system alterations in Alzheimer's disease: A systematic review of human studies. *Brain Research*, *1749*, 147135.

Billakota, S., Devinsky, O., & Marsh, E. (2019). Cannabinoid therapy in epilepsy. *Current Opinion in Neurology*, *32*(2), 220–226.

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Bilsland, L. G., Dick, J. R., Pryce, G., Petrosino, S., Di Marzo, V., Baker, D., & Greensmith, L. (2006). Increasing cannabinoid levels by pharmacological and genetic manipulation delay disease progression in SOD1 mice. *The FASEB Journal*, *20*(7), 1003–1005.

Bisogno, T., Howell, F., Williams, G., Minassi, A., Cascio, M. G., Ligresti, A., & Doherty, P. (2003). Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *The Journal of Cell Biology*, *163*(3), 463–468.

Bisogno, T., Martire, A., Petrosino, S., Popoli, P., & Di Marzo, V. (2008). Symptom-related changes of endocannabinoid and palmitoylethanolamide levels in brain areas of R6/2 mice, a transgenic model of Huntington's disease. *Neurochemistry International*, *52*(1–2), 307–313.

Bjornevik, K., Cortese, M., Healy, B. C., Kuhle, J., Mina, M. J., Leng, Y., & Ascherio, A. (2022). Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. *Science*, *375* (6578), 296–301.

Blankman, J. L., Long, J. Z., Trauger, S. A., Siuzdak, G., & Cravatt, B. F. (2013). ABHD12 controls brain lysophosphatidylserine pathways that are deregulated in a murine model of the neurodegenerative disease PHARC. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(4), 1500–1505.

Blankman, J. L., Simon, G. M., & Cravatt, B. F. (2007). A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chemistry & Biology*, *14*(12), 1347–1356.

Blázquez, C., Chiarlone, A., Sagredo, O., Aguado, T., Pazos, M. R., Resel, E., & Guzmán, M. (2011). Loss of striatal type 1 cannabinoid receptors is a key pathogenic factor in Huntington's disease. *Brain*, *134*(1), 119–136.

Bouchard, J., Truong, J., Bouchard, K., Dunkelberger, D., Desrayaud, S., Moussaoui, S., & Muchowski, P. J. (2012). Cannabinoid receptor 2 signaling in peripheral immune cells modulates disease onset and severity in mouse models of Huntington's disease. *The Journal of Neuroscience*, *32*(50), 18259–18268.

Cabranes, A., Pryce, G., Baker, D., & Fernández-Ruiz, J. (2006). Changes in CB1 receptors in motor-related brain structures of chronic relapsing experimental allergic encephalomyelitis mice. *Brain Research*, *1107*(1), 199–205.

Cao, J. K., Kaplan, J., & Stella, N. (2019). ABHD6: Its place in endocannabinoid signaling and beyond. *Trends in Pharmacological Sciences*, *40*(4), 267–277.

Cao, X., Liang, L., Hadcock, J. R., Iredale, P. A., Griffith, D. A., Menniti, F. S., & Papa, S. M. (2007). Blockade of cannabinoid type 1 receptors augments the antiparkinsonian action of levodopa without affecting dyskinesias in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated rhesus monkeys. *The Journal of Pharmacology and Experimental Therapeutics*, *323*(1), 318–326.

Carrier, E. J., Kearn, C. S., Barkmeier, A. J., Breese, N. M., Yang, W., Nithipatikom, K., & Hillard, C. J. (2004). Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonylglycerol, which increases proliferation via a CB2 receptor-dependent mechanism. *Molecular Pharmacology*, *65*(4), 999–1007.

Carroll, C. B., Bain, P. G., Teare, L., Liu, X., Joint, C., Wroath, C., & Zajicek, J. P. (2004). Cannabis for dyskinesia in Parkinson disease: A randomized double-blind crossover study. *Neurology*, *63*(7), 1245–1250.

Celorrio, M., Fernández-Suárez, D., Rojo-Bustamante, E., Echeverry-Alzate, V., Ramírez, M. J., Hillard, C. J., & Aymerich, M. S. (2016). Fatty acid amide hydrolase inhibition for the symptomatic relief of Parkinson's disease. *Brain, Behavior, and Immunity*, *57*, 94–105.

Centonze, D., Bari, M., Rossi, S., Prosperetti, C., Furlan, R., Fezza, F., & Maccarrone, M. (2007). The endocannabinoid system is dysregulated in multiple sclerosis and in experimental autoimmune encephalomyelitis. *Brain*, *130*(10), 2543–2553.

Chan, D. C. (2020). Mitochondrial dynamics and its involvement in disease. *Annual Review of Pathology*, *15*, 235–259.

Chen, D. H., Naydenov, A., Blankman, J. L., Mefford, H. C., Davis, M., Sul, Y., & Bird, T. D. (2013). Two novel mutations in ABHD12: Expansion of the mutation spectrum in PHARC and assessment of their functional effects. *Human Mutation*, *34*(12), 1672–1678.

Chen, R., Zhang, J., Wu, Y., Wang, D., Feng, G., Tang, Y. P., & Chen, C. (2012). Monoacylglycerol lipase is a therapeutic target for Alzheimer's disease. *Cell Reports*, *2*(5), 1329–1339.

Chen, X. Q., & Mobley, W. C. (2019). Alzheimer disease pathogenesis: Insights from molecular and cellular biology studies of oligomeric Aβ and tau species. *Frontiers in Neuroscience*, *13*, 659.

Chen, Y., Liu, X., Vickstrom, C. R., Liu, M. J., Zhao, L., Viader, A., & Liu, Q. S. (2016). Neuronal and astrocytic monoacylglycerol lipase limit the spread of endocannabinoid signaling in the cerebellum. *eNeuro*, *3*(3), ENEURO.0048-16.2016.

Cheng, A., Jia, W., Kawahata, I., & Fukunaga, K. (2021). A novel fatty acid-binding protein 5 and 7 inhibitor ameliorates oligodendrocyte injury in multiple sclerosis mouse models. *eBioMedicine*, *72*, 103582.

Chiarlone, A., Bellocchio, L., Blázquez, C., Resel, E., Soria-Gómez, E., Cannich, A., & Guzmán, M. (2014). A restricted population of CB1 cannabinoid receptors with neuroprotective activity. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(22), 8257–8262.

Chicca, A., Nicolussi, S., Bartholomäus, R., Blunder, M., Aparisi Rey, A., Petrucci, V., & Gertsch, J. (2017). Chemical probes to potently and selectively inhibit endocannabinoid cellular reuptake. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(25), 5006–5015.

Chiurchiù, V., van der Stelt, M., Centonze, D., & Maccarrone, M. (2018). The endocannabinoid system and its therapeutic exploitation in multiple sclerosis: Clues for other neuroinflammatory diseases. *Progress in Neurobiology*, *160*, 82–100.

Clarke, L. E., Liddelow, S. A., Chakraborty, C., Münch, A. E., Heiman, M., & Barres, B. A. (2018). Normal aging induces A1-like astrocyte reactivity. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(8), E1896–E1905.

Coiret, G., Ster, J., Grewe, B., Wendling, F., Helmchen, F., Gerber, U., & Benquet, P. (2012). Neuron to astrocyte communication via cannabinoid receptors is necessary for sustained epileptiform activity in rat hippocampus. *PLoS One*, *7*(5), e37320.

Consroe, P., Laguna, J., Allender, J., Snider, S., Stern, L., Sandyk, R., & Schram, K. (1991). Controlled clinical trial of cannabidiol in Huntington's disease. *Pharmacology, Biochemistry, and Behavior*, *40*(3), 701–708.

Cravatt, B. F., Demarest, K., Patricelli, M. P., Bracey, M. H., Giang, D. K., Martin, B. R., & Lichtman, A. H. (2001). Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(16), 9371–9376.

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*(6604), 83–87.

Cristino, L., Bisogno, T., & Di Marzo, V. (2020). Cannabinoids and the expanded endocannabinoid system in neurological disorders. *Nature Reviews. Neurology*, *16*(1), 9–29.

Curtis, A., Mitchell, I., Patel, S., Ives, N., & Rickards, H. (2009). A pilot study using nabilone for symptomatic treatment in Huntington's disease. *Movement Disorders*, *24*(15), 2254–2259.

De Meij, J., Alfanek, Z., Morel, L., Decoeur, F., Leyrolle, Q., Picard, K., Carrier, M., Aubert, A., Séré, A., Lucas, C., Laforest, G., Helbling, J.-C., Tremblay, M.-E., Cota, D., Moisan, M.-P., Marsicano, G., Layé, S., & Nadjar, A. (2021). Microglial cannabinoid type 1 receptor regulates

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

brain inflammation in a sex-specific manner. *Cannabis and Cannabinoid Research*, *6*(6), 488–507.

Dean, D. C., Hurley, S. A., Kecskemeti, S. R., O'Grady, J. P., Canda, C., Davenport-Sis, N. J., & Bendlin, B. B. (2017). Association of amyloid pathology with myelin alteration in preclinical Alzheimer disease. *JAMA Neurology*, *74*(1), 41–49.

Denovan-Wright, E. M., & Robertson, H. A. (2000). Cannabinoid receptor messenger RNA levels decrease in a subset of neurons of the lateral striatum, cortex and hippocampus of transgenic Huntington's disease mice. *Neuroscience*, *98*(4), 705–713.

Devane, W. A., Dysarz, F. A., Johnson, M. R., Melvin, L. S., & Howlett, A. C. (1988). Determination and characterization of a cannabinoid receptor in rat brain. *Molecular Pharmacology*, *34*(5), 605–613.

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., & Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*, *258*(5090), 1946–1949.

Dewan, R., Chia, R., Ding, J., Hickman, R. A., Stein, T. D., Abramzon, Y., & Consortium, P. (2021). Pathogenic huntingtin repeat expansions in patients with frontotemporal dementia and amyotrophic lateral sclerosis. *Neuron*, *109*(3), 448–460.

Di Filippo, M., Pini, L. A., Pelliccioli, G. P., Calabresi, P., & Sarchielli, P. (2008). Abnormalities in the cerebrospinal fluid levels of endocannabinoids in multiple sclerosis. *Journal of Neurology, Neurosurgery, and Psychiatry*, *79*(11), 1224–1229.

Di Marzo, V. (2018). New approaches and challenges to targeting the endocannabinoid system. *Nature Reviews. Drug Discovery*, *17*(9), 623–639.

Di Marzo, V., Hill, M. P., Bisogno, T., Crossman, A. R., & Brotchie, J. M. (2000). Enhanced levels of endogenous cannabinoids in the globus pallidus are associated with a reduction in movement in an animal model of Parkinson's disease. *The FASEB Journal*, *14*(10), 1432–1438.

Dinh, T. P., Freund, T. F., & Piomelli, D. (2002). A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. *Chemistry and Physics of Lipids*, *121*(1–2), 149–158.

Dowie, M. J., Bradshaw, H. B., Howard, M. L., Nicholson, L. F., Faull, R. L., Hannan, A. J., & Glass, M. (2009). Altered CB1 receptor and endocannabinoid levels precede motor symptom onset in a transgenic mouse model of Huntington's disease. *Neuroscience*, *163*(1), 456–465.

Dudok, B., Barna, L., Ledri, M., Szabó, S. I., Szabadits, E., Pintér, B., & Katona, I. (2015). Cell-specific STORM super-resolution imaging reveals nanoscale organization of cannabinoid signaling. *Nature Neuroscience*, *18*(1), 75–86.

Duffy, S. S., Hayes, J. P., Fiore, N. T., & Moalem-Taylor, G. (2021). The cannabinoid system and microglia in health and disease. *Neuropharmacology*, *190*, 108555.

Ehrhart, J., Obregon, D., Mori, T., Hou, H., Sun, N., Bai, Y., & Shytle, R. D. (2005). Stimulation of cannabinoid receptor 2 (CB2) suppresses microglial activation. *Journal of Neuroinflammation*, *2*, 29.

Eljaschewitsch, E., Witting, A., Mawrin, C., Lee, T., Schmidt, P. M., Wolf, S., & Ullrich, O. (2006). The endocannabinoid anandamide protects neurons during CNS inflammation by induction of MKP-1 in microglial cells. *Neuron*, *49*(1), 67–79.

Espejo-Porras, F., Fernández-Ruiz, J., & de Lago, E. (2018). Analysis of endocannabinoid receptors and enzymes in the post-mortem motor cortex and spinal cord of amyotrophic lateral sclerosis patients. *Amyotroph Lateral Scler Frontotemporal Degener*, *19*(5–6), 377–386.

Espejo-Porras, F., García-Toscano, L., Rodríguez-Cueto, C., Santos-García, I., de Lago, E., & Fernandez-Ruiz, J. (2019). Targeting glial cannabinoid CB2 receptors to delay the progression of the pathological phenotype in TDP-43 (A315T) transgenic mice, a model of amyotrophic lateral sclerosis. *British Journal of Pharmacology*, *176*(10), 1585–1600.

Espejo-Porras, F., Piscitelli, F., Verde, R., Ramos, J. A., Di Marzo, V., de Lago, E., & Fernández-Ruiz, J. (2015). Changes in the endocannabinoid signaling system in CNS structures of TDP-43 transgenic mice: Relevance for a neuroprotective therapy in TDP-43-related disorders. *Journal of Neuroimmune Pharmacology*, *10*(2), 233–244.

Esposito, G., Scuderi, C., Savani, C., Steardo, L., De Filippis, D., Cottone, P., & Cuomo, V. (2007). Cannabidiol in vivo blunts beta-amyloid induced neuroinflammation by suppressing IL-1beta and iNOS expression. *British Journal of Pharmacology*, *151*(8), 1272–1279.

Esposito, G., Scuderi, C., Valenza, M., Togna, G. I., Latina, V., De Filippis, D., & Steardo, L. (2011). Cannabidiol reduces Aβ-induced neuroinflammation and promotes hippocampal neurogenesis through PPARγ involvement. *PLoS One*, *6*(12), e28668.

Estrada, J. A., & Contreras, I. (2020). Endocannabinoid receptors in the CNS: Potential drug targets for the prevention and treatment of neurologic and psychiatric disorders. *Current Neuropharmacology*, *18*(8), 769–787.

Feliú, A., Bonilla Del Río, I., Carrillo-Salinas, F. J., Hernández-Torres, G., Mestre, L., Puente, N., & Guaza, C. (2017). 2-Arachidonoylglycerol reduces proteoglycans and enhances remyelination in a progressive model of demyelination. *The Journal of Neuroscience*, *37*(35), 8385–8398.

Fernandez-Espejo, E., Caraballo, I., de Fonseca, F. R., El Banoua, F., Ferrer, B., Flores, J. A., & Galan-Rodriguez, B. (2005). Cannabinoid CB1 antagonists possess antiparkinsonian efficacy only in rats with very severe nigral lesion in experimental parkinsonism. *Neurobiology of Disease*, *18*(3), 591–601.

Fernández-Suárez, D., Celorrio, M., Riezu-Boj, J. I., Ugarte, A., Pacheco, R., González, H., & Aymerich, M. S. (2014). Monoacylglycerol lipase inhibitor JZL184 is neuroprotective and alters glial cell phenotype in the chronic MPTP mouse model. *Neurobiology of Aging*, *35*(11), 2603–2616.

Finsterwald, C., Magistretti, P. J., & Lengacher, S. (2015). Astrocytes: New targets for the treatment of neurodegenerative diseases. *Current Pharmaceutical Design*, *21*(25), 3570–3581.

Fiskerstrand, T., H'mida-Ben Brahim, D., Johansson, S., M'zahem, A., Haukanes, B. I., Drouot, N., & Knappskog, P. M. (2010). Mutations in ABHD12 cause the neurodegenerative disease PHARC: An inborn error of endocannabinoid metabolism. *American Journal of Human Genetics*, *87*(3), 410–417.

Foerster, S., Guzman de la Fuente, A., Kagawa, Y., Bartels, T., Owada, Y., & Franklin, R. J. M. (2020). The fatty acid binding protein FABP7 is required for optimal oligodendrocyte differentiation during myelination but not during remyelination. *Glia*, *68*(7), 1410–1420.

Gao, Y., Vasilyev, D. V., Goncalves, M. B., Howell, F. V., Hobbs, C., Reisenberg, M., & Doherty, P. (2010). Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. *The Journal of Neuroscience*, *30*(6), 2017–2024.

Garabadu, D., Agrawal, N., Sharma, A., & Sharma, S. (2019). Mitochondrial metabolism: A common link between neuroinflammation and neurodegeneration. *Behavioural Pharmacology*, *30*(8), 642–652.

García-Arencibia, M., García, C., Kurz, A., Rodríguez-Navarro, J. A., Gispert-Sáchez, S., Mena, M. A., & Fernández-Ruiz, J. (2009). Cannabinoid CB1 receptors are early downregulated followed by a further upregulation in the basal ganglia of mice with deletion of specific park genes. *Journal of Neural Transmission*, *73*, 269–275.

García-Arencibia, M., González, S., de Lago, E., Ramos, J. A., Mechoulam, R., & Fernández-Ruiz, J. (2007). Evaluation of the neuroprotective effect of cannabinoids in a rat model of Parkinson's disease: Importance of antioxidant and cannabinoid receptor-independent properties. *Brain Research*, *1134*(1), 162–170.

García-Gutiérrez, M. S., Ortega-Álvaro, A., Busquets-García, A., Pérez-Ortiz, J. M., Caltana, L., Ricatti, M. J., & Manzanares, J. (2013). Synaptic plasticity alterations associated with memory impairment induced by deletion of CB2 cannabinoid receptors. *Neuropharmacology*, *73*, 388–396.

Giacoppo, S., Bramanti, P., & Mazzon, E. (2017). Sativex in the management of multiple sclerosis-related spasticity: An overview of the last

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

decade of clinical evaluation. *Multiple Sclerosis and Related Disorders*, *17*, 22–31.

Glass, M., Dragunow, M., & Faull, R. L. (1997). Cannabinoid receptors in the human brain: A detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience*, *77*(2), 299–318.

Glass, M., Faull, R. L., & Dragunow, M. (1993). Loss of cannabinoid receptors in the substantia nigra in Huntington's disease. *Neuroscience*, *56*(3), 523–527.

Godlewski, G., Offertáler, L., Wagner, J. A., & Kunos, G. (2009). Receptors for acylethanolamides-GPR55 and GPR119. *Prostaglandins & Other Lipid Mediators*, *89*(3–4), 105–111.

Gomez, O., Arevalo-Martin, A., Garcia-Ovejero, D., Ortega-Gutierrez, S., Cisneros, J. A., Almazan, G., & Molina-Holgado, E. (2010). The constitutive production of the endocannabinoid 2-arachidonoylglycerol participates in oligodendrocyte differentiation. *Glia*, *58*(16), 1913–1927.

Gomez, O., Sanchez-Rodriguez, A., Le, M., Sanchez-Caro, C., Molina-Holgado, F., & Molina-Holgado, E. (2011). Cannabinoid receptor agonists modulate oligodendrocyte differentiation by activating PI3K/Akt and the mammalian target of rapamycin (mTOR) pathways. *British Journal of Pharmacology*, *163*(7), 1520–1532.

Gomez, O., Sanchez-Rodriguez, M. A., Ortega-Gutierrez, S., Vazquez-Villa, H., Guaza, C., Molina-Holgado, F., & Molina-Holgado, E. (2015). A basal tone of 2-arachidonoylglycerol contributes to early oligodendrocyte progenitor proliferation by activating phosphatidylinositol 3-kinase (PI3K)/AKT and the mammalian target of rapamycin (MTOR) pathways. *Journal of Neuroimmune Pharmacology*, *10*(2), 309–317.

Gómez-Gálvez, Y., Palomo-Garo, C., Fernández-Ruiz, J., & García, C. (2016). Potential of the cannabinoid CB(2) receptor as a pharmacological target against inflammation in Parkinson's disease. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *64*, 200–208.

Grieco, M., De Caris, M. G., Maggi, E., Armeli, F., Coccurello, R., Bisogno, T., & Businaro, R. (2021). Fatty acid amide hydrolase (FAAH) inhibition modulates amyloid-beta-induced microglia polarization. *International Journal of Molecular Sciences*, *22*(14), 7711.

Gubellini, P., Picconi, B., Bari, M., Battista, N., Calabresi, P., Centonze, D., & Maccarrone, M. (2002). Experimental parkinsonism alters endocannabinoid degradation: Implications for striatal glutamatergic transmission. *The Journal of Neuroscience*, *22*(16), 6900–6907.

Gulyas, A. I., Cravatt, B. F., Bracey, M. H., Dinh, T. P., Piomelli, D., Boscia, F., & Freund, T. F. (2004). Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. *The European Journal of Neuroscience*, *20*(2), 441–458.

Gutiérrez-Rodríguez, A., Bonilla-Del Río, I., Puente, N., Gómez-Urquijo, S. M., Fontaine, C. J., Egaña-Huguet, J., & Grandes, P. (2018). Localization of the cannabinoid type-1 receptor in subcellular astrocyte compartments of mutant mouse hippocampus. *Glia*, *66*(7), 1417–1431.

Gutiérrez-Rodríguez, A., Puente, N., Elezgarai, I., Ruehle, S., Lutz, B., Reguero, L., & Grandes, P. (2017). Anatomical characterization of the cannabinoid CB1 receptor in cell-type-specific mutant mouse rescue models. *The Journal of Comparative Neurology*, *525*(2), 302–318.

Haj-Dahmane, S., Shen, R. Y., Elmes, M. W., Studholme, K., Kanjiya, M. P., Bogdan, D., & Kaczocha, M. (2018). Fatty-acid-binding protein 5 controls retrograde endocannabinoid signaling at central glutamate synapses. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(13), 3482–3487.

Han, J., Kesner, P., Metna-Laurent, M., Duan, T., Xu, L., Georges, F., & Zhang, X. (2012). Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. *Cell*, *148*(5), 1039–1050.

Häring, M., Enk, V., Aparisi Rey, A., Loch, S., Ruiz de Azua, I., Weber, T., & Lutz, B. (2015). Cannabinoid type-1 receptor signaling in central serotonergic neurons regulates anxiety-like behavior and sociability. *Frontiers in Behavioral Neuroscience*, *9*, 235.

Hasel, P., Rose, I. V. L., Sadick, J. S., Kim, R. D., & Liddelow, S. A. (2021). Neuroinflammatory astrocyte subtypes in the mouse brain. *Nature Neuroscience*, *24*(10), 1475–1487.

Hashem, J., Hu, M., Zhang, J., Gao, F., & Chen, C. (2021). Inhibition of 2-arachidonoylglycerol metabolism alleviates neuropathology and improves cognitive function in a tau mouse model of Alzheimer's disease. *Molecular Neurobiology*, *58*(8), 4122–4133.

Hebert-Chatelain, E., Desprez, T., Serrat, R., Bellocchio, L., Soria-Gomez, E., Busquets-Garcia, A., & Marsicano, G. (2016). A cannabinoid link between mitochondria and memory. *Nature*, *539*(7630), 555–559.

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. *The Journal of Neuroscience*, *11*(2), 563–583.

Herkenham, M., Lynn, A. B., Little, M. D., Johnson, M. R., Melvin, L. S., de Costa, B. R., & Rice, K. C. (1990). Cannabinoid receptor localization in brain. *Proceedings of the National Academy of Sciences of the United States of America*, *87*(5), 1932–1936.

Herrmann, N., Ruthirakuhan, M., Gallagher, D., Verhoeff, N. P. L. G., Kiss, A., Black, S. E., & Lanctôt, K. L. (2019). Randomized placebo-controlled trial of nabilone for agitation in Alzheimer's disease. *The American Journal of Geriatric Psychiatry*, *27*(11), 1161–1173.

Hickman, S., Izzy, S., Sen, P., Morsett, L., & El Khoury, J. (2018). Microglia in neurodegeneration. *Nature Neuroscience*, *21*(10), 1359–1369.

Hickman, S. E., Kingery, N. D., Ohsumi, T. K., Borowsky, M. L., Wang, L. C., Means, T. K., & El Khoury, J. (2013). The microglial sensome revealed by direct RNA sequencing. *Nature Neuroscience*, *16*(12), 1896–1905.

Howlett, A. C. (2002). The cannabinoid receptors. *Prostaglandins & Other Lipid Mediators*, *68-69*, 619–631.

Hsu, K. L., Tsuboi, K., Adibekian, A., Pugh, H., Masuda, K., & Cravatt, B. F. (2012). DAGLβ inhibition perturbs a lipid network involved in macrophage inflammatory responses. *Nature Chemical Biology*, *8*(12), 999–1007.

Huerga-Gómez, A., Aguado, T., Sánchez-de la Torre, A., Bernal-Chico, A., Matute, C., Mato, S., & Palazuelos, J. (2021). Δ9 -tetrahydrocannabinol promotes oligodendrocyte development and CNS myelination in vivo. *Glia*, *69*(3), 532–545.

Jean-Gilles, L., Feng, S., Tench, C. R., Chapman, V., Kendall, D. A., Barrett, D. A., & Constantinescu, C. S. (2009). Plasma endocannabinoid levels in multiple sclerosis. *Journal of the Neurological Sciences*, *287*(1–2), 212–215.

Jimenez-Blasco, D., Busquets-Garcia, A., Hebert-Chatelain, E., Serrat, R., Vicente-Gutierrez, C., Ioannidou, C., & Marsicano, G. (2020). Glucose metabolism links astroglial mitochondria to cannabinoid effects. *Nature*, *583*(7817), 603–608.

Jung, K. M., Astarita, G., Yasar, S., Vasilevko, V., Cribbs, D. H., Head, E., & Piomelli, D. (2012). An amyloid β42-dependent deficit in anandamide mobilization is associated with cognitive dysfunction in Alzheimer's disease. *Neurobiology of Aging*, *33*(8), 1522–1532.

Kaczocha, M., Glaser, S. T., & Deutsch, D. G. (2009). Identification of intracellular carriers for the endocannabinoid anandamide. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(15), 6375–6380.

Kaczocha, M., Glaser, S. T., Maher, T., Clavin, B., Hamilton, J., O'Rourke, J., & Thanos, P. K. (2015). Fatty acid binding protein deletion suppresses inflammatory pain through endocannabinoid/N-acylethanolamine-dependent mechanisms. *Molecular Pain*, *11*, 52.

Kaczocha, M., Rebecchi, M. J., Ralph, B. P., Teng, Y. H., Berger, W. T., Galbavy, W., & Ojima, I. (2014). Inhibition of fatty acid binding proteins elevates brain anandamide levels and produces analgesia. *PLoS One*, *9*(4), e94200.

Kalifa, S., Polston, E. K., Allard, J. S., & Manaye, K. F. (2011). Distribution patterns of cannabinoid CB1 receptors in the hippocampus of APPswe/PS1ΔE9 double transgenic mice. *Brain Research*, *1376*, 94–100.

Kamizato, K., Sato, S., Shil, S. K., Umaru, B. A., Kagawa, Y., Yamamoto, Y., & Miyazaki, H. (2019). The role of fatty acid binding

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

protein 7 in spinal cord astrocytes in a mouse model of experimental autoimmune encephalomyelitis. *Neuroscience*, *409*, 120–129.

Kano, M., Ohno-Shosaku, T., Hashimotodani, Y., Uchigashima, M., & Watanabe, M. (2009). Endocannabinoid-mediated control of synaptic transmission. *Physiological Reviews*, *89*(1), 309–380.

Kawamura, Y., Fukaya, M., Maejima, T., Yoshida, T., Miura, E., Watanabe, M., & Kano, M. (2006). The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. *The Journal of Neuroscience*, *26*(11), 2991–3001.

Kipp, M., Gingele, S., Pott, F., Clarner, T., van der Valk, P., Denecke, B., & Beyer, C. (2011). BLBP-expression in astrocytes during experimental demyelination and in human multiple sclerosis lesions. *Brain, Behavior, and Immunity*, *25*(8), 1554–1568.

Koppel, J., Vingtdeux, V., Marambaud, P., d'Abramo, C., Jimenez, H., Stauber, M., & Davies, P. (2014). CB2 receptor deficiency increases amyloid pathology and alters tau processing in a transgenic mouse model of Alzheimer's disease. *Molecular Medicine*, *20*, 29–36.

Kornek, B., & Lassmann, H. (2003). Neuropathology of multiple sclerosis-new concepts. *Brain Research Bulletin*, *61*(3), 321–326.

Kozak, K. R., Prusakiewicz, J. J., & Marnett, L. J. (2004). Oxidative metabolism of endocannabinoids by COX-2. *Current Pharmaceutical Design*, *10*(6), 659–667.

Kuhns, L., Kroon, E., Colyer-Patel, K., & Cousijn, J. (2021). Associations between cannabis use, cannabis use disorder, and mood disorders: Longitudinal, genetic, and neurocognitive evidence. *Psychopharmacology*. https://doi.org/10.1007/s00213-021-06001-8. (Online ahead of print).

Lanz, T. V., Brewer, R. C., Ho, P. P., Moon, J. S., Jude, K. M., Fernandez, D., & Robinson, W. H. (2022). Clonally expanded B cells in multiple sclerosis bind EBV EBNA1 and GlialCAM. *Nature*, *603*(7900), 321–327.

Laprairie, R. B., Warford, J. R., Hutchings, S., Robertson, G. S., Kelly, M. E., & Denovan-Wright, E. M. (2014). The cytokine and endocannabinoid systems are co-regulated by NF-κB p65/RelA in cell culture and transgenic mouse models of Huntington's disease and in striatal tissue from Huntington's disease patients. *Journal of Neuroimmunology*, *267*(1–2), 61–72.

Lassmann, H. (2014). Mechanisms of white matter damage in multiple sclerosis. *Glia*, *62*(11), 1816–1830.

Lastres-Becker, I., Molina-Holgado, F., Ramos, J. A., Mechoulam, R., & Fernández-Ruiz, J. (2005). Cannabinoids provide neuroprotection against 6-hydroxydopamine toxicity in vivo and in vitro: Relevance to Parkinson's disease. *Neurobiology of Disease*, *19*(1–2), 96–107.

Leng, F., & Edison, P. (2021). Neuroinflammation and microglial activation in Alzheimer disease: Where do we go from here? *Nature Reviews. Neurology*, *17*(3), 157–172.

Lin, M. T., & Beal, M. F. (2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature*, *443*(7113), 787–795.

Long, J. Z., Li, W., Booker, L., Burston, J. J., Kinsey, S. G., Schlosburg, J. E., & Cravatt, B. F. (2009). Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. *Nature Chemical Biology*, *5*(1), 37–44.

López, A., Aparicio, N., Pazos, M. R., Grande, M. T., Barreda-Manso, M. A., Benito-Cuesta, I., & Romero, J. (2018). Cannabinoid CB 2 receptors in the mouse brain: Relevance for Alzheimer's disease. *Journal of Neuroinflammation*, *15*(1), 158.

López-Sendón Moreno, J. L., García Caldentey, J., Trigo Cubillo, P., Ruiz Romero, C., García Ribas, G., Alonso Arias, M. A., de Yébenes, G., & Prous, J. (2016). A double-blind, randomized, cross-over, placebo-controlled, pilot trial with Sativex in Huntington's disease. *Journal of Neurology*, *263*(7), 1390–1400.

Loría, F., Petrosino, S., Mestre, L., Spagnolo, A., Correa, F., Hernangómez, M., & Docagne, F. (2008). Study of the regulation of the endocannabinoid system in a virus model of multiple sclerosis reveals a therapeutic effect of palmitoylethanolamide. *The European Journal of Neuroscience*, *28*(4), 633–641.

Lutz, B. (2020). Neurobiology of cannabinoid receptor signaling. *Dialogues in Clinical Neuroscience*, *22*(3), 207–222.

Lutz, B., Marsicano, G., Maldonado, R., & Hillard, C. J. (2015). The endocannabinoid system in guarding against fear, anxiety and stress. *Nature Reviews. Neuroscience*, *16*(12), 705–718.

Maas, A. I., Murray, G., Henney, H., Kassem, N., Legrand, V., Mangelus, M., & Investigators, P. T. (2006). Efficacy and safety of dexanabinol in severe traumatic brain injury: Results of a phase III randomised, placebo-controlled, clinical trial. *Lancet Neurology*, *5*(1), 38–45.

Maccarrone, M., Guzmán, M., Mackie, K., Doherty, P., & Harkany, T. (2014). Programming of neural cells by (endo)cannabinoids: From physiological rules to emerging therapies. *Nature Reviews. Neuroscience*, *15*(12), 786–801.

Maccarrone, M., Totaro, A., Leuti, A., Giacovazzo, G., Scipioni, L., Mango, D., & Oddi, S. (2018). Early alteration of distribution and activity of hippocampal type-1 cannabinoid receptor in Alzheimer's disease-like mice overexpressing the human mutant amyloid precursor protein. *Pharmacological Research*, *130*, 366–373.

Manterola, A., Bernal-Chico, A., Cipriani, R., Canedo-Antelo, M., Moreno-García, Á., Martín-Fontecha, M., & Mato, S. (2018). Deregulation of the endocannabinoid system and therapeutic potential of ABHD6 blockade in the cuprizone model of demyelination. *Biochemical Pharmacology*, *157*, 189–201.

Manterola, A., Bernal-Chico, A., Cipriani, R., Ruiz, A., Pérez-Samartín, A., Moreno-Rodríguez, M., & Mato, S. (2018). Re-examining the potential of targeting ABHD6 in multiple sclerosis: Efficacy of systemic and peripherally restricted inhibitors in experimental autoimmune encephalomyelitis. *Neuropharmacology*, *141*, 181–191.

Maresz, K., Pryce, G., Ponomarev, E. D., Marsicano, G., Croxford, J. L., Shriver, L. P., & Dittel, B. N. (2007). Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB1 on neurons and CB2 on autoreactive T cells. *Nature Medicine*, *13*(4), 492–497.

Marinelli, S., Pacioni, S., Bisogno, T., Di Marzo, V., Prince, D. A., Huguenard, J. R., & Bacci, A. (2008). The endocannabinoid 2-arachidonoylglycerol is responsible for the slow self-inhibition in neocortical interneurons. *The Journal of Neuroscience*, *28*(50), 13532–13541.

Marisca, R., Hoche, T., Agirre, E., Hoodless, L. J., Barkey, W., Auer, F., & Czopka, T. (2020). Functionally distinct subgroups of oligodendrocyte precursor cells integrate neural activity and execute myelin formation. *Nature Neuroscience*, *23*(3), 363–374.

Markovà, J., Essner, U., Akmaz, B., Marinelli, M., Trompke, C., Lentschat, A., & Vila, C. (2019). Sativex. *The International Journal of Neuroscience*, *129*(2), 119–128.

Maroso, M., Szabo, G. G., Kim, H. K., Alexander, A., Bui, A. D., Lee, S. H., & Soltesz, I. (2016). Cannabinoid control of learning and memory through HCN channels. *Neuron*, *89*(5), 1059–1073.

Marques, S., Zeisel, A., Codeluppi, S., van Bruggen, D., Mendanha Falcão, A., Xiao, L., & Castelo-Branco, G. (2016). Oligodendrocyte heterogeneity in the mouse juvenile and adult central nervous system. *Science*, *352*(6291), 1326–1329.

Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., & Lutz, B. (2003). CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science*, *302*(5642), 84–88.

Marsicano, G., Wotjak, C. T., Azad, S. C., Bisogno, T., Rammes, G., Cascio, M. G., & Lutz, B. (2002). The endogenous cannabinoid system controls extinction of aversive memories. *Nature*, *418*(6897), 530–534.

Mato, S., Alberdi, E., Ledent, C., Watanabe, M., & Matute, C. (2009). CB1 cannabinoid receptor-dependent and -independent inhibition of depolarization-induced calcium influx in oligodendrocytes. *Glia*, *57*(3), 295–306.

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Mato, S., Del Olmo, E., & Pazos, A. (2003). Ontogenetic development of cannabinoid receptor expression and signal transduction functionality in the human brain. *The European Journal of Neuroscience*, *17*(9), 1747–1754.

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*(6284), 561–564.

Mazzola, C., Micale, V., & Drago, F. (2003). Amnesia induced by beta-amyloid fragments is counteracted by cannabinoid CB1 receptor blockade. *European Journal of Pharmacology*, *477*(3), 219–225.

Mecha, M., Carrillo-Salinas, F. J., Feliú, A., Mestre, L., & Guaza, C. (2016). Microglia activation states and cannabinoid system: Therapeutic implications. *Pharmacology & Therapeutics*, *166*, 40–55.

Mecha, M., Feliú, A., Carrillo-Salinas, F. J., Rueda-Zubiaurre, A., Ortega-Gutiérrez, S., de Sola, R. G., & Guaza, C. (2015). Endocannabinoids drive the acquisition of an alternative phenotype in microglia. *Brain, Behavior, and Immunity*, *49*, 233–245.

Mecha, M., Feliú, A., Machín, I., Cordero, C., Carrillo-Salinas, F., Mestre, L., & Guaza, C. (2018). 2-AG limits Theiler's virus induced acute neuroinflammation by modulating microglia and promoting MDSCs. *Glia*, *66*(7), 1447–1463.

Mechoulam, R. (1970). Marihuana chemistry. *Science*, *168*(3936), 1159–1166.

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N. E., Schatz, A. R., & Compton, D. R. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochemical Pharmacology*, *50*(1), 83–90.

Mesnage, V., Houeto, J. L., Bonnet, A. M., Clavier, I., Arnulf, I., Cattelin, F., & Agid, Y. (2004). Neurokinin B, neurotensin, and cannabinoid receptor antagonists and Parkinson disease. *Clinical Neuropharmacology*, *27*(3), 108–110.

Min, R., & Nevian, T. (2012). Astrocyte signaling controls spike timing-dependent depression at neocortical synapses. *Nature Neuroscience*, *15*(5), 746–753.

Moldrich, G., & Wenger, T. (2000). Localization of the CB1 cannabinoid receptor in the rat brain. *An immunohistochemical study*. *Peptides*, *21*(11), 1735–1742.

Molina-Holgado, F., Molina-Holgado, E., Guaza, C., & Rothwell, N. J. (2002). Role of CB1 and CB2 receptors in the inhibitory effects of cannabinoids on lipopolysaccharide-induced nitric oxide release in astrocyte cultures. *Journal of Neuroscience Research*, *67*(6), 829–836.

Monory, K., Massa, F., Egertová, M., Eder, M., Blaudzun, H., Westenbroek, R., & Lutz, B. (2006). The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron*, *51*(4), 455–466.

Morello, G., Imperatore, R., Palomba, L., Finelli, C., Labruna, G., Pasanisi, F., & Cristino, L. (2016). Orexin-a represses satiety-inducing POMC neurons and contributes to obesity via stimulation of endocannabinoid signaling. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(17), 4759–4764.

Moreno-García, Á., Bernal-Chico, A., Colomer, T., Rodríguez-Antigüedad, A., Matute, C., & Mato, S. (2020). Gene expression analysis of astrocyte and microglia endocannabinoid signaling during autoimmune demyelination. *Biomolecules*, *10*(9), 1228.

Moreno-Martet, M., Espejo-Porras, F., Fernández-Ruiz, J., & de Lago, E. (2014). Changes in endocannabinoid receptors and enzymes in the spinal cord of SOD1(G93A) transgenic mice and evaluation of a Sativex (®) -like combination of phytocannabinoids: Interest for future therapies in amyotrophic lateral sclerosis. *CNS Neuroscience & Therapeutics*, *20*(9), 809–815.

Morozov, Y. M., Ben-Ari, Y., & Freund, T. F. (2004). The spatial and temporal pattern of fatty acid amide hydrolase expression in rat hippocampus during postnatal development. *The European Journal of Neuroscience*, *20*(2), 459–466.

Mulder, J., Zilberter, M., Pasquaré, S. J., Alpár, A., Schulte, G., Ferreira, S. G., & Harkany, T. (2011). Molecular reorganization of endocannabinoid signalling in Alzheimer's disease. *Brain*, *134*(4), 1041–1060.

Munro, S., Thomas, K. L., & Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, *365*(6441), 61–65.

Nakane, S., Oka, S., Arai, S., Waku, K., Ishima, Y., Tokumura, A., & Sugiura, T. (2002). 2-Arachidonoyl-sn-glycero-3-phosphate, an arachidonic acid-containing lysophosphatidic acid: Occurrence and rapid enzymatic conversion to 2-arachidonoyl-sn-glycerol, a cannabinoid receptor ligand, in rat brain. *Archives of Biochemistry and Biophysics*, *402*(1), 51–58.

Navarrete, F., García-Gutiérrez, M. S., Aracil-Fernández, A., Lanciego, J. L., & 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*(2), 459–469.

Navarrete, F., Rodríguez-Arias, M., Martín-García, E., Navarro, D., García-Gutiérrez, M. S., Aguilar, M. A., & Manzanares, J. (2013). Role of CB2 cannabinoid receptors in the rewarding, reinforcing, and physical effects of nicotine. *Neuropsychopharmacology*, *38*(12), 2515–2524.

Navarrete, M., & Araque, A. (2010). Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. *Neuron*, *68*(1), 113–126.

Navia-Paldanius, D., Savinainen, J. R., & Laitinen, J. T. (2012). Biochemical and pharmacological characterization of human α/β-hydrolase domain containing 6 (ABHD6) and 12 (ABHD12). *Journal of Lipid Research*, *53*(11), 2413–2424.

Naydenov, A. V., Horne, E. A., Cheah, C. S., Swinney, K., Hsu, K. L., Cao, J. K., & Stella, N. (2014). ABHD6 blockade exerts antiepileptic activity in PTZ-induced seizures and in spontaneous seizures in R6/2 mice. *Neuron*, *83*(2), 361–371.

Nielsen, S., Germanos, R., Weier, M., Pollard, J., Degenhardt, L., Hall, W., & Farrell, M. (2018). The use of cannabis and cannabinoids in treating symptoms of multiple sclerosis: A systematic review of reviews. *Current Neurology and Neuroscience Reports*, *18*(2), 8.

Nomura, D. K., Morrison, B. E., Blankman, J. L., Long, J. Z., Kinsey, S. G., Marcondes, M. C., & Cravatt, B. F. (2011). Endocannabinoid hydrolysis generates brain prostaglandins that promote neuroinflammation. *Science*, *334*(6057), 809–813.

Novotna, A., Mares, J., Ratcliffe, S., Novakova, I., Vachova, M., Zapletalova, O., & Group, S. S. S. (2011). A randomized, double-blind, placebo-controlled, parallel-group, enriched-design study of nabiximols* (Sativex(®)), as add-on therapy, in subjects with refractory spasticity caused by multiple sclerosis. *European Journal of Neurology*, *18*(9), 1122–1131.

Oddi, S., Scipioni, L., & Maccarrone, M. (2020). Endocannabinoid system and adult neurogenesis: A focused review. *Current Opinion in Pharmacology*, *50*, 25–32.

Okamoto, Y., Morishita, J., Tsuboi, K., Tonai, T., & Ueda, N. (2004). Molecular characterization of a phospholipase D generating anandamide and its congeners. *The Journal of Biological Chemistry*, *279*(7), 5298–5305.

Ortega-Gutiérrez, S., Molina-Holgado, E., Arévalo-Martín, A., Correa, F., Viso, A., López-Rodríguez, M. L., & Guaza, C. (2005). Activation of the endocannabinoid system as therapeutic approach in a murine model of multiple sclerosis. *The FASEB Journal*, *19*(10), 1338–1340.

Palazuelos, J., Aguado, T., Pazos, M. R., Julien, B., Carrasco, C., Resel, E., & Galve-Roperh, I. (2009). Microglial CB2 cannabinoid receptors are neuroprotective in Huntington's disease excitotoxicity. *Brain*, *132*(11), 3152–3164.

Palazuelos, J., Davoust, N., Julien, B., Hatterer, E., Aguado, T., Mechoulam, R., & Galve-Roperh, I. (2008). The CB(2) cannabinoid receptor controls myeloid progenitor trafficking: Involvement in the

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

pathogenesis of an animal model of multiple sclerosis. *The Journal of Biological Chemistry*, *283*(19), 13320–13329.

Peball, M., Krismer, F., Knaus, H. G., Djamshidian, A., Werkmann, M., Carbone, F., & Innsbruck, C. (2020). Non-motor symptoms in Parkinson's disease are reduced by Nabilone. *Annals of Neurology*, *88*(4), 712–722.

Pertwee, R. G., Howlett, A. C., Abood, M. E., Alexander, S. P., Di Marzo, V., Elphick, M. R., & Ross, R. A. (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: Beyond CB_1 and CB_2. *Pharmacological Reviews*, *62*(4), 588–631.

Peters, A., & Folger, C. (2013). A website entitled "the fine structure of the aging brain". *The Journal of Comparative Neurology*, *521*(6), 1203–1206.

Pihlaja, R., Takkinen, J., Eskola, O., Vasara, J., López-Picón, F. R., Haaparanta-Solin, M., & Rinne, J. O. (2015). Monoacylglycerol lipase inhibitor JZL184 reduces neuroinflammatory response in APdE9 mice and in adult mouse glial cells. *Journal of Neuroinflammation*, *12*, 81.

Piomelli, D. (2003). The molecular logic of endocannabinoid signalling. *Nature Reviews. Neuroscience*, *4*(11), 873–884.

Piro, J. R., Benjamin, D. I., Duerr, J. M., Pi, Y., Gonzales, C., Wood, K. M., & Samad, T. A. (2012). A dysregulated endocannabinoid-eicosanoid network supports pathogenesis in a mouse model of Alzheimer's disease. *Cell Reports*, *1*(6), 617–623.

Pisani, A., Fezza, F., Galati, S., Battista, N., Napolitano, S., Finazzi-Agrò, A., & Maccarrone, M. (2005). High endogenous cannabinoid levels in the cerebrospinal fluid of untreated Parkinson's disease patients. *Annals of Neurology*, *57*(5), 777–779.

Poursharifi, P., Madiraju, S. R. M., & Prentki, M. (2017). Monoacylglycerol signalling and ABHD6 in health and disease. *Diabetes, Obesity & Metabolism*, *19*(1), 76–89.

Pryce, G., Ahmed, Z., Hankey, D. J., Jackson, S. J., Croxford, J. L., Pocock, J. M., & Baker, D. (2003). Cannabinoids inhibit neurodegeneration in models of multiple sclerosis. *Brain*, *126*(10), 2191–2202.

Pryce, G., Cabranes, A., Fernández-Ruiz, J., Bisogno, T., Di Marzo, V., Long, J. Z., & Baker, D. (2013). Control of experimental spasticity by targeting the degradation of endocannabinoids using selective fatty acid amide hydrolase inhibitors. *Multiple Sclerosis*, *19*(14), 1896–1904.

Raman, C., McAllister, S. D., Rizvi, G., Patel, S. G., Moore, D. H., & Abood, M. E. (2004). Amyotrophic lateral sclerosis: Delayed disease progression in mice by treatment with a cannabinoid. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders*, *5*(1), 33–39.

Renton, A. E., Chiò, A., & Traynor, B. J. (2014). State of play in amyotrophic lateral sclerosis genetics. *Nature Neuroscience*, *17*(1), 17–23.

Reynolds, J. M., Liu, Q., Brittingham, K. C., Liu, Y., Gruenthal, M., Gorgun, C. Z., & Suttles, J. (2007). Deficiency of fatty acid-binding proteins in mice confers protection from development of experimental autoimmune encephalomyelitis. *Journal of Immunology*, *179*(1), 313–321.

Riva, N., Mora, G., Sorarù, G., Lunetta, C., Ferraro, O. E., Falzone, Y., & Group, C. S. (2019). Safety and efficacy of nabiximols on spasticity symptoms in patients with motor neuron disease (CANALS): A multicentre, double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet Neurology*, *18*(2), 155–164.

Robin, L. M., Oliveira da Cruz, J. F., Langlais, V. C., Martin-Fernandez, M., Metna-Laurent, M., Busquets-Garcia, A., & Marsicano, G. (2018). Astroglial CB1 receptors determine synaptic D-serine availability to enable recognition memory. *Neuron*, *98*(5), 935–944.

Rodriguez, J. J., Mackie, K., & Pickel, V. M. (2001). Ultrastructural localization of the CB1 cannabinoid receptor in mu-opioid receptor patches of the rat caudate putamen nucleus. *The Journal of Neuroscience*, *21*(3), 823–833.

Rodríguez-Arias, M., Navarrete, F., Blanco-Gandia, M. C., Arenas, M. C., Aguilar, M. A., Bartoll-Andrés, A., & Manzanares, J. (2015). Role of CB2 receptors in social and aggressive behavior in male mice. *Psychopharmacology*, *232*(16), 3019–3031.

Rodríguez-Cueto, C., García-Toscano, L., Santos-García, I., Gómez-Almería, M., Gonzalo-Consuegra, C., Espejo-Porras, F., & de Lago, E. (2021). Targeting the CB2 receptor and other endocannabinoid elements to delay disease progression in amyotrophic lateral sclerosis. *British Journal of Pharmacology*, *178*(6), 1373–1387.

Rodríguez-Cueto, C., Gómez-Almería, M., García Toscano, L., Romero, J., Hillard, C. J., de Lago, E., & Fernández-Ruiz, J. (2021). Inactivation of the CB2 receptor accelerated the neuropathological deterioration in TDP-43 transgenic mice, a model of amyotrophic lateral sclerosis. *Brain Pathology*, *31*(6), e12972.

Rojo-Bustamante, E., Abellanas, M. A., Clavero, P., Thiolat, M. L., Li, Q., Luquin, M. R., & Aymerich, M. S. (2018). The expression of cannabinoid type 1 receptor and 2-arachidonoyl glycerol synthesizing/degrading enzymes is altered in basal ganglia during the active phase of levodopa-induced dyskinesia. *Neurobiology of Disease*, *118*, 64–75.

Rossi, S., De Chiara, V., Musella, A., Cozzolino, M., Bernardi, G., Maccarrone, M., & Centonze, D. (2010). Abnormal sensitivity of cannabinoid CB1 receptors in the striatum of mice with experimental amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosis*, *11*(1–2), 83–90.

Ruiz-Calvo, A., Bajo-Grañeras, R., Maroto, I. B., Zian, D., Grabner, G. F., García-Taboada, E., & Guzmán, M. (2019). Astroglial monoacylglycerol lipase controls mutant huntingtin-induced damage of striatal neurons. *Neuropharmacology*, *150*, 134–144.

Ruiz-Pérez, G., de Martín, R., Esteban, S., Marqués, S., Aparicio, N., Grande, M. T., Benito-Cuesta, I., & Palenzuela, R. (2021). Potentiation of amyloid beta phagocytosis and amelioration of synaptic dysfunction upon FAAH deletion in a mouse model of Alzheimer's disease. *Journal of Neuroinflammation*, *18*(1), 223.

Saab, A. S., & Nave, K. A. (2017). Myelin dynamics: Protecting and shaping neuronal functions. *Current Opinion in Neurobiology*, *47*, 104–112.

Saft, C., von Hein, S. M., Lücke, T., Thiels, C., Peball, M., Djamshidian, A., & Seppi, K. (2018). Cannabinoids for treatment of dystonia in Huntington's disease. *J Huntingtons Dis*, *7*(2), 167–173.

Sagredo, O., González, S., Aroyo, I., Pazos, M. R., Benito, C., Lastres-Becker, I., & Fernández-Ruiz, J. (2009). Cannabinoid CB2 receptor agonists protect the striatum against malonate toxicity: Relevance for Huntington's disease. *Glia*, *57*(11), 1154–1167.

Sanchez-Rodriguez, M. A., Gomez, O., Esteban, P. F., Garcia-Ovejero, D., & Molina-Holgado, E. (2018). The endocannabinoid 2-arachidonoylglycerol regulates oligodendrocyte progenitor cell migration. *Biochemical Pharmacology*, *157*, 180–188.

Savinainen, J. R., Saario, S. M., & Laitinen, J. T. (2012). The serine hydrolases MAGL, ABHD6 and ABHD12 as guardians of 2-arachidonoylglycerol signalling through cannabinoid receptors. *Acta Physiologica (Oxford, England)*, *204*(2), 267–276.

Schlosburg, J. E., Blankman, J. L., Long, J. Z., Nomura, D. K., Pan, B., Kinsey, S. G., & Cravatt, B. F. (2010). Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. *Nature Neuroscience*, *13*(9), 1113–1119.

Shin, M., Buckner, A., Prince, J., Bullock, T. N. J., & Hsu, K. L. (2019). Diacylglycerol lipase-β is required for TNF-α response but not CD8. *Cell Chemical Biology*, *26*(7), 1036–1041.

Sieradzan, K. A., Fox, S. H., Hill, M., Dick, J. P., Crossman, A. R., & Brotchie, J. M. (2001). Cannabinoids reduce levodopa-induced dyskinesia in Parkinson's disease: A pilot study. *Neurology*, *57*(11), 2108–2111.

Sigel, E., Baur, R., Rácz, I., Marazzi, J., Smart, T. G., Zimmer, A., & Gertsch, J. (2011). The major central endocannabinoid directly acts at GABA(a) receptors. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(44), 18150–18155.

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Solas, M., Francis, P. T., Franco, R., & Ramirez, M. J. (2013). CB2 receptor and amyloid pathology in frontal cortex of Alzheimer's disease patients. *Neurobiology of Aging*, *34*(3), 805–808.

Soria-Gomez, E., Pagano Zottola, A. C., Mariani, Y., Desprez, T., Barresi, M., Bonilla-Del Río, I., & Bellocchio, L. (2021). Subcellular specificity of cannabinoid effects in striatonigral circuits. *Neuron*, *109*(9), 1513–1526.

Sorosina, M., Clarelli, F., Ferrè, L., Osiceanu, A. M., Unal, N. T., Mascia, E., & Martinelli Boneschi, F. (2018). Clinical response to nabiximols correlates with the downregulation of immune pathways in multiple sclerosis. *European Journal of Neurology*, *25*(7), 934–970.

Stella, N. (2004). Cannabinoid signaling in glial cells. *Glia*, *48*(4), 267–277.

Stella, N. (2009). Endocannabinoid signaling in microglial cells. *Neuropharmacology*, *56*(1), 244–253.

Stella, N. (2010). Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. *Glia*, *58*(9), 1017–1030.

Stempel, A. V., Stumpf, A., Zhang, H. Y., Özdoğan, T., Pannasch, U., Theis, A. K., & Schmitz, D. (2016). Cannabinoid type 2 receptors mediate a cell type-specific plasticity in the hippocampus. *Neuron*, *90*(4), 795–809.

Stumpf, A., Parthier, D., Sammons, R. P., Stempel, A. V., Breustedt, J., Rost, B. R., & Schmitz, D. (2018). Cannabinoid type 2 receptors mediate a cell type-specific self-inhibition in cortical neurons. *Neuropharmacology*, *139*, 217–225.

Tanaka, M., Sackett, S., & Zhang, Y. (2020). Endocannabinoid modulation of microglial phenotypes in neuropathology. *Frontiers in Neurology*, *11*, 87.

Tanimura, A., Yamazaki, M., Hashimotodani, Y., Uchigashima, M., Kawata, S., Abe, M., & Kano, M. (2010). The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase alpha mediates retrograde suppression of synaptic transmission. *Neuron*, *65*(3), 320–327.

Tsuboi, K., Zhao, L. Y., Okamoto, Y., Araki, N., Ueno, M., Sakamoto, H., & Ueda, N. (2007). Predominant expression of lysosomal N-acylethanolamine-hydrolyzing acid amidase in macrophages revealed by immunochemical studies. *Biochimica et Biophysica Acta*, *1771*(5), 623–632.

Urbi, B., Broadley, S., Bedlack, R., Russo, E., & Sabet, A. (2019). Study protocol for a randomised, double-blind, placebo-controlled study evaluating the efficacy of cannabis-based medicine extract in slowing the disease progression of amyotrophic lateral sclerosis or motor neurone disease: The EMERALD trial. *BMJ Open*, *9*(11), e029449.

Valdeolivas, S., Pazos, M. R., Bisogno, T., Piscitelli, F., Iannotti, F. A., Allarà, M., & Fernández-Ruiz, J. (2013). The inhibition of 2-arachidonoyl-glycerol (2-AG) biosynthesis, rather than enhancing striatal damage, protects striatal neurons from malonate-induced death: A potential role of cyclooxygenase-2-dependent metabolism of 2-AG. *Cell Death & Disease*, *4*, e862.

van der Poel, M., Ulas, T., Mizee, M. R., Hsiao, C. C., & Miedema, S. S. M. (2019). Transcriptional profiling of human microglia reveals grey-white matter heterogeneity and multiple sclerosis-associated changes. *Nature Communications*, *10*(1), 1139.

van der Stelt, M., Fox, S. H., Hill, M., Crossman, A. R., Petrosino, S., Di Marzo, V., & Brotchie, J. M. (2005). A role for endocannabinoids in the generation of parkinsonism and levodopa-induced dyskinesia in MPTP-lesioned non-human primate models of Parkinson's disease. *The FASEB Journal*, *19*(9), 1140–1142.

van der Stelt, M., Mazzola, C., Esposito, G., Matias, I., Petrosino, S., De Filippis, D., & Di Marzo, V. (2006). Endocannabinoids and beta-amyloid-induced neurotoxicity in vivo: Effect of pharmacological elevation of endocannabinoid levels. *Cellular and Molecular Life Sciences*, *63*(12), 1410–1424.

Van Laere, K., Casteels, C., Lunskens, S., Goffin, K., Grachev, I. D., Bormans, G., & Vandenberghe, W. (2012). Regional changes in type 1 cannabinoid receptor availability in Parkinson's disease in vivo. *Neurobiology of Aging*, *33*(3), 620–628.

Van Sickle, M. D., Duncan, M., Kingsley, P. J., Mouihate, A., Urbani, P., Mackie, K., & Sharkey, K. A. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science*, *310*(5746), 329–332.

Vázquez, C., Tolón, R. M., Grande, M. T., Caraza, M., Moreno, M., Koester, E. C., & Romero, J. (2015). Endocannabinoid regulation of amyloid-induced neuroinflammation. *Neurobiology of Aging*, *36*(11), 3008–3019.

Verkhratsky, A., & Nedergaard, M. (2018). Physiology of Astroglia. *Physiological Reviews*, *98*(1), 239–389.

Viader, A., Blankman, J. L., Zhong, P., Liu, X., Schlosburg, J. E., Joslyn, C. M., & Cravatt, B. F. (2015). Metabolic interplay between astrocytes and neurons regulates endocannabinoid action. *Cell Reports*, *12*(5), 798–808.

Viader, A., Ogasawara, D., Joslyn, C. M., Sanchez-Alavez, M., Mori, S., Nguyen, W., & Cravatt, B. F. (2016). A chemical proteomic atlas of brain serine hydrolases identifies cell type-specific pathways regulating neuroinflammation. *eLife*, *5*, e12345.

Walter, L., Dinh, T., & Stella, N. (2004). ATP induces a rapid and pronounced increase in 2-arachidonoylglycerol production by astrocytes, a response limited by monoacylglycerol lipase. *The Journal of Neuroscience*, *24*(37), 8068–8074.

Walter, L., Franklin, A., Witting, A., Moller, T., & Stella, N. (2002). Astrocytes in culture produce anandamide and other acylethanolamides. *The Journal of Biological Chemistry*, *277*(23), 20869–20876.

Walter, L., Franklin, A., Witting, A., Wade, C., Xie, Y., Kunos, G., & Stella, N. (2003). Nonpsychotropic cannabinoid receptors regulate microglial cell migration. *The Journal of Neuroscience*, *23*(4), 1398–1405.

Walter, L., & Stella, N. (2003). Endothelin-1 increases 2-arachidonoyl glycerol (2-AG) production in astrocytes. *Glia*, *44*(1), 85–90.

Weber, M., Goldman, B., & Truniger, S. (2010). Tetrahydrocannabinol (THC) for cramps in amyotrophic lateral sclerosis: A randomised, double-blind crossover trial. *Journal of Neurology, Neurosurgery, and Psychiatry*, *81*(10), 1135–1140.

Wilkerson, J. L., Ghosh, S., Bagdas, D., Mason, B. L., Crowe, M. S., Hsu, K. L., & Lichtman, A. H. (2016). Diacylglycerol lipase β inhibition reverses nociceptive behaviour in mouse models of inflammatory and neuropathic pain. *British Journal of Pharmacology*, *173*(10), 1678–1692.

Witting, A., Weydt, P., Hong, S., Kliot, M., Moller, T., & Stella, N. (2004). Endocannabinoids accumulate in spinal cord of SOD1 G93A transgenic mice. *Journal of Neurochemistry*, *89*(6), 1555–1557.

Wu, J., Hocevar, M., Foss, J. F., Bie, B., & Naguib, M. (2017). Activation of CB2 receptor system restores cognitive capacity and hippocampal Sox2 expression in a transgenic mouse model of Alzheimer's disease. *European Journal of Pharmacology*, *811*, 12–20.

Yiangou, Y., Facer, P., Durrenberger, P., Chessell, I. P., Naylor, A., Bountra, C., & Anand, P. (2006). COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord. *BMC Neurology*, *6*, 12.

Zajicek, J., Ball, S., Wright, D., Vickery, J., Nunn, A., Miller, D., & Group, C. i. (2013). Effect of dronabinol on progression in progressive multiple sclerosis (CUPID): A randomised, placebo-controlled trial. *Lancet Neurology*, *12*(9), 857–865.

Zhang, H. Y., Gao, M., Liu, Q. R., Bi, G. H., Li, X., Yang, H. J., & Xi, Z. X. (2014). Cannabinoid CB2 receptors modulate midbrain dopamine neuronal activity and dopamine-related behavior in mice. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(46), 5007–5015.

Zhang, J., & Chen, C. (2018). Alleviation of neuropathology by inhibition of monoacylglycerol lipase in APP transgenic mice lacking CB2 eeceptors. *Molecular Neurobiology*, *55*(6), 4802–4810.

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Zhang, J., Hu, M., Teng, Z., Tang, Y. P., & Chen, C. (2014). Synaptic and cognitive improvements by inhibition of 2-AG metabolism are through upregulation of microRNA-188-3p in a mouse model of Alzheimer's disease. *The Journal of Neuroscience*, *34*(45), 14919–14933.

Zhao, P., Ignacio, S., Beattie, E. C., & Abood, M. E. (2008). Altered presymptomatic AMPA and cannabinoid receptor trafficking in motor neurons of ALS model mice: Implications for excitotoxicity. *The European Journal of Neuroscience*, *27*(3), 572–579.

Zimmermann, T., Maroso, M., Beer, A., Baddenhausen, S., Ludewig, S., Fan, W., & Leschik, J. (2018). Neural stem cell lineage-specific cannabinoid type-1 receptor regulates neurogenesis and plasticity in the adult mouse hippocampus. *Cerebral Cortex*, *28*(12), 4454–4471.

How to cite this article: Bernal-Chico, A., Tepavcevic, V., Manterola, A., Utrilla, C., Matute, C., & Mato, S. (2023). Endocannabinoid signaling in brain diseases: Emerging relevance of glial cells. *Glia*, *71*(1), 103–126. https://doi.org/10.1002/glia.24172

10981136, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/glia.24172 by Universidad Del Pais Vasco, Wiley Online Library on [09/02/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License