**Astrocyte reactivity in Alzheimer’s Disease; Therapeutic Opportunities to Promote Repair**

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**Abstract**

Astrocytes are fast climbing the ladder of importance in neurodegenerative disorders, particularly in Alzheimer’s disease (AD), with the prominent presence of reactive astrocytes surrounding amyloid β- plaques, together with activated microglia. Reactive astrogliosis, implying morphological and molecular transformations in astrocytes, seems to precede neurodegeneration, suggesting a role in the development of the disease. Single-cell transcriptomics have recently demonstrated that astrocytes from AD brains are different from “normal” healthy astrocytes, showing dysregulations in areas such as neurotransmitter recycling, including glutamate and GABA, and impaired homeostatic functions. However, recent data suggests that the ablation of astrocytes in mouse models of amyloidosis results in an increase in amyloid pathology as well as in the inflammatory profile and reduced synaptic density, indicating that astrocytes mediate neuroprotective effects. The idea that interventions targeting astrocytes may have great potential for AD has therefore emerged, supported by a range of drugs and stem cell transplantation studies that have successfully shown a therapeutic effect in mouse models of AD. In this article, we review the latest reports on the role and profile of astrocytes in AD brains and how manipulation of astrocytes in animal models has paved the way for the use of treatments enhancing astrocytic function as future therapeutic avenues for AD.

Keywords: astrocyte, glial cells, Alzheimer’s disease, amyloid

1. Introduction
2. Astrocytes in AD brain
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5. **Introduction**

Astrocytes represent 20 % of cells in the human brain, although this proportion depends on the technique and marker used for quantification, with an increasing ratio of astrocytes to neurons with primate evolution [1]. Also known as astroglia, they were named after the star-like cells by Michael von Lenhossek in 1891 [2], even though there were previous descriptions and visualizations of astroglial cells by Otto Deiters dating back to 1865 [3]. Alongside their presence in the Central Nervous System (CNS), a population of astrocyte-like cells can be found beneath the intestinal epithelial cells, which are known as enteric glial cells (EGCs) [4].

Brain astrocytes are highly diverse, and their differences seem to be region-specific. Interestingly, human astrocytes are structurally larger with a threefold larger diameter and ten times the number of primary processes [5], and appear functionally more complex than those found in rodents. Initially, astrocytes were classified according to their morphology into protoplasmic (in grey matter, with highly branched bushy processes) and fibrous (in white matter, presenting straight and long processes). However, other more specialized astrocytic types have been described, such as Bergmann glia in the cerebellum, involved in structural support, and stem cells known as radial glia in the subventricular and subgranular zones, which are multipotent cells that can give rise to neurons [6].

Subtypes of astrocytes have also been defined according to their response to CNS insults. In the past, these “reactive states” were defined as analogous to those described for microglia or macrophages [7]. After an injury, prominent morphological changes take place, some of which include cell swelling and enlargement (hypertrophy), retraction of processes, proliferation, necrosis and formation of inclusions (Figure 1) [8]. Gene expression patterns are also modified to accommodate the physiological requirements of activated astrocytes [9, 10, 11]. In recent years, with the development of transcriptomics, astrocytes were classified into A1 and A2 states, whereby A1 presents a harmful, pro-inflammatory phenotype, whereas A2 promotes repair [12]. However, as it is the case for the M1 and M2 microglial types, this seems to be an overly simplistic classification, as in reality astrocytes are much more diverse. Because of all these controversies, a recent review including a consensus on the definition and nomenclature of the reactive astrocytes states was published, which includes guidelines to define the process of astrocyte remodelling as reactive astrogliosis [13]. This does not refer to astrocyte proliferation or “glial scar” formation other than in instances of physical trauma or blood-brain barrier (BBB) disruption. In addition, it was recommended that reactive astrogliosis should be assessed as changes in combinations of various molecular markers, not only overexpression of glial fibrillary acidic protein (GFAP) and morphological alterations, because not all astrocytes are GFAP positive [13].

The functions of astrocytes are as diverse as their morphology. They are involved in the modulation of synapses, regulating synaptogenesis and synaptic pruning. They also participate in adult neurogenesis [14, 15] and have been implicated in the recycling of neurotransmitters, such as glutamine [16]. Furthermore, astrocytes act as a potassium reservoir, maintaining a good supply of potassium, which is important for action potential. Astrocytic processes have been shown to be linked through gap junctions that facilitate inter-glia communication via propagation of calcium signals between coupled cells [17, 18], also known as astrocytic calcium waves [19, 20, 21]. Moreover, these gap junctions facilitate intercellular diffusion of ions, second messengers and metabolites such as glucose and ATP [21, 22, 23].

Astrocytes have additionally a metabolic function, producing lactate, cholesterol and Apolipoprotein E (ApoE) and have the capacity to store glycogen. Furthermore, astroglia form an integral part of the blood brain barrier (BBB), participating in its maintenance [15], regulating metabolic waste clearance and providing nutrients for neurons. Astrocytic endfeet are capable of regulating blood flow in response to neuronal activity, in a process also referred to as neurovascular coupling, by releasing vasoactive substances that control local blood flow [24, 25, 26, 27, 28].

Notably, astrocytes have a neuroprotective function, by producing neurotrophic factors and anti-inflammatory cytokines and are implicated in the modulation of oxidative stress [29]. Finally, they provide structural support and are involved in neuronal migration during development.

Most recently, single-cell RNA sequencing has identified five transcriptomically distinct astrocyte subtypes in the adult mouse cortex and hippocampus. Distinct gene expression profiles were found across all major astrocyte functions along with differential spatial distributions and morphologies [30].

Importantly, astrocyte dysfunction has been linked to many neurodevelopmental and psychiatric disorders [31, 32, 33]. Perturbation of the many protective and supportive functions of glial cells can have severe consequences in the brain. Therefore, it is crucial that these glial cells execute their normal functions without causing damage to surrounding cells and tissues or leading to chronic inflammation by excessive or prolonged activation [34]. Under certain neurodegenerative conditions or in situations of brain damage, astrocytes form an “astrocytic scar” or barrier surrounding the wounded area. In addition, they increase the secretion of pro-inflammatory mediators, such as nitric oxide and reactive oxygen species (ROS) via the accumulation of superoxide dismutase. However, similar to what is seen in microglia, this effort by reactive astrocytes to eliminate potentially damaging molecules entering the brain and halt their spread from pathology or injury sites to surrounding tissue could be eventually deleterious, if left unresolved and in chronic disease.

In this review, we analyse and summarise what has been reported on the role of astrocytes in AD brains, from anatomical studies and transcriptomics in the human brain, to the manipulation of astrocytes using animal models. In addition, we will discuss potential avenues for therapies targeting astrocytes.

1. **Astrocytes in AD brain**

The pathological potential of astrocytes in Alzheimer’s disease (AD) was originally suggested by Alois Alzheimer over a century ago [35]. Accumulation of hypertrophic reactive astrocytes around senile plaques is seen in post-mortem human tissue from AD patients (Reviewed in [36]) and in animal models of the disease (Figure 1) [37]. Astrocytes express receptors, such as RAGE, that directly bind to amyloid-β (Aβ) and contribute to their activation. Early histological findings interpreted the increased presence of astrocytes in acute lesions as evidence for their division and proliferation. In several experimental studies, reactive astrogliosis associated with amyloid plaques were shown to involve increased hypertrophy of resting astrocytes in addition to upregulation and intracellular accumulation of GFAP, rather than the proliferation or migration of these cells [38,39, 40]. The question of enhanced prominence versus absolute numerical increase based on the morphologically altered astrocytes and the apparent increase in numbers seen in AD brains has recently been investigated. Studies using human brain tissue demonstrated higher numbers of GFAP-expressing astrocytes in AD patients compared with non-demented controls, although both groups had similar numbers of total astrocytes double-labelled with GFAP and another astrocyte-specific marker, such as aldehyde dehydrogenase 1 L1 (ALDH1L1) or glutamine synthetase (GS) [41, 42]. Furthermore, investigations in animal models have reported either no increase or a modest increase in astrocyte proliferation [42, 43,44, 45]. Conversely, other studies have found an increase in astrocytic hypertrophy, without an increase in numerical density, only in the population associated with amyloid plaques [37]. Thus, this effect might be different depending on the location of astrocytes and the marker used to identify them.

In AD, the function of astrocytes has been found to be both neuroprotective and detrimental, depending on the disease stage. Astrocytes have been involved in the clearance and degradation of Aβ, by secreting neprilysin, insulin degrading enzymes, ApoE and also by phagocytosing Aβ [46]. Furthermore, they appear surrounding amyloid plaques, forming an astrocytic barrier, protecting the surrounding tissue from pro-inflammatory mediators. It was originally thought that this involved the migration of astrocytes to these sites, although this has been proven inaccurate [40]. In addition, astrocytes have been involved in the phagocytosis of dystrophic neurites [47], contributing to the clearance of synaptic debris.

Conversely, prolonged activation of astrocytes could also have adverse effects on neurons by inducing the secretion of ROS, nitric oxide, and other neurotoxic products, such as glutamate, as well as pro-inflammatory cytokines and chemokines [48]. Based on this, the abundance of reactive microglia and astrocytes in the vicinity of Aβ plaques could exacerbate the already neurotoxic environment and trigger further inflammatory responses causing even more neuronal damage or death [29]. Studies in primary astrocytic cultures treated with synthetic Aβ resulted in an enhanced calcium ion-wave signalling [49] and this has also been observed in animal models [50]. This effect may increase the levels of transmitters including glutamate, D-Serine, ATP, and Gamma aminobutyric acid (GABA), leading to glutamate-mediated excitotoxicity [51].

It is worth noting that there is evidence that astrocytes, as well as neurons, could be involved in the generation of Aβ. Astrocytes also express the amyloid precursor protein (APP) and the amyloidogenic β-APP cleaving enzyme (BACE1) [52,53], especially under inflammatory conditions, increasing the expression of both proteins.

A better understanding of reactive astrogliosis and its influence on the surrounding tissue would be beneficial to developing approaches that allow modulation of inflammatory responses since astrocytic responses seem to occur in a disease-, severity- and time-specific manner, and may or may not be detrimental to repair.

Recently, a quantitative mass spectrometry (MS)-based proteomic study of 2000 brains has shed light on the effects of AD protein network changes. These studies have helped identify potentially new microglial and astroglial biomarkers for AD in both the brain and CSF. Among the different modules, the authors found strong AD trait associations of the module or cluster of astrocyte/microglial metabolism and its enrichment in AD genetic risk factors and proved that these were linked more strongly with AD than with other biological processes, such as normal aging [54].

Investigations using single-cell RNA sequencing in AD brains suggest that major transcriptional changes appear early in the pathological progression of AD. The AD-pathology-associated astrocyte subpopulation showed preferential expression of GLUL and the AD risk factor clusterin, which have been shown to be upregulated in reactive astrocytes in response to neurodegeneration [55]. Another study analysing RNAs from 69,496 nuclei from the prefrontal cortical samples of AD patients and healthy controls revealed a dysregulation of neuroprotective glial cells in AD, which may contribute to impaired neurotransmitter recycling in astrocytes and an increase in the levels of glutamate secretion [56]. Furthermore, another report showed differential expression of genes between AD and healthy individuals, depending on the cell line. APOE was downregulated in Alzheimer’s disease oligodendrocyte precursor cells, oligodendrocyte and astrocyte subclusters while it was upregulated in a microglial AD subcluster [57].

Studies analysing the profile of astrocytes extracted from AD mouse brains suggest the presence of dysfunctional astrocytes in AD mouse models compared with wild-type controls, releasing increased levels of GABA [58], showing a pro-inflammatory phenotype [59] and reduced expression of genes involved in neuronal communication. Single-cell mRNA sequencing experiments in mouse brain revealed that certain astrocytic genes are up-regulated with ageing, including the reactive-astrocyte markers Serpina3n and Osmr, a well-known AD risk gene (*Apoe*) and a gene encoding a component of the complement cascade (*C4b*). Moreover, *Snca* (synuclein-α) and *Sncg* (synuclein-γ) were also upregulated throughout aging, adopting the reactive phenotype of neuroinflammatory A1-like reactive astrocytes [60].

1. **Manipulation of astrocytes in animal models of AD**

To investigate the potential functions of astrocytes in AD and the way whereby they change at different stages of the disease, different types of genetic and pharmacological manipulations have been performed in animal models of AD.

**3.1 GFAP knockout mice**

Glial reactivity is most identifiable through hypertrophy and upregulation of intermediate filament (IF) GFAP (glial fibrillary acidic protein) [61]. While the function of GFAP, beyond being a component of the glial cytoskeleton, is not fully understood, it is thought to be vital for cell motility [62], migration [63] and signal transduction [64], functions that are supported by studies *in vitro*. GFAP overexpression gives rise to internal aggregates and produces a severe neurodegenerative phenotype akin to what is seen in neurodevelopmental disorders such Alexander’s disease [65]. Nevertheless, harnessing gliosis through the targeting of GFAP expression has been an attractive method to investigate the role of astrocytes in models of AD.

The GFAP knockout mouse was first generated by Pekny and colleagues in 1995 [66], whereby full deletion of GFAP was accomplished through a targeted mutation at the embryonic stem cell stage. They found that the deletion of GFAP in mice does not cause any obvious phenotypic changes in movement, learning or memory nor in the histological architecture of the brain. The reason for there being no obvious change in phenotype may be due to the continued presence of Vimentin (Vim), another IF, although Vim is unable to fully compensate for the loss of GFAP. However, Vim up-regulation was noted in this model following induced brain injury in a pattern that was qualitatively similar to GFAP expression seen in WT animals exposed to the same trauma [66].

However, it was later reported, by Xu et al., that GFAP KO mice are less able to restrict Aβ plaques through formation of “mesh like aggregates” as compared to GFAP+/+ mice indicating that glial barrier formation and plaque interaction was impaired in these knockout mice [67].

**3.2 GFAP/Vim double knockouts**

Various studies have also been carried out in mice that are genetically deficient in both GFAP and Vim. Vim is expressed in astrocytes earlier in development than GFAP, but both undergo increased expression during reactive astrogliosis [68]. GFAP/Vim double-knockout mice display a number of physiological changes related to responses to trauma and CNS injury, showing reduced hypertrophy and glial barrier formation. However, loss of both GFAP and Vim also gave rise to an improved regeneration profile following injury with lower scar formation and greater regeneration [69, 70], overall highlighting the dichotomy of gliosis in an injury context.

However, when this double knockout model was further crossed with APP/PS1 mice to investigate the loss of astrogliosis in the context of AD, mice displayed a near two-fold increase in Aβ plaque formation at 8 and 12 months of age [71]. While no changes were seen in APP processing or degradation, there was evident neuritic dystrophy along with a far lower level of astrocyte interaction with surrounding amyloid plaques as compared to WT animals. The ability of astrocytes to hypertrophy, penetrate and interact with amyloid plaques likely aids in containing the growth of plaques. Reduced astrocyte interactions with amyloid plaques were also shown by Kamphius et al., although in this study overall plaque load was not altered in APP/PS1 mice lacking GFAP and Vim [72]. Despite conflicting results between these two studies, it is apparent that loss of GFAP and Vim context leads to astrocytes having a reduced ability to form protective barriers around amyloid deposits, a feature that is considered protective in early stages of AD. This phenomenon was inferred in earlier studies in GFAP knockout mice that show how integral IFs are to astrocyte motility and how they interact with their surrounding environment.

* 1. **Ablation of proliferative astrocytes (GFAP-TK model)**

Because the GFAP knockout models target GFAP+ astrocytes during their complete lifespan, another approach is to selectively ablate reactive astrocytes at specific time points. This can be achieved through the use of GFAP-TK mice, which express the herpes simplex virus thymidine kinase (HSV-TK) targeted to astrocytes via the GFAP promoter [73]. Treatment with the antiviral drug ganciclovir (GCV), which is metabolised by the TK enzyme into toxic nucleoside analogues, results in the termination of DNA synthesis and death of proliferating GFAP positive cells. Our group recently published that double transgenic APP23/GFAP-TK mice treated with GCV at 9 months of age, when they experienced early amyloid deposition and memory loss, showed increased levels of monomeric Aβ in cortex compared with APP23/GFAP-TK treated with vehicle (without changes in the density, number and size of amyloid plaques). In addition, these changes appeared alongside reductions in clearance factors ApoE and Neprilysin, but not on Aβ generation, suggesting a role for astrocytes in amyloid clearance. Furthermore, reactive astrocyte ablation resulted in reduced synaptic and neuronal density and led to poorer performances in hippocampal-dependent behavioural tests [74]. These effects could have been mediated either by the increase in Aβ or by the exacerbation of the pro-inflammatory profile observed in the APP23/GFAP-TK treated with GCV mice. The role of proliferating astrocytes has been questioned in AD, however, this study suggests that they are overall beneficial and that loss of functioning astrocytes on the progression of AD pathogenesis and cognition is largely deleterious.

**3.4 Pharmacological elimination of Astrocytes**

The approaches mentioned above, using the GFAP-TK mice or GFAP knockout animals, only allowed investigating the role of reactive astrocytes and affected only GFAP positive cells. Another approach to ablate astrocytes involves the use of toxins that specifically eliminate astrocytes. The selective astrocytic toxin L-alpha-aminoadipate (L-AAA) is a glutamate analogue that inhibits the glial glutamate transport specifically in astrocytes, causing oxidative stress leading to programmed cell death and apoptosis [75]. Results from our lab have shown that the elimination of astrocytes in *ex vivo* organotypic cultures of 5XFAD mice resulted in an increase in Aβ expression and a reduction of key degradation and clearance factors Neprilysin, ApoE and IDE, while triggering an inflammatory profile of microglial cells and a deterioration of dendritic spines in the hippocampus [76]. These results are in line with the data obtained in the GFAP-TK model.

* 1. **Deletion of Aquaporin 4**

The clearance of brain solutes via aquaporin-4 (AQP4) in astrocytic endfeet has also been targeted in the study of other key functions of astrocytic activity in AD. Perivascular astrocytes are highly involved in the clearance of solutes, but also importantly Aβ, from the brain parenchyma and they orchestrate this movement through the activity of water channel AQP4 present in their end-feet. Reactive astrocytes are known to have altered levels of AQP4, and its expression has been shown by some to change in AD [77]. AQP4 gene knockout in the APP/PS1 transgenic mouse model led to reduced neuroinflammation and caused increased brain Aβ accumulation, subsequently exacerbating synaptic protein loss, astrocyte atrophy and cognitive dysfunction [78].

**3.6 GFAP-associated ApoE knockout**

Astrocytic clearance roles have also been subject to investigation in ApoE knockout models, as astrocytes are the main source of CNS-related ApoE. ApoE can, however, be produced by other cell types such as neurons and microglia/macrophages under certain circumstances. ApoE has become an interesting player in the development and progression of AD due to the varying risk levels associated with ApoE isoforms E2, E3 and E4. However, in order to shed light on astrocyte-related ApoE and its role as a risk factor for AD, selective deletion of ApoE in astrocytes was carried out in the APP/PS1 mouse model for AD [79]. Zheng et al. generated APP-ApoE and APP-GFAP-ApoE knockouts (whole body and astrocyte specific respectively) and found a stark amelioration of AD pathology with both whole body and astrocyte specific ApoE knockout, along with improvements in spatial learning and memory deficits. Furthermore, they concluded that the improvements in AD pathology were due to the impairment of the TGF-β/Smad2/STAT3 pathway signalling, as reversal of this inhibition caused the opposite effects in pathology.

**3.7 Reductions of astrocyte reactivity by targeting JAK2-STAT3 pathway**

JAK2-STAT3 has been reported to be a master regulator of astrocyte reactivity in vivo. A recent study has suggested that this pathway is involved in the induction and long-term maintenance of reactive astrocytes. Interestingly, its inhibition by viral astrocytic injection reduced amyloid load, improved spatial learning and restored synaptic function in AD mouse models [80].

1. **Treatments targeting astrocytes to promote repair**
	1. **Therapeutic window to target astrocytes in AD**

As mentioned earlier, the vast majority of therapeutic efforts remain focused on the neurocentric model of AD, targeting either Aβ plaque or tau pathology [81]. Given the presence of sustained neuroinflammation in many neurodegenerative disorders including AD, it is puzzling that only a limited number of studies to date have explored potential treatments directly targeting glial function and reactivity. This is of interest as alterations in macro and microglial reactivity are often, if not always, observed following disease-alleviating treatments in transgenic animal models of AD. Arguably, changes in inflammatory profiles may be secondary to Alzheimer’s proteinaceous burden and pathogenicity; however, the possibility that astrocytic changes may precede or drive the human disease cannot be ruled out given their physiological properties and essential functions. Some of these include their evolutionary conserved abundance and heterogeneity in the brain, and involvement in the tripartite synapse, network oscillations, homeostatic regulation and altered activation states in several neuropathologies [82, 83, 84, 85, 86, 87]. The immense complexity of the astrocyte response in AD and similar disorders may underlie the difficulty researchers face in developing a targeted therapy.

The onset and temporal progression of cerebral reactive astrogliosis in AD patients remains elusive due to lack of clinically established biomarkers. A small number of studies detected higher levels of CSF GFAP in patients with AD compared with controls [88, 89], which was significantly and inversely correlated with cognitive integrity, in terms of both executive functions and processing speed [90]. More recently, higher plasma GFAP levels were reported in cognitively normal older adults with elevated brain Aβ load and at risk of developing AD, compared to participants with low brain Aβ [91], suggesting that astrocytic markers can already differentiate populations at risk within the optimal therapeutic time window prior to synaptic and neuronal loss. High CSF and plasma GFAP levels are believed to be indicative of early astrocytic damage and atrophy in AD patients, similar to increased CSF and plasma levels of neurofilament light chain (NfL), a biomarker for neurodegeneration [92,93]. Contrary to traditional views, the astrocytic response is neither homogenous throughout the brain nor unidirectional during disease progression. In fact, several lines of evidence document region-specific astroglial atrophy prior to Aβ deposition as early as 1 month of age in several mouse models of AD, and chronic activation and senescence particularly in close proximity to Aβ plaques in later stages of disease [37, 94, 95, 84]. The latter has been confirmed by the use of bioluminescence lines, which has allowed detection of *in vivo* age- and transgene-dependent increases in the cerebral reactive astrogliosis, which correlated with the onset of Aβ deposition in the brain of transgenic APP mice [85].

 It is also worth noting that early in the course of disease, reactive astrogliosis may be beneficial since its inhibition exacerbated disease severity in mouse models of AD [71,75] and spinocerebellar ataxia [96]. Therefore, promoting ‘astroprotection’, reversing ‘astrosenescence’ and preventing the transition of reactive astrocytes from a neuroprotective to neurotoxic state in chronic disease could prove to be most beneficial (reviewed in [97].

**4.2 Targeting glutamate buffering by astrocytes**

Numerous lines of evidence point to disruptions of glutamate homeostasis in the AD brain. Increased extracellular glutamate concentrations can lead to overstimulation of glutamatergic NMDA receptors, synaptic dysfunction, neuronal damage and ultimately cognitive impairment [98]. Interestingly, astrocytes play a major role in extracellular glutamate transport via excitatory amino acid transporters, also known as EAATs [99], thereby preventing glutamate-induced neuronal excitotoxicity [100]. GLT-1 or EAAT2 is the main glutamate transporter in the brain, expressed predominantly by astrocytes and in low levels by neurons [101,102], and its levels are reduced both in ageing and in AD [103]. In co-cultures of primary neuron and astrocyte, Aβ has been shown to directly and significantly downregulate GLT-1 expression levels [104]. Notably, examination of donated brains from cognitively normal subjects with similar AD pathology to patients with dementia revealed activated astrocytes with elevated GLT-1 expression along with a higher number of neurons and synapses compared with the brains of demented patients [105]. Therefore, restoration of astrocytic GLT-1 expression may be beneficial in AD. The FDA-approved drug ceftriaxone, a representative of beta-lactam antibiotics, was shown to stimulate GLT-1 expression in astrocytes and exert neuroprotection [106]. In the 3xTg AD mouse model, pharmacological upregulation of GLT-1 by ceftriaxone ameliorated tau pathology, restored synaptic proteins and rescued cognitive decline with minimal effects on Aβ pathology [104]. Ceftriaxone was also shown to upregulate GLT-1 levels and improve cognitive function in APP/PS1 mice [107], whereby the beneficial effects of the drug on cognition were inhibited by GLT-1 knockdown in the same mouse model of AD [108].

**4.3 Astrocyte-targeted therapeutic gene delivery**

Cell type-specific gene delivery approaches have proven instrumental for identification of novel treatment targets in various neurological diseases. A clear example of this is the targeted viral delivery of calcium-sensitive Kir4.1 channel to striatal astrocytes in a Huntington’s disease mouse model, which resulted in restoration of homeostatic astrocyte functions and increased astrocytic GLT-1 expression, along with prolonged survival and behavioral improvements [109]. Interestingly, alterations in astrocytic Kir4.1 have also been identified in AD, Parkinson’s disease, Multiple sclerosis, amyotrophic lateral sclerosis, epilepsy, and autism spectrum and mood disorders [110, 111, 112]. Yet, it remains to be investigated whether manipulation of similar astrocytic ion channels holds therapeutic potential in AD.

Studies on intrahippocampal AAV vector delivery of APOE ε2 allele in the PDAPP mouse model of AD resulted in substantial reductions of insoluble Aβ1–40 and Aβ 1-42 (~60 and 70%, respectively), soluble Aβ1–42 (~30%) and oligomeric Aβ (~40%) at 8 weeks post injection [113]. The same group also demonstrated that APOE2 gene delivery could ameliorate APOE4-dependent amyloid pathology in the APP/PS1/TRE4 mouse model of AD [113]. In contrast to the primary physiological source of APOE in the brain, in this study APOE2 was not expressed solely in astrocytes. To overcome this, previously developed adeno-associated virus (AAV)-ApoE vectors driving expression under the GFAP or other astrocyte-specific promoters could be used [114]. Due to the close association between astrocytes and GFAP expression, the GFAP promoter has been regularly used in other astrocyte targeted interventions; however, there are some important caveats to employing GFAP as an astrocyte specific promoter, including the fact that GFAP is not expressed by all astrocytes [13], and is more closely associated with reactive states in neurodegenerative disorders and proliferative states following brain or spinal injury [115]. Moreover, GFAP is also expressed by other cells types in the CNS such as in neural and glial progenitor cells [116,117]and constitutive loss of GFAP may have implications beyond astrocytes. To overcome this limitation, other viral vectors using astrocyte-specific promoters such as ALDH1L1, GLAST, GLT-1 or Gfa2 could be used in future studies [118].

Other therapeutic avenues to explore include the enhancement of brain- and glial-derived neurotrophic factors such as BDNF and GDNF, respectively, using astrocyte-targeting viral vectors. In relation to this, restoration of BDNF level in the P301L tauopathy model of AD, using AAV-GFAP-BDNF, improved cognitive deficits and exhibited both synapto- and neuroprotective properties [119], without affecting tau pathology. Similar benefits were observed in the 5XFAD model of amyloidosis [120], whereby conditional BDNF production in astrocytes resulted in memory improvement and increase in synaptic markers. Astrocyte-targeted GDNF gene therapy was also found to be protective against cognitive decline in both aging rats and 3xTg AD mice, without affecting Aβ or tau levels in the latter [121, 122].

Studies of factors involved in cytokine production and glial phenotype switching in neurodegenerative disorders are growing. Targeting hippocampal astrocytes by localized injection of adeno-associated virus (AAV) vectors driving the expression of VIVIT under the astrocyte-specific Gfa2 promoter reduced amyloid pathology, synaptic dysfunction, and cognitive deficits in middle-aged APP/PS1 mice [123]. VIVIT is a peptide that interferes with the immune/inflammatory calcineurin/NFAT (nuclear factor of activated T cells) signalling pathway and reduces astrocyte activation [124].

**4.4 I2-Imidazoline receptor ligands**

An unconventional target for modulation of reactive astrocytosis and inflammatory processes in AD has also emerged. I2-Imidazoline receptors (I2-IRs) are found in the outer membrane of mitochondria in astrocytes [125,126,127], and have been proposed to regulate astrocytic GFAP expression [128]. We recently observed that treatment with the I2-IRs ligand BU224 increased levels of both GFAP and the glutamate to glutamine converting enzyme, GS, in 5XFAD mice [129].

Interestingly, sub-chronic and chronic administration of various I2-IRs ligands have produced promising results in mouse models of AD and aging, improving or restoring several cognitive and memory functions [129, 130, 131, 132, 133], with diverse effects regarding amyloid deposition, which may depend on the length of the treatment [129, 133].

The exact mechanisms driving I2-IR-mediated neuroprotection remain to be fully elucidated. However phenotypically, treatment with I2-IR ligands reduced pro-inflammatory markers of microglial activation including Iba1, Il-1β and TNFα across these studies [129,130,131, 133]. Moreover, anti-apoptotic and neuroprotective effects, as well as improved synaptic integrity, were also observed [129,130]. I2-IR ligands including the endogenous ligand agmatine were also reported to exert neuroprotection against glutamate-induced neurotoxicity [134-136]. Since some of these ligands were shown to increase levels of GS [129], it remains to be investigated whether the beneficial effects of such compounds are mediated by more effective glutamate buffering via increased astrocytic expression of transporters such as GLT-1. In support of this, treatment with LSL60101 was previously shown to increase GLT-1 immunoreactivity in the adult rat cortex [137]. Additionally, I2R ligands can exert neuroprotection by blockade of NMDA receptor channels. Studies carried out in our lab on calcium signalling suggest that BU224 may restore Aβ-induced alterations of NMDA function [129].

**4.5 Physical exercise and astrocyte reactivity**

Considering that localized reactive astrogliosis occurs in close proximity to plaques and astrocytic atrophy in more distal regions in AD, a global increase in brain GFAP levels along with a shift in activation state toward a more neuroprotective phenotype may, in fact, be favourable. In line with this, exposure of aged mice to physical activity or environmental enrichment (EE) resulted in higher hippocampal GFAP levels as well as increased morphological complexity in astrocytes that also coincided with cognitive improvement [138].

Long-term exposure to voluntary physical exercise or EE also increased GFAP levels in hippocampal astrocytes in both 3xTg and WT mice, leading to morphologically indistinguishable hippocampal astrocytes between both groups [139]. Similarly, long-lasting physical stimulation by voluntary exercise in 5XFAD mice also ameliorated behavioural impairments related to nest building behaviour and anxiety, and improved hippocampal-dependent learning and memory [140]. In these transgenic mice, voluntary exercise increased levels of hippocampal GFAP, BDNF and post-synaptic marker PSD95, without altering either Aβ pathology, overall astrocytic density, microgliosis, neurogenesis or neuronal survival. Interestingly, GFAP-positive astrocytes also displayed exercise-induced morphological alterations including enhanced branching in 5XFAD mice, specifically in plaque-associated astrocytes [140]. Conversely, others have reported a reduction in GFAP levels following long-term exposure to voluntary exercise in APP/PS1 mice [141,142], indicating that the effects of exercise or environmental enrichment on astrocytic markers or reactivity may depend on the age of animals, length and intensity of stimulation, disease model, brain region and method of analysis. These studies further highlight the dynamic complexity of astrocytic phenotypes and activation states.

**4.6 Targeting astrocyte-driven GABA inhibition by MAO-B inhibitors**

Excessive levels of GABA, synthesized by monoamine oxidase-b (MAO-B) in reactive astrocytes, can contribute to memory deficits in animal models of AD [143,144]. Increased expression of MAO-B in reactive astrocytes was reported in both postmortem brains from AD patients [145,146] and in animal models of AD [140,144]. Considering this and the detrimental effects of tonic GABAergic inhibition on synaptic transmission and consequential impairment of synaptic plasticity and memory formation [143,147], it is thus not surprising that astroglial MAO-B has also been suggested as a therapeutic target for AD treatment. To date, short-term but not long-term treatment with selegiline, a MAO-B inhibitor, has shown promise in both preclinical [143] and clinical studies [148,149]. The irreversible nature of the drug and recovery of GABA levels by compensatory mechanisms has been proposed to underlie the lack of long-term effects [150]. Accordingly, the search for more potent and reversible MAO-B inhibitors is ongoing and such drug candidates have so far produced encouraging results in animal models of AD [150].

* 1. **Transplantation of glial progenitor cells and enteric glial engrafts**

As astrocytes have been involved in regulation of synaptic formation and memory, some groups have explored the potential of transplantation of human precursors of astrocytes on memory function in mice. Engraftment of human glial progenitor cells into neonatal immunodeficient mice resulted in an enhancement of long-term potentiation in the human glial chimeric mice, as well as in cognition [151]. In 3xTg mice, transplanted human nervous system stem cells initially differentiated into neural stem cells in the brain and subsequently into immature neurons and glial cells [152]. Synaptic density and memory consolidation but not learning ability were improved in these mice, without changes in levels of Aβ or tau protein. Other studies have directly transplanted astrocytes isolated from adult and neonatal mice into the hippocampi of transgenic APPSwe1PS1dE9 (APPdE9) mice, showing that murine astrocytes can internalize human Aβ deposits [153]. In addition, studies in a mouse model of human tauopathy also supported the neuroprotective role of astrocytes by transplanting neural precursor cell-derived astrocytes, showing a reduction in neuronal death in cortical areas [154].

Alternatively, autologous engraftment of enteric glial cells (EGCs) isolated from the gastrointestinal nervous system into the brain of an Aβ1-42-induced AD rat model revealed functional similarities between these cells and resident astrocytes in response to increasing plaque burden [155]. EGCs were shown to migrate to sites of amyloid deposition and significantly reduce fibrillar Aβ load, neurofibrillary tangle burden and levels of pro-inflammatory markers in the brains of these animals, relative to Aβ-infused control rats that did not undergo EGC transplantation. Interestingly, no signs of immune-related graft rejection were observed, while elevated levels of NGF, BDNF, GDNF and AQP-4 were also reported [155]. Furthermore, EGC engraftment improved both learning spatial memory skills in these animals. Although promising, the long-term consequences, safety and efficacy of this and similar approaches in humans are unknown and demand further research.

1. **Conclusions and future directions**

Reactive astrogliosis is common across several CNS pathologies and in AD it is initially beneficial and protective for brain tissue. Astrocytes carry out vital roles in ionic homeostasis, neurotransmission, inflammation, degradation and clearance of pathological material and misfolded proteins, and in some instances glial scar formation and structural tissue repair. Models with loss-of-function astrocytes have confirmed that certain reactive astrocyte states are highly beneficial and supportive in pathological conditions. However, during chronic disease, reactive astrogliosis and in some cases astroglial atrophy ultimately leads to dysfunction [156]. This fact is highlighted by the alleviation of certain aspects of disease, particularly in relation to propagation of inflammation and neurotoxic pathology with the loss of reactive astrocytes.

However, the study of astrocytes provides a unique challenge in part due to their highly interactive nature and bidirectional communication with surrounding neurons and microglia. In particular, great potential lies in understanding their interactive role with microglia via the “astrogliosis-microgliosis axis” [156]. Nevertheless, due to the limited number of studies employing astrocyte-based AD models and the somewhat conflicting evidence in reports, further research with these models is crucial. Determining the point beyond which astrocytes transform from reactive to dysfunctional warrants further investigation and the development and employment of additional astrocyte-focused animal models may shed light on this topic.

Potential interventions targeting astrocytes may have great prospects for AD. For instance, recently it was highlighted the role of circadian rhythm in astrocytes [157]. Circadian clock transcription factor BMAL1 was found significantly elevated in cerebral astrocytes from patients with AD and has been linked to impaired aerobic glycolysis in astrocytes [158]. It is important to note that in a non-AD context, deletion of astrocyte-specific Bmal1 induced reactive astrogliosis, promoted neuronal and astrocytic death *in vitro* [159] and lead to similar phenotypic changes in addition to glutamate excitotoxicity, impaired metabolism, cognitive deficits and shorter lifespan [160,161]. For instance, the physiological consequences of targeting circadian clock proteins and their downstream targets are most likely disease- and context-dependent, thus requiring transient, reversible and/or appropriately dosed therapeutic interventions.

Additionally, targeting astrocytes at the neurovascular unit, and in particular regulating the expression and polarization of AQP4, would be worth investigating in the future. Deficits in astroglial facilitation of water transport due to miss-localization of AQP4 in reactive astrocytes may also negatively impact the paravascular glymphatic system, involved in both Aβ and tau clearance, that is impaired in AD and several other neurological condition and affects the clearance of Aβ. Interestingly, AQP4 levels are regulated during sleep, linking the clearance of Aβ by astrocytic endfeet with alterations in the circadian rhythm [162].

Overall, most evidence hint at the neuroprotective role of astrocytes in AD, particularly in promoting amyloid clearance and increased synaptic plasticity, suggesting that enhancing the beneficial functions of these glial cells, would provide an exciting new strategy to treat AD pathology and symptoms.

**Abbreviations**

AAV, Adeno-associated virus; AD, Alzheimer’s disease; Aβ, amyloid-β; APP, amyloid precursor protein; ApoE, Apolipoprotein E; AQP4, Aquaporin 4; BBB, Blood-brain barrier; BACE, beta-APP cleaving enzyme; BDNF, brain-derived growth factor; EGCs, enteric glial cells; GABA, Gamma aminobutyric acid; GCV, ganciclovir; GDNF, glial-derived nerve factor; GFAP, glial fibrillary acidic protein; GLT-1, Glutamate transporter-1; GS, glutamine synthase; I2Rs, I2 imidazoline receptors; Iba1, ionized calcium binding adaptor molecule 1; IDE, insulin degrading enzyme; MAO-B, monoamine oxidase-B; NGF, nerve growth factor; PS1, presenilin-1; ROS, reactive oxygen species; TK, thymidine kinase; Vim, vimentin; WT, wild-type.

**Conflict of Interest**

The authors have declared that no conflict of interest exists.

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**Figure legends**

Figure 1. Phenotype difference of astrocytes in wild-type and transgenic AD mice. Staining in FAST-clear section of hippocampus of wild-type and 5XFAD mice. A) Less reactive ‘wisp-like’ astrocytes and ramified microglia in WT. B) Aβ plaques surrounded by reactive astrocytes in 5xFAD. (GFAP; cyan), microglia (iba1;red) and Aβ plaques (ThioS; green). 100µm thick z-stack; x63 objectives.

Figure 2. Schematic diagrams of the neuroinflammatory response in AD brain. A) Homeostatic state of astrocytes and microglia in the healthy brain parenchyma. B) Release of pro-inflammatory cytokines and chemokines as well as proteins involved in the clearance and degradation of Aβ by reactive astrocytes (hypertrophic) and activated microglia (amoeboid morphology), which are often found in the vicinity of Aβ plaques, as are intraneuronal inclusions of hyperphosphorylated tau known as neurofibrillary tangles. C) Homeostatic regulation of neurovascular coupling in the healthy brain, which is essential for meeting neuro-metabolic demand and tightly regulated by a selective network of specialized endothelial cells making up the blood brain barrier and surrounded by pericytes and astrocytic end-feet. D) Pathologically driven neurovascular uncoupling or dysfunction in the AD brain is associated with a heightened inflammatory profile in astrocytes and microglia, detachment of astrocytic endfeet from blood vessels, pericyte loss, metabolic deficits, neurodegeneration, blood-brain barrier leakage and infiltration of peripheral immune cells.