REVIEW

Interplay Between Astroglial Endocannabinoid System and the Cognitive Dysfunction in Alzheimer's Disease

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Summary
Cannabinoid CB1 receptors have been shown to regulate wide array of functions ranging from homeostasis to the cognitive functioning but recent data support the hypothesis that astrocytes also operate as a mediator of synaptic plasticity and contribute to cognition and learning. The receptor heterogeneity plays a key role in understanding the molecular mechanisms underlying these processes. Despite the fact that the majority of CB1 receptors act on neurons, studies have revealed that cannabinoids have direct control over astrocytes, including energy generation and neuroprotection. The tripartite synapse connects astrocytes to neurons and allows them to interact with one another and the astrocytes are key players in synaptic plasticity, which is associated with cognitive functions. This review focuses on our growing understanding of the intricate functions of astroglial CB1 that underpin physiological brain function, and in Alzheimer's disease.

Key words
Alzheimer's disease • Cannabinoid • Astrocytes • Cognition

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Introduction
Alzheimer's disease (AD) is a progressive neurodegenerative disorder with cognitive and memory impairments. Pathological deposition of amyloid-β peptides, hyper phosphorylation of tau as paired helical filaments and neurofibrillary tangles were all reported, along with synaptic dysfunction, neuronal loss, enhanced neuroinflammation, and oxidative stress [1]. In turn, inflammation and oxidative stress also contribute to AD progression [2]. Astroglia have long been viewed as a passive and supportive cells, while microglia have recently received a lot more attention and are recognized to be implicated in the neuroinflammation of AD [3,4]. Recent evidence suggests that astrocytes play a paramount role in the progression of Alzheimer's disease (Table 1) [5,6,7]. In astrocytes a large number of genes are expressed, and more than 40 genetic loci have been linked to the risk of Alzheimer's disease. Endocytosis, metabolism, and inflammation have all been identified as new potential therapeutic targets in genome-wide association studies (GWAS) [8]. Interestingly, a recent transcriptome analysis revealed that in AD mice, 33 age-up genes were upregulated in astrocytes, while 53 age-down genes were downregulated [9].

Morphobiology of astrocytes
Astrocytes are star-shaped cells with branches that have specialized structures at the end of their processes called “end feet”. These are also known to regulate a variety of functions in the central nervous system (CNS).

Astrocyte heterogeneity
Based on their morphology and location astrocytes were previously classified as protoplasmic or
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<th>Impaired</th>
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The type of stimuli that activate astrocytes differs. For example, inflammation activates A1 astrocytes, which are neurotoxic, while ischemia activates A2 astrocytes, which are protective. Atrophic astrocytes and reactive astrocytes are the two types of astrocytes that are traditionally classified. Atrophic astrocytes have a lower volume and fewer processes, whereas reactive astrocytes have a larger volume and thicker processes [6]. The cytoarchitecture of astrocytes differs in vitro and in vivo, with cultured astrocytes having fewer ramifications than in vivo [12]. These glial cells undergo morphological and functional alterations under pathological conditions, transforming them into reactive astrocytes. These reactive astrocytes express certain cytoskeletal component proteins such as glial fibrillary acidic protein (GFAP), vimentin, nestin [13]. Furthermore, these reactive astrocytes also known to express increased levels of the enzyme monoamine oxidase-B (MAO-B) [6].

**Characteristic of astrocytes**

A complex, diverse larger astrocytes are generally found in humans. The main structural features of astrocytes include the presence of gap junctions due to connexins, filament bundles, glycogen granules, astrocyte extensions around blood vessels, synapses, presence of the aquaporins, receptors and the ion channels while, the main functional features include the non-excitability, high negative membrane potential, cell signaling mediated by Ca^{2+} ions, neurotransmitter uptake by various transporters. Astrocytes regulate energy metabolism, homeostasis, inflammatory axis, and various signaling pathways involving calcium signaling, purinergic signaling, Jak-Stat3, MAPK, TGF-β, cholesterol production and in growth factor (IGF-1, BDNF, CCL5, NGF) functions [10,14]. Thus, astrocytes play a crucial role to preserve neurological function. Any changes in their physiological functions may lead to various neurological diseases. One such disease is Alzheimer's disease (AD).

**Astrocyte functions**

Astrocytes perform more functions than any other sort of nervous system cell. These astrocytes form the scaffold for the entire nervous system and occupy a huge amount of the space of the CNS and form the majority of the structure that comprises the brain and spinal cord. They provide structural support as well as a home for all other cells, such as neurons and glia [3,10,15]. If the CNS is injured, these astrocytes proliferate and generate more astrocytes, which migrate to the affected location and undergo hypertrophy, where they develop into much larger, thicker, and longer...
processes that surround the damage and form a thick layer of scar tissue between them [10,15]. The entire process of astrocytes reacting to injury is termed as gliosis/astrogliosis/astrocytosis/reactive astrocytosis, and the scar tissue formed is known as a glial scar. Astrocytes also help in maintenance of homeostasis, specifically the homeostasis of the interstitial fluid, which is critical since neurons require a very finely tuned, even-keeled environment to function effectively. Astrocytes constantly monitor the interstitial fluid to keep ion concentrations at their optimal levels (K+ buffering), as well as release lactate and provide energy to neurons [15]. The contribution of astrocytes to the blood-brain barrier is another key role. Astrocytes attach their feet to the blood vessels that flow through the CNS, preventing numerous big molecules from exiting the bloodstream and entering the brain. The clearing out of synapses between neurons is another important function of astrocytes; it is vital to reset the synapse so that it can be used for communication again. Besides these functions, astrocytes have an effect on neurons and other glial cells, and vice versa, through the exchange of various chemicals [10].

Fig. 1. Astrocyte physiological functions and the formation of tripartite synapse with intercellular communications. Arrows in black indicates facilitatory affect. The image was created on Biorender.com under paid subscription.

Astrocyte communications

Gap junctions and purine signaling are used by astrocytes to communicate with other astrocytes, emphasizing astrocyte communication in the brain. Apart from direct communication via gap junctions, astrocytes use hemichannels to send signals to neighboring neurons, microglia, and oligodendrocytes, allowing for crosstalk. Astrocytes communicate with pre- and post-synaptic neurons, forming a tripartite synapse (a communication between pre- and post-synaptic neurons and astrocyte processes) (Fig. 1), and interact intercellularly via glutamate and ATP release via hemichannels (connexin 43), modulating synaptic function [10]. The peri synaptic astrocyte processes (PAPs) are the thin membranes surrounding synapses, and these express some lamellar proteins such as glutamate transporters (EAAT), neurotransmitter machinery, cholinergic receptors (α7nicotinic, muscarinic) while the post-synaptic astrocytes express CB1 cannabinoid, purinergic, TNF receptors, and the lactate metabolite transporter.
Apart from these, astrocytes release different molecules that act on synapses such as neurotransmitters: glutamate, GABA, acetylcholine, ATP and adenosine, metabolic substances such as lactate, and tissue hormones such as growth factors, TNF-α, which affect different facets of plasticity and this process is termed as gliotransmission. Gap junction coupling between adjacent glial cells is mediated by astrocytic-astrocyte extensive connections, which form a cellular network known as the astrocytic 'syncytium'. Astrocytes detect neural activity and react by increasing intracellular Ca$^{2+}$ levels and releasing transmitters. The hippocampus CA1 LTP, for example, requires D-serine, which is released by astrocytes. The release of D-serine raises NMDAR occupancy to a threshold level, triggering the downstream processes necessary for LTP induction. Astrocytes also express a variety of pumps and transporters, including the GLUT1 and MCT1 transporters, which deliver energy to astrocytes and neurons. The astrocyte has a lot of potassium (Kir 4.1) and calcium channels that help with ion influx and efflux across the membrane. GLT-1 and GLAST are astrocyte-specific glutamate transporters that contribute in the rapid clearance of neurotransmitters produced into the synaptic cleft. Astrocytes communicate with blood-brain barrier through the water channel aquaporin 4 which is required for the regulation of cell volume.

**Introduction to the endocannabinoid system**

Cannabinoid receptors (CBR) are seven-transmembrane spanning metabotropic receptors that attach to tetrahydro cannabinoil (THC), the main psychotropic component in marijuana. These CBR bind to endogenous ligands called endocannabinoids (eCBs), which include anandamide and 2-AG. They are the signalling molecules that maintain homeostasis after pathogenic insults. The CNR1 and CNR2 genes, which have been mapped to human chromosomes 6q14-15 and 1p36, encode the CB1 and CB2 receptors.

CB1R is located mainly in the cingulate gyrus, hippocampus, cerebellum, cortex, and basal ganglia. CB2R are found in the spleen, circulating immune cells macrophage-derived cells including microglia, astrocytes, tonsils, osteocytes, liver cells and also found in hippocampus, entorhinal cortex, hypothalamus, thalamus, striatum.

Endocannabinoids like anandamide (AEA) and 2-arachidonylglycerol (2-AG) are synthesized in the body under physiological conditions. AEA shows high affinity for CB1R while 2-AG exerts moderate to low affinity for both receptor subtypes. AEA acts as a partial agonist at CB1R while 2-AG acts as a full agonist for both receptors.

**Dual role of astrocytes in generation and elimination of amyloid-β peptides**

Astrocytes play a bidirectional role in the production and clearance of amyloid-β peptide, and the astroglial dysfunction contributes directly to the pathogenesis of AD. Increased oxidative stress and the generation of reactive oxygen species can trigger detrimental pathways in astrocytes, while reactive astrocytes that were supposed to eliminate or degrade β-amyloid become distorted and contribute to Aβ aggregation. Although astrocytic Aβ production is lower than that of neurons, due to their greater number, astrocytic Aβ production is known to have a significant impact on AD progression. The reactive astrocytes release number of inflammatory cytokines.

The inhibitory gliotransmitter GABA's uncontrolled release impairs hippocampus memory functions, whereas the astrocyte's inability to control the excitatory transmitter glutamate causes a loss of balance in frontal brain function. In Alzheimer's disease, pro-inflammatory cytokines are increased, and initial defensive inflammatory responses by reactive astrocytes against Aβ plaque development gradually contribute to amyloid load as a result of cellular and molecular stresses causing enzymatic alterations inside microglia and astrocytes. Interferon-γ, TNF-α, and IL-1β treatment of primary mouse astrocytes resulted in enhanced astrocytic production of amyloid precursor protein, as well as BACE1 and γ-secretase. Hence, the levels of AD biomarkers were progressively elevated due to exposure to pro-inflammatory factors, indicating that sustained exposure to inflammatory mediators leads to not only metabolic dysfunction but also increased amyloid expression. In contrast, Astrocytes help in the clearance of amyloid plaques via phagocytic and enzymatic pathways. In response to the presence of monocyte chemoattractant protein 1 (MCP1) in the lesions, astrocytes bind to Aβ proteins and elevate calcium and magnesium divalent ion exposure. The presence of additional binding molecules on Aβ, such as...
the receptor for advanced glycation end products (RAGE), proteoglycans, and low-density lipoprotein receptors, can cause astrocytes and amyloid-β to bind [38,39].

**Cannabinoid receptors and signaling in astrocytes**

The endocannabinoid system in astrocytes is fully functional, with the ability to produce and inactivate endocannabinoids. CB1 receptors are found in the spinal cord [40], the hippocampus [41], the neocortex [42], and the caudate putamen [43]. Mostly cannabinoid 1 receptors are expressed in the neurons [44], microglia [4] and other glial cells. CB1 receptors in the brain mediate a variety of metabolic and cellular events [45], influencing metabolic and behavioral activities, as evidenced by research from knock-out mice [46].

The endocannabinoid signaling involves a retrograde pathway in which the endocannabinoid mobilized from the post synaptic terminal activates the presynaptic CB1 receptors [47], which inhibit calcium channels (voltage-gated), hence limiting neurotransmitter release [48].

Because CB1 receptors are GPCRs, they are activated by ligand binding [49]. In astrocytes, these receptors are coupled to Gq/11 G-proteins, which activate the phospholipase C and diacyl glycerol pathway, generating inositol 1,4,5-trisphosphate and calcium mobilization from intracellular stores. This activation of CB1R elevates astrocyte Ca^{2+} concentrations, which evoke glutamate-mediated signaling in tripartite synapse [47].

Calcium signaling independent of the CB1R has also been proposed, which is known to be carried out by the transient receptor potential A1 (TRPA1) channels and reduces GABA release and D-serine [50]. Even though this process is not dependent on CB1R, it is modulated by endocannabinoids [45], and the cannabinoid system activates to protect neurons from excitotoxic injury by acting “on demand” [51].

The slender perisynaptic astrocytic processes engage physically with synapses in an active manner that is influenced by activity-induced plasticity signals, implying the role of astrocytes in physical synapse stabilization [15]. Because of the high K⁺ concentrations, astrocytes have a negative resting membrane potential and a high K⁺ permeability. The coupled astrocyte syncytium has “isopotentiality” due to its high baseline K⁺ conductance and extensive gap junctional coupling, which reduces activity-induced astrocyte membrane potential variations [52].

Astrocytes serve as a bridge for inter neuronal communication [47] and the cannabinoid mediated signaling plays an essential role in learning and memory. Gliotransmission involves various events that occur when the astrocytes release different bioactive molecules in a calcium dependent manner. Following depolarization, synaptic depression is induced and termed as the depolarization-induced suppression of excitation (DSE) in glutamatergic synapses [53] while in the GABAergic synapses it is depolarization-induced suppression of inhibition (DSI) [45].

Endocannabinoids activate the CB1 receptors during neuronal depolarization, which is crucial for resetting the synapse so that it can be used for communication again. Finally, astrocytes assist in the release of a neurotransmitter that are distant from the depolarized neurons. Thus, endocannabinoids activate a long-range signal involving astrocyte calcium mobilization and glutamate release, which results in synaptic potentiation of distant synapses indirectly. This process is known as lateral synaptic regulation, and it was initially discovered in the hippocampus [54].

The released glutamate activates CA1 pyramidal neurons as well as inhibitory GABAergic neurons when the Schaffer collaterals are activated. GABA activates GABA-B receptors in astrocytes, which triggers astrocyte calcium signaling and releases ATP, which converts to adenosine, resulting in unstimulated synaptic depression [55], thus the CB1R activation facilitates LTP by inducing LTD [56].

Lateral synaptic regulation is also known to be involved in the striatum, where striatal astrocytes respond to the calcium mobilization by endocannabinoids released from the GABAergic medium-spiny neurons. CB1R stimulation activates glutamnergic (NMDA) pre-synaptic receptors, resulting in circuit-specific lateral synaptic potentiation [57]. The stimulation of CB1 receptors by endocannabinoids causes purine release in neurons in the amygdala area.

Neurons in the amygdala region are also activated by the CB1 receptor stimulation upon binding of the endocannabinoids, which leads to the purine release. The released purine binds to the adenosine receptors inducing synaptic regulation in distant neurons. Purines released from the astrocyte activation bind to the purinergic A1 receptors of the presynaptic terminals and also stimulate presynaptic A_{2A} receptors.
endocannabinoids control synapses and modulate excitation/inhibition [45]. Long-term lateral potentiation is produced when astrocyte-to-pre-synapse communication occurs simultaneously with retrograde nitric oxide release signaling. The endocannabinoids-induced astrocyte lateral modulation has been shown to contribute to synaptic plasticity [58].

**Role of astrocytes in synaptic plasticity**

The endocannabinoid system has been known to be associated with the long-term depression and long-term potentiation of the synaptic plasticity. Long-term potentiation is induced by astrocyte induced release of the neurotransmitter when endocannabinoids are activated simultaneously with postsynaptic activity [59]. This is due to a sustained increase in the short-term potentiation of cannabinoid astrocyte induced release of the neurotransmitter.

Certainly, the cannabinoid induced LTP requires the correlation of cannabinoid-induced glutamate signaling and nitric oxide release in the post synaptic transmission. The activation of presynaptic glutaminergic and protein kinase C is induced by the sequential coincidence of these signals, resulting in persistent potentiation for neurotransmitter release. So, the long-term potentiation requires synchronized activity of the elements of the tripartite synapse, such as cannabinoid-induced calcium mobilization, which releases glutamate, nitric oxide generation, and protein C activation [54,59]. The endocannabinoids act as retrograde signals, depressing neurotransmission in the synapses and act as lateral messengers, inducing LTP in distant heteroneuronal synapses via astrocyte network activation [59].

Induction of LTP requires the stimulation of CB1R by the endocannabinoids on the astrocyte membrane and induces the release of D-serine which is required for the formation of NMDA dependent LTP [22] and this plays important role in the formation of recognition memory.

LTD is an extensive synaptic plasticity phenomenon that involves several events with different underlying mechanisms. The induction of LTD may depend on NMDAR, mGluR, presynaptic A1 and A2 receptors or on retrograde signaling via presynaptic cannabinoid (CB1) receptors. By actively exchanging signals with synaptic components of neurons, astrocytes help to modulate Spike-Timing Dependent Plasticity (STDP). This induction approach can result in STDP, in which the direction tLTD (Timing-dependent Long-Term Depression) and magnitude of plasticity are determined by the order and interval of the two spikes [60].

Timing-dependent LTP is produced by Ca2+ influx through postsynaptic NMDA receptors, similar to LTP in the hippocampus and other synapses, at the L4-to-L2/3 (spiny stellate cells) synapse. tLTD, on the other hand, is more complicated, requiring the activation of postsynaptic metabotropic glutamate receptors (mGluRs), postsynaptic voltage gated Ca2+ channels, presynaptic NMDA receptors, and, most importantly, cannabinoid receptors, which are activated by endocannabinoids produced in the postsynaptic cell.

Min and Nevian employed patch clamp recording for the assessment of tLTD mechanisms in rodent barrel cortex [68]. They found that tLTD inducing CB receptors were located on astrocytes and their activation increases calcium spiking for the induction of tLTD. The recurrent activation of astroglial CB1Rs gradually elevated astrocyte Ca2+ levels and led to astrocytic glutamate mobilization, which activated presynaptic NMDA receptors, implying that astrocytes activation is adequate for the induction of synaptic depression. In this way they act as a memory buffer, integrating associated neuronal activity via calcium accumulation [61]. This endocannabinoid (CB1R) facilitated tLTD is vital for the sensory neocortex development. They also presented that cannabinoid receptor blockade or synthesis inhibition or loading with calcium chelators prevents the calcium spiking and eventually the induction of tLTD (Fig. 2). CB1R agonists alone do not cause long-term plasticity without concurrent presynaptic activity [62]. It was reported that when direct astrocyte stimulation is paired with presynaptic afferent activity, LTD is elicited. This is prevented by NMDA blockade [61] (Fig. 2).

**Molecular interplay between endocannabinoid signaling in astrocytes and cognitive dysfunction in AD**

Glial changes are often known to precede the formation of AD pathology [12]. In post-mortem brains of AD, reactive astrocytes are classically observed in region with high Aβ and tau pathology. Activation of microglia can also cause astrocyte reactivity [6]. Reactive astrocytes contribute to neuroinflammatory alterations in Alzheimer's disease by producing cytokines, inflammatory factors, nitric oxide (NO), and reactive oxygen species (ROS) and raising the redox imbalance.
Astrocytes undergo structural and functional changes in AD [63,74], and the morphological changes arise in a region-specific pattern. Atrophy is first identified in the entorhinal cortex, followed by the prefrontal cortex, and finally the hippocampus at the pre-plaque stage [20].

Memory acquisition is sufficient to increase memory retrieval following pharmacogenetic or optogenetic activation of astroglial Gq signaling in the hippocampus, suggesting that astrocytes can improve cognition [64]. Astrocytic dysfunction may thus play a role in disease phenotype, but the extent to which it does so is uncertain. CB1Rs in astrocytic cells, not neuronal cells, were found to be key mediators of synaptic plasticity and behavioral effects in knock-out mouse models [46]. Despite the fact that there have been few studies on the cognitive and memory impairments in relation to astrocyte cannabinoids, it was reported that ablation of DAGL alters endocannabinoid concentrations and, eventually, behavior in a mouse model. It was demonstrated that heterosynaptic depression was increased by the CB1 agonist [65]. It was also reported that cannabis affected connexin 43 dysregulation via activating the CB1 receptor in astrocytes [66] and CB1 receptors also decreased glutamate release from glutaminergic neurons [67].

Recent research has shown that astrocytes...
respond to the endocannabinoids released during the induction process by elevating the levels of calcium and then releasing D-serine, which is required for LTP induction in vivo, as well as recognition memory consolidation [68,69,22]. In another study, it was reported that astroglial CB1R activation produced purines which induced LTP [70].

Anandamide reversed AMPA-induced neurotoxicity in cultured astrocytes and down regulated GLT-1 and GLAST transporters in CB1 dependent pathway [71]. Similarly, THC protected against MDMA toxicity and prevented astrogliosis via CBR dependent-mechanism [72]. Besides these cannabinoids also exert anti-inflammatory activities that can mitigate neuroinflammation in AD and other neurodegenerative disorders.

Anandamide inhibited inflammation in astrocytes via CB1 cannabinoid receptors. UCM707, a selective anandamide (AEA) uptake inhibitor, inhibited the generation of proinflammatory mediators by LPS-stimulated astrocytes. UCM707 decreased NO release, iNOS expression, TNF-α and IL-1β levels, and increased IL-6 levels [51]. Another CB agonist, WIN55,212-2 inhibited their release of NO, TNF-α, in human cultured astrocytes [73].

Conclusions
Much research is required to elucidate the detailed mechanisms underlying the astrocyte cannabinoid system in Alzheimer’s disease. Astrocytes are promising therapeutic target because of their pathogenic potential, excitotoxicity-mediated neuronal death in AD caused by increasing extracellular glutamate levels, GLT1 loss, and GABA release from astrocytes. Pathological astrocyte remodelling that alters their homeostatic and neuroprotective roles and the development of glia-targeting therapeutics is still in its early stages, with the most difficult task being to find astrocyte specific and therapeutically relevant compounds. However, pharmacogenetic manipulation of astrocytes and astrogial glutamate uptake could be a way to mitigate excitotoxicity or another way is to target astrocyte genes.

Conflict of Interest
There is no conflict of interest.

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Abbreviations

References


