


## SPECIAL ISSUE ARTICLE

## Endocannabinoid signaling in astrocytes

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## Abstract

The study of the astrocytic contribution to brain functions has been growing in popularity in the neuroscience field. In the last years, and especially since the demonstration of the involvement of astrocytes in synaptic functions, the astrocyte field has revealed multiple functions of these cells that seemed inconceivable not long ago. In parallel, cannabinoid investigation has also identified different ways by which cannabinoids are able to interact with these cells, modify their functions, alter their communication with neurons and impact behavior. In this review, we will describe the expression of different endocannabinoid system members in astrocytes. Moreover, we will relate the latest findings regarding cannabinoid modulation of some of the most relevant astroglial functions, namely calcium ( $\text{Ca}^{2+}$ ) dynamics, gliotransmission, metabolism, and inflammation.

## KEYWORDS

astrocytes, calcium, cannabinoids, gliotransmission, metabolism, neuroinflammation

## 1 | ASTROCYTES AS INTEGRATORS OF INFORMATION

Astrocytes are a population of glial cells representing approximately 10%–20% of all the cells in the central nervous system (CNS) (Sun et al., 2017; Verkhratsky et al., 2017). Their unique morphological complexity, with intricate arborisation and specialized structures that contact other CNS elements such as other glial cells, blood vessels, and synapses, make astrocytes fundamental players in brain physiology, as well as in the development of several pathological processes (Valori et al., 2021). Many diverse functions have been attributed to these cells, from providing metabolic support for neurons to the exciting new concepts of astrocyte-mediated de novo memory enhancement and active involvement in higher-brain functions (Adamsky et al., 2018; Kastanenka

et al., 2020; Mederos et al., 2021; Mederos & Perea, 2019; Navarrete et al., 2019; Zhou et al., 2021). Considering this, it has become clear that the studies of CNS physiology need to address the role of astrocytes (and probably other glial cells) to get a full picture of how the brain elements are interconnected and work together.

Due to their singular morphology, one single astrocyte is in contact with thousands of synapses through its thin distal processes (Bushong et al., 2002; Oberheim et al., 2009). Astrocytes are thus able to sense neuronal activity, adapting their physiology to neuronal demands and even participating in the synaptic function through the release of gliotransmitters (Allen & Eroglu, 2017; Araque et al., 2014; Durkee & Araque, 2019; Perea et al., 2009). On top of that, astrocytes express different neuromodulator receptors that are able to sense global environmental stimuli, integrating both synaptic

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and network information to finely tune neuronal connectivity (Ding et al., 2013; Paukert et al., 2014; Zhang et al., 2021). Thus, in response to both synaptic and extrasynaptic inputs, astrocytes are able to modify their physiology at different time points to orchestrate neuronal functions, being key for the proper function of brain physiology (Pacholko et al., 2020).

This review will address how the presence and the activity of one of these neuromodulator inputs, the endocannabinoid system (ECS), can impact astrocyte signaling and modulate key brain functions.

## 2 | THE ENDOCANNABINOID SYSTEM IN ASTROCYTES

The ECS is a widely distributed, polyfunctional signaling system that is virtually involved in all brain functions (Fride, 2005). This signaling system was named after the discovery of the G-protein coupled receptors (GPCRs) activated by the main active compound of Cannabis, ( $-$ )- $\Delta^9$ -tetrahydrocannabinol (THC), and the endogenous lipidic substances with cannabimimetic effects, so called endocannabinoids (eCBs) (Di Marzo, 1998). Thus, the ECS is classically considered to be composed by type 1 and type 2 cannabinoid receptors (CB1, CB2), anandamide (AEA), and 2-arachidonoylglycerol (2-AG), which are the best characterized eCBs derived from arachidonic acid (AA), and the enzymes responsible for their synthesis and degradation (for reviews on the ECS: Castillo et al., 2012; Hu & Mackie, 2015, 2015; Zou & Kumar, 2018; Cristino et al., 2020).

The boundaries of the ECS, however, are not well defined. Several reports have been pointing towards others cannabinoid-like receptors and cation channels that are activated by eCBs or other biochemically related molecules (Di Marzo, 2018). Moreover, in addition to the “classical” AA-derived AEA and 2-AG, other endogenous ligands have been recently described as cannabinoid receptor modulators. For instance, the lipids Lipoxin A4 (Pamplona et al., 2012) and pregnenolone (Vallee et al., 2014) have been shown to act as positive and negative allosteric modulators of CB1 receptors, whereas a family of peptides (so-called Pepcans) has been proposed to regulate cannabinoid signaling in the brain (Bauer et al., 2012). These additional elements introduce new layers of complexity that transform our perceptions on this signaling system, giving rise to the concept of an expanded ECS (Cristino et al., 2020; Di Marzo, 1998; Veilleux et al., 2019).

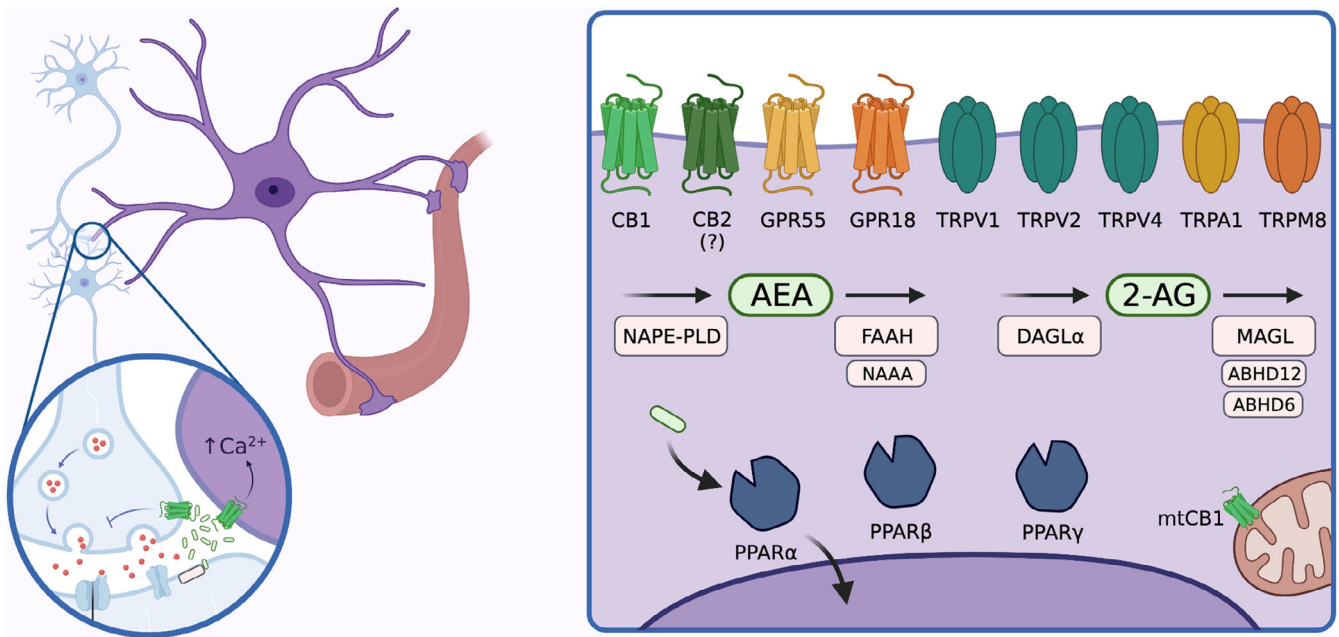
Functionally, a wide-spread well-characterized and powerful way through which the ECS regulates brain functions is the retrograde control of synaptic transmission and plasticity (Castillo et al., 2012; Kano et al., 2009). Briefly, this model indicates that the stimulation of postsynaptic neurons induces the mobilization of eCBs that travel retrogradely through the synaptic cleft, binds to presynaptic CB1 receptors and thereby reduces synaptic transmission and triggers synaptic plasticity (Piomelli, 2003). This can occur at many different types of synapses (excitatory, inhibitory, modulatory), thereby providing a very

fine-tuned control of interneuronal communication. However, besides this well-described mechanism of action of the ECS, studies during the last decades revealed several additional ways through which the ECS impacts brain functions. One of the most unexpected and intriguing ones is the relatively recent discovery that astrocytes express several eCB-related proteins, playing a key role in the control of their activity and functions (Busquets-Garcia et al., 2018; Covelo et al., 2021; Oliveira da Cruz et al., 2016) (Figure 1).

The first evidence suggesting the expression of cannabinoid-like receptors in astrocytes resulted from the observation that AEA treatment reduced the connection of cultured striatal astrocytes by gap junctions (Stella, 2010; Venance et al., 1995). Importantly, this effect seemed to be CB1-independent, as it was not mimicked by CB1 synthetic agonists (CP55940 and WIN55) nor reverted by the CB1 antagonist rimonabant. Indeed, CB1 expression in astrocytes was initially controversial and early studies using cultured astrocytes addressing this question stated contradictory results. In the same way, the identification of astroglial CB1 by ultrastructural studies was ambiguous (Katona et al., 1999; Moldrich & Wenger, 2000; Rodríguez et al., 2001; Salio et al., 2002). However, CB1 expression on astrocytes was functionally demonstrated in the context of the hippocampal tripartite synapse, where local application of cannabinoids induced astroglial  $\text{Ca}^{2+}$  increases, an effect that was reverted by the CB1 selective antagonist AM251 (Navarrete & Araque, 2008, 2010). Complementarily, electron microscopic analyzes of double immunostaining of the astroglial marker glial fibrillary acid protein (GFAP) and CB1 in wild-type and null CB1 mutant mice (CB1-KO) provided definitive anatomical evidence for the existence of astroglial CB1, although in lower levels than neurons (Gutiérrez-Rodríguez et al., 2018; Han et al., 2012).

The expression of CB1 receptors in astrocytes is rather complex. Besides the widely explored localization of CB1 in astrocytic plasma membranes close to synaptic terminals (Covelo et al., 2021), this receptor can be found in other subcellular localizations such as in mitochondria (Gutiérrez-Rodríguez et al., 2018; Jimenez-Blasco et al., 2020) and the plasmalemma of astrocytic perivascular end-feet (Moldrich & Wenger, 2000; Rodríguez et al., 2001). Indeed, recent evidence has shown that activation of mitochondrial CB1 (mtCB1) in astrocytes via cannabinoids has a polyfunctional role that impacts glucose metabolism,  $\text{Ca}^{2+}$  signaling and behavior (Jimenez-Blasco et al., 2020; Serrat et al., 2021).

Contrary to CB1, CB2 was initially considered to be absent from the brain (Atwood & Mackie, 2010). However, this receptor was later found to be present and functionally relevant in both microglia and possibly neurons (Jordan & Xi, 2019). Regarding astrocytes, CB2 presence seems to be questionable (Benyó et al., 2016; Dowie et al., 2014; Núñez et al., 2008; Stella, 2004). There are a few studies suggesting that CB2 is expressed in astrocytic cultures (Cassano et al., 2017; Köfalvi et al., 2016; Molina-Holgado et al., 2002) but this can be a result of the impact that different culture protocols have on the astroglial transcriptome (Lange et al., 2012). Overall, there is a current lack of robust evidence for the presence of astroglial CB2 receptors in physiological conditions, although astrocytes may express this



**FIGURE 1** Endocannabinoid system elements in astrocytes. Upon depolarization, endocannabinoids (eCBs) are synthesized “on demand” and released from the postsynaptic terminal. eCBs will travel retrogradely to the presynaptic neuron to inhibit neurotransmitter release but also to the astrocytic thin processes, regulating astrocyte physiology. One of the most well-known consequences of eCBs effects on astrocytes is the increase in cytosolic  $\text{Ca}^{2+}$  signals through cannabinoid (CB1) activation. However, astrocytes express other receptors associated to eCB signaling, which, upon activation may modulate several astroglial functions, such as release of gliotransmitters, metabolism or immune response via different signaling pathways. Of note, besides CB1 (and maybe CB2), other potential cannabinoid receptors have been described in astrocytes, such as GPR55 and GPR18 as well as different transient receptor potential (TRPs) such as TRPV1, TRPV2, TRPV4, TRPA1 or TRPM8. Moreover, the intracellular receptors peroxisome proliferator-activated receptors (PPARs) ( $\alpha$ ,  $\beta$  and  $\gamma$ ) have been described as potential cannabinoid receptors in astrocytes. Furthermore, functional CB1 receptors were found associated to astroglial mitochondria. Finally, astrocytes also express all the enzymes that take part in synthesis and catabolism of both anandamide (AEA) (N-arachidonylethanolamine phospholipase D [NAPE-PLD] for synthesis and fatty acid amide hydrolase (FAAH) and N-acyl ethanolamine acid amidase [NAAA] for degradation) and 2-AG (diacylglycerol lipase  $\alpha$  [DAGL $\alpha$ ] for synthesis and monoacylglycerol lipase [MAGL], Alpha/beta-hydrolase domain containing 12 [ABHD12], Alpha/beta-hydrolase domain containing 6 [ABHD6] for catabolism), suggesting its implication in eCBs production and degradation.

receptor under some pathological conditions. For instance, it is widely accepted that CB2 is upregulated in glial cells in response to some insults (Benito et al., 2008; Jordan & Xi, 2019). However, while some reports found this response to be specific to microglia (Núñez et al., 2008; Schmöle et al., 2015), others reported CB2 expression in astrocytes. For instance, CB2 was reported to be present on astrocytes from the spinal cord of a canine model for amyotrophic lateral sclerosis (Fernández-Trapero et al., 2017). Moreover, CB2 is also present in human astrocytes derived from human fetal brain tissues (Sheng et al., 2005) and from postmortem studies of multiple sclerosis, spinocerebellar ataxia patients, and suicide victims (García-Gutiérrez et al., 2018; Rodríguez-Cueto et al., 2014; Zhang et al., 2011).

Besides CB1 and CB2, other GPCRs such as GPR55 and GPR18 have been proposed as putative cannabinoid receptors (Boczek & Zylinska, 2021; Cristino et al., 2020). These receptors have been identified in astrocytes, at both mRNA- and protein-level (García-Gutiérrez et al., 2018; Grabiec et al., 2019; Gutiérrez-Rodríguez et al., 2018; Sawzdargo et al., 1999), however, their functional significance in astrocytes remains largely unexplored.

Additionally to GPCRs, receptors from other classes have also been associated to eCB signaling, namely a subset of transient receptor

potential (TRP) channels and some isoforms of peroxisome proliferator-activated receptors (PPARs) (Muller et al., 2019; O'Sullivan, 2016). TRPs are a superfamily of transmembrane cation channels that exhibit a remarkable diversity of activation mechanisms, including chemical and physical stimuli (Venkatchalam & Montell, 2007). In many cases, a single TRP channel can be modulated by distinct stimuli that can modify each other's responses, thereby acting as multiple signal integrators (Venkatchalam & Montell, 2007). Amongst TRP channels, there is evidence that cannabinoids can modulate the activity of TRP channels from the vanilloid (TRPV), ankyrin (TRPA), and melastatin (TRPM) subfamilies, namely TRPV1-4, TRPA1 and TRPM8 (Boczek & Zylinska, 2021; Muller et al., 2019). In astrocytes, these so-called “ionotropic cannabinoid receptors” are mainly associated to spatiotemporal coordination of  $\text{Ca}^{2+}$  and sodium ( $\text{Na}^{+}$ ) transients, that further transduce in various internal signaling cascades with multiple outcomes (Verkhatsky et al., 2014). The astrocytic expression and functional relevance of TRPV1, TRPV2, TRPV4, and TRPA1 is widely accepted (Benfenati et al., 2011; Luo et al., 2020; Shibasaki et al., 2013; Shigetomi et al., 2013; Tóth et al., 2005; Yang et al., 2019). Additionally, a recent study using primary cultures derived from piglet brains suggested the presence of TRPM8 in astrocytes (Fedinec et al., 2021).

Finally, even though TRPV3 is known to be present in the brain, there is no evidence so far for its expression in astrocytes (Luo et al., 2020; Xu et al., 2002). However, the impact of (endo)cannabinoids on astroglial functions through this family of receptors is still to be established.

Regarding PPARs, these proteins constitute a family of nuclear hormone receptors that regulate gene expression by binding to DNA sequences called PPAR response elements upon ligand-dependent activation (Bugge & Mandrup, 2010). All three subtypes of PPARs ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) have been associated to some of the CB1/CB2-independent cannabinoid effects (O'Sullivan, 2016; Sun & Bennett, 2007). In mice, astrocytes have been reported to express PPAR $\alpha$  and PPAR $\gamma$ , but no PPAR $\beta$  (Warden et al., 2016). Interestingly, the expression of these receptors varies according to the brain region, with a lower expression in the ventral tegmental area compared to the prefrontal cortex, nucleus accumbens or amygdala, suggesting the idea of an area-specific or circuit-specific astrocytic cannabinoid signaling. Moreover, these receptors exhibit different subcellular distributions: while PPAR $\alpha$  is localized in the astrocytic cell body and processes, astroglial PPAR $\gamma$  is mostly present in the cell body and scarce in processes (Warden et al., 2016). This suggests a potential for a PPAR-mediated diversity of function of cannabinoids in astrocytes yet to be unveiled.

Importantly, astrocytes are not only passive eCB "receivers" and some evidence has shown that these cells participate in eCB metabolism. First, astrocytes have a well-recognized role on eCB degradation. In the context of retrograde signaling, astrocytes and neurons contribute synergistically to 2-AG signaling termination through monoacylglycerol lipase (MAGL), its main degrading enzyme (Chen et al., 2016; Liu et al., 2016; Viader et al., 2015). Alpha/beta-hydrolase domain containing 6 (ABHD6) and Alpha/beta-hydrolase domain containing 12 (ABHD12) also contribute for astrocytic 2-AG degradation, what can be particularly relevant in adaptative responses to certain pathological conditions (Marrs et al., 2010; Moreno-García et al., 2020; Viader et al., 2016). Regarding AEA degradation, astrocytes dynamically express its main catabolic enzyme, fatty acid amide hydrolase (FAAH), which is upregulated in certain neuroinflammatory responses (Benito et al., 2007; Kallendrusch et al., 2012; Moreno-García et al., 2020; Núñez et al., 2008). Moreover, astrocytes also express N-acyl ethanolamine acid amidase (NAAA), an alternative AEA degradation enzyme, although in lower levels when compared to FAAH (Kallendrusch et al., 2012; Moreno-García et al., 2020).

Finally, although it was early demonstrated that cultured astrocytes can produce both AEA and 2-AG (Walter et al., 2002, 2004; Walter & Stella, 2003), there is an ongoing debate about the functional significance of astroglial-derived eCBs (Covelo et al., 2021). Nonetheless, the expression of the main enzymes responsible for 2-AG and AEA production, diacylglycerol lipase  $\alpha$  (DAGL $\alpha$ ) and N-arachidonylethanolamine phospholipase D (NAPE-PLD) respectively, was characterized in cultured astrocytes (Kallendrusch et al., 2012; Viader et al., 2016) and their mRNA can also be detected *ex vivo* (Schüle et al., 2021). Interestingly, this machinery seems to be sensitive to changes in the environment, such as during maternal caloric restriction in rats (Tovar et al., 2021).

### 3 | ROLE OF ENDOCANNABINOID SYSTEM IN ASTROGLIAL CALCIUM SIGNALING

#### 3.1 | Calcium signaling in astrocytes

Being electrically nonexcitable cells, astrocytes communicate with other cell types through different mechanisms that are largely regulated by intracellular Ca<sup>2+</sup> levels. These Ca<sup>2+</sup> fluctuations in astrocytes are then modulated by environmental conditions. Ca<sup>2+</sup> increase is mediated either by Ca<sup>2+</sup> entry from the extracellular space through the plasma membrane or by Ca<sup>2+</sup> release from the intracellular Ca<sup>2+</sup> stores such as the endoplasmic reticulum (ER) and mitochondria. The resulting Ca<sup>2+</sup> events spread within the cell by passive diffusion and/or by regenerative mechanisms (Semyanov, 2019).

Plasma membrane entrance pathways consist in ligand-activated cation permeable ionotropic receptors, such as N-methyl-D-aspartate (NMDA), purinergic P2X(1/5) receptors (Lalo et al., 2006; Palygin et al., 2010), TRP channels that sense various changes in the environment (Verkhatsky et al., 2014), store operated Ca<sup>2+</sup> entrance (Sakuragi et al., 2017), voltage-gated Ca<sup>2+</sup> channels (VGCCs) (Carmignoto et al., 1998), and the sodium/calcium exchanger (NCX) operating in reverse mode (Kirischuk et al., 2012). Note that these sources can work together with intracellular Ca<sup>2+</sup> stores. For instance, it has been proposed that Ca<sup>2+</sup> entry through VGCCs is very small in astrocytes, but it can trigger Ca<sup>2+</sup> release from the ER (Carmignoto et al., 1998). The Ca<sup>2+</sup> events are terminated by the inactivation of the Ca<sup>2+</sup> source and by Ca<sup>2+</sup> extrusion through the plasma membrane and/or uptake to the intracellular Ca<sup>2+</sup> stores. The infinite possibilities of combinations result in a high diversity of Ca<sup>2+</sup> events, whose decoding is still far to be achieved.

In astrocytes, Ca<sup>2+</sup> release from ER is mediated by the Inositol triphosphate Receptor (IP3R) (Kirischuk et al., 1999; Ullah et al., 2006). Ca<sup>2+</sup> and IP3 are co-agonists of IP3Rs (Bezprozvanny et al., 1991; Dupont & Goldbeter, 1993; Mak et al., 1998). Ca<sup>2+</sup> elevations reaching the IP3R activation threshold can trigger a release of Ca<sup>2+</sup> from the ER (Shinohara et al., 2011; Wu et al., 2019), a process known as Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release. IP3Rs activation threshold depends on the level of IP3: when it increases, IP3Rs become more sensitive to Ca<sup>2+</sup>, which enhances their opening probability, and consequently IP3R-mediated amplification and propagation of Ca<sup>2+</sup> events (Mak et al., 1998; Shinohara et al., 2011). Astrocytes express several types of Gq-protein coupled receptors which can trigger IP3 production via phospholipase C (PLC) activation: metabotropic glutamate receptor 5 (mGluR5) which can be activated following synaptic glutamate release (Bradley & Challiss, 2012; Nakahara et al., 1997; Umpierre et al., 2019), Gq-protein coupled purinergic P2Y receptors (Fumagalli et al., 2003; Helen et al., 1992), serotonergic 5HT2A, adrenergic  $\alpha$ 1AR Gq-protein coupled receptors (O'Donnell et al., 2015; Porter-Stransky et al., 2019; Xu & Pandey, 2000) and CB1 (Navarrete & Araque, 2008). Of note, IP3R activity is regulated by cytosolic Ca<sup>2+</sup> following a bell shape dependency: low concentrations of Ca<sup>2+</sup> (50 nM–1  $\mu$ M) increase the opening probability of IP3R, whereas

higher concentrations (more than 1  $\mu\text{M}$ ) lower it. It has been proposed that high concentrations of  $\text{Ca}^{2+}$  at the mouth of the channel might prevent the propagation of the  $\text{Ca}^{2+}$  signal by inhibiting the channel activity (Foskett & Mak, 2010).

Other important players in astrocytic  $\text{Ca}^{2+}$  dynamics, and potentially astrocytes regulation of synaptic function, are mitochondria (Stephen et al., 2014). Mitochondria act globally as  $\text{Ca}^{2+}$  stores. Mitochondrial  $\text{Ca}^{2+}$  uptake is mostly mediated by the mitochondrial  $\text{Ca}^{2+}$  uniporter (MCU) and  $\text{Ca}^{2+}$  release by mitochondrial NCX,  $\text{H}^+/\text{Ca}^{2+}$  exchanger, and the permeability transition pore (PTP) (O-Uchi et al., 2012; Rizzuto et al., 2012). These transport mechanisms are located in the inner mitochondrial membrane, whereas the outer mitochondrial membrane is highly permeable to soluble molecules smaller than 5 kDa, including  $\text{Ca}^{2+}$ . MCU drives the entry of large flux of  $\text{Ca}^{2+}$  into mitochondria, but it operates at micromolar cytosolic  $\text{Ca}^{2+}$  levels, which are usually only reached at the mouth of  $\text{Ca}^{2+}$  sources (Boyman & Lederer, 2020; Williams et al., 2013). When mitochondria are located in close proximity to the ER at the level of mitochondria/ER contacts (MERCs), MCU can contribute to the removal of  $\text{Ca}^{2+}$  released by IP3Rs (Boyman & Lederer, 2020; Marchi & Pinton, 2014; Rizzuto et al., 2012). MERCs have been observed in astrocytes processes and endfeet (Göbel et al., 2020), and the expression of some MERCs-constitutive proteins induces  $\text{Ca}^{2+}$  transfer from the ER to mitochondria (Eraso-Pichot et al., 2017). However, it is not clear how mitochondria  $\text{Ca}^{2+}$  uptake affects cytosolic  $\text{Ca}^{2+}$  transients in astrocytes. Some studies have reported that the impairment of the mitochondrial function increases the propagation and the duration of  $\text{Ca}^{2+}$  signals, which would suggest that mitochondria modulate negatively  $\text{Ca}^{2+}$  waves propagation (Boitier et al., 1999; Jackson & Robinson, 2015; Reyes & Parpura, 2008). On the other hand, other studies have observed that amplification sites for  $\text{Ca}^{2+}$  waves are characterized by accumulations of ER  $\text{Ca}^{2+}$  signaling proteins (calreticulin and IP3R), and by the presence of mitochondria (Simpson et al., 1997). Hence, ER-mitochondria  $\text{Ca}^{2+}$  transfer could be needed for the propagation of cytosolic  $\text{Ca}^{2+}$  waves in astrocytes. Recently, we have shown that pharmacological inhibition or overexpression of a dominant negative mutant of MCU hamper the propagation of cytosolic  $\text{Ca}^{2+}$  events (Serrat et al., 2021). Altogether, these data suggest that mitochondria might prevent IP3R self-inhibition by buffering the released  $\text{Ca}^{2+}$  at the mouth of the channel in astrocytes. Hence, depending on the studies, mitochondria appear as downregulators or enhancers of cytosolic  $\text{Ca}^{2+}$  signals. Discrepancies could originate from the different stimuli used (physiological synaptic stimulation vs pharmacological [ATP] or mechanical stimuli) and the type of mitochondrial drugs used (mitochondrial uncoupler or diverse MCU inhibitors).

### 3.2 | Calcium patterns modulation following synaptic events

Astrocytes exhibit local and short  $\text{Ca}^{2+}$  events under resting conditions. The precise mechanisms by which those spontaneous  $\text{Ca}^{2+}$

transients are generated remain debated. Although it has been proposed that they could be linked to spontaneous synaptic vesicle release (Sun et al., 2014), astrocytes are still able to generate spontaneous  $\text{Ca}^{2+}$  events when neuronal and astrocytic vesicular release is blocked by bafilomycin A1 (Bowser & Khakh, 2007; Nett et al., 2002). Alternatively, stochastic opening of IP3Rs could underline spontaneous  $\text{Ca}^{2+}$  transients (Foskett & Mak, 2010). However, knockout of astrocytic IP3R type 2 still display spontaneous  $\text{Ca}^{2+}$  events (Sherwood et al., 2017; Srinivasan et al., 2015). Finally, a recent report has suggested that brief openings of PTP can be responsible for astrocytic spontaneous  $\text{Ca}^{2+}$  events in the absence of IP3R-mediated  $\text{Ca}^{2+}$  release (Agarwal et al., 2017). Most likely, they are generated by stochastic  $\text{Ca}^{2+}$  fluxes through multiple pathways. Interestingly, changes in the properties of  $\text{Ca}^{2+}$  events (frequency, duration, and spread) occur in response to changes in environment, such as increased neuronal activity or high metabolic demand.

Extensive literature has shown that synaptic activity induces intracellular  $\text{Ca}^{2+}$  transients in astrocytes (Araque et al., 2001; Haydon & Carmignoto, 2006; Nedergaard et al., 2003; Panatier et al., 2011; Perea et al., 2009; Perea & Araque, 2005; Porter & McCarthy, 1996; Volterra & Meldolesi, 2005), through the activation of astroglial neurotransmitter receptors coupled to second messenger pathways. In turn,  $\text{Ca}^{2+}$  elevations cause the release of different signaling molecules, termed gliotransmitters, that modulate neuronal excitability and synaptic transmission (Araque et al., 1999; Beattie et al., 2002; Perea & Araque, 2007). Different patterns of neuronal activity are encoded in spatial and temporal properties of astroglial  $\text{Ca}^{2+}$  events. Thus, astrocytes display a highly complex repertoire of subcellular and intercellular  $\text{Ca}^{2+}$  signals that can be generated by different routes and involve different subregions of the cells.

For instance, spatial propagation of  $\text{Ca}^{2+}$  signals has been observed following application of mGluR agonists and low or high frequency stimulation of Schaffer collaterals (Navarrete & Araque, 2008; Sun et al., 2014; Wu et al., 2014). Propagation of  $\text{Ca}^{2+}$  transients is hypothesized to allow astrocytes to convey messages to distant synapses (Semyanov, 2019). This modulation of  $\text{Ca}^{2+}$  event properties was abolished by mGluR antagonists, by IP3 buffering via overexpression of an IP3 sponge (modified ligand-binding domain of the IP3R) or by knocking out the astrocyte-specific IP3R (Rungta et al., 2016; Sherwood et al., 2017; Srinivasan et al., 2015; Tanaka et al., 2013). The IP3R is the ideal support for the spatial propagation of  $\text{Ca}^{2+}$  transients upon neuronal stimulation. Indeed, glutamate, ATP, noradrenaline and eCBs can activate GPCRs and thereby increase the production of diacylglycerol and IP3 by PLC. The resulting high cytosolic IP3 levels increase the probability that spontaneous  $\text{Ca}^{2+}$  fluctuations are amplified by  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release. This amplification can convert spatially restricted  $\text{Ca}^{2+}$  transient into propagating events. The central role of IP3R in the propagation of  $\text{Ca}^{2+}$  transient in astrocytes was illustrated in a couple of studies using KO mice for IP3R2 in astrocytes. Spontaneous  $\text{Ca}^{2+}$  fluctuations still occur in those mice, but they can no longer be amplified by  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release (Rungta et al., 2016; Sherwood et al., 2017; Srinivasan et al., 2015; Tanaka et al., 2013). Recent work from our team also

suggests that neuronal activity can modulate IP3R activity by tuning the  $\text{Ca}^{2+}$  uptake into mitochondria (Serrat et al., 2021). Interestingly, mtCB1 is involved in this mechanism, as will be discussed below.

### 3.3 | Cannabinoid modulation of calcium signals in astrocytes

The most studied example of how cannabinoids may modulate neuronal communication through astrocytic  $\text{Ca}^{2+}$  signals so far is the CB1 receptor. In 2008, Alfonso Araque's group demonstrated that CB1 expressed in hippocampal astrocytes could be activated by exogenous cannabinoids as well as by eCBs released by neurons following neuronal stimulation. CB1 activation increased astrocyte  $\text{Ca}^{2+}$  levels through PLC-dependent  $\text{Ca}^{2+}$  release from the ER, ultimately inducing cytosolic  $\text{Ca}^{2+}$  increases in astrocytes soma and processes. This CB1-induced  $\text{Ca}^{2+}$  increase can spread along the cell and induce the release of gliotransmitters at distal regions (Navarrete & Araque, 2008). This propagation of astrocytic  $\text{Ca}^{2+}$  signals has been shown to induce short-term (Covelo & Araque, 2018; Navarrete & Araque, 2010) or long-term potentiation (Gómez-Gonzalo et al., 2015) at distant synapses. In this aspect, astroglial CB1 act through specific signaling pathways that differ from the canonical neuronal ones that occur at short distances and result in a presynaptic inhibition of neurotransmitter release (Kano et al., 2009). Interestingly, other studies have shown that the physiological or the pharmacological activation of CB1 produces astrocytic  $\text{Ca}^{2+}$  increase (Hegyí et al., 2018; Kószeghy et al., 2015; Robin et al., 2018). However, in these works, the mechanisms involved in the propagation of the  $\text{Ca}^{2+}$  signal were not explored.

At subcellular level, CB1 receptors in astrocytes have been observed not only at the plasma membrane but also associated with intracellular organelles like mitochondria (Gutiérrez-Rodríguez et al., 2018; Jimenez-Blasco et al., 2020). Contrary to other pools of CB1, astroglial mtCB1 activation has been associated with  $G_{\alpha i/o}$  proteins, inhibition of mitochondrial soluble adenylyl cyclase (sAC) and reduction of mitochondrial respiration through the regulation of specific mitochondrial respiratory subunits (Hebert-Chatelain et al., 2016; Jimenez-Blasco et al., 2020). Our recent paper shows that the physiological spreading of astrocytic  $\text{Ca}^{2+}$  events in the context of lateral potentiation requires mtCB1 signaling (Serrat et al., 2021). Indeed, lateral potentiation is virtually abolished in mice expressing DN22 CB1, a mutant version of this receptor that is excluded from mitochondria (Hebert-Chatelain et al., 2016; Soria-Gomez et al., 2021). We hypothesize that the activation of mtCB1 enhances the transfer of  $\text{Ca}^{2+}$  between ER and mitochondria, removing the inhibition of IP3R by buffering the released  $\text{Ca}^{2+}$  at the mouth of the channel. Indeed, in cultured astrocytes, almost 100% of mitochondria are located less than 100 nm from ER, a distance that permit  $\text{Ca}^{2+}$  exchanges between the two organelles (Csordás et al., 2018). In addition, activation of mtCB1 receptors induces ER-mitochondria  $\text{Ca}^{2+}$  transfer. mtCB1 has also been identified in astrocytic processes, in the vicinity of synapses (less than 1  $\mu\text{M}$  away [Gutiérrez-Rodríguez et al., 2018]).

Hence, all the machinery needed for mtCB1-regulated ER-mitochondria  $\text{Ca}^{2+}$  transfer is present in astrocytic processes. In addition, in our hand, MCU inhibition affects the spatial and temporal spreading of subcellular  $\text{Ca}^{2+}$  events following strong stimulation of Schaffer collaterals in hippocampal slices, a protocol known to induce lateral potentiation (Serrat et al., 2021).

Interestingly, other members of the ECS are potential modulators of  $\text{Ca}^{2+}$  signals in astrocytes. Astrocytes express a huge variety of TRP channels that may control astrocytic  $\text{Ca}^{2+}$  signals (Verkhatsky et al., 2014) and are potentially modulated by both endogenous and exogenous cannabinoids (Muller et al., 2019). For instance, TRPA1, which has been implicated in the regulation of astrocyte resting  $\text{Ca}^{2+}$  (Shigetomi et al., 2013), has shown also cannabinoid-mediated activity (Muller et al., 2019). Also, in *Drosophila melanogaster*, the TRP analog water witch (WTRW) has been seen to control  $\text{Ca}^{2+}$  dynamics in astrocytes, modulating neuronal activity and behavior (Ma et al., 2016). Interestingly, this same channel in astrocytes and neurons is modulated by AEA metabolites and protects against seizures (Jacobs & Sehgal, 2020), thus opening the door for further effects of cannabinoids on astrocyte  $\text{Ca}^{2+}$  regulation in a CB1-independent manner.

### 3.4 | Cannabinoid modulation of gliotransmission

The cannabinoid regulation of  $\text{Ca}^{2+}$  signals was linked to the astroglial release of a number of gliotransmitters. These active molecules, including ATP, glutamate, D-Serine, and GABA, released in many different brain regions, such as the hippocampus, amygdala, striatum or spinal cord (Carlsen et al., 2021; Covelo & Araque, 2018; Martín et al., 2015; Martín-Fernandez et al., 2017; Navarrete & Araque, 2008; Robin et al., 2018) were shown to modulate a number of short- and long-term effects on synaptic transmission at both inhibitory and excitatory synapses (for recent review see [Covelo et al., 2021]). While the role of glial cells on cannabinoid-mediated synaptic effects is well described, the molecular and cellular mechanisms of gliotransmitter release from astrocytes are yet to be fully understood.

One of the general mechanisms used by eukaryotic cells to export their contents to the extracellular space is through vesicular release dependent on SNARE proteins. These proteins, also expressed in astrocytes, were suggested to be major mediators of gliotransmission (Araque et al., 2000; Navarrete et al., 2019; Schwarz et al., 2017). Likewise, it was proposed that they are involved in a mechanism by which cannabinoid-activated astrocytic  $\text{Ca}^{2+}$ -signaling affects synaptic function. Min and Nevian (2012) showed that eCB mediated spike timing-dependent depression (t-LTD) in the rat barrel cortex relies on SNARE-dependent exocytosis of astroglial glutamate. Another study found that CB1 receptor activation triggers the release of D-serine and ATP from neocortical astrocytes by SNARE-dependent mechanism (Rasooli-Nejad et al., 2014). Interestingly, one alternative, non-vesicular mechanism was recently proposed to rely on astroglial hemichannels (Labra et al., 2018). In agreement with this hypothesis,

it was previously shown that AEA may facilitate the opening of astrocytic hemichannels in the healthy brain, suggesting that the eCB might participate in the mechanisms of ATP release (Vázquez et al., 2015). More in depth investigation of different mechanisms governing gliotransmission in the future might help to disentangle the complex eCB-mediated effects on synaptic transmission.

In the classical model of cannabinoid signaling, eCBs are released from postsynaptic neurons upon neuronal activation and travel across synapses, activating CB1 on presynaptic axons and glia to modulate neurotransmission. Thus, so far, the majority of research on the cannabinoid effects on glia–neuron communication focused on CB1-regulated gliotransmission. However, recently accumulating evidence suggests that glial cells are not only affected by cannabinoids, but, as we mentioned before, they also possess functional machinery to produce and release them. Early evidence showing that ATP stimulation increased 2-AG production in astrocytes *in vitro* (Walter et al., 2004; Witting et al., 2004), was followed by recent work that demonstrated that cultured spinal astrocytes co-express CB1 and the 2-AG synthesizing enzyme, DAGL $\alpha$ , and that upon cannabinoid stimulation astrocytes produce 2-AG in a Ca<sup>2+</sup>-dependent manner (Hegyí et al., 2018). In line with this, (Schüle et al., 2021) showed that a subpopulation of astrocytes in the adult mouse brain expresses DAGL $\alpha$  and that genetic deletion of this enzyme in glutamate/aspartate transporter-expressing astrocytes resulted in altered affective behaviors in female mice. Furthermore, one recent study suggested that astrocyte-derived eCBs are involved in transient heterosynaptic depression in the hippocampus (Smith et al., 2020). Taken together, these results point out to a potential role of astrocyte-derived eCBs in the regulation of brain functions, an interesting direction for future studies.

## 4 | ENDOCANNABINOID SYSTEM REGULATION OF ASTROCYTE METABOLISM

### 4.1 | Astrocyte metabolism

One of the key and best studied functions of astrocytes is their contribution to brain metabolism in general and to neuronal metabolism in particular. In that sense, metabolic compartmentalization between astrocytes and neurons has risen to a complex picture in which many metabolic pathways start in one cell type and end in the other (Bélanger et al., 2011). The prototypical example of this compartmentalization is the well-known astrocyte–neuron lactate shuttle (ANLS). The ANLS claims that astrocytes are capable to perform aerobic glycolysis and release lactate that will be in turn used by neighboring neurons as a metabolic or signaling molecule (Barros, 2013; Bélanger et al., 2011; Pellerin & Magistretti, 1994).

The discovery of the ANLS led to the idea that astrocytes are mainly glycolytic cells, while neurons rely on their mitochondrial metabolic activity to sustain their high energy demands. However, some recent studies propose that astrocytes show a more complex and

versatile metabolism than previously thought. As an example, astrocytes are able to perform fatty acid oxidation (Ebert et al., 2003; Eraso-Pichot et al., 2018; Fecher et al., 2019) although the contribution of this pathway to the general brain metabolism is still a matter of debate (Schönfeld & Reiser, 2013, 2021).

In addition, although to a lower level than some peripheral tissues such as the liver, astrocytes present glycogen reserves, which can be rapidly metabolized to obtain ATP and lactate (Bak et al., 2018). Finally, other metabolic pathways have been proposed to mediate this astrocyte–neuron metabolic coupling and add layers to astrocyte metabolic complexity, such as the oxidation of glutamate and the glutamate–glutamine cycle (Schousboe et al., 2014).

Interestingly, the metabolic complexity of astrocytes depends on environmental conditions. The best-known example of astrocytic adaptation to neuronal demands is the aforementioned ANLS, where neuronal activity through glutamate release is able to inhibit astrocytic mitochondrial activity and increase aerobic glycolysis and lactate release (Pellerin et al., 2007). However, many other metabolic pathways in astrocytes have been demonstrated to be plastic upon neuronal activity such as the glycogenolysis (Magistretti et al., 1981), the oxidation of fatty acids (Eraso-Pichot et al., 2018) or the glutamate–glutamine cycle (Schousboe et al., 2014).

Cannabinoids are amongst the signaling molecules that may serve as environmental cues to induce changes in astrocytic metabolism, and could help explain how astroglial metabolic adaptations are necessary for brain physiology. Interestingly, exogenous and endogenous cannabinoids exert an effect on brain glucose levels that point to them as regulators of astrocytic metabolism (Brett et al., 2001; Margulies & Hammer, 1991; Nogueiras et al., 2009; Volkow et al., 1991).

### 4.2 | Endocannabinoid impact on astrocyte metabolism

Evidence for the different roles of cannabinoids in astrocytic metabolism came mostly from the use of exogenous cannabinoids like THC to change astrocytic metabolic pathways. For instance, one of the earliest hints of the direct impact of cannabinoids in astrocyte glucose metabolism was provided by the observation that THC stimulates glucose utilization possibly through CB1 receptors or other alternative noncanonical pathways in cultured astrocytes (Sánchez et al., 1998). On the other hand, pharmacological activation of cannabinoid receptors induced suppression of glucose metabolism in the astrocytic tricarboxylic acid (TCA) cycle in hippocampal slices, blocked by a specific CB1 antagonist (AM251) (Duarte et al., 2012).

Recent studies revealed the link between astrocytic mtCB1 receptors, astrocyte glucose metabolism and THC-induced defects in social behavior. In that study, 24 h THC treatment induced a decrease in complex I stability and function, eventually leading to a decrease of lactate production that resulted in impaired social behaviors. These metabolic changes were mediated by mtCB1 and were due to a decrease of phosphorylation of the Ser 173 residue of NADH:

ubiquinone oxidoreductase subunit S4 (NDUFS4) by intra-mitochondrial protein kinase A (PKA)/cAMP signaling (Jimenez-Blasco et al., 2020). The changes in mitochondrial complex I affected not only mitochondrial respiration, but also reduced astrocytic mitochondrial reactive oxygen species (mROS). Astrocytes produce a higher amount of mROS than neurons, which plays a key role on the physiological regulation of glucose utilization and neuronal survival (Vicente-Gutierrez et al., 2019). The transcription factor hypoxia-inducible factor 1 (HIF-1) senses mROS and regulates glycolysis, impacting the conversion of glucose to lactate (Kim et al., 2006). THC treatment decreased the expression of the alpha subunit of HIF-1, which resulted in a decrease of lactate production, both in cultured astrocytes and in vivo, in an mtCB1 dependent manner, affecting social behaviors in mice (Jimenez-Blasco et al., 2020). In summary, this study showed that exogenous cannabinoid-induced bioenergetic stress in the astrocytes directly impacts neuronal functions in vivo.

In relation to eCBs, one study focusing on brain ischemia suggested a possible relation between 2-AG and astrocytic glucose metabolism regulation: the authors showed that after short glucose and oxygen deprivation, treatment of primary astrocytes with 2-AG increased their survival via STAT3 (Wang et al., 2016). STAT3 is a transcription factor that regulates aerobic glycolysis and ROS production in astrocytes (Sarafian et al., 2010) and while the mechanisms of the action of 2-AG remain unexplored, this evidence could suggest a link between this endogenous messenger and astrocytic metabolism.

CB2 receptors have also been studied in the context of astrocytic metabolism, although scarcely. In vitro, activation of CB2 in cultured cortical astrocytes with the selective agonists GP1a and JWH133 led to an increase of glucose transport, which was blocked by AM630, a selective CB2 antagonist. Moreover, the inhibition of cyclooxygenase 2, an enzyme that, amongst other functions, have been shown to participate in the degradation of AEA, induced an increase in glucose uptake in ex vivo hippocampal slices, which was blocked by CB2 activation (Köfalvi et al., 2016). However, it is possible that this effect is not due to the direct stimulation of glucose metabolism by CB2 but more because of a change on transporter localization dynamics in the plasma membrane, since the CB1/CB2 agonist WIN55,212-2 decreases mitochondrial oxidative glucose metabolism through CB1 receptors in astrocytes (Duarte et al., 2012).

As mentioned above, glutamate metabolism is another of the astrocytic functions that contribute to brain energy homeostasis and neuronal functions. Although glutamate is mostly metabolized into lactate in the TCA cycle, about 30% of the glutamate is transformed to glutamine to then be transported to neurons (Schousboe et al., 1993). When THC is injected in the brain of rats, the expression of the enzyme glutamine synthetase (GS) – that metabolizes synaptic glutamate into glutamine – is reduced (Suárez et al., 2002). Interestingly, endogenous 2-AG protects against GS changes induced by lipopolysaccharide (LPS) activation of cultured primary astrocytes: during the early phase of the treatment, GS shows an upregulation linked to p38 phosphorylation that can be blocked by 2-AG, an effect that is not present after treatment with AM630, a CB2 receptor antagonist. Moreover, during the late phase of activation via LPS, extracellular

signal-regulated kinases 1/2 (ERK1/2) phosphorylation mediates a downregulation of GS, which is reversed by 2-AG. This effect is partially blocked by both AM281 and AM630 suggesting the role of both CB1 and CB2 receptors in this process (Wang et al., 2018). Although underexplored, this evidence suggests a role for eCB modulation of glutamate/glutamine metabolism in astrocytes.

Regarding cannabinoid-modulation of other metabolic pathways in astrocytes, THC has been seen to activate carnitine palmitoyltransferase 1 (CPT1) and ketogenesis in cultured astrocytes in a CB1-dependent manner (Blazquez et al., 1999). This effect, together with other observations in other organs showing a CB1-dependent modulation of fatty acid oxidation (Jourdan et al., 2012), suggests a role for cannabinoids in the modulation of astrocytic fatty acid oxidation, which may be complementary to their effect in glucose usage. Finally, a work from our lab established an indirect regulation of glycogen content mediated by CB1 in astrocytes (Bosier et al., 2013), again opening new ways of cannabinoid regulation of astrocytic metabolism.

In summary, the impact of eCBs on astrocyte metabolism remains highly unexplored, but evidence of exogenous cannabinoids and receptor pharmacology points to a complex regulation of multiple pathways in these glial cells, which might link regulation of metabolism to brain functions and behavior.

## 5 | ROLE OF THE ENDOCANNABINOID SYSTEM IN ASTROGLIAL INFLAMMATORY PROCESSES

### 5.1 | Astrocyte implication in inflammation

Astrocytes are known to play key roles in the CNS inflammation in response to innate and adaptive immune responses. First, astrocytes are major players in the maintenance and permeability of the blood-brain barrier, thus controlling immune infiltration in the brain. Second, astrocytes are immune-competent cells, meaning that they are able to respond to danger signals through the release of cytokines and chemokines, activating adaptive immune defense (Colombo & Farina, 2016).

Like many elements of these pathways, the role of astrocytes in the development and maintenance of inflammation in the CNS is multiple. For instance, depending on the nature of the stimulus as well as the phase of the inflammation process, astrocytic immune activation may be detrimental or protective (Sofroniew, 2020).

One of the most-studied and best-known indicators of astrocytic responses to danger, linked to the astrocyte contribution to CNS inflammation, is astrogliosis. Astrogliosis has been broadly studied for over a century as a hallmark of brain disease conditions, and it was considered to be a morphological change occurring in astrocytes through the increased expression of GFAP, a major protein constituent of astrocytic intermediate filaments. However, nowadays, and thanks to the contributions of years of research in different labs, its definition has changed to a complex process whereby astrocytes, in



response to pathology, “engage in molecularly defined programs involving changes in transcriptional regulation, as well as biochemical, morphological, metabolic, and physiological remodeling, which ultimately result in gain of new function(s) or loss or upregulation of homeostatic ones” (Escartin et al., 2021). Interestingly, this process depends on the injury as well as the area affected and it can be protective or detrimental depending on the population, the nature of the danger or the state of the inflammation, so it seems meaningless to consider astrogliosis as a whole as a friend or a foe of the inflammatory processes (Escartin et al., 2021).

Interestingly, both endogenous and exogenous cannabinoids have been shown to be key players in the inflammatory responses of the brain, even being considered as potential targets in the treatment of some diseases that involve inflammatory processes (Kasatkina et al., 2021). The cellular mediators of these effects, however, are not clear yet. Given their role in the control of inflammatory responses, astrocytes are potential players in the modulation of inflammation by cannabinoids; however, the direct link is still missing. Here we will present some findings that point to astrocytes as possible mediators of the cannabinoid-mediated effects on CNS inflammation.

## 5.2 | Cannabinoid impact on astrocyte-mediated inflammation

Evidence on the role of cannabinoids in astrocyte-mediated inflammation came from studies in astrocytic cultures. In these early works, AEA was shown to modulate astrocytic immune responses to different stimuli, such as infection with Theiler's murine encephalomyelitis virus (TMEV) or treatment with LPS. In these works, AEA treatment decreases nitric oxide and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) release in response to these insults (Molina-Holgado et al., 1997), as well as to increase the release of interleukin-6, considered to be anti-inflammatory and immunosuppressive, in astrocytic cultures infected with TMEV (Molina-Holgado et al., 1998). Moreover, AEA and the synthetic cannabinoid analog CP-55940 reduced nitric oxide production induced by LPS stimulation in a CB1 and CB2 dependent mechanism (Molina-Holgado et al., 2002). In the same line, application of the synthetic cannabinoid WIN55,212-2 was shown to inhibit the generation of inflammatory mediators in interleukin-1 $\beta$ -stimulated human astrocytes (Sheng et al., 2005). Finally, UCM707, a potent and selective AEA uptake inhibitor, was able to reduce the production of proinflammatory molecules in LPS-treated astrocytes also in a CB1 and CB2 dependent manner, thus emphasizing a role of astroglial eCBs in this process (Ortega-Gutiérrez et al., 2005). These early works suggested a role of cannabinoids in the modulation of astrocytic inflammatory responses via different signaling mechanisms.

Interestingly, contrary to the other astrocytic functions explained in this review, the regulation of the astrocytic inflammatory processes by cannabinoids has been described to be modulated through different mechanisms. In all these early works, many of the cannabinoid effects were found to be modulated, but not completely blocked by CB1 and CB2 antagonism, suggesting an involvement of other ECS

members. As an example, recent evidence point to a protective role of palmitoylethanolamide (PEA), which can be considered as an “extended endocannabinoid” (Cristino et al., 2020), in different models of Alzheimer's disease (Beggiato et al., 2018; Bronzuoli et al., 2018; Scuderi et al., 2011). PEA has been demonstrated to exert some of its effects in astrocytes, reducing the release of inflammatory mediators and thus promoting neuronal survival (Beggiato et al., 2020). Interestingly, PEA seems to act through PPAR $\alpha$  receptors, which are expressed in astrocytes but are not the classic cannabinoid receptors (Scuderi et al., 2012).

Another mechanism by which astrocytes contribute to inflammatory processes seems to be the degradation of the eCB signals. In fact, it has been demonstrated that astrocytes play key roles in terminating the cannabinoid signals from neurons via degradation enzymes such as MAGL (Viader et al., 2015). In fact, astrocytic-specific deletion of MAGL attenuates LPS-induced neuroinflammation. Given that MAGL is the enzyme responsible of degradation of 2-AG to AA, a precursor of prostaglandin synthesis, the authors suggest that the reduction in the inflammation is due to the decrease in the synthesis of prostaglandins in the brain, rather than by an increased eCB tone (Grabner et al., 2016). Anyhow, the link between the eCBs and inflammatory molecules and their shared pathways in astrocytes needs to be addressed in more detail and seems also a promising candidate for the cannabinoid regulation of astrocytic-mediated inflammatory processes.

Nowadays, different cannabis-derived cannabinoids have gained interest due to their anti-inflammatory effects in different pathological models (Graczyk et al., 2021). Given the relevant role of astrocytes in brain inflammation, it seems logic that some of these effects of cannabinoids are mediated by astrocytes. As reviewed here, some evidences have found this link between cannabinoids, astrocytes, and inflammation. Interestingly, many of these effects seem to be mediated by other receptors than CB1 such as CB2 or nonclassical cannabinoid receptors, opening new possibilities by which cannabinoids may regulate the other astrocytic functions. However, the exact mechanism of the different cannabinoids in regulating CNS inflammation through astrocytes -especially in vivo- are still lacking and represent a great target for future studies and application of cannabinoids as brain anti-inflammatory agents.

## 6 | CONCLUDING REMARKS

Astrocytes emerged as active players in multiple CNS functions, from memory encoding to the immune defense of the brain. Interestingly, some of these functions have been discovered only recently, suggesting that we may have uncovered only the tip of the iceberg of astrocytic implication in brain processes. At the same time, although the study of cannabinoids has traveled a longer way, astrocytic implication in some of the effects of cannabinoids is just starting to emerge. As seen in this review, most of the studies so far regarding the cannabinoid modulation of astroglial functions are focused on the CB1 receptor. However, astrocyte express other ECS members which are

gaining more interest since they may be targets of the newly-discovered promising therapeutic components of the *cannabis sativa* plant, such as cannabidiol or cannabigerol. Thus, the field should address the potential modulation of astroglial functions by these new components through other ECS members, to have a complete picture of the role of cannabinoids in astrocytes in physiology and pathology. Also, cannabinoids might be implicated in other astrocytic functions with therapeutic interest not addressed in this review, such as in neurotransmitter or ionic uptake (Egaña-Huguet et al., 2021). To summarize, the field is just starting to address the role of astrocytes in some cannabinoid-mediated effects in the CNS, as seen in some recent discoveries discussed in this review. These studies, together with the possible implications of other ECS members in astrocyte physiology, suggest new research paths to understand how (endo)cannabinoid effects and functions can be exerted through astrocytes and, conversely, how astrocytes can contribute to higher-brain functions via cannabinoid signaling.”

## AUTHOR CONTRIBUTIONS

All authors conceived, wrote and edited this review.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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